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ABSTRACT

While hypertension has been linked to various modifiable and fixed risk factors, the exact mechanisms behind blood pressure (BP) regulation and hypertension onset remain elusive. The rapid development in scientific branches studying genome, proteome, metabolome, and metagenome, coined collectively as 'omics', offer robust methods for studying complex biological phenomena in large population samples.

The motivation of the current study was to assess the relation of the metabolome and metagenome with high BP in large Finnish cohorts. The specific aims were to estimate the association between gut microbiota and hypertension, to study the plasma metabolic profile of hypertension, and to elucidate if a family of circulating polyunsaturated fatty acid derived small molecule regulators of systemic inflammation, eicosanoids, are associated with BP.

This and previous population studies demonstrate that gut microbiota is associated with human hypertension. Biologically reasonable pathophysiological mechanisms, including ones related to sodium intake, have been proposed to explain the phenomenon. However, the significance of these effects on population BP and health remains unclear and warrants further research.

In this thesis, we demonstrate a strong association between circulating eicosanoids and BP in humans. We also use conventional statistical methods and multivariable machine learning models to define a metabolic profile of hypertension and blood pressure change using high abundance serum metabolic measures. Our results suggest that particularly serum lipids, and particularly low density lipoprotein-derived and very low density lipoprotein-derived cholesterol measures, and glucose metabolism abnormalities are associated with hypertension onset.

Our studies improve the current knowledge on the associations of gut microbiota and circulating metabolites with BP. Metabolomics and metagenomics offer novel approaches to improve hypertension risk prediction and to discover potential targets for therapeutic intervention of elevated BP.

KEYWORDS: blood pressure, dietary salt, epidemiology, hypertension, lactobacillus, LC–MS, metabolomics, metagenomics, NMR, shotgun sequencing

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
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TIIVISTELMÄ

Verenpainetaudin ilmaantuminen on yhteydessä lukuisiin riskitekijöihin, joista osaan voimme vaikuttaa elintavoilla ja joista osa on synnynnäisiä. Tästä huolimatta ymmärryksemme verenpainetaudin synnystä on vielä puutteellinen. Viime vuosina tutkijoiden käyttöön on tullut laaja joukko uusia biomolekyylien tutkimusmenetelmiä, joita kutsutaan yhteisnimellä 'omiikat'. Tämän kehityksen myötä myös suurten kansallisten tutkimusaineistojen analysointi on muuttunut kustannustehokkaaksi.

Väitöskirjani keskeisenä tutkimuskysymyksenä oli selvittää, tarjoavatko nämä biomolekyylien tutkimusmenetelmät uutta tietoa kohonneesta verenpaineesta suurissa suomalaisissa väestöaineistoissa. Väitöskirjani ensimmäisessä osassa tutkimme suolistobakteerien yhteyttä verenpainetautiin ja ravinnon suolaan. Väitöskirjani toisessa osassa tutkimme verenkierron aineenvaihduntatuotteiden yhteyttä verenpainetautiin.

Tuloksemme yhdessä aikaisemman tutkimustiedon kanssa tukee käsitystä, että verenpaineen ja suolistobakteerien välillä on yhteys. Kykenemme myös jo kertyneen tiedon valossa esittämään hypoteeseja yhteyden tautiopillisesta perustasta. Erityisesti ravinnon suola vaikuttaa isäntälajin lisäksi myös suolistobakteerien elinolosuhteisiin. Emme kuitenkaan vielä kykene arvioimaan, onko suolistobakteerien ja verenpaineen välisellä yhteydellä kansanterveystieteellistä merkitystä, mutta odotamme tulevaisuuden tutkimusten vielä tuovan tähän kysymykseen vastauksen.

Väitöskirjani jälkimmäinen puoli tukee verenkierrossa kulkevien pienten rasvaliukoisten tulehdusvälittäjäaineiden, eikosanoidien, yhteyttä verenpainetautiin. Tutkimme myös sekä perinteisiä tilastollisia menetelmiä että koneoppimismalleja käyttäen seerumin aineenvaihduntatuotteiden profiilia verenpainetaudissa. Tulostemme perusteella seerumin rasva- ja sokeriaineenvaihdunnan häiriöt, erityisesti korkea LDL- ja VLDL-kolesteroli, kytkeytyvät verenpainetaudin ilmaantuvuuteen.

Väitöskirjani tuotti uutta tieteellistä tietoa suolistobakteerien ja verenkierron aineenvaihduntatuotteiden yhteydestä verenpainetautiin. Metabolomiikka ja metagenomiikka tarjoavat joukon tehokkaita uusia menetelmiä tutkimukselle, jonka tavoitteena on korkean verenpaineen ehkäiseminen tai hoitaminen.

AVAINSANAT: epidemiologia, laktobasilli, LC–MS, metabolomiikka, metagenomiikka, NMR, suola, shotgun sequencing, verenpaine, verenpainetauti

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Abbreviations

AA	Arachidonic acid
ACE	Angiotensin-converting enzyme
ADMA	Asymmetric dimethylarginine
Apo	Apolipoprotein
ATC	Anatomical Therapeutic Chemical classification
ATR	Angiotensin II type 1 receptor
BM	Bone marrow
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
CI	Confidence interval
COX	Cyclooxygenase
CRP	C-reactive protein
CYP	Cytochrome P450
DHA	Docosahexaenoic acid
DILGOM	Dietary, Lifestyle and Genetic determinants of Obesity and Metabolic syndrome study
DNA	Deoxyribonucleic acid
EC_{50}	Half maximal effective concentration
EET	Epoxy-eicosatrienoic acid
FDR	False discovery rate
FHS	Framingham Heart Study
FIMM	Institute of Molecular Medicine Finland
FITC	Fluorescein isothiocyanate-dextran
FMT	Fecal microbiota transplantation
GPCR	G-protein-coupled receptor
Gper1	G-protein coupled estrogen receptor
Gpr41	G protein-coupled receptor 41
GWAS	Genome-wide association study
HDL	High density lipoprotein
HETE	Hydroxyeicosatetraenoic acid

hs-CRP	High-sensitivity C-reactive protein
ICD	International classification of diseases
IDL	Intermediate-density lipoprotein
IL	Interleukin
IL-6R	Interleukin-6 receptor
KO	Kyoto Encyclopedia of Genes and Genomes Orthology group
LA	Linoleic acid
LC–MS	Liquid chromatography–mass spectrometry
LDL	Low-density lipoprotein
LOX	Lipoxygenase
LT	Leukotriene
m/z	Mass-to-charge ratio
MAP	Mean arterial pressure
MDS	Multidimensional scaling
MLN	Mesenteric lymph nodes
MR	Mendelian randomization
MUFA	Monounsaturated fatty acids
NADPH	Nicotinamide adenosine dinucleotide phosphate
NMR	Nuclear magnetic resonance spectroscopy
Olfr78	Olfactory receptor 78
OR	Odds ratio
OTU	Operational taxonomic units
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PG	Prostaglandins
PGI_2	Prostaglandin I_2, prostacyclin
PERMANOVA	Permutational multivariate analysis of variance
PUFA	Polyunsaturated fatty acids
PVN	Paraventricular nucleus of hypothalamus
PWV	Pulse wave velocity
RAG	Recombinase-activating gene
RAS	Renin-angiotensin system
RMSE	Root-mean-square error
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
ROS	Reactive oxygen species
SCFA	Short-chain fatty acids
SFA	Saturated fatty acids
sICAM-1	Intercellular adhesion molecule-1
SHR	Spontaneously hypertensive rat

SNP	Single-nucleotide polymorphisms
SNS	Sympathetic nervous system
T_H	T helper cell
TG	Triglycerides
THL	Finnish Institute for Health and Welfare
TMA	Trimethylamine
TMAO	Trimethylamine-oxide
TNFα	Tumor necrosis factor alpha
Treg	Regulatory T cell
TXA_2	Thromboxane A2
VLDL	Very-low-density lipoprotein
WKY	Wistar Kyoto rat
WGS	Whole genome shotgun sequencing

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Palmu J, Lahti L, Niiranen TJ. Targeting Gut Microbiota to Treat Hypertension: A Systematic Review. *International Journal of Environmental Research and Public Health*, 2021, 18: 1248.

II Palmu J, Salosensaari A, Havulinna AS, Cheng S, Inouye M, Jain M, Salido RA, Sanders JG, Brennan C, Humphrey GC, Sanders JG, Vartiainen E, Laatikainen T, Jousilahti P, Salomaa V, Knight R, Lahti L, Niiranen TJ. Association Between the Gut Microbiota and Blood Pressure in a Population Cohort of 6953 Individuals. *Journal of the American Heart Association*, 2020, 9: e016641.

III Palmu J, Watrous JD, Mercader K, Havulinna AS, Lagerborg KA, Salosensaari A, Inouye M, Larson MG, Rong J, Vasan RS, Lahti L, Allen A, Cheng S, Jousilahti P, Salomaa V, Jain M, Niiranen TJ. Eicosanoid Inflammatory Mediators Are Robustly Associated With Blood Pressure in the General Population. *Journal of the American Heart Association*, 2020, 9: e017598.

IV Palmu J, Tikkanen E, Havulinna AS, Vartiainen E, Lundqvist A, Ruuskanen MO, Perola M, Ala-Korpela M, Jousilahti P, Würtz P, Salomaa V, Lahti L, Niiranen T. Comprehensive biomarker profiling of hypertension in 36 985 Finnish individuals. *Journal of Hypertension*, 2021.

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1 Introduction

Hypertension is the leading modifiable cause of cardiovascular disease, disease-adjusted life years, and premature death worldwide (GBD 2017 Risk Factor Collaborators, 2018). Hypertension is a prevalent and inadequately treated condition observed approximately in one of third adults globally while current treatment and control rates are generally unsatisfactory regardless of the income level of the distinct countries (Zhou et al., 2021). While hypertension has been linked to various modifiable and fixed risk factors, the exact mechanisms behind blood pressure (BP) regulation and hypertension onset remain elusive. Our incomplete understanding of the pathogenesis of hypertension is also reflected in drug development where the focus over the last decade has been in fixed-dose combinations of the established antihypertensive medications rather than in the discovery of novel treatment options (Ali et al., 2017; Saklayen & Deshpande, 2016).

The rapid development in scientific branches studying genome, proteome, metabolome, and metagenome, coined collectively as 'omics', offer robust methods for studying complex biological phenomena in large population samples. The research protocols of the Finnish cohort studies FINRISK 1997–2012, Health 2000/2011, and FinHealth 2017 included the collection of wide range of biological samples (plasma, serum, urine, and stool) that were stored in foresight with the future development of the research methodology. Today, linking the data acquired from these biological samples with information from health questionnaires, physical measurements, and longitudinal register-based follow-up, enables the researcher to execute cutting-edge epidemiological studies in well-phenotyped, large-scale human cohorts.

Changes in gut microbiota have recently been linked to various chronic diseases such as obesity (Petriz, 2014), metabolic syndrome (Fändriks, 2017), diabetes mellitus (Qin et al., 2012), cardiovascular disease (Wang et al., 2011), and even 15-year mortality risk (Salosensaari et al., 2021). Pioneering animal models implementing fecal microbiota transplants and studying genetic deletions and direct metagenomics analyses have suggested that gut microbiota is linked with host BP (Vijay-Kumar et al., 2010; Yang Tao et al., 2015; Adnan et al., 2016). Intriguingly, high salt intake, a classic risk factor for both hypertension and cardiovascular

disease, has been demonstrated to modulate mural gut microbiota particularly depleting *Lactobacillus* species (Wilck et al., 2017). Consistently, oral administration of *L. murinus*, the most strongly modulated *Lactobacillus* strain isolated from the mural feces, prevented the development of salt-sensitive hypertension in mice (Wilck et al., 2017). In Study I of this thesis, we performed a systematic literature review to summarize the current knowledge of the link between gut microbiota and BP. In Study II, we analyzed the cross-sectional associations between gut microbiota, dietary sodium intake, and BP in FINRISK 2002.

Metabolites are the intermediate and end products of the numerous biological processes occurring in living organisms. In addition to host metabolism, gut microbiota derived metabolites such as short-chain fatty acids (SCFAs) have the ability to enter the host circulation affecting the host homeostasis (Al Khodor et al., 2017; Pluznick, 2017). The measurement of circulating metabolites and metabolic biomarkers provides functional information about the host physiology offering a natural method to study BP regulation and hypertension onset. To date, most studies reporting results for the link between the circulating or urine metabolome and BP have been performed in small cohorts and with a small number of metabolites (Islam, 2017). Recent development in high-throughput nuclear magnetic resonance spectroscopy (NMR) and liquid chromatography mass spectrometry (LC–MS) offer two cost-effective methods with distinct strengths and weaknesses to study large human cohorts (Nikolic et al., 2014). In Study III, we analyzed the cross-sectional associations between BP and comprehensive panel of >500 distinct high-quality upstream eicosanoids and related oxylipin mediators in FINRISK 2002 using LC–MS. In Study IV, we analyzed the cross-sectional and longitudinal metabolic profile of hypertension in the FINRISK 1997–2012, Health 2000/2011, and FinHealth 2017 cohort studies using NMR measured high abundance serum biomarkers.

2 Literature Review

2.1 Gut microbiota and hypertension

Technical discoveries have foreshadowed our understanding of the microbes from microscopy in 1670, microbial cultivation in 1857, mass spectrometry in 1911, polymerase chain reaction (PCR) technique in 1983, next-generation sequencing in 2005, and to third-generation sequencing in 2008 (Berg et al., 2020). The scientific branches studying the genome, proteome, metabolome, and metagenome, coined collectively as 'omics', offer robust method for studying microbial communities (Figure 1). In particular, metagenomics offers information about the presence and abundance of microbes in biological sample (including microbes that would be difficult to culture) and metabolomics offers information about active functional processes in the microbial community. The collection of microbes in a biological system is called microbiota, and microbiota in context with the biological, physical, and chemical properties of the microbial habitats is called the microbiome (Berg et al., 2020). We summarize in following paragraphs the main tools used in metagenomics and review the studies reporting associations between gut microbiota and BP.

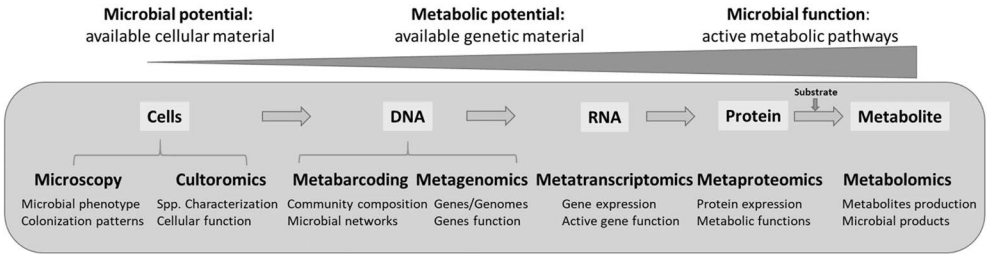

Figure 1. The description of different methods available to study microbial communities*.

* Released under Creative Commons Attribution license (Berg et al., 2020).

2.1.1 Sequencing gut microbiota

Gut microbiota is composed of bacteria, viruses, bacterial viruses, and fungi that reside in the gastrointestinal tract. Rectal swapping and collecting stool samples are noninvasive and convenient methods that allow large scale and longitudinal gut microbial study designs (Claesson et al., 2017; Knight et al., 2018; Quince et al., 2017). While these two collection methods offer limited control over sampling sites, contain dead bacteria, include bacteria from unspecified sites, and may underrepresent important microbial colonies, both methods are regarded as an acceptable proxy for distal gut microbiota (Claesson et al., 2017).

Appropriate delivery and storage protocol for stool samples is required to preserve the microbial deoxyribonucleic acid (DNA). Sample deterioration can be prevented using commercially available preservation kits. The main benefit of a kit is extended ambient temperature storage and reduced number of freezing-thawing cycles (Anderson et al., 2016). For studies, in particular, performed without preservation kits, uniform storage protocols, the ambient temperature exposure, and freezing conditions should be reported and evaluated (Vogtmann et al., 2017).

Extracting microbial DNA from stool samples is complicated by the presence of host DNA and various substances present in the gastrointestinal tract. Most commercial applications are based on chemical and mechanical cell lysis techniques performed in buffers that protect the liberated nucleic acids (Persson et al., 2011). The extracted and cleaned DNA can be sequenced using either 16S ribosomal RNA (rRNA) sequencing or shotgun metagenomic sequencing.

2.1.1.1 16S rRNA sequencing

Ribosomes are cellular particles that translate the messenger ribonucleic acid (RNA) into sequence of amino acids complying the central dogma of molecular biology: DNA makes RNA, and RNA makes protein. The 16S rRNA is a component of the prokaryotic ribosome and therefore ubiquitous in bacteria (Claesson et al., 2017). The 16S rRNA gene consists of nine hypervariable regions flanked by more conserved sequences (Martinez-Porchas et al., 2017). PCR primers can be targeted to the conserved regions of the 16S rRNA gene while the bacteria can be identified reading the sequence of the hypervariable region (Figure 2). PCR primers can also be labeled with specific barcode sequences that identify different samples (hosts).

The sequence reads can be clustered into operational taxonomic units (OTUs). The usual clustering threshold is 97% similarity between sequences (Claesson et al., 2017). The nucleotide sequences of different OTUs can be compared with reference databases of previously sequenced bacteria to infer likely taxonomic classification (Johnson et al., 2019). The conventional 16S rRNA is often accurate at the genus level while full-length 16S rRNA sequence data could potentially provide taxonomic

resolution of bacterial species and strain level (Johnson et al., 2019). However, amplicon sequence variants could replace OTUs in future offering finer sequencing resolution (Callahan et al., 2016). Because viruses and fungi do not possess 16S rRNA gene, the choice of this particular marker gene limits the observed results to bacteria and archaea.

2.1.1.2 Shotgun metagenomic sequencing

Instead of selecting specific marker genes for PCR, in whole genome shotgun sequencing (WGS) the complete extracted DNA is randomly sheered into desired fragment sizes for processing (Claesson et al., 2017). The short sequence reads of 150–400 base pairs (in second generation sequencing) are often assembled to longer sequences using reference genomes or de novo methods (Figure 2). The assembled DNA sequences are grouped by their likely host genomes and assigned taxonomy using reference databases (Claesson et al., 2017). Using databases with genes annotated with, for instance, KEGG Orthology groups, allows calculating functional profiles for potential molecular functions present in microbiota (Hillmann et al., 2018).

Compared to 16S rRNA, WGS is more costly and requires more fine-tuned quality control in addition to excluding the host DNA from analyses (Claesson et al., 2017). However, incorporating barcodes in primers in WGS allows simultaneous high-throughput sequencing of multiple samples; barcoded DNA fragments allow simultaneous sequencing of multiple samples in process coined shallow WGS which makes the method cost-effective alternative for 16S rRNA in large studies (Hillmann et al., 2018). Nevertheless, the benefits of WGS compared to 16S rRNA that include greater microbial resolution and detection of non-bacterial microbes, require the contribution from a skilled bioinformatician and ability to perform computationally intensive calculations in cluster computing environment (Claesson et al., 2017).

2.1.2 Gut microbiota data analysis

2.1.2.1 Microbiota data are compositional

High-throughput sequencing produces a random sample of the relative abundances of the observed microbes because the total microbial load (number of microbes) is unknown (Gloor et al., 2017). Therefore, microbial data are inherently compositional and changes in few species can alter the observed counts for all species and changes in total microbial load make different scenarios indistinguishable (Figure 3). Relative abundance data can be studied using appropriate statistical methods or estimating the microbial load using additional biochemical analyses (Morton et al., 2019).

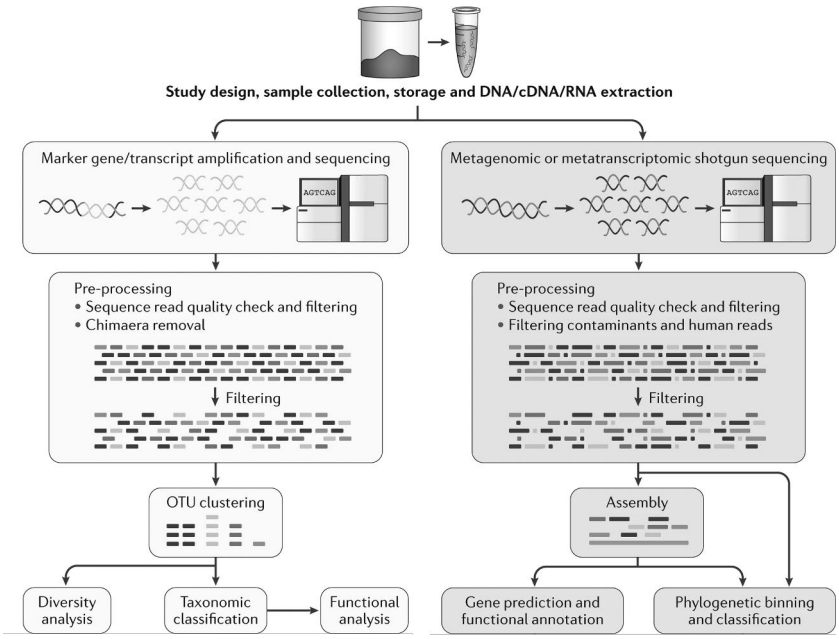

Figure 2. The two main sequencing methods in the metagenomics*.

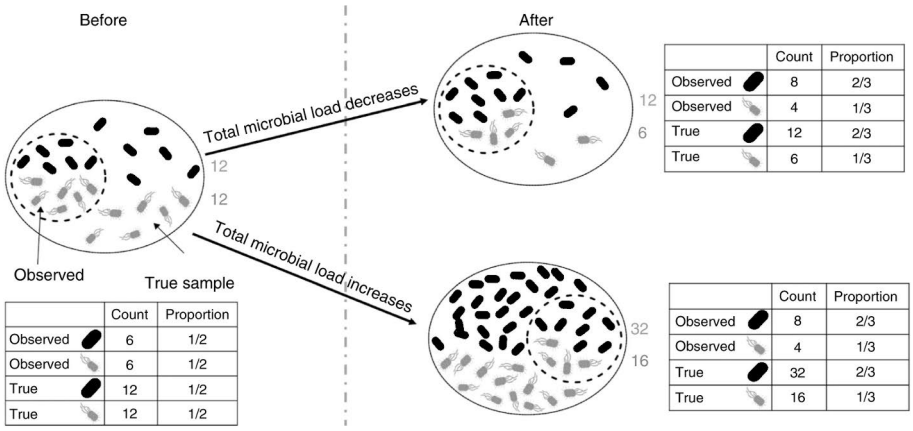

Figure 3. Microbial data is inherently compositional†.

2.1.2.2 Taxonomic diversity

While diversity is straightforward concept, formulating its mathematical definition has proved difficult in practical applications (Daly et al., 2018). Natural communities

* Adapted by permission from Springer Nature (Claesson et al., 2017).
† Licensed under Creative Commons Attribution license (Morton et al., 2019).

can be described using two levels of diversity: within-sample variance called alpha diversity and between-sample variance called beta diversity (Whittaker, 1960). These ecological concepts, originally intended to characterize vegetation in large geological areas, can also be applied in metagenomics.

The straightforward definition for alpha diversity is the number of distinct taxa present in a sample, information that is independent from the properties of all other samples. A more sophisticated and popular choice for alpha diversity is the Shannon's index (Daly et al., 2018). Shannon's index was originally developed to measure information content in data. In metagenomics, the index quantifies the difficulty to identify randomly picked taxon from all taxa in sample. The index is low in samples with few taxa and high in samples with large number of taxa with varying abundances; observation from limited sample may also underestimate the diversity.

Beta diversity describes one-on-one differences between samples. Two straightforward definitions for beta diversity are Manhattan distance and Euclidian distance: respectively the sum of the absolute and squared differences between number of each taxon in the two studied samples (Legendre & De Cáceres, 2013). Bray-Curtis dissimilarity, commonly used indices for beta-diversity, scales the Manhattan distances by the total number of taxa observed in the two compared samples (Legendre & De Cáceres, 2013). The dissimilarity between two samples increases as the differences between proportions of shared taxa increase ranging zero to one (Qian et al., 2020).

2.1.2.3 Comparing microbial abundances

There are multiple benchmarked and consistent methods to study differences between distinct microbial abundances (Schurch et al., 2016; Weiss et al., 2017). The small number of replicates and large number of low prevalence taxa often observed in metagenomics, require sophisticated methodology to reach adequate statistical power in analyses and to avoid biases.

DESeq2 is commonly used library in metagenomics to study differences in fold-changes, the ratios between observed counts, between studied groups or conditions (Love et al., 2014). DESeq2 performs internal normalization for microbial counts which accounts for varying microbial load. DESeq2 also uses Bayesian dispersion estimate to reliably estimate the variances in low-abundance taxa (Figure 4). This shrinkage procedure reduces the chance of false positives in taxa with abnormally low dispersion and false negatives in taxa with moderately high dispersion (Love et al., 2014). To reduce the variance in fold-changes of low count taxa, DESeq2 uses zero biased Bayesian method to shrink fold-changes based on the amount of information that was present in the model. The Bayesian method also provides

standard errors for the observed associations. DESeq2 uses Wald test for significance testing.

For a more detailed explanation, DESeq2 models sequenced count data K_{ij} using negative binomial distribution $K_{ij} \sim \mathrm{NB}(\mu_{ij}, \alpha_i)$ for taxon i and sample j, where μ_{ij} represents fitted means and α_i the dispersion parameters. Fitted means $\mu_{ij} = s_j q_{ij}$ are scaled using size factors s_j that is based on median of the geometric mean of the microbial counts. Linear predictors are mapped to model variable using link function $\log_2 q_{ij} = x_j \beta_i$ where x_j are model covariates and β_i the estimated parameters for covariates. DESeq2 performs analyzes in four steps: first size factors s_j are estimated using geometric means, second dispersions α_i are estimated using Bayesian model, third negative binomial generalized linear model is fitted for β_j, and finally significance is tested using Wald statistics with false discovery rate (FDR) correction. FDR correction is a method to compensate for performing large number of statistical tests; using uncorrected *P*-values would inflate the number of false positive associations observed at $P<0.05$.

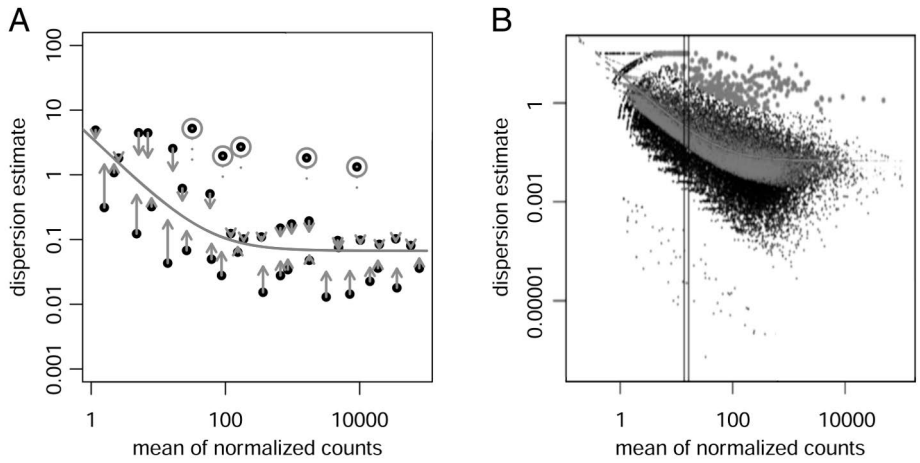

Figure 4. DESeq2 uses shrinkage estimation for dispersion to reduce model biases*.

The choice of negative binomial distribution in DESeq2 is reasonable (Y. Chen et al., 2014). While the read counts could be modeled using binomial distribution $\mathrm{Bin}(n, p)$, the calculations would be straining due to large number of sequencing reads. Poisson distribution $\mathrm{Pois}(\lambda)$ with $\lambda = np$ could be used to approximate $\mathrm{Bin}(n, p)$ when $n \to \infty$. However, due to single parameter fixing both mean and variance, Poisson distribution can only estimate technical variation of repeated

* Licensed under Creative Commons Attribution license (Love et al., 2014).

measures from single sample. Estimating the λ parameter with Gamma distribution, a family of positive valued continuous probability distributions, allows the model to account for the biological variation in Poisson parameters between samples. Calculating compound probability distribution of Poisson distribution with Gamma distribution produces the negative binomial distribution. The choice of negative binomial distribution can also be justified studying the mean-variance relationship of the sequence data (Y. Chen et al., 2014).

2.1.3 Animal studies

2.1.3.1 Fecal microbiota transplantation

The first metagenomics study analyzing gut microbiota and BP was a cecal transplantation in Dahl rats reaching family level resolution using 16S rRNA (Mell et al., 2015). Salt-sensitive (S) and salt-resistant (R) Dahl rats were fed high-salt diet, gavaged antibiotics to ablate the native microbiota, and transplanted with S or R rat cecal contents. R to S rats had higher BP and shorter life-span compared to S to S rats after single bolus of gavaged cecal content. No differences were observed between S to R and R to R groups. To study whether the effect was introduced by antibiotic treatment or fecal microbiota transplantation (FMT), the authors compared the BP between S rats with and without antibiotic treatment. In this study, the BP in two groups did not differ indicating that BP change in FMT observed in S rats was not due to the loss of the original gut microbial composition. However, R to S had higher circulating acetate and reduced urinary sodium excretion compared to S to S giving possible mechanism for host-microbial interactions in salt-sensitive hypertension.

In another rodent study, FMT from spontaneously hypertensive rats (SHR) to normotensive Wistar Kyoto rats (WKY) increased BP compared to FMT from WKY to WKY (Adnan et al., 2016). In another study, FMT from SHR to SKY increased T cell activation in mesenteric lymph nodes (MLN), circulating T cells, aortic T cell infiltration, and impaired endothelial function in addition to previously reported increase in BP (Toral, Robles-Vera, de la Visitación, Romero, Sánchez, et al., 2019). FMT from WKY to SHR also reduced the production of reactive oxygen species production and proinflammatory cytokines in the paraventricular nucleus (PVN) of the hypothalamus indicating that gut microbiota may modulate sympathetic response of the host (Toral, Robles-Vera, de la Visitación, Romero, Yang, et al., 2019). Notably, a cross-species FMT from hypertensive individuals to mice resulted to higher BP compared to FMT from a normotensive individual at 10 weeks post-transplantation (Li et al., 2017). Previous FMTs also induced changes in gut microbiota of the rodents, as would be reasonable to expect.

2.1.3.2 Gut wall permeability

Chronic angiotensin II infusion in SHRs has been demonstrated to induce changes in gut wall permeability (Santisteban et al., 2017). Hypertensive rodents had reduced number of goblet cells and tight junction proteins, and stunted villi. Intestinal permeability was assessed measuring plasma levels of gavaged fluorescein isothiocyanate-dextran; SHRs had increased fluorescent marker levels compared to normotensive WKYs indicating increased functional permeability in hypertension that are consistent with the histopathological changes. The decreased level of tight junction protein in young prehypertensive SHRs compared to normotensive age-controls suggests that gut wall pathology could precede or at least have close temporal connection to the onset of hypertension.

In addition to previous histopathological changes, SHRs have reduced levels of mucin and increased levels of circulating endotoxins compared to normotensive WKYs (Robles-Vera et al., 2020). Mucin is a barrier protein produced in goblet cells that protects gut wall against pathogens. Reduced gut wall permeability could lead increased levels of bacterial wall components such as lipopolysaccharides (endotoxins) to enter host circulation where they can activate toll-like receptors promoting oxidative stress and vascular inflammation (Liang et al., 2013).

In SHRs, candesartan treatment increased both the expression of genes encoding tight junction proteins and ameliorated depletion of *Lactobacillus* species induced by hypertension (Wu et al., 2019). Another renin-angiotensin system (RAS) inhibitor, captopril, also demonstrated attenuated gut wall pathology and dampened posterior pituitary neuronal activity in SHRs maintained over prolonged withdrawal (T. Yang et al., 2019). Vasopressin is released from the posterior pituitary.

2.1.3.3 Short-chain fatty acids

SCFAs are gut microbial fermentation products of dietary fibers that can be absorbed in the host circulation where they can potentially modulating host homeostasis (Pluznick, 2017). While the gut microbiota may not be the only contributor to host SCFA production, germ-free mice have been reported to possess negligible endogenous production of the three primary SCFAs, acetate, propionate, and butyrate (Perry et al., 2016). The plasma concentration of the three primary SCFAs were 30–400 μmol/l and dry weight fecal molality 10–300 μmol/g in mice (Perry et al., 2016). The serum levels of SCFAs have consistently been positively associated with dietary fiber levels in mice (Trompette et al., 2014). In summary, gut microbiota contributes to SCFA production and only proportion of SCFAs enter circulation.

Recognizing the ligand profile of a family of renin release and glomerular filtration rate modulating G-protein-coupled receptor (GPCRs) led to the discovery that SCFAs act as ligands for GPCRs (Pluznick, 2017). Olfactory receptor 78

(Olfr78) mediates renin secretion in juxtaglomerular apparatus (Pluznick et al., 2013). Both Olfr78 and G protein-coupled receptor 41 (Gpr41) are expressed in the muscle cells of resistance vessels (Pluznick et al., 2013). In knock-out animal models, $Gpr41^{-/-}$ knockout mice developed hypertension and $Olfr78^{-/-}$ knockout mice hypotension compared to wild type mice (Pluznick et al., 2013; Natarajan et al., 2016). Notably, Gpr41 has a lower half maximal effective concentration than Olfr78, and, therefore, Gpr41 could be active at basal concentrations, whereas the activation of Olfr78 could help to balance the effect (hypotension) at high concentrations (Pluznick, 2017). OR51E2 is human ortholog with murine Olfr78 (Pluznick, 2017). In summary, SCFAs modulate host homeostasis binding to GPCRs (Table 1).

Table 1. G-protein-coupled receptors binding SCFAs associated with hypertension.

	G protein-coupled receptor 41 (Gpr41)	**Olfactory receptor 78 (Olfr78)**	**Olfactory receptor 51E2 (OR51E2)**
Species	Humans, mice	Mice	Humans
Ligands	Acetate, butyrate, propionate	Acetate, propionate, lactate	Acetate, propionate
Location	Vascular epithelium	Renal afferent arteriole, vascular smooth muscle cells	Gastrointestinal tract
Null mice	Gpr41-/- mice are hypertensive	Olfr78-/- mice are hypotensive	
References	(Natarajan et al., 2016)	(Pluznick et al., 2013)	(Pluznick, 2017) (R. Muralitharan & Marques, 2021)

Hypertension has been linked with reduced numbers of SFCA producing bacteria and decreased circulating SCFAs (Robles-Vera et al., 2018). Treatment with *Bifidobacterium* and *Lactobacillus* probiotics has been linked with increased butyrate-producing bacteria while direct treatment with acetate and butyrate alleviated the gut-wall pathology in SHRs compared to normotensive WKYs (Robles-Vera et al., 2020). Acetate supplementation has also been associated with reduced cardiac fibrosis, left ventricular hypertrophy, and hypertension in mineralocorticoid-excess treated mice compared to mice fed control diet (Marques et al., 2017). High dietary salt was associated with increased fecal acetate, propionate, and isobutyrate levels in rats (Bier et al., 2018). Intervention with candesartan, an angiotensin II type 1 receptor blocker, was associated with improved gut wall permeability, as well as increased fecal acetate, propionate, and butyrate levels in SHRs compared to controls (Wu et al., 2019).

2.1.3.4 Gut modulated sympathetic activity

A seminal work in mice with genetic deletion of the recombinase-activating gene (RAG-$1^{-/-}$) demonstrated that T cell activation has a role in hypertension onset (Guzik et al., 2007). RAG-$1^{-/-}$ mice lack T and B cells and have a blunted response to angiotensin II infusion. However, adoptive transfer of T but not B cells restored the hypertensive response to angiotensin II. In particular, angiotensin II also increased the T cell tissue homing into the perivascular adipose tissue and increased the nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase activity in wild-type mice.

Like other hematopoietic cells, T cells are created in the bone marrow. In a rodent study, ablation of the bone marrow (BM) in normotensive WKYs followed by reconstructing of the BM from SHR promoted neuroinflammation and hypertension in chimeric rats (Santisteban et al., 2015). BM transplantation also increased the number of circulating pro-inflammatory and increased microglial activity in the PVN of the hypothalamus. Consistently, oral administration of minocycline, an inhibitor of microglial activation, reduced blood pressure in SHRs compared to SHR controls. The effect was later demonstrated to be anti-inflammatory rather than antimicrobial using chemically modified tetracycline-3 that is deficient in antibacterial properties but retains minocycline's anti-inflammatory properties (Sharma et al., 2019).

An in situ decerebrated artificially-perfused rat preparation model of phrenic nerve activity patterns revealed elevated sympathetic sensitivity in SHRs compared to normotensive WKYs (Santisteban et al., 2017). Using pseudorabies virus expressing green fluorescent protein retrograde labeling applied in the colon and small intestine of rats demonstrated significant fluorescent labeling of neurons in the PVN of hypothalamus in of SHRs and angiotensin II infused WKYs but not WKY controls. SHRs had also increased levels of tyrosine hydroxylase immunoreactivity in small intestine compared to WKYs indicating increased norepinephrine generation in SHRs.

2.1.3.5 Inflammation and dietary sodium

Table 2 summarizes the role of T cells in hypertension (Wenzel et al., 2016). The high dietary salt was recently associated with changes in gut microbiota and host inflammation response, in addition to, previously well-documented deleterious effect on cardiovascular health (Wilck et al., 2017).

In vitro, the half maximal growth inhibition for *Lactobacillus* species was 0.6 mol/L sodium at 37 °C under aerobic conditions (Wilck et al., 2017). In vivo, high dietary sodium (4% dietary and 1% drinking water salt) resulted in colonic sodium concentration of 0.3 mol/L and normal dietary salt (0.5% dietary salt) of 0.1 mol/L. Consistently, high dietary salt was associated with changes in gut microbiota and particularly depleting *L. murinus* in mice compared to controls fed normal dietary

salt. However, *L. murinus* treatment prevented the development of salt-induced hypertension and increase in IL-17A producing $CD4^+$ T_H17 in gastrointestinal lymphocytes (Wilck et al., 2017).

The changes in gut microbiota in hypertension have been associated with increased T helper 17 (T_H17) to regulatory T (Treg) ratio in mesenteric lymph nodes and aorta (Toral, Robles-Vera, de la Visitación, Romero, Sánchez, et al., 2019). Similarly, salt-induced hypertension has been demonstrated to increase the number of immune cells in mesenteric arterial arcade and FMT from hypertensive mice to germ-free mice resulted to increased circulating interleukin (IL)-6 and IL-17 levels (Ferguson et al., 2019). The prohypertensive effects transmitted in FMTs appear to be connected to B7-dependant activation of T cells and the effect is at least partially governed by the T_H17/Treg ratio, the ratio of pro- and anti-inflammatory cells (Toral, Robles-Vera, de la Visitación, Romero, Sánchez, et al., 2019).

Table 2. Summary for the role of different T cells in hypertension*.

Variable	T helper cell 1 (TH1)	T helper cell 17 (TH17)	Regulatory T cell (Treg)
Function	Cell mediated autoimmunity	Cell mediated autoimmunity	Downregulation of the immune response
Experimental hypertension models	Increased IFN-γ production in Ang II-induced hypertension	Increased IL-17a production in Ang II-induced hypertension. Increased number of TH17 cells in kidney of hypertensive mice.	Treg correlates with the amount of injury
Effect of knockout	IFN-γ has either no effect or reduced BP	L-17a and IL-6 deficiency reduces BP	Knockout is lethal
Effect of overexpression	IFN-γ induces vascular dysfunction	IL-17a induces hypertension	
Effect of administration		IL-17 increases BP	Treg decreases BP

2.1.4 Human studies

Previous animal studies have suggested that gut dysbiosis and hypertension could be causally related. In animal models, both hypertension and gut microbiota were linked to gut wall permeability regulation. Gut microbiota has in experimental models also been demonstrated to modulate host sympathetic activity and produce bioactive metabolites that can enter host circulation. In particular, *Lactobacillus* species were

* Adapted by permission from American Society of Nephrology (Wenzel et al., 2016).

associated with both salt-induced hypertension and host inflammatory response. However, even optimally performed preclinical trials may fail to replicate in humans (Worp et al., 2010). These translation problems can be partially explained by the differences in disease manifestation, pharmacokinetics, pharmacodynamics, and immune response in different species (Pound & Ram, 2020). Therefore, the previously discussed preclinical results still require observational and interventional human studies to validate the role of gut microbiota in human hypertension. The gold standard for causality in clinical studies are randomized controlled trials while observational studies generally produce only information about associations. However, epidemiological studies can also provide information about causality in observational data using Mendelian randomization where genetic variants fixed at conception mimic the randomization performed in interventional studies (Sekula et al., 2016; Davies et al., 2018).

2.1.4.1 Gut microbiota and human hypertension

While numerous scientific studies have addressed the association between gut microbiota and hypertension, only few studies have been performed in a representative sample of >500 individuals (Jackson et al., 2018; Sun et al., 2019; Verhaar, Collard, et al., 2020). However, in small study of Chinese individuals (N = 129), genus level differences were observed between hypertension, isolated systolic and diastolic hypertension, pre-hypertension, and normotension (Dan et al., 2019). A small American pilot study (N = 52) suggested that gut microbiota may contribute the differences observed in hypertension between black and white individuals (Walejko et al., 2018). Hypertension in black patients may be accompanied by increased oxidative stress and insulin resistance compared to white individuals (Walejko et al., 2018). In summary, different subtypes of hypertension may have accompanying differences in gut microbiota and the genomics of the host may influence the metagenomics of the gut.

Three large cross-sectional studies had reported associations between gut microbiota and human hypertension excluding Study II. In the TwinsUK (N = 2,737; 89% women, age 60 ± 12; 16S rRNA) study, the researchers explored the connection between gut microbiota and 38 common diseases and 51 medications (Jackson et al., 2018). However, self-declared hypertension was not associated with 68 microbiota markers (Jackson et al., 2018). In the Coronary Artery Risk Development in Young Adults (CARDIA; N = 529; 54% women, age 55 ± 3; 16S rRNA) study, microbial alpha diversity and *Robinsoniella*-genus were negatively associated with systolic BP (Sun et al., 2019). In the Healthy Life In an Urban Setting (HELIUS; N = 4,672; 52% women, age 50 ± 12; 16S rRNA) study, authors used gradient boosting machine learning model and self-defined formula

for the proportion of variance to estimate the overall effect between gut microbiota and hypertension (Verhaar, Collard, et al., 2020). Gut microbiota explained 4.4% of the overall unadjusted systolic BP variance and 2.2% of the residual systolic BP adjusted for age, sex, and BMI. However, in stratified analyses, only residual BP had consistent R^2 within the six ethnicities.

Observational studies are susceptible to various biases including confounding and reverse causation. In metagenomics studies medications can independently influence both gut microbiota and host pathophysiology (Jackson et al., 2018). While statistical models should be adjusted for relevant covariates, non-adherence to antihypertensive medication, in particular, is common and can potentially result in misclassification of study participants (Tomaszewski et al., 2014). Access to register based information of regular drug purchases or direct measurement of drug metabolites in biofluids such as urine could potentially improve quality of observational studies. Mendelian randomization models would also be less likely affected by the conventional biases of the observational studies and provide information about causality (Davies et al., 2018).

2.1.4.2 Dietary salt in human hypertension

The previously discussed study reporting results for dietary salt and *Lactobacillus* species in mice included a human pilot study (Wilck et al., 2017). In a moderate salt challenge, 12 healthy men received 6 g slow-releasing sodium chloride in addition to their accustomed diets for total daily salt intake 13.8 ± 2.6 g. The challenge was linked with nocturnal BP increase from baseline 106/60 mmHg to 111/67 mmHg and 1.8-fold increase in circulating $CD4^+IL\text{-}17A^+TNF\text{-}\alpha^+$ T_H17 cells. Participants also had reduced survival of *Lactobacillus* species (Figure 5) compared to published time course data from 121 individuals not undergoing any intervention.

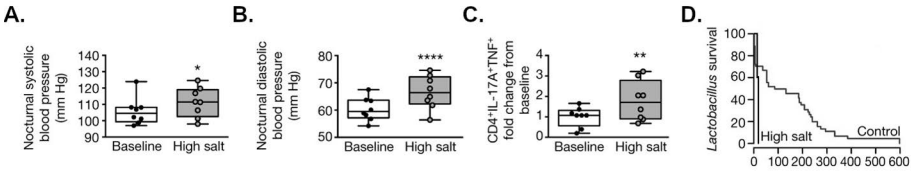

Figure 5. The effect of dietary sodium intervention to BP, T_H17 cells and Lactobacillus survival*.

* Reprinted by permission from Springer Nature (Wilck et al., 2017).

2.1.4.3 SCFAs are altered in human hypertension

Gut microbial fermentation of the indigestible foods in cecum and ascending colon as well as hepatic de novo synthesis are the main sources of SCFAs in humans (Overby & Ferguson, 2021). While multiple bacterial species produce SFCAs (Table 3), our understanding about SCFA production and pathophysiology is still partial. Hypertension has been associated with increased levels of fecal SCFAs in Spanish (N = 61), Colombians (N = 441), Belgians (N = 54), and Dutch (N = 200) individuals (Calderón-Pérez et al., 2020; de la Cuesta-Zuluaga et al., 2018; Huart et al., 2019; Verhaar, Collard, et al., 2020). Abundances of multiple SCFA producing bacteria such as *Ruminococcaceae*, *Roseburia*, and *Faecalibacterium* have been negatively associated with human hypertension (Verhaar, Prodan, et al., 2020). Few studies have reported on the links between hypertension and circulating SCFA levels; however, negative associations were observed between BP and plasma SCFAs levels in Spanish (N = 61) and American (N = 40) individuals (Calderón-Pérez et al., 2020; Kim et al., 2018).

In a moderately sized randomized controlled cross-over trial (N = 145, 34% women), sodium reduction was associated with increased circulating levels of eight SCFAs, including butyrate (L. Chen et al., 2020). Notably, subgroup-analysis revealed that results were sex-differentiated and significant SCFA increases in response to sodium reduction were observed in women only. In summary, hypertension and high dietary sodium have been associated with changes in 1) gut microbiota, including in SCFA producing bacteria; 2) gastrointestinal tract function; and 3) fecal and circulating SCFA levels.

Table 3. The main SCFA producing bacterial genera present in human gut microbiota.

SCFA	Main producers	Source
Acetate	*Bifidobacteria, Lactobacillus, Prevotella, Ruminococcus*	(Baxter et al., 2019; Franke & Deppenmeier, 2018; Moens et al., 2017)
Propionate	*Akkermansia, Alistipes, Bacteroides, Blautia, Coprococcus, Dialister, Eubacterium, Phascolarctobacterium, Prevotella, Roseburia*	(Louis & Flint, 2017)
Butyrate	*Anaerostipes, Clostridium, Coprococcus, Eubacterium, Faecalibacterium, Roseburia, Subdoligranulum*	(Louis & Flint, 2017; Parada Venegas et al., 2019)

2.1.4.4 Host inflammatory response

Human hypertension has been linked to increased endotoxemia and increased expression of peripheral blood T_H17 cells (Wilck et al., 2017; Kim et al., 2018). Immunohistochemical analysis on tissue samples from a biobank revealed that hypertensive individuals have also increased infiltration of T cells and macrophages in the intestinal wall compared to normotensive controls (Ferguson et al., 2019). Hypertension may therefore be modulated by immune-mediated signals that are present in both gastrointestinal tract and peripheral blood. SCFAs can similarly induce changes in host homeostasis: for example, they can locally modulate gastrointestinal permeability and remotely bind to SCFA receptors. However, the translation of the results from animal studies to humans is still ongoing.

2.2 Circulating metabolites and hypertension

2.2.1 Introduction to the circulating metabolome

Metabolites are low-molecular weight components observed within various cells, tissues, and biofluids. Unlike amino acids in proteins or nucleotides in DNA, metabolites do not carry information encoded using repeating subunits, but the information is rather confined in the thousands of the metabolites themselves (Nikolic et al., 2014). The varying chemical properties and concentrations of metabolites make their identification and quantification difficult.

Metabolomics studies are usually classified to targeted and untargeted analysis designs. Targeted analysis focuses on known sets of metabolites and untargeted analysis aims to identify new molecular compounds. In the following section, we

will review common analytical methods used in metabolomics and review the literature for metabolites associated with hypertension.

2.2.2 Analytical technologies

2.2.2.1 Mass spectrometry

The modern mass spectrometric device consists of multiple components that are varied to meet the needs of the study at hand (Pitt, 2009; Alsaleh et al., 2019). We focus our review on the liquid chromatography mass spectrometry system (LC–MS), because solvent phase is particularly suitable in studying plasma and urine samples.

A liquid chromatograph is used to separate aqueous biological samples into separate components (Figure 6). First, samples are injected into a moving stream of liquid solvent called mobile phase. Then, analytes are transported into a liquid chromatographic column that is packed with granular solid material called stationary phase. The differences in the affinity of the distinct analytes in the studied sample with the mobile and stationary phases results in varying travel times along the column. Different columns are used in different applications, and in some columns, a pneumatic pump is used to maintain the flow along the column constant instead of relying on gravity to sustain it. The choice of phase materials affects the travel time and should preferably be reported to allow the replication of the analyses.

Notably, different analytes arrive to MS device at different times from LC. The analytes are ionized and transformed into gaseous state to allow further spectroscopic analysis using high precision electromagnetic fields. In biological samples, soft ionization methods that produce adduct ions without causing fragmentation, are preferred. After ionization, the analytes pass through a vacuum interface into a mass analyzer.

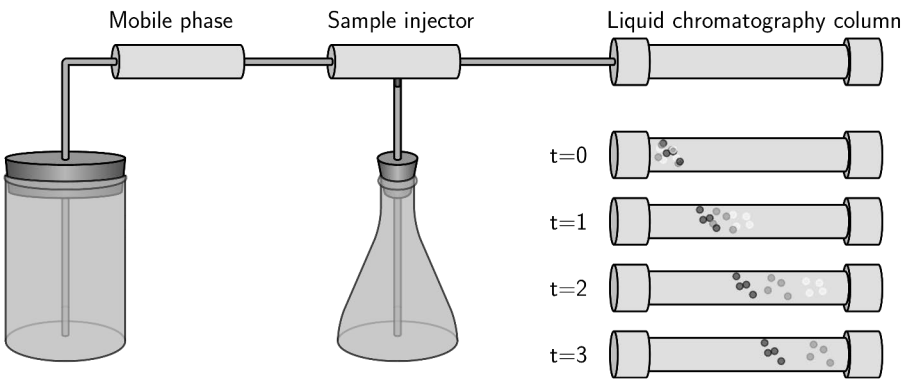

Figure 6. Liquid chromatography column[*].

Electric quadrupoles are typically used to filter charged analytes based on their mass-to-charge (m/z) ratio (Figure 7). Different trajectories near specific m/z values can be stabilized in the time-varying quadrupolar field by adjusting constant voltages and varying radio frequency voltages applied to the quadrupole rods. Finally, information about retention time in LC and m/z in MS can be linked with the measured abundance of analytes reaching detector.

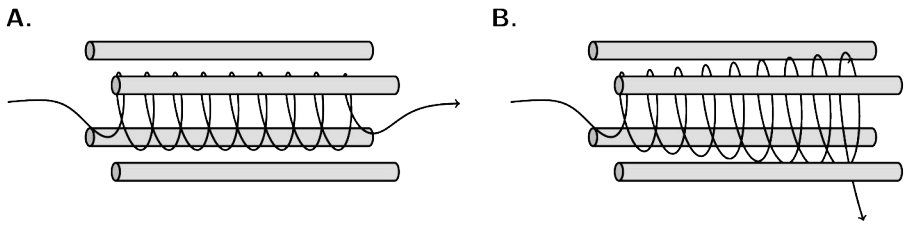

Figure 7. Left panel illustrates stable trajectory for analytes with specific mass-to-charge ratio compared to instable trajectory in the right panel[†].

The liquid chromatography mass spectrometer analysis has multiple steps and sources of batch-to-batch variation making reproducible and quantitative determination of absolute responses difficult (Pitt, 2009). Calibration standards with distinguishable chemical properties can be used in spectroscopy to link the measured

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abundances with known standard concentrations. Internal standards are mixed within the study samples and external standards are studied separate from the samples. In brief, internal standards can be used to estimate molar concentrations for analytes and external standards to correct for the batch-to-batch variation. If systematic signal drifts and patch effects are not properly compensated with peak alignment and quality control, the measurement data is unreliable (Figure 8).

While quality control steps can be often automated, the identification of analytes is a common bottleneck of LC–MS. Biofluids contain large number of distinct metabolites and a single metabolite can produce multiple distinct spectral peaks due to differences in ionization. When studying few analytes using targeted analysis design, authentic standards could be used in identification. However, in an untargeted approach, the analytes are not known a priori and even with strong hypotheses, the use of authentic standards is limited by feasibility, cost, and commercial availability. Unknown analytes can be characterized using information about their spectroscopic features such as retention time and mass-to-charge ratio.

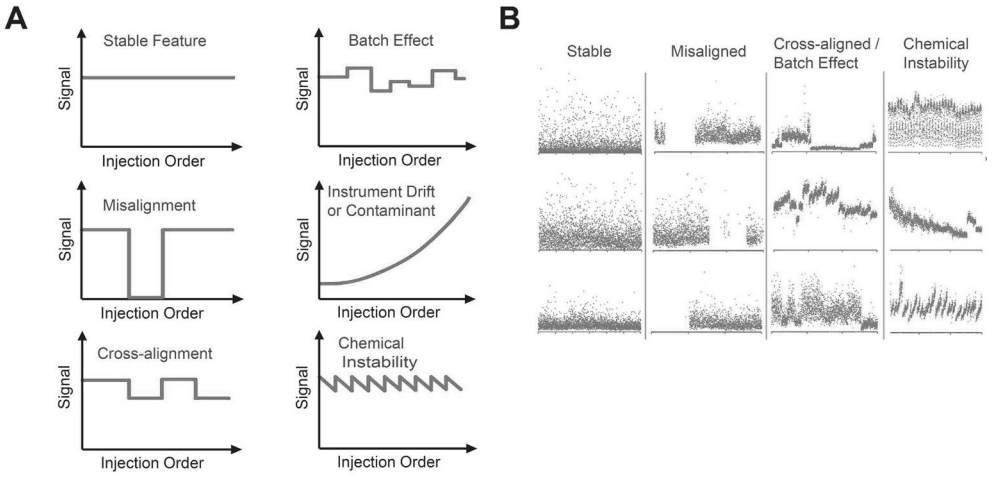

Figure 8. Liquid chromatography mass spectrometry analysis has multiple error sources*.

2.2.2.2 Nuclear magnetic resonance spectrometry

Nuclear magnetic resonance (NMR) is a physical phenomenon that offers two medical applications: NMR imaging and NMR spectroscopy (Tognarelli et al., 2015). A nucleus with an odd mass number has a half-integer nuclear spin, and a nucleus with an even mass number has an integer spin. Nuclei with half-integer (non-

* Adapted with permission from American Chemical Society (Watrous et al., 2017).

zero) spin have a non-zero magnetic moment that interacts with external magnetic fields.

Hydrogen-1, the most abundant atom in biological molecules, has a half-integer nuclear spin. When a biological sample is placed in a constant external magnetic field, the degenerate energy levels of hydrogen nuclei (protons) split. This means the nuclei will be left with differing energy levels depending on whether the spin is parallel or antiparallel with the external magnetic field (Figure 9). However, each hydrogen nucleus within the biological sample experiences perturbations in the applied magnetic field from chemical bonds close to the nucleus. Therefore, the energy levels of hydrogen nuclei will also contain information about its local chemical structure. We summarize in the following paragraphs the principles of ^{1}H-NMR spectrometry.

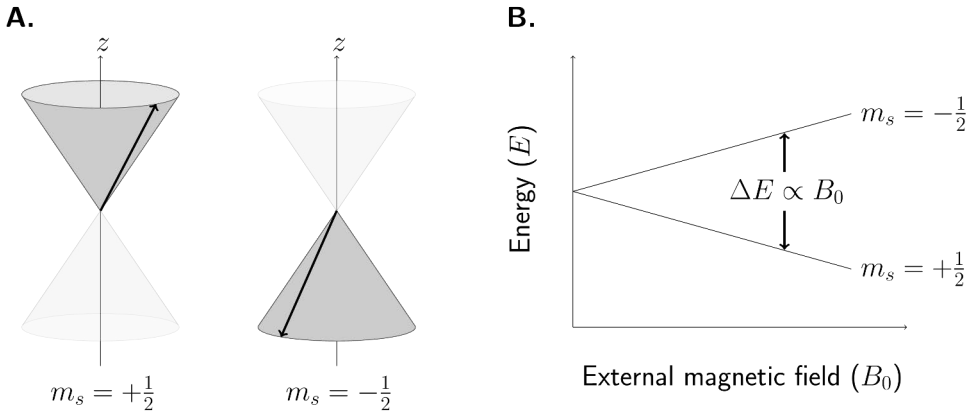

Figure 9. The nuclear energy states of hydrogen split in magnetic field*.

In an NMR spectroscopy device, the sample is placed inside a strong magnetic field and a short, intense radio frequency pulse is imposed to excite some of the nuclei to their higher energy state (Figure 10). When the radio frequency field is turned off, the nuclei tend to return to the low energy state, emitting photons in process. This free induction decay is the superimposition of all signals in the sample and is captured using detector coil. The chemical shifts imposed by the local environment of each hydrogen atom gives rise to a spectrum that contains information about chemical structures observed in the sample. NMR spectral profiles have been well categorized, making metabolite identification easy and reproducibility high.

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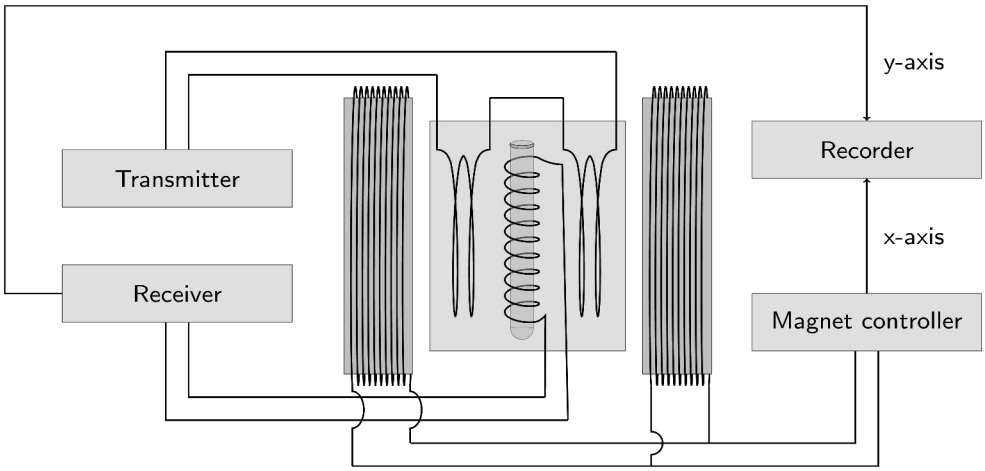

Figure 10. Schematic for nuclear magnetic resonance spectroscopy*.

Notably, the NMR spectroscopy neither degrades the samples nor do the samples leave residue in the device because the applied radiation is non-ionizing and no physical contact is required between the analyte and the device. Inclusion of gradient magnetic field (field with varying strength) across the sample would permit the location of the emission, information that is used in NMR imaging.

In summary, LC–MS and NMR can be used to study various biological samples and the two methods have distinct strengths and weaknesses (Nikolic et al., 2014). NMR has low sensitivity of <100 analytes per run but high reproducibility and easy analyte identification while untargeted LC–MS has high sensitivity of >1000 metabolites per run but is laborious and requires non-trivial analyte identification. Research groups performing spectroscopy analyses should include skilled analytical chemist to allow, in particular, proper quality control of the results and analyte identification.

2.2.3 Human lipidome

Lipids are biomolecules that are soluble in nonpolar solvents (Quehenberger & Dennis, 2011). Because plasma is mostly composed out of water, the circulating lipids are solubilized and dispersed in carrier proteins such as albumin and lipoprotein particles. Lipids are the largest group of biological molecules in plasma and different lipid molecules have large structural diversity (Figure 11). We will review in following sections the current literature of human lipidome, the collection

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of all lipids in organism, and focus in more detail on a small group of bioactive signaling molecules, eicosanoids.

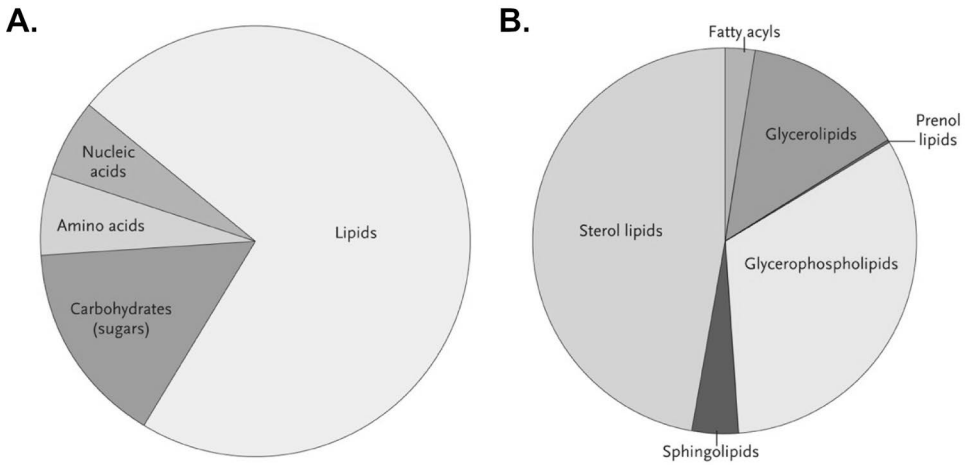

Figure 11. The distribution of biological molecules (A; g/dl) and lipids (B; mol) in human plasma*.

2.2.3.1 Lipoprotein particles

While LDL cholesterol has log-linear association with coronary artery disease (CAD), nearly half (49.5%) of the patients hospitalized for CAD in large cross-sectional study had LDL cholesterol level under recommended levels <100mg/dl or <2.6 mmol/l (Sachdeva et al., 2009). A meta-analysis of individuals under statin therapy in seven placebo controlled trials, revealed that risk reduction of statin therapy was more closely related to reduction of apolipoprotein B compared to low-density lipoprotein (LDL) cholesterol (Thanassoulis et al., 2014). While one copy of apolipoprotein B is found on multiple lipoprotein particles including LDL, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and lipoprotein(a), most (>90%) apolipoprotein B in plasma is associated with LDL due to particles long plasma residence time (Varvel et al., 2015). The LDL particle number and apolipoprotein B concentrations are therefore highly correlated ($R^2 = 0.79$) and both measures offer stronger predictor for future cardiovascular events than the current lipid therapy treatment target, LDL cholesterol concentration (Varvel et al., 2015).

A few large cohort studies report associations between circulating lipid measures and the development of hypertension. In the Women's Health Study (N = 17,527;

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aged 48.2–59.6), the average VLDL particle size, apolipoprotein B and total triglycerides were positively and the average LDL particle size negatively associated with incident hypertension (Paynter et al., 2011). Apolipoprotein B and total triglycerides had positive and association with the development of hypertension (Paynter et al., 2011). In the Brisighella Heart Study (N = 1,864; 49.1% women, aged 50.8 ± 11.4), baseline LDL cholesterol level was positively related to rate of hypertension onset (Cicero et al., 2014). In a study of non-hypertensive Japanese men (N = 14,215, aged 38 ± 9), total cholesterol, LDL cholesterol (calculated using Friedewald formula), and non-HDL cholesterol were positively associated with development of hypertension (Otsuka et al., 2016).

In the Women's Health Study, high-density lipoprotein (HDL) cholesterol, large HDL particle concentration, and average HDL particle size were negatively associated with incident hypertension (Paynter et al., 2011). In contrast, in cross-sectional study of healthy Japanese (N = 2,953; 38.9% women, aged 49.7 ± 9.0), HDL cholesterol was positively associated with hypertension (Oda & Kawai, 2011). U-shaped association between HDL cholesterol and hypertension onset was observed in 14 215 (aged 38 ± 9) normotensive men (Otsuka et al., 2016). However, several disease processes (including diabetes, coronary artery disease, and chronic kidney disease) may lead to HDL particle dysfunction that promotes impaired endothelial repair, increased proinflammatory activation, and increased BP (Lüscher et al., 2014; Shimizu et al., 2017). In a cross-sectional study of elderly Japanese men (N = 477, aged 65.4 ± 2.6), positive association between HDL cholesterol and hypertension was observed only in a subsample of participants with high levels of CD34-positive circulating endothelial progenitor cells (Shimizu et al., 2017). While the exact role between CD34-positive cells and HDL cholesterol is still unclear, high level of CD34 cells could indicate reduced endothelial anti-inflammatory ability of HDL particles.

2.2.3.2 Free fatty acids

Fatty acids are aliphatic chains of carbon atoms with carboxyl group (R-COOH). Free fatty acids are used to build more complex lipids such as sphingolipids, phospholipids, and glycerolipids such as triglycerides (Figure 11). In a cross-sectional study of Chinese individuals (N = 2,447, 52.9% women, aged 35–79), polyunsaturated fatty acids (PUFA) and omega-3 PUFAs were negatively and saturated fatty acids (SFA) positively associated with hypertension (B. Yang et al., 2016). In two small studies of Japanese men (N = 315, 69.7% women, aged 52.2 ± 7.3) and of South Africans (N = 300, aged 53.1 ± 9.8), a small number of omega-6 PUFAs, including linolenic acid, were negatively associated with hypertension (Tsukamoto & Sugawara, 2018; Zec et al., 2019).

2.2.3.3 Eicosanoids

Eicosanoids are fatty acids with 20 carbon atoms that are enzymatically generated from ω-3 and ω-6 PUFAs (Khanapure et al., 2007). Compared to circulating free fatty acids, eicosanoids are paracrine signaling molecules that act locally in nanomolar concentrations rather than conventional fatty acid substrates of anabolism and catabolism (Capra et al., 2015). Arachidonic acid (AA) is a 20:4(ω-6) PUFA abundantly located in the membrane phospholipids that serves as an essential substrate of eicosanoid synthesis (Capra et al., 2015). Notably, the calcium dependent activation of phospholipase A_2 and following release of AA from membrane phospholipids is the rate limiting factor in eicosanoid production (Mitchell et al., 2021).

2.2.3.3.1 Biosynthesis of eicosanoids

Eicosanoids are produced from AA and other PUFAs following three major enzymatic pathways (Figure 12). Cyclooxygenase (COX) pathway produces prostaglandins (PG) that are named with letter denoting the components of the five-member prostane ring (A-K) and subscript denoting the number of double bonds in the PG (Buczynski et al., 2009). COX enzyme has two functional isoforms that have minor differences in activity sites that, in particular, allows COX-2 metabolize dihomo-γ-linolenic and eicosapentaenoic acid in addition to AA. COX-1 is constitutively expressed in most cells while COX-2 is constitutively expressed only in selected locations in brains, gut, thymus, lungs, and kidneys (Mitchell et al., 2021). However, COX-2 is rapidly induced in sites of inflammation or cancer (Mitchell et al., 2021). The catalytic activity of COX on AA produces highly unstable PGH_2 (Capra et al., 2015). Specific synthase enzymes convert PHG_2 to prostacyclin (PGI_2), thromboxane A2 (TXA_2), and other bioactive PGs (Capra et al., 2015). Circulating PGs interact with transmembrane GPCRs (Capra et al., 2015).

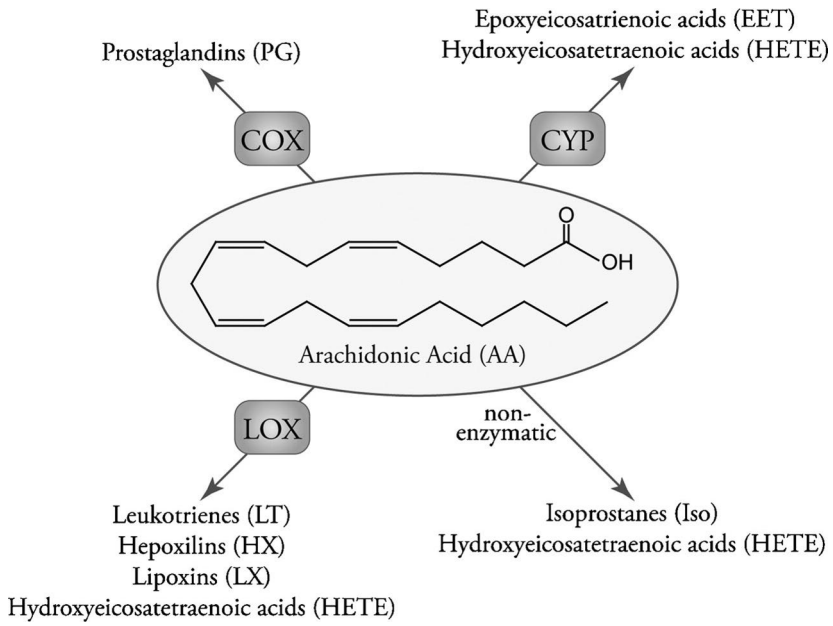

Figure 12. Three enzymatic pathways of eicosanoid synthesis*.

Lipoxygenase (LOX) pathway produces another large family of eicosanoids, leukotrienes, hydroxyeicosatetraenoic acids (HETE), and lipoxins (Figure 12). 5-lipoxygenase (5-LOX) is expressed in myeloid cells, where it catalyzes the formation of leukotriene (LT) A_4 from AA (Buczynski et al., 2009). Leukotrienes follow similar nomenclature as PGs: letter indicates the structure and subscript the number of double bonds in the molecule (Samuelsson & Hammarström, 1980). LTA_4 is further catalyzed into more stable LTB_4, LTC_4, and LXA_4. 12-LOX pathway produces, in particular, HETEs and, in concert with 5-LOX, hepoxilinis (Buczynski et al., 2009).

Cytochrome P450 (CYP) pathway catalyzes AA to hydroxyeicosatetraenoic acids (HETEs) and epoxy-eicosatrienoic (EET) acids (Figure 12). While CYP enzymes are present in all forms of life, the function of the enzyme is highly varied under single amino acid mutations resulting unique set of CYP enzymes in each species (Buczynski et al., 2009). Remarkably, the catalyzation of unstable intermediates of eicosanoid synthesis (LTA_4 and PGH_2) into final compounds can result from transcellular biosynthesis from nearby cells (Capra et al., 2015).

* Published under Creative Commons Attribution license (Buczynski et al., 2009).

2.2.3.3.2 Pathophysiological roles of eicosanoids

Prostaglandins promote and suppress platelet activation. PGI_2 is produced in endothelial blood vessels and released PGI_2 binds to platelet receptors suppressing platelet adhesion, aggregation, and granule secretion (Crescente et al., 2019). Another prostaglandin, TXA_2 produced in activated platelets promotes thrombogenesis; TXA_2 amplify local platelet activation, TXA_2 formation, and platelet aggregation (Crescente et al., 2019). TXA_2 also induces proliferation and vasoconstriction in vascular smooth muscle cell (Crescente et al., 2019). Constitutive COX-2 activity regulates kidney homeostasis, including renal hemodynamics, sodium excretion, and angiotensin II formation (Mitchell et al., 2021). Notably, COX-2 enzyme is the treatment target of the non-steroidal anti-inflammatory drugs and COX-2 inhibition has been linked with increased BP (Mitchell & Kirkby, 2019). Current hypothesis suggest that inhibition of COX-2 pathway removes the inhibition of methylarginine pathway and results in increased production of potent nitric oxide synthase inhibitor, asymmetric dimethylarginine (ADMA); a knockout rat model indicated PGI_2 as possible end product of the constitutive COX-2 synthesis that limits ADMA production (Mitchell & Kirkby, 2019).

CYP pathway derived 20-HETE and EETs modulate BP homeostasis. EETs are produced in vascular endothelial cells where they have anti-inflammatory effect and promote vasodilation (Crescente et al., 2019). 20-HETE increasing renal vascular resistance inducing contraction in the afferent arterioles and inhibits sodium reabsorption in proximal tubule and thick ascending loop of Henle (Elshenawy et al., 2017). The afferent vasoconstriction is partly mediated by the COX enzymes that catalyze 20-HETE to PGs (Elshenawy et al., 2017). CYP pathway derived EETs are produced in endothelial cells, kidney, and heart (Imig, 2015). EETs dilate preglomerular afferent arterioles and inhibit sodium transport in the proximal tubule and the cortical collecting duct (Imig, 2015). Remarkably, mutation in the CYP4A11 and CYP4F2 enzymes has been linked to elevated BP in humans (Fan et al., 2015). Consistently, deficiency in the formation of 20-HETE and EETs have been linked with salt-sensitive hypertension (Fan et al., 2015).

5-LOX pathway derived LTs promote bronchoconstriction and leucocyte recruitment during inflammation, asthma, and allergy (Dennis & Norris, 2015). LTs, and particularly LTB_4, have been linked with pulmonary arterial hypertension offering potential treatment option using anti-LT therapy (Tian et al., 2014).

Finally, the biosynthesis of eicosanoids is markedly increased in response to inflammatory stimuli (Dennis & Norris, 2015). PGs and LTs allow the innate immune reaction against bacteria while bacterial pathogens are able to release virulence factors altering the expression of eicosanoid biosynthesis enzymes (Sheppe & Edelmann, 2021). PGE_2, in particular, enhances inflammasome activation, secretion of distinct interleukins, and formation of insoluble components

that are able to trap bacteria inside macrophages (Sheppe & Edelmann, 2021). Eicosanoids have been reported to express both pro- and anti-inflammatory functions (Dennis & Norris, 2015).

2.2.4 Amino acids

A few recent studies have reported on the associations between circulating amino acids and hypertension. In the Dutch Prevention of Renal and Vascular End-stage Disease study (N = 4,169; 54.4% women; aged 49.2 ± 10.3) and in a Japanese study (N = 5,541; 51.9% women), circulating branched amino acids, isoleucine, leucine, and valine, were positively associated with hypertension (Flores-Guerrero et al., 2019; Mahbub et al., 2020). In another cross-sectional study of Japanese (N=8,115; 52.7% women; aged 56.5 ± 15.1) circulating glycine, glutamine, and histidine were negatively and alanine positively associated with hypertension (Yamaguchi et al., 2017).

2.2.5 Energy metabolism-related measures

In healthy individuals, postprandial insulin secretion promotes changes in circulating metabolites reflecting switch from catabolism to anabolism (Shaham et al., 2008). Increased lactate indicates increases glycolysis, decreased glycerol indicates decreased lipolysis, decreases beta-hydroxybutyrate indicates decreased ketogenesis, and decreased amino acids indicate decreases proteolysis. In the Bogalusa Heart Study (N=1,249; 58.8 women; aged 48.2 ± 5.3), fasting glucose was positively associated with systolic BP (He et al., 2020). In an American study (N=5,554; 54.9% women; aged 61.9 ± 5.5), lactate was positively associated with hypertension onset in women only (Juraschek, 2015).

2.2.6 Fluid balance related-metabolic measures

Albumin increases vascular colloid-osmotic pressure and transports hormones, drugs, amino acids, and free fatty acids (Høstmark et al., 2005). In the Oslo Health Study (N=5,171; 61.9% women; aged 30–75) and in the Neuroprotective Model for Healthy Longevity among the Malaysian Elderly study (N=2,322; 52.0% women), albumin was positively associated with BP (Eshkoor et al., 2016; Høstmark et al., 2005). However, in a retrospective study of normotensive Japanese (N=2,240; 38.2% women, aged 49.8 ± 8.7), albumin was negatively associated with hypertension onset (Oda, 2014). The cross-sectional positive association could possibly indicate greater vascular volume and negative longitudinal association indicate other properties albumin imposes or transmits (Oda, 2014).

2.2.7 Inflammation markers

Hypertension has been linked with chronic inflammation in animal and human studies (Harrison et al., 2011; Barrows et al., 2019). Indirect immunofluorescence technique and high-resolution confocal microscopy revealed existence of C-reactive protein (CRP) deposits associated in atherosclerotic plaques in human coronary artery sections from 68 autopsies (Y. X. Zhang et al., 1999). In the cross-sectional Physicians' Health Study (N=508), two inflammation markers, intercellular adhesion molecule-1 and IL-6, were positively associated with BP adjusted for cardiac risk factors in men (Chae et al., 2001). In the Women's Health Study (N=20,525; median follow-up 7.8 years), baseline CRP was positively associated with incident hypertension adjusted for baseline coronary risk factors in women (Sesso et al., 2003). In moderate sized study, high-sensitivity CRP (hs-CRP) was independent predictor of pulse wave velocity, the gold-standard measure of arterial stiffness (Mahmud & Feely, 2005).

Mendelian randomization meta-analysis of 47 epidemiological studies (N= 194,418) examined the causal relationship between circulating CRP concentration and coronary heart disease (Collaboration (CCGC), 2011). Information was available on four SNPs (rs3093077, rs1205, rs1130864, rs1800947) that were associated with CRP and were unrelated to traditional cardiovascular risk factors. In conclusion, individual SNPs and genetically predicted CRP level were not significantly associated with coronary heart disease. Therefore, the association between CRP and CAD appears to be non-causal and because hypertension is a well-established risk factor for CAD, the association between CRP and BP is probably likewise non-causal.

Nonsynonymous mutation of amino acid position Asp358Ala on the main cleavage site of IL-6 receptor (IL-6R) affects IL-6 signaling (Revez et al., 2013). Minor allele of the IL-6R gene (rs2228145, A>C) has been demonstrated to increase the expression of soluble isoform of IL-6R and reduce the classical IL-6 signaling (Stephens et al., 2012). Meta-analysis of 82 studies (N>200000) demonstrated that each 358Ala copy inherited was associated with increased soluble IL-6R, reduced CRP, and reduced CAD risk independent of the classical risk factors (IL6R Genetics Consortium Emerging Risk Factors Collaboration et al., 2012). Therefore, while CRP is not causally associated with coronary artery disease, previous study supports causal IL-6 mediated inflammation hypothesis in cardiovascular disease.

Harrison et al. proposed a 2-step inflammation hypothesis where the early pre-hypertensive elevation of BP caused by dietary habits and other factors brings about an inflammatory response causing sustained hypertension (Harrison et al., 2011). The inflammatory response recruits T cells and macrophages into the perivascular fat and kidney where the interplay of released cytokines, catecholamines, reactive

oxygen species, and angiotensin II promote vasoconstriction, vascular remodeling, and sodium retention (Harrison et al., 2011).

2.2.8 Other metabolites associated with hypertension

The metabolites in human circulation have three origins: they can be host derived, microbiota derived, or host-microbiota derived. Alterations in carbohydrate, lipid, amino acid, tri-carboxylic acid, and ketone metabolism have been linked with hypertension (Islam, 2017; Chakraborty et al., 2020). Microbiota can produce in addition to SCFAs other metabolites that are also produced in human metabolism (Chakraborty et al., 2020).

Bile acids and trimethylamine (TMA) regulate BP and are produced in interplay between host and microbiota (Chakraborty et al., 2020). The microbial TMA is oxidized in liver to trimethylamine-oxide (TMAO) that has been positively linked with stroke and CAD events (Nie et al., 2018; Jaworska et al., 2019). Meta-analysis of 11750 individuals revealed positive association between TMAO and BP (Ge et al., 2020). However, the significance of the roles between TMA and TMAO is not currently fully understood (Jaworska et al., 2019). Hydrogen sulfide (H_2S) is endogenously produced gaseous signaling molecule (van Goor et al., 2016). In animal models, inhibition of enzymes in H_2S production has been reported to promote hypertension and administration of H_2S has resulted to decreased BP (van Goor et al., 2016).

2.3 Summary

Metagenomics and metabolomics offer practical and cost-effective methods to analyze samples from large human cohorts to study the pathophysiology and correlates of chronic diseases. We reviewed in this chapter the basic methodologies of metagenomics and metabolomics and synthetized the relevant literature related to hypertension in this domain.

Animal models have built weight of evidence linking gut microbiota with host hypertension. Working hypotheses have also been formulated for the observed findings. Gut microbiota could affect BP, in particular, by modulating sympathetic activity, gut wall permeability, and producing signal molecules in circulation. Dietary sodium, a classic risk factor for hypertension, could also affect gut microbiota potentially by depleting beneficial microbial species. However, much of our knowledge is still based solely on animal models and only few large-scale human studies have been published to date. In particular, the clinical significance of the observed associations to human hypertension are unclear.

The circulating human lipidome is complex. A large number of prior studies have focused on lipoprotein particles. While the results for atherogenic lipoprotein particles, such as LDL, are mostly consistent, the role of HDL cholesterol to CAD and hypertension is currently not fully understood. There also exists a large family of bioactive fatty acids, eicosanoids, that have been linked to thrombogenesis and BP modulation in mainly small animal and studies. In addition to lipids, other metabolites and metabolic biomarkers, including amino acids, glycolysis-related metabolites, and inflammation markers, have been associated with hypertension.

While our current knowledge is based on promising results from animal studies, the utilization of third generation sequencing and study designs combining data from multiple omics fields have potential to transition the research focus from the experimental models to human studies in near future. Emergence of openly available data, modeling software, and analysis code, and the shift to open-access publishing has potential to increase the availability, quantity, and quality of future omics studies.

3 Aims

This thesis was designed to study novel correlates of hypertension using contemporary metagenomics and metabolomics.

The specific aims were:

1. To review the current knowledge on the association between gut microbiota and hypertension in animal and human studies (I).
2. To study the links between gut metagenome, dietary salt, and BP (II).
3. To investigate the associations between eicosanoids, the PUFA-derived small molecule activators and suppressors of systemic inflammation, and BP (III).
4. To investigate the cross-sectional and prospective associations between high abundance metabolite biomarkers and BP (IV).

4 Materials and Methods

4.1 Systematic literature review

We performed a systematic literature review for original research articles using three scientific databases: Medical Literature Analysis and Retrieval System Online (MEDLINE), Excerpta Medica database EMBASE), and Cochrane Library. Our search terms aimed to capture the intersection of studies reporting results for essential hypertension and metagenomics (Table 4). While our study focused on gut microbiota, the exclusion of unrelated metagenomics studies was left to screening.

Table 4. The search terms used in three scientific databases.

Database	Search terms
MEDLINE	("Blood Pressure"[MeSH] OR "Hypertension"[MESH] OR "blood pressure"[TI] OR "hypertension"[TI] OR "blood pressure"[OT] OR "hypertension"[OT]) AND ("Gastrointestinal Microbiome"[MeSH] OR "microbiota"[tiab] OR "microbiome"[tiab] OR "metagenomics"[tiab])
EMBASE	('blood pressure':ti OR 'hypertension':ti OR 'blood pressure':kw OR 'hypertension':kw) AND ('intestine flora'/exp OR 'microbiota':ti,ab OR 'microbiome':ti,ab OR 'metagenomics':ti,ab)
Cochrane Library	((blood pressure):ti,kw OR (hypertension):ti,kw) AND ((microbiome):ti,ab,kw OR (metagenomics):ti,ab,kw OR (microbiota):ti,ab,kw)

MEDLINE, Medical Literature Analysis and Retrieval System Online; EMBASE, Excerpta Medica database.

Our literature review followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines (Liberati et al., 2009). We included in the review original research articles reporting results for essential hypertension and sequenced gut microbiota. Interventional studies were limited to stool transfers, orally administrated probiotics, dietary sodium, antihypertensive medications, and short chain fatty acids, and genomic knockout models. Duplicate search results were automatically detected using Digital Object Identifiers. Manual screening was performed by single author using first titles, second abstracts, and finally full-text.

4.2 Study samples

4.2.1 FINRISK (II–IV)

The Finnish Institute for Health and Welfare has performed population surveys every five years since 1972 to monitor the development of cardiovascular risk factors in the Finnish population (Borodulin et al., 2018). The FINRISK 1997–2012 study samples consist of participants randomly drawn from the national population register from up to six geographical areas stratified by sex, region and 10-year age group. The six areas are Helsinki and Vantaa, Turku and Loimaa, North Karelia, Northern Savo, Northern Pohjanmaa and Kainuu, and Lapland. Lapland was included in FINRISK 2002 with health examination and in FINRISK 2007 with self-reporting questionnaire. In recent FINRISK studies the age range of invited individuals has broaden to ages 25-74 in all areas, while only individuals aged 25-64 were invited in Northern Savo, Turku and Loimaa, and Oulu areas in FINRISK 1997 and 2002. The participation rates to health examination in FINRISK 1997–2012 was 56–72%, the mean age of the participants was 45.2-45.7 years, and the proportion of women was 52.6–54.0%. In FINRISK 2002, the study protocol included the collection of stool samples and urinary sodium samples (Study II) in addition to venous blood samples (studies II-IV). The FINRISK study was approved by the Ethics Committee of the Hospital District of Southwest Finland.

The participants of FINRISK 2007 were invited to participate in the DIetary, Lifestyle and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) study (Konttinen et al., 2018). All DILGOM 2007 participants alive at the end of 2013 were invited to participate in DILGOM 2014. In 2014, the participants from the two southern study areas were invited to health examination while the participant (Study IV) from the three northern study areas provided self-reported physical measurements. The flowchart of the DILGOM study is presented in Figure 13.

4.2.2 Health 2000-2011 (IV)

Health 2000-2011 is a multidisciplinary epidemiological survey of individuals aged ≥30 years living in mainland Finland (Heistaro, 2008; Lundqvist & Mäki-Opas, 2016). The study was carried out by the Finnish Institute for Health and Welfare in collaboration with multiple research and funding agencies and the main aim of the study was to gather information about major health problems in population ≥30 years, health service needs, and working capacity. Study population was stratified by five university hospital districts: Helsinki, Turku, Tampere, Kuopio, and Oulu. From each university hospital regions, 16 health care districts were sampled. All living participants were invited to follow-up examination in 2011 (Figure 14). The

studies were approved by the Coordinating Ethics Committee of the Helsinki University Hospital District.

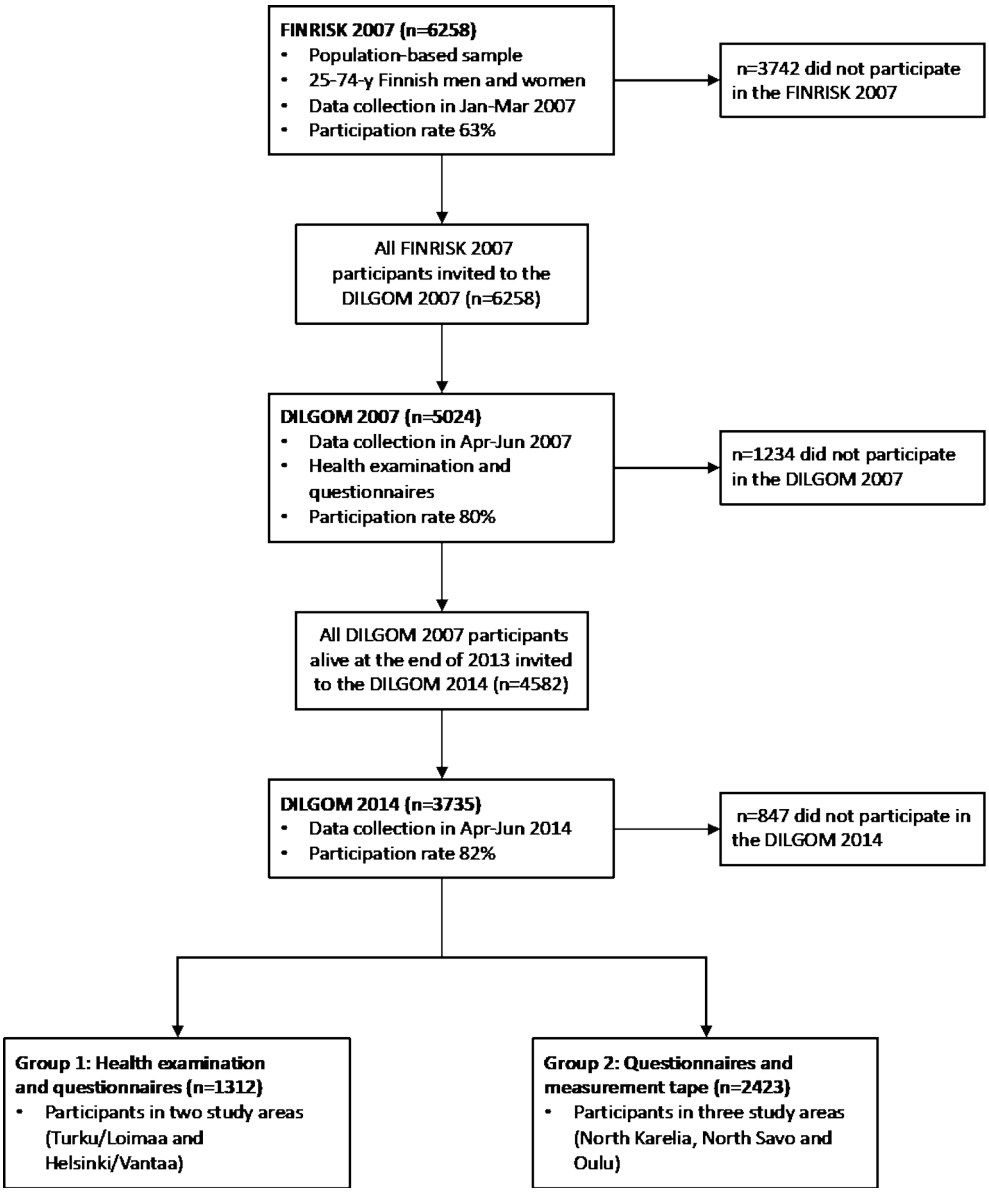

Figure 13. Participant flow chart of the DILGOM study*.

* Reprinted by permission from Springer Nature (Konttinen et al., 2018).

4.2.3 FinHealth 2017 (IV)

After FINRISK 2012, the Finnish Institute for Health and Welfare merged the National FINRISK study and Health 2000-2011 survey protocols to National FinHealth Study (Borodulin & Sääksjärvi, 2019). The aim of FinHealth is to obtain information on health, functional capacity and well-being of adults (aged ≥18) residing in Finland. In FinHealth 2017 study, mainland Finland was stratified in 50 health centre districts: 15 largest cities and seven randomly selected health centre districts from each university hospital regions. The participation rate in the health examination was 58%. The FinHealth 2017 study was approved by Coordinating Ethics Committee of the Helsinki University Hospital District.

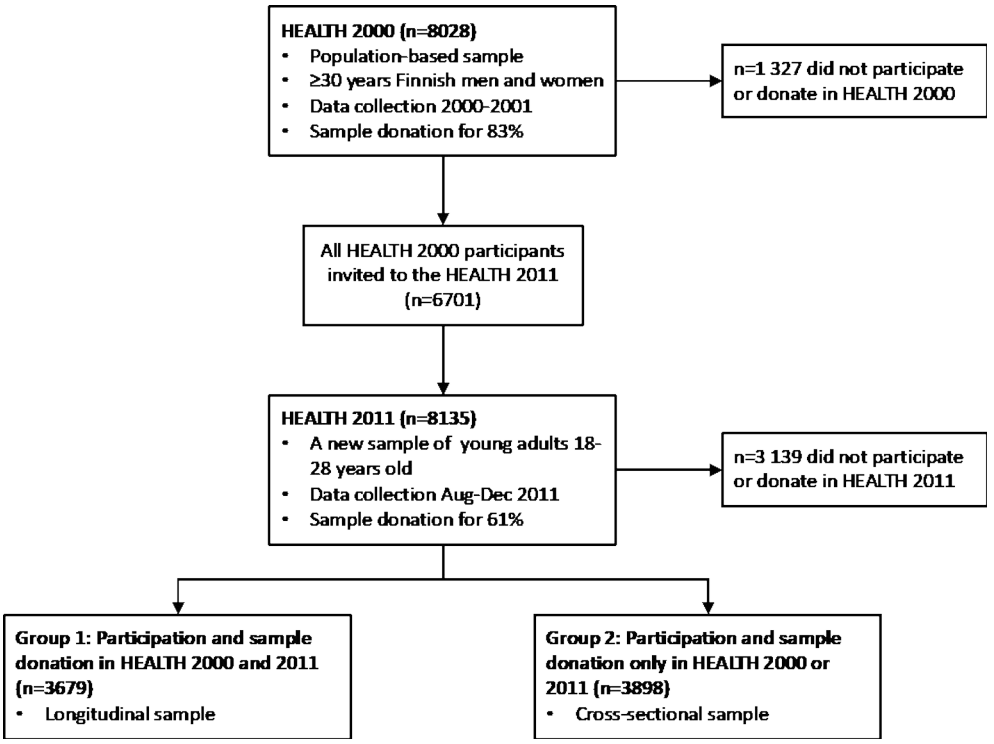

Figure 14. Participant flow chart of the Health 2000-2011 study.

4.2.4 Framingham Heart Study (III)

The first-generation (i.e. the 'Original') cohort of the Framingham Heart Study (FHS) included a random sample of two thirds of the adult population of Framingham, MA who were enrolled in a longitudinal community-based cohort study in 1948. The children and children's spouses of the couples of first-generation

FHS were invited to participate in FHS Offspring study (Kannel et al., 1979). The objective of FHS Offspring study is to obtain information about cardiovascular disease risk factors and family patterns of cardiovascular disease. The participants of the FHS Offspring study have been re-examined every four-to-eight years since the first examination in 1971. In the eighth examination cycle of the study in 2005–2008, serum samples were collected in addition to physical examination. FHS Offspring was approved by Boston University Medical Center's Institutional Review Board.

4.3 Study flow

In the FINRISK and FinHealth 2017 studies (Borodulin et al., 2018; Borodulin & Sääksjärvi, 2019), after filling in a questionnaire on sociodemographic information, lifestyles, medications, and medical history at home, the participants attended a physical examination at a local study site. The participants underwent measurements for height and weight and blood samples were drawn mainly after a minimum of 4 hours of fasting.

In the Health 2000–2011 study (Heistaro, 2008; Lundqvist & Mäki-Opas, 2016), participants were interviewed by centrally trained interviewers on sociodemographic information, lifestyle, medications, and medical history 1-6 weeks before attending physical examination at local study sites. The participants underwent measurements for height and weight. Overnight fasting blood samples were drawn.

According to FHS Offspring Cycle 8 study protocol (Kannel et al., 1979), body weight was measured without shoes optionally wearing a gown and the height was measured barefoot or wearing thin socks using vertical mounted stadiometer rounding down to the nearest quarter inch. Technicians recorded participant's regular medication at on home visit using medication bottles or nursing home charts. Medical history, including diabetes status and smoking, was obtained using standardized interview forms.

4.3.1 Blood pressure measurement (II–IV)

The European Health Examination Surveys recommends that BP should be measured after five minutes of rest taking three measurements one minute apart (Tolonen, 2016). Study participants should fast for >4 hours and avoid smoking and vigorous exercise for one hour before examination (Tolonen, 2016). FINRISK, Health, FHS, and FinHealth studies performed sequential blood pressure measurements using a mercury column sphygmomanometer on seated participants but some difference remains due to contemporary guidelines (Table 5). In FINRISK and FinHealth 2017 studies, a study nurse measured sitting BP three times from the

right arm using a mercury sphygmomanometer with an appropriately sized cuff (Borodulin et al., 2018; Borodulin & Sääksjärvi, 2019). In Health 2000-2011 study, a study nurse measured sitting BP two times from the right arm using a mercury sphygmomanometer and a 15 x 43 cm sized cuff; a larger cuff was used when needed (Heistaro, 2008; Lundqvist & Mäki-Opas, 2016). In FHS study, blood pressure was measured by average of two measurements using manual mercury sphygmomanometer using with appropriate cuff size (Kannel et al., 1979).

Table 5. Blood pressure measurements in FINRISK, FHS, Health, and FinHealth.

Study	N	Cuff size (proximal arm circumference)	Rest for first measurement	Rest between measurements
FINRISK 1997	2	13 cm x 42 cm	>5 min	
Health 2000*	2	12 cm x 35 cm for ≤35 cm 15 cm x 43 cm for >35 cm	>5 min	2 min
FINRISK 2002	3	14 cm x 36 cm	>5 min	>1 min
FHS OFFSPRING 8th cycle	2	Adult thigh cuff Adult large cuff Adult regular cuff Pediatric cuff	>5 min	>30 sec
FINRISK 2007	3	14 cm x 36 cm	>5 min	>1 min
Health 2011†	2	12 cm x 35 cm for ≤35 cm 15 cm x 43 cm for >35 cm	>5 min	>1 min
FINRISK 2012	3	14 cm x 36 cm	>5 min	> 1min
FinHealth 2017‡	3	small cuff for <24 cm medium cuff for 24– 32 cm large cuff for 32–48 cm extra large cuff for >48 cm	>5 min	>1 min

Information is combined from reported FINRISK (Borodulin et al., 2018), Health 2000 (Heistaro, 2008), FHS (Kannel et al., 1979), Health 2011 (Lundqvist & Mäki-Opas, 2016), and FinHealth 2017 (Borodulin & Sääksjärvi, 2019) study protocols. The Finnish language study protocols were consulted with respect to rest between BP measurements (ISBN 951-740-073-X, ISBN 951-740-356-9, ISBN 978-951-740-911-7, ISBN 978-952-302-052-8).
*Participants asked not to eat, smoke, and avoid physical exertion.
†Participants asked to avoid heavy exercise, cola drinks, coffee, tea, eating, and smoking before
‡Participant advised to refrain from heavy exercise, eating and drinking prior the examination.

4.3.2 Blood samples (II–IV)

In the FINRISK studies, blood samples were drawn after a minimum of 4 hours of fasting, the samples were centrifuged at the field surveys sites (Borodulin et al., 2018). In FINRISK 1997 and 2002, fresh samples were sent daily to THL and from FINRISK 2007, sera were frozen after separation at field surveys sites and

transported to THL once a week. Blood samples were stored at -70°C. In FHS, samples were drawn after 12-hour fast, centrifuged 22 min at 4°C, and separated plasma was stored at -80°C within 90 minutes of collection (Kannel et al., 1979). In the Health 2000 study, blood samples were drawn after a minimum of 4 hours of fasting, serum samples were frozen on site at -20°C within 90 minutes from sampling and stored at -70°C within 1-2 week after sampling (Heistaro, 2008). In the FinHealth 2017 study, blood samples were drawn after a minimum of 4 hours of fasting, the samples were frozen at -20°C within 120 minutes from sampling and stored at -70°C within 1-2 week after sampling (Borodulin & Sääksjärvi, 2019).

4.3.3 Stool samples (II)

In the FINRISK 2002 study, stool samples were collected from all voluntary participants (Borodulin et al., 2018; L. Valsta, personal communication, 2019). At the final stage of the health examination, a research nurse informed participants about the stool sample protocol and provided stool sample kits. Participants were instructed to collect stool at home and send the stool samples to THL in 50 ml Falcon tubes using provided mailing packages. The samples were then frozen in -20°C and kept unthawed until 2017, when they were sent to the University of California San Diego for microbiome sequencing.

4.3.4 Urine samples (II)

In the FINRISK 2002 study, 10-year age group and sex stratified subsample of 2240 individuals aged 25–64 years was drawn from North Karelia, southwestern Finland, and Helsinki area for 24-hour urine collection (Laatikainen et al., 2006). Participants were instructed to start the 24-hour urine collection on a Sunday morning and return the container the following day to the examination site. The purpose of the urine collection to assess the dietary salt measurement was not specifically mentioned to the participants. At the examination site, a study nurse mixed the sample, measured total urine volume and took a sample of urine to central laboratory. The samples were frozen at -20°C for storage and later analyzed using an ion-selective electrode (Optima analyzer, Thermo Electron Oy, Vantaa, Finland). Daily urine sodium excretion was calculated as the product of 24-hour urine sodium concentration and volume.

Of the 2240 invited individuals, 1564 participated and 919 returned the urine specimen. Of the 919 returned urine collections, 10 were deemed incomplete due to creatinine ≤5.0 mmol/day or creatinine ≤6.0 mmol/day with volume <1000 ml for final urinary sodium subsample of 909. Out of the 909 participants with completed

urinary collection, 63 were excluded due to missing stool collection, and 17 for missing relevant covariates for final urinary sodium subsample of 829 participants.

4.4 Shotgun metagenomics (II)

Microbiota analyses for stool samples collected in FINRISK 2002 were performed at the University of California San Diego using whole genome untargeted shallow shotgun metagenomic sequencing following previously published protocol (Salosensaari et al., 2021). Illumina-compatible libraries were prepared from isolated DNA and normalized to 5 ng input per sample. DNA was ligated with iTru dual-indexing system allowing sample pooling (Glenn et al., 2019). Barcoded and amplified libraries were then pooled in approximately equimolar ratios and sequenced using Illumina Hi-Seq 4000 for paired-end 150 bp reads. Sequenced reads were mapped against taxonomy using SHOGUN v1.0.5 (Hillmann et al., 2018) against NCBI RefSeq database version 82 (O'Leary et al., 2016).

Functional profiling was obtained using Kyoto Encyclopedia of Genes and Genomes Orthology group (KO) annotations for RefSeq-derived genes following the default parameters of SHOGUN tool (Hillmann et al., 2018). To improve the accuracy of low-abundance genes, the KO profiles were also estimated using reference genomes to predict the presence of unsampled genes within the observed genome. The final KO table represents the average of directly observed and weighted predicted KO profiles.

Out of the 8799 individuals who took part in FINRISK 2002, we excluded 1568 participants who did not provide stool samples, 20 participants due to low sequencing depth (<50000 reads), and 258 participants due to missing relevant covariates for a final study sample of 6953 individuals who were included in the analysis. The average read count was approximately 900,000 reads per sample (Salosensaari et al., 2021).

4.5 Genotyping (III)

The genome-wide single-nucleotide polymorphisms (SNP) genotyping and quality control have been previously described in detail (Abraham et al., 2016). The participants of FINRISK 2002 were genotyped in two batches together with FINRISK 1992-1997 participants: Illumina HumanHap610 platform was used in first and Illumina CoreExome genotyping array in second batch. Genotype calls were generated at the Institute of Molecular Medicine Finland (FIMM) and genotype imputation was performed using Sequencing Initiative Suomi v3 reference panel (Pärn, Fontarnau, et al., 2018; Pärn, Isokallio, et al., 2018).

4.6 Spectroscopy

4.6.1 Eicosanoid profiling (III)

We used high-throughput measure of bioactive lipids using directed non-targeted LC–MS approach described previously in detail (Fendt & Lunt, 2019; Watrous et al., 2019). Using a directed non-targeted LC–MS approach in conjunction with computational chemical networking of spectral fragmentation patterns, we identified 545 eicosanoids and related oxylipins in the FINRISK. Metabolite data were adjusted for technical variation in off-plate pooled plasma samples and in spike-in internal standards. We adjusted for patch variation normalizing each metabolite measurement in each plate using formula (raw peak intensity - plate median peak intensity)/(median absolute deviation of plate). Missing values were replaced with minimum value for each eicosanoid abundance. While observed metabolites were labeled using mass-to-charge ratios and retention times, we were prepared to manually match a subset of all metabolites between FINRISK and FHS comparing their LC–MS profiles, reference standards, and online databases.

After participants with missing relevant covariates were excluded, we had eicosanoid and related oxylipins profiles available for 8099 participants in FINRISK 2002 (discovery cohort) and 2859 participants in FHS (replication cohort). The group of signals we defined as eicosanoids, were highly consistent with known and putative eicosanoids and related oxylipins in human plasma (Watrous et al., 2019).

4.6.2 NMR metabolic measures (IV)

We had access to metabolomics analyses performed using 1H-NMR spectroscopy on highly automated platform from serum samples by Nightingale Health Ltd, Helsinki, Finland (Soininen et al., 2015; Würtz et al., 2017). Frozen serum samples were thawed over night at +4°C. Automatic liquid handler inserted buffer to serum samples and moved the aliquots to 96-format racks. Three molecular windows were available using 500 MHz and 600 MHz spectrometers: broad signals arising from lipoproteins, low-molecular-weight metabolites, and lipids. The validity of this approach in detection of lipoprotein cholesterol, triglycerides, glucose, circulating fatty-acids, and beta-hydroxybutyrate has been demonstrated compared to routine clinical assays, gas chromatography, and enzymatic method (Würtz et al., 2017).

We included in our analyses 53 circulating biomarkers and 97 lipoprotein subclass measures for cross-sectional sample of 36985 and longitudinal sample of 4197 FINRISK 1997–2012, Health 2000-2011, and FinHealth 2017 participants.

4.7 Definitions

4.7.1 Blood pressure measurements

We used in all studies the mean of the first two BP measurements to define systolic and diastolic BP. We defined hypertension as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or use of antihypertensive medication. Study II used register-based and studies III and IV self-reported information about antihypertensive medication use. We defined pulse pressure as systolic minus diastolic BP and mean arterial pressure as [(2 x diastolic BP) + systolic BP] / 3.

4.7.2 Questionnaire based definitions

Smoking was defined by self-reported current daily smoking. Leisure time physical activity was self-reported from four options: (i) sedentary, (ii) light activity for over four hours per week, (iii) fitness training or other strenuous exercise for over three hours per week, and (iv) competitive sports. In Study IV, diabetes, antihypertensive medication use, and lipid medication use was self-reported.

4.7.3 Anthropomorphic measures

Body mass index (BMI) was defined as weight (kg) divided by the square of the body height (m).

4.7.4 Register and laboratory-value based definitions

The information about medication use was retrieved from Finnish national Drug Purchase Register using Anatomical Therapeutic Chemical classification (ATC) codes (The Social Insurance Institution of Finland, 2012; WHO Collaborating Centre for Drug Statistics, 2018). Finnish pharmacies fill prescriptions for a maximum of three months and medication use was determined as a drug purchase occurring within the four months preceding baseline. Information about different comorbidities was acquired from nationwide Care Register for Health Care using International Classification of Diseases (ICD) codes (Finnish Institute for Health and Welfare, 2021; Lääkintöhallitus, 1986).

In studies II, antihypertensive medication was defined using four ATC classes: diuretics (C03*), beta blockers (C07*), calcium channel blockers (C08*), and renin–angiotensin system inhibitors (C09*). In studies II and III, prevalent diabetes was defined as self-reported diabetes, previous diagnostic code (ICD-10 codes E10-E14 or ICD-8/9 code 250), a previous diabetes medication purchase (ATC code A10*),

or special reimbursement code for diabetes medications in the Drug Reimbursement Register. However, in FHS Offspring, prevalent diabetes was defined as a fasting plasma glucose ≥7.0 mmol/l or self-reported use of glucose-lowering medications.

4.8 Statistical analyses

We used R version 3.6 for all statistical analyses and published the source code for the analyses under open license in open-access repository Zenodo.

4.8.1 Study I

The preliminary literature review did not reveal any consistent numerical features that could be used to perform meta-analysis.

4.8.2 Study II

Unless otherwise noted, we adjusted the analyses for the well-established correlates of hypertension: age, sex, BMI, smoking, exercise, diabetes mellitus, diuretic use, beta blocker use, calcium channel blocker use, and renin–angiotensin system inhibitor. We calculated alpha diversity using Shannon's index on species level data. We used Bray-Curtis dissimilarity indices on compositional microbial species-level abundance for beta diversity and to perform Principal Coordinates Analysis (PCoA). We analyzed the proportion of variance BP explains about microbial beta diversity using multivariate analysis of variance (PERMANOVA) with 999 permutations. We defined common microbial genera to be prevalent in at least 1% of sample population with a relative abundance over 0.1%. We used DESeq2 with Benjamini-Hochberg correction to study the associations between microbial abundances and BP (Benjamini & Hochberg, 1995; Love et al., 2014). First, we studied the associations between common microbial genera and blood pressure indices. Second, we studied the associations between *Lactobacillus* species with (1) blood pressure indices and (2) 24-hour urinary sodium excretion. We used abundances from all available species to estimate size factors to be uses in analyses with *Lactobacillus* species. We studied the association between log(x+1) transformed KO groups and systolic BP using linear regression with Benjamini-Hochberg correction.

4.8.3 Study III

Unless otherwise noted, we adjusted all analyses for age, sex, BMI, current smoking, diabetes, antihypertensive medication, and technical variable (mass spectrometry batch). We corrected for missing LC–MS data imputing missing values with

minimum observed values for each feature. All features were centered to zero and standardized to unit variance without other transformations. We used linear and logistic regression models to study the associations between eicosanoids and BP. We adjusted for multiple testing using Bonferroni correction to minimize the type I error. We studied the Spearman correlation between eicosanoid significantly associated with systolic BP in combination with hierarchical cluster analysis with complete linkage method. We studied the multivariable association between eicosanoids and systolic BP using forward stepwise regression with inclusion threshold of P=0.05/545. We defined eicosanoid risk score using effect sizes from forward selection model according to the formula $\beta_1 \cdot X_1 + \beta_2 \cdot \mathrm{X}_2 + \cdots + \beta_\mathrm{n} \cdot \mathrm{X}_\mathrm{n}$ with X_i denoting the standardized value for the i:th eicosanoid abundance, and β_i denoting the effect size in the forward selection model. Finally, we calculated the association between eicosanoid risk score and BP. We replicated these analyses in FHS Offspring using the eicosanoid abundances in FHS and the regression coefficients from FINRISK.

To analyze the causative role of the eicosanoid risk score, we performed Mendelian randomization (MR). To account for ordered patterns in genetic data, we calculated multidimensional scaling based on raw Hamming distances using PLINK version 1.9. We performed genome-wide association study (GWAS) for the continuous eicosanoid risk scores and the autosomes using SNPTEST version 2.5.2 adjusted for age, sex, batch, and first ten MDS-axes. In brief, we used FINRISK data to find SNPs associated with eicosanoid risk score and UK Biobank data to find the associations between SNPs and BP. We performed the MR using TwoSampleMR with SNPs that had Hardy–Weinberg equilibrium >1E-6, P < 5E-8, and minor allele frequencies >0.01. We estimated the causative roles using five regression method.

4.8.4 Study IV

Unless otherwise noted, we adjusted all analyses for age, sex, BMI, smoking, diabetes, leisure-time physical activity, antihypertensive medication, lipid medication, and cohort. Cohort was included to account for the time-dependent differences in the diagnosis and management of hypertension. Antihypertensive medication was not used in models where outcome was hypertension. All features were centered to zero and standardized to unit variance. We adjusted for multiple testing using Benjamini-Hochberg correction (Benjamini & Hochberg, 1995). We studied the associations between systolic BP, diastolic BP, and hypertension in the cross-sectional sample using linear and logistic regression models. To study the age- and sex-related differences in metabolite-BP associations, we performed similar analyses in groups divided by median age (50.5 years) and sex. In the longitudinal sample, we defined systolic BP change as follow-up BP minus baseline BP. We

studied associations between the baseline metabolites and systolic BP change using linear models; we included baseline systolic BP among the covariates in the longitudinal model.

We used gradient boosting machine learning algorithm XGBoost to assess the multivariable associations of the 53 circulating biomarkers with BP (T. Chen & Guestrin, 2016). We used root-mean-square error (RMSE) to estimate model fit. We performed leave-one-out cross-validation in FINRISK 1997-2002 and Health 2011 and five-fold cross-validation for Health 2000–2011. We used FinHealth 2017 and FINRISK 2007–2014 for testing. We tuned the hyperparameters using Bayesian optimization using R package 'mlrMBO' (Bischl et al., 2017). We compared the model fit using three set of covariates: (1) clinical covariates, (2) metabolic measures, (3) the combination of clinical covariates and metabolic measures. We studied marginal associations between distinct metabolic measures and BP using partial dependency plots using pdp-package (Greenwell, 2017).

5 Results

5.1 Literature review for gut microbiota (I)

The systematic literature search for original research studies reporting results between gut microbiota and essential hypertension was performed on 4 September 2020 without language and publication date restrictions. The search utilized three medical databases and it identified 669 potential original research articles (Figure 15). In initial screening, we excluded 180 duplicate search results, 264 results based on title and 132 results based on abstract.

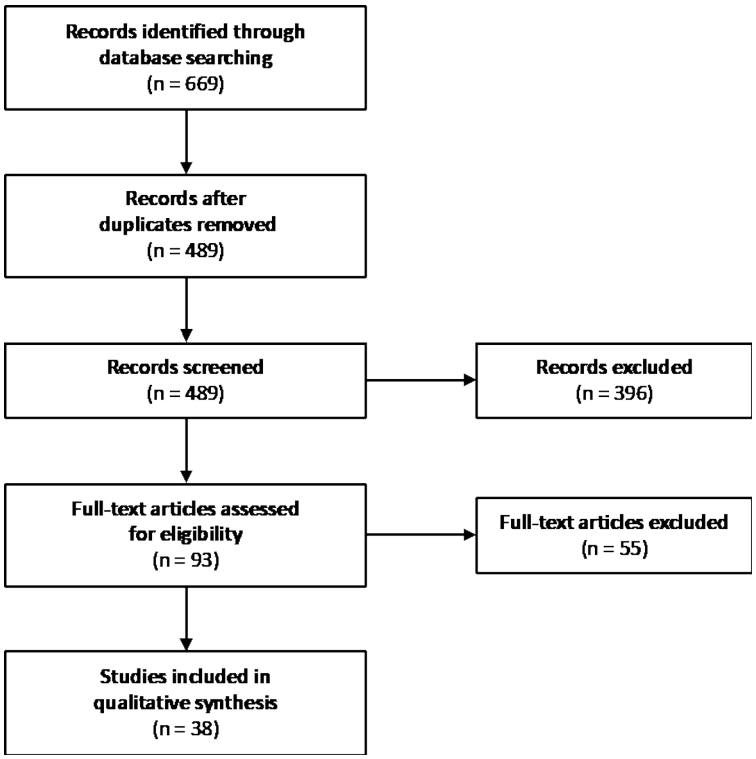

Figure 15. Flow chart for the original research articles identified the systematic literature review.

In total, 93 manuscripts were assessed for eligibility in full-text screening. Four manuscripts were excluded as review articles. While reporting results, 20 manuscripts did not perform gut microbiota sequencing and 27 manuscripts performed interventions outside of the scope of our systematic literature review. The 38 original research articles included in our systematic literature review reported results for 22 animal studies, 13 small-scale human studies, four large-scale human studies, and one interventional pilot study in humans.

The animal studies contained comprehensive set of interventional research designs aimed to study the association between BP and gut microbiota in rodents. We summarized the animal studies in table identifying the studied animals, sequencing method, intervention type, the positive and negative associations the intervention had with circulating biomarkers, vasculature, organs significant in BP regulation, and the positive and negative associations the intervention had with gut microbiota and fecal SCFAs (Study I, Table 1).

In human studies, except one interventional pilot study, all original research articles reported cross-sectional associations between BP and hypertension. We summarized the human studies in the table identifying the study population, gut microbiota sequencing methods, the taxa with positive association with BP, the taxa with negative association with BP, the associations SCFAs had with hypertension, and the association between taxa and dietary salt (Study I, Table 2).

In summary, animal studies have suggested that gut microbiota and hypertension are linked. However, the evidence from human studies is still incomplete as only four large-scale human studies have been published to date. Future studies could be improved by 1) using more accurate methods of BP measurement, 2) performing deep metagenomic sequencing, and 3) utilizing interventional designs.

5.2 Gut microbiota and blood pressure (II)

The main sample of Study II included 6953 participants (mean age 49.2 ± 12.9 years, 54.9% women) and 24-hour urinary sodium subsample 829 participants (mean age 47.2 ± 10.9 years, 55.5% women). We observed 91 common microbial genera (4.7% of all available genera) and 134 *Lactobacillus* species.

5.2.1 Microbial diversity and BP

In models adjusted for age and sex, a standard deviation increase in microbiota alpha diversity was inversely associated with systolic blood pressure (effect size -0.54 mmHg; 95% confidence interval [CI], -0.96 to -0.12; $P = 0.012$), diastolic blood pressure (-0.31 mmHg; 95% CI, -0.56 to -0.06; $P = 0.016$), and hypertension (odds

ratio [OR] 0.91; 95% CI, 0.86 to 0.96; P<0.001). However, alpha diversity was not significantly associated with BP in the fully adjusted models (Study II, Figure 1).

Consistently, we observed associations between beta diversity and BP indices in age- and sex-adjusted models (P≤0.04 for all). The coefficients of determination (R^2) for the BP indices and beta diversity varied between 0.0002 and 0.0006. In multivariable-adjusted models, only diastolic blood pressure (R^2 = 0.0002, P = 0.032) was significantly related to beta diversity (Study II, Figure 1). The results for all diastolic BP model covariates are presented in Table 6. Due to the implementation of the PERMANOVA method, the covariate order affects the observed R^2 favoring the first introduced covariates. The first three PCoA axes explained 31.3% of the variation in bacterial abundances (Study II, Figure 2).

Table 6. The proportion of gut microbial beta diversity explained by diastolic BP and model covariates.

	Age and sex adjusted model		**Multivariable-adjusted model**	
Model covariates	***R²***	***P***	***R²***	***P***
Age at baseline	0.49%	0.001	0.49%	0.001
Women	0.45%	0.001	0.45%	0.001
BMI			0.29%	0.001
Smoker			0.23%	0.001
Exercise			0.10%	0.001
Diabetes			0.06%	0.001
Diuretic			0.04%	0.003
Beta blocker			0.03%	0.002
Calcium channel blocker			0.02%	0.151
Agents acting on the RAS			0.03%	0.019
Diastolic BP	0.05%	0.001	0.02%	0.032
Residuals	99.00%		98.24%	

Multivariable-adjusted model was adjusted for age, sex, BMI, smoking, exercise, diuretics, beta blockers, calcium channel blockers, and renin-angiotensin system blockers. Analysis of variance for beta diversity was calculated using 999 permutations. BP, blood pressure; RAS, renin–angiotensin system; R^2, proportion of variation.

5.2.2 Common microbial genera and BP

We observed 122 significant associations between 45 distinct gut microbial genera and BP in fully adjusted model with FDR-corrected P<0.05 (Study II, Figure 3). A subset of genera that are significantly associated with both systolic BP and hypertension is presented in Table 7. We studied the number of significant associations for hypertension when third BMI was introduced to the age- and sex-adjusted model and observed that the number of significant associations was reduced from 39 to 23 (59%).

Table 7. Bacterial genera associated with both hypertension and systolic BP.

	Systolic BP		**Hypertension**	
	Log2FC ± SE	***P***	**Log2FC ± SE**	***P***
Anaerostipes	0.06 ± 0.02	0.015	0.16 ± 0.04	<0.001
Anaerotruncus	-0.05 ± 0.01	<0.001	-0.06 ± 0.02	0.037
Blautia	0.04 ± 0.01	0.015	0.11 ± 0.03	<0.001
Coprobacillus	-0.12 ± 0.02	<0.001	-0.20 ± 0.04	<0.001
Coprococcus	0.05 ± 0.01	0.004	0.10 ± 0.03	0.012
Dielma	0.23 ± 0.03	<0.001	0.28 ± 0.07	0.001
Enterobacter	0.25 ± 0.04	<0.001	0.89 ± 0.09	<0.001
Fournierella	-0.02 ± 0.01	0.022	-0.07 ± 0.02	0.002
Holdemania	0.10 ± 0.02	<0.001	0.20 ± 0.04	<0.001
Megasphaera	0.19 ± 0.03	<0.001	0.23 ± 0.07	0.008
Phascolarctobacterium	0.10 ± 0.03	0.011	0.22 ± 0.07	0.007
Ruthenibacterium	0.07 ± 0.02	0.004	0.12 ± 0.05	0.034

Models are adjusted for age, sex, BMI, smoking, exercise, diuretics, beta blockers, calcium channel blockers, and renin–angiotensin system blockers. Log2FC, fold change in logarithmic scale to base 2; SE, standard error.

5.2.3 *Lactobacillus* species and BP

We observed 41 significant associations between 19 distinct *Lactobacillus* species and BP indices in the fully adjusted models with FDR-corrected P<0.05 (Study II, Figure 3). Of the 41 observed associations, 12 had positive and 29 negative association with BP indices. A subset of *Lactobacillus* species also associated with systolic BP is presented in Table 8. Notably, *Lactobacillus* genus was not significantly associated with BP in the fully adjusted models which could be explained by the presence of both positive and negative associations observed in

species-level analyses. In the age- and sex-adjusted model and in most three covariate models, *Lactobacillus* genus had a negative association with hypertension.

Table 8. Lactobacillus species associated with systolic BP.

	Systolic BP		Hypertension	
	Log2FC ± SE	**P**	**Log2FC ± SE**	**P**
L. aviarius	-0.13 ± 0.03	0.001		
L. farciminis	-0.33 ± 0.05	<0.001	-0.60 ± 0.10	<0.001
L. hominis	-0.26 ± 0.04	<0.001	-0.42 ± 0.09	<0.001
L. iners	-0.18 ± 0.05	0.013	-0.45 ± 0.11	0.001
L. kalixensis	0.23 ± 0.07	0.013		
L. kefiranofaciens	0.20 ± 0.06	0.025		
L. paracasei	-0.15 ± 0.04	0.020		
L. sakei	0.15 ± 0.04	0.009		

Models are adjusted for age, sex, BMI, smoking, exercise, diuretics, beta blockers, calcium channel blockers, and renin–angiotensin system blockers. BP, blood pressure. Log2FC, fold change in logarithmic scale to base 2; SE, standard error.

5.2.4 *Lactobacillus* species, dietary salt, and BP

In 24-h urinary sodium subsample (N = 829), the mean sodium excretion was 142.3 ± 62.9 mmol. We observed 15.5% *Lactobacillus* prevalence at the detection limit of 0.1% relative abundance. 24-hour urinary sodium excretion was not associated with *Lactobacillus* genus. We, however, observed two significant associations between 24-hour urinary sodium excretion and species level *Lactobacillus* abundances (Study II, Figure 4). *L. paracasei* had a negative (Log2FC -0.018 ± 0.002, P<0.001) and *L. salivarius* (Log2FC 0.007 ± 0.002, P = 0.004) a positive association with urinary sodium excretion.

5.2.5 Functional analysis of gut microbiota

We observed 481 associations between KO groups and systolic BP with FDR-corrected P<0.05. The most prominent observed pathways were related to lipid metabolism, gluconeogenesis, and xenobiotic metabolism (Study II, Figure S3). The KO groups provide information about potential metabolic pathways available for the observed gut microbiota; the information about gut microbial gene expression and the presence of different metabolic end products could be measured using transcriptomics and metabolomics methods.

5.3 Eicosanoids and BP (III)

The main discovery sample of Study III included 8099 participants (FINRISK 2002, mean age 48.0 ± 13.1 years, 53.1% women) and replication sample 2859 participants (FHS, mean age 66.3 ± 8.9 years, 54.7% women). We observed 545 eicosanoids and related oxylipin mediators using a high-throughput directed non-targeted LC–MS approach (Watrous et al., 2019). Oxylipins are collection of oxygenated lipids and eicosanoids are an important subgroup of oxylipins in mammals (Noverr et al., 2003); we use in the following paragraphs the term eicosanoids while potentially referring also to other closely related non-eicosanoid PUFAs and PUFA derivates.

5.3.1 Association between eicosanoids and BP

We used systolic BP as our main outcome variable due to its well-established association with cardiovascular diseases and linear correlation with age. We observed 187 (34.3%) significant associations between distinct eicosanoids and systolic BP with $P<0.05/545$ (Study III, Figure 1). The majority of the observed features were positively (N = 175, 93.6%) associated with systolic BP. Spearman's rank correlations revealed strong overall correlations between eicosanoids but only minor clustering when ordered using hierarchical cluster analysis with complete linkage method (Study III, Figure 2).

5.3.2 Defining an eicosanoid risk score

We used a fully adjusted forward selection linear regression model with a Bonferroni-corrected inclusion threshold $P<0.05/545$ to find multivariable association between set of eicosanoids and systolic BP in the discovery cohort. The model resulted to six eicosanoids that were identified using reference standards and online databases (Table 9). We rounded the effect sizes to two decimals and defined eicosanoid risk score using the formula $0.88 \cdot X_1 + 0.91 \cdot \mathrm{X}_2 + 1.00 \cdot \mathrm{X}_3 + 1.32 \cdot \mathrm{X}_4 + 1.35 \cdot \mathrm{X}_5 + 0.83 \cdot \mathrm{X}_6$, where X_i refers to the eicosanoid at i:th row or the Table 9.

The spectroscopic profile of the six eicosanoids from discovery cohort were matched in replication cohort with four eicosanoids. Two of the eicosanoids, 11-dehydro-2,3-dinor-TXB2 and 295.2279/4.89, could not be detected in the plasma samples of the replication cohort. While the metabolite identification in LC–MS is non-trivial, the matching of features between cohorts was robust. The abundances of the missing eicosanoids were treated zero valued in replication cohort giving rise to effective eicosanoid risk formula $0.91 \cdot \mathrm{X}_2 + 1.32 \cdot \mathrm{X}_4 + 1.35 \cdot \mathrm{X}_5 + 0.83 \cdot \mathrm{X}_6$ (Table 9).

Feature selection regression model and single eicosanoid models in both discovery and replication cohorts gave consistent results. Remarkably, all captured associations had positive association with systolic BP.

Table 9. Results for feature selection model and corresponding association of features with systolic BP in FINRISK and FHS.

	Feature selection	Single eicosanoid model in FINRISK	Single eicosanoid model l n FHS
Metabolite	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
11-dehydro-2,3-dinor-TXB2	0.88 (0.47–1.30)	1.60 (1.20–2.01)	
12-HHTrE	0.91 (0.52–1.30)	1.12 (0.74–1.50)	1.38 (0.77–1.98)
295.2279/4.89*	1.00 (0.60–1.39)	1.96 (1.58–2.34)	
5,6-EET	1.32 (0.81–1.83)	2.74 (2.37–3.11)	1.42 (0.82–2.02)
Adrenic Acid	1.35 (0.81–1.89)	2.77 (2.37–3.16)	1.26 (0.63–1.88)
Tetranor-12(R)-HETE	0.83 (0.44–1.23)	1.43 (1.05–1.81)	1.89 (1.28–2.49)

All models were adjusted for age, sex, BMI, current smoking, diabetes mellitus, antihypertensive medication, and batch. Asterisk (*) denotes putative eicosanoid. FHS, Framingham Heart Study; HHTrE, hydroxyhepta-decatrenoic acid; TXB2, thromboxane B2; HETE, hexadecatrienoic acid.

5.3.3 Eicosanoid risk score and systolic BP

Individuals at the highest quartile of risk score in discovery cohort had 9.0 mmHg (95% CI 8.0-10.1 mmHg) and in replication cohort 6.8 mmHg (95% CI 5.1-8.5 mmHg) higher systolic BP compared to individuals in the lowest quartile (Study III, Figure 4). A 1-SD increase in risk score was associated with 3.6 mmHg (95% CI 3.2–3.9, P<0.001) and 2.2 mmHg (95% CI 1.6–2.8, P<0.001) higher systolic BP in the discovery and replication cohorts, respectively.

5.3.4 Two-sample Mendelian randomization

In GWAS, 222 SNPs were associated with the eicosanoid risk score. 132 SNPs were located in chromosome 1 and 90 in chromosome 11. To account for nonrandom associations between alleles of different loci, the linkage-disequilibrium, only the SNP with lowest P-value was retained in each 10kb window (Table 10).

To increase statistical power, we used two-sample approach in Mendelian randomization where features are linked with SNPs in one sample and SNPs are linked with outcome in another sample. UK Biobank (Cardiff University, United Kingdom) reports association between GWAS results and systolic BP for 436419 individuals. However, two-sample Mendelian randomization between the three

SNPs in FINRISK 2002 and automated systolic BP measurement in UK Biobank did not produce significant results ($P > 0.26$). Therefore, we were unable to provide evidence for the causality of the observed associations in the current study.

Table 10. The SNPs associated with eicosanoid risk score in genome-wide association study after adjusting for linkage-disequilibrium.

SNP	Chromosome	Alleles	MAF	Effect size ± SE	P
rs72681939	1	A/G	13.6%	0.136 ± 0.025	3.5E-08
rs7523082	1	A/T	34.3%	0.116 ± 0.018	8.1E-11
rs174536	11	A/C	42.8%	-0.174 ± 0.017	2.6E-24

Models were adjusted for age, sex, batch. MAF, minor allele frequency; SE, standard error; SNP, Single-nucleotide polymorphisms.

5.4 Biomarker profile of hypertension (IV)

The cross-sectional sample of Study IV included 36985 participants (FINRISK 1997–2012, Health 2000, FinHealth 2017; mean age 50.5 ± 14.2 years, 53.1% women) and longitudinal sample 4197 participants (FINRISK 2007–DILGOM 2014, Health 2000–2011; mean age 49.4 ± 11.8 years, 55.3% women). We included in our core analyses 53 circulating metabolite biomarkers measures using high-throughput NMR. We also studied in more detail 97 lipoprotein measures related lipoprotein particle subclasses.

5.4.1 Cross-sectional associations

In the conventional linear regression models of the cross-sectional sample, only two amino acids, histidine, and valine, of all 53 circulating biomarkers included in our analysis were not associated with BP (Study IV Figure 1). We also performed sex- (Study IV Figure S3) and median age-stratified (Study IV Figure S4) analyses. Acetate was negatively associated with hypertension in women only and acetoacetate positively associated with hypertension in men only. The significant association between total cholesterol, LDL cholesterol, esterified cholesterol, and HDL cholesterol with hypertension was not observed in older than median age participants. Men compared to women and younger participants compared to older participants had in some cases slightly larger effect sizes. Large and extremely large HDL fractions were negatively and medium and small HDL fractions positively associated with hypertension (Study IV Figure S5). The cross-sectional associations were highly consistent across study cohorts supporting data pooling (Figure 16).

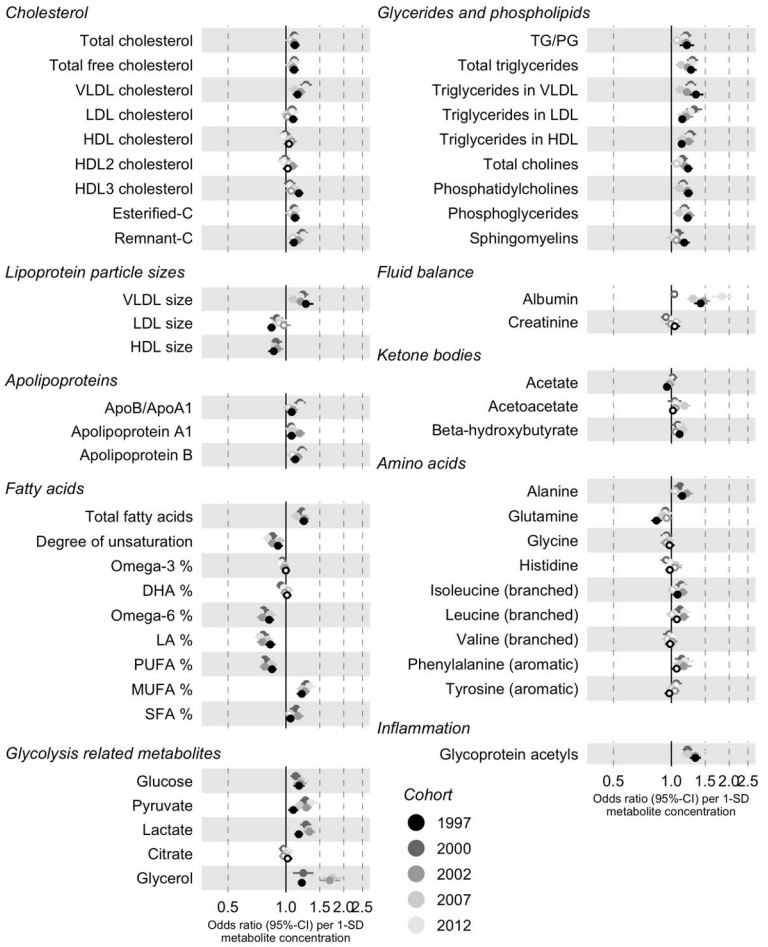

Figure 16. Cross-sectional associations between metabolic measures and hypertension performed separately in each study cohort*.

5.4.2 Longitudinal associations

We studied the associations between baseline metabolic measures and systolic BP change between baseline and a follow-up of 7–11 years (Study IV, Figure 2). We observed that LDL cholesterol (β = 0.74 mmHg per 1-SD metabolite concentration; 95% CI 0.28–1.20 mmHg; $P = 0.01$), remnant cholesterol (β = 0.62 mmHg; 95% CI 0.14–1.10 mmHg; $P = 0.03$), apolipoprotein B (β = 0.63 mmHg; 95% CI 0.14–1.11 mmHg; $P = 0.03$), and acetate (β = 0.83 mmHg; 95% CI 0.25–1.41 mmHg; $P = 0.02$) were positively and average HDL particle size (β = -0.89; 95% CI -1.46 to -0.32

* Figure published under Creative Commons Attribution license (Palmu et al., 2021).

mmHg; $P = 0.01$) negatively associated with systolic BP change between baseline and follow-up examinations. Large and extremely large HDL fractions were negatively and other lipoprotein fractions mostly positively associated with systolic BP change (Study IV, Figure S6).

5.4.3 Metabolic profile of hypertension

We used multivariable gradient boosting machine learning models to study the cross-sectional and longitudinal metabolic profile of hypertension. We compared the predictive accuracy between three set of model covariates: (1) clinical covariates, (2) metabolic measures, (3) the combination of clinical covariates and metabolic measures. We estimated the model performance in test models using FinHealth 2017 and FINRISK 2007–DILGOM 2014 cohorts that were not used in model training. In cross-sectional and longitudinal samples, information about clinical characteristic gave better model prediction than only using information about the 53 core metabolic measures (Table 11). However, the full model containing information about clinical characteristics and metabolic measures gave the best model estimate (Table 11).

In the cross-sectional model, glucose, albumin, and triglycerides in LDL had the highest importance scores with systolic BP (Study IV Figure 3). Glucose, albumin, and triglycerides in LDL had approximately positive linear relation with systolic BP in partial dependence plot (Study IV Figure 4).

In the longitudinal model, glycerol, average VLDL size, and acetoacetate had the highest importance scores with future systolic BP (Study IV Figure 3). Glycerol, average VLDL size, and acetoacetate had a stepwise association with future systolic BP each demonstrating additional peak from monotone relationship in partial dependence plot (Study IV Figure 4). However, following the general shape of the graphs, glycerol had positive association and average VLDL size and acetoacetate negative associations with future systolic BP.

Table 11. Root-mean-square error for multivariable gradient boosting model fit.

Sample	Step	Clinical characteristics	Metabolic measures	Full model
Cross-sectional	Training	16.95 mmHg	16.36 mmHg	15.50 mmHg
Cross-sectional	Test	16.70 mmHg	18.03 mmHg	16.27 mmHg
Longitudinal	Training	14.84 mmHg	16.46 mmHg	13.22 mmHg
Longitudinal	Test	18.52 mmHg	19.78 mmHg	17.61 mmHg

Clinical characteristics were sex, BMI, current smoking, diabetes, antihypertensive medication, exercise, and lipid medication, and baseline SBP (only in longitudinal model). Metabolic measures were the core 53 circulating biomarkers. Full model was adjusted for both clinical covariates and metabolic measures.

6 Discussion

6.1 Gut microbiota and BP (I–II)

In Study I, we performed a systematic literature review on hypertension research related to gut microbiota. We reviewed 17 observational human studies and 22 animal studies, using pre-defined criteria for the included interventional designs. In Study II, we investigated the link between hypertension, gut microbiota, and dietary salt in a cross-sectional human cohort using shallow shotgun sequencing while adjusting for relevant confounding factors. The major improvements to previous research were the objectively measured BP, register based medication information, and large cohort size.

Our literature review included several prior animal studies that have reported how fecal microbiota transplantation and various interventions targeting dietary salt, antihypertensive medication, probiotics and SCFAs affect the gut microbiota and BP of the host. These animal studies have invoked hypotheses for the pathophysiological mechanisms explaining the effect of gut microbiota on hypertension and motivated the implementation of large-scale epidemiological human studies. In addition to our Study II, three other large cohort studies have reported associations between gut microbiota and human hypertension.

In the following paragraphs, we first summarize the main findings from the human cohort studies and then review in more detail the evidence for the association between BP with the overall gut microbial composition and distinct microbial abundances. In the TwinsUK study, no associations were observed between 68 microbiota markers and self-declared hypertension after correcting for multiple testing (Jackson et al., 2018). However, the low prevalence of self-reported hypertension (27.6%) in TwinsUK cohort compared to expected European prevalence (43.5%) and the lack of objective blood pressure measurements could explain these non-significant results (Jama et al., 2018). In the CARDIA study, a negative association was observed between objectively measured systolic blood pressure with alpha diversity and *Robinsoniella*-genus (Sun et al., 2019). In our study (FINRISK 2002; Study II), 45 microbial genera and 19 *Lactobacillus* species were associated with objectively measured BP. In the 24-hour urinary sodium subsample of FINRISK 2002, *L. paracasei* was negatively and *L. salivarius* positively

associated with both BP and urinary sodium, a proxy for dietary sodium intake (Study II). In the HELIUS study, gut microbiota explained 4.4% of the objectively measured systolic BP variance and 2.2% of the residual systolic BP adjusted with age, sex, and BMI (Verhaar, Collard, et al., 2020). *Roseburia*, *Clostridium*, *Romboutsia*, and *Ruminococcaceae* were the best negative predictors and *Streptococcus* the best positive predictor of systolic BP (Verhaar, Collard, et al., 2020).

6.1.1 Overall gut microbial composition and BP

6.1.1.1 Alpha diversity

Three of the four previous studies report associations between alpha diversity and hypertension. In the CARDIA study, Shannon's diversity index was negatively associated with systolic BP (β = -1.33 per 1-SD change, 95% CI -2.60 to -0.05, P = 0.04) adjusted for age, sex, race, antihypertensive medication, BMI, education, dietary quality score, physical activity, smoking status, clinical field center, and sequence run (Sun et al., 2019). In the FINRISK 2002 study, Shannon's index was negatively associated with systolic BP adjusted for age and sex, but the significance was lost when adjusting additionally for BMI, smoking, exercise, diuretics, beta blockers, calcium channel blockers, and renin–angiotensin system blockers (Study II). In the HELIUS study, Spearman's correlation between Shannon's diversity index and systolic BP was -0.1 with P<0.01 (Verhaar, Collard, et al., 2020). Therefore, alpha diversity correlated negatively with BP in the three previous studies and, assuming normal distribution and using 68–95–99.7 rule, the difference between high or low alpha diversity would be 2–5 mmHg at the population level. Based on the results of all these studies, the lack of the significant results in the fully adjusted models may indicate that alpha diversity does not have independent role in the association with BP or that the effect size for the association is small.

6.1.1.2 Beta diversity

Of the four cohort studies, only the FINRISK 2002 study reported results for the association between gut microbial beta diversity and hypertension (Study II). The used PERMANOVA model is sensitive to the order of studied covariates, favoring previously introduced covariates. Therefore, the BP variables were included in the models last. Beta diversity explained 0.05% of the variance of diastolic BP in the age- and sex-adjusted model and 0.02% in the multivariable-adjusted model. Also, other relevant clinical covariates including age, sex, and BMI also had R^2 of <0.5% with beta diversity. In particular, beta diversity explained 0.06% of the variance of

diabetes, whereas diabetes and metformin use has been associated with changes in gut microbiota (Forslund et al., 2015). In summary, beta diversity had only minor R^2 with all clinical covariates and the R^2 for diastolic BP appeared to be one order of magnitude lower than those observed for age, sex, and BMI. Also, only significant association in fully adjusted models was observed with diastolic BP.

6.1.1.3 Multivariable gradient boosting model

Instead of ecological diversity measures, the general gut microbial profile can also be studied using multivariable gradient boosting machine learning models. The HELIUS study was the only study that employed such modeling to estimate the proportion of variance gut microbiota explains about BP (Verhaar, Collard, et al., 2020). The authors defined an *ad hoc* formula for the proportion of variance defined as $R^2 = 1 - \mathrm{Var}(\mathrm{y} - \hat{\mathrm{y}})/\,\mathrm{Var}(\mathrm{y})$, where y is the measured and $\hat{\mathrm{y}}$ the estimated BP. To adjust the analyses for the main clinical covariates, the authors also studied residual BP obtained after fitting a linear regression model for BP with age, sex, and BMI. The characteristics and main results of the HELIUS study are presented in Table 12.

Table 12. Overall population and ethnic subsamples of HELIUS study.

Ethnicity	N	Age	Women	BMI	HT	HTX	SBP	Res SBP	DBP	Res DBP
Overall	4672	50 ± 12	52%	27 ± 5	42%	22%	4%	2%	4%	2%
Dutch	1328	51 ± 13	48%	25 ± 4	34%	16%	5%	0.6%	0.4%	n.a.
South Asian Surinamese	575	52 ± 11	52%	27 ± 5	49%	30%	n.a.	0.6%	n.a.	0.1%
African Surinamese	1128	52 ± 11	60%	28 ± 5	53%	31%	n.a.	0.7%	n.a.	0.1%
Ghanaian	462	48 ± 9	55%	28 ± 5	59%	29%	n.a.	n.a.	n.a.	n.a.
Moroccan	605	46 ± 11	46%	28 ± 5	24%	8%	0.8%	0.4%	n.a.	0.6%
Turkish	436	44 ± 11	51%	29 ± 5	29%	15%	n.a.	n.a.	0.5%	0.6%

BMI, body mass index; HT, hypertension; HTX, antihypertensive medication; n.a., model lacked predictive power; Res, residual variance adjusted for age and sex.

While age, sex, and BMI had consistent characteristics between ethnicities in the HELIUS study, notable differences were observed in hypertension (24–59%), antihypertensive medications (8–31%), diabetes (5–24%), and antidiabetic medication (2–20%). The observed associations for unadjusted systolic and diastolic

BP in subgroup analyses were highly variable. However, the R^2 with BP residuals appeared more consistent, varying 0.4–0.7% for residual systolic BP and 0.1–0.6% for residual diastolic BP.

The large number of negative values (marked n.a. in Table 12) observed for R^2 in the HELIUS study indicates that the *ad hoc* formula used may not be a generally suitable definition for the R^2. The study also adjusted for clinical covariates using residual BP as opposed to including the covariates in the model; furthermore, the study did not include all relevant confounders of elevated BP. Although not ideal, this remains a sensible definition for R^2 in gradient boosting until a more robust definition is formulated. Although estimators such as RMSE or c-statistic allow comparing different models, they do not provide information about the clinical significance of the differences found. Even considering the limitations, the HELIUS study provided a reasonable estimation for the link between overall gut microbiota and BP.

6.1.2 Associations for distinct genera and species

Gut microbiota can be studied at different levels of taxonomic ranks (domain > kingdom > phylum > class > order > family > genus > species). In addition to the overall gut microbial composition, associations between distinct taxa with BP can be studied using metagenomics libraries, including DESeq2.

In the CARDIA and HELIUS studies, a negative association was reported between *Robinsoniella*, *Roseburia*, *Clostridium*, *Romboutsia*, and *Ruminococcaceae* and systolic BP (Sun et al., 2019; Verhaar, Collard, et al., 2020). *Robinsoniella* and *Romboutsia* were not part of the common microbial genera in FINRISK 2002 (Study II). *Clostridium*, *Roseburia*, and *Ruminococcus* were also detected in FINRISK 2002, but these genera were not associated with BP. Notably, CARDIA and HELIUS used 16S rRNA sequencing, and therefore reported results for distinct OTUs rather than particular genera (as in shotgun metagenomics), which partially explains why these associations were not observed in FINRISK 2002.

In FINRISK 2002, we observed 45 distinct microbial genera associated with BP indices. A total of 27 of these 45 genera belong to the *Firmicutes*, a phylum that has been previously associated with obesity, diabetes, and chronic kidney disease (Tang et al., 2017). More specifically, BP was associated with a large number of SCFA producing bacteria (Table 3). *Anaerostipes*, *Bacteroides*, *Blautia*, *Coprococcus*, *Dialister*, and *Phascolarctobacterium* were positively and *Prevotella* negatively associated with BP. The genus *Lactobacillus* has been linked with acetate production, and we observed 12 positive and 29 negative associations for 19 distinct *Lactobacillus* species and BP. In particular, *L. paracasei* was negatively associated with both systolic BP ($P = 0.02$) and urinary sodium excretion ($P<0.001$), and *L.*

salivarius was positively associated with both pulse pressure ($P<0.001$) and urinary sodium excretion ($P = 0.004$).

In a subsample (N = 200) of the HELIUS study, fecal acetate and propionate were positively associated with systolic BP (Verhaar et al., 2020). In our Study IV, circulating acetate was negatively associated with systolic BP and hypertension, and positively associated with diastolic BP and the systolic BP change in follow-up.

6.1.3 Summary for gut microbiota and BP

Multiple epidemiological study designs have demonstrated consistently that different characteristics of gut microbiota are associated with BP. While the ecological diversity may be too generally defined to capture practical information for epidemiological research, the multivariable gradient boosting method was able to give a reasonable estimate for the magnitude of the effect. Additionally, distinct gut microbial taxa have been both positively and negatively associated with BP. Some of the bacteria associated with BP have also been linked to dietary sodium and SCFA production. However, different studies have mostly found associations for disjoint sets of gut microbial taxa, potentially due to the differences in sequencing techniques used. The low number of studies combined with potential ethnic and geographic differences in microbial taxa may also contribute to the disparity.

6.2 The circulating metabolites and BP (III–IV)

In Study III, we investigated the association BP had with 545 low-abundance plasma eicosanoids and related oxylipins, family of metabolites that have been previously linked to numerous pathophysiological processes that are central to BP regulation (see section 2.2.3.3). Additionally, an eicosanoid risk formula defined in the discovery cohort (FINRISK 2002) was associated with BP in the replication cohort (FHS). In Study IV, we examined the link between BP and high-abundance serum metabolic measures. Our multivariable gradient boosting modeling revealed that baseline serum lipid, and particularly LDL-derived and VLDL-derived cholesterol measures, and glucose metabolism abnormalities were associated with follow-up BP. The information about baseline metabolic measures improved the prediction of follow-up BP compared to a model that used only common clinical covariates.

6.2.1 Human lipidome

In cross-sectional analyses, most PUFAs were negative associated with BP (Study IV). Our results were consistent with previously published large cohort studies, including Women's Health Study and Brisighella Heart Study, and provided new

information about cross-sectional and longitudinal multivariable associations between BP and lipids in large, well-phenotyped cohorts (Paynter et al., 2011; Cicero et al., 2014).

Our Study III is the first publication that comprehensively examines the association between eicosanoids and BP in humans using high-throughput LC–MS. Although prior studies have been limited by the number of metabolites studied, they have been able to demonstrate that a small set of eicosanoids are associated with renal function, vascular tone, and hypertension (Laffer et al., 2003; Minuz et al., 2008; Taddei et al., 2006; Ward et al., 2005, 2008).

Our eicosanoid risk score included both intermediate and potentially terminal metabolites. 11-dehydro-2,3-dinor-TXB_2 can be measured from urine indicating that the eicosanoid is a terminal metabolite (DeFilippis et al., 2013). In a small human study, individuals with prior myocardial infarction compared to healthy controls demonstrated differences in TXA_2 production assessed by measuring urine 11-dehydro-2,3-dinor-TXB_2 (DeFilippis et al., 2013). 12-HHTrE is a non-enzymatic degradation product of TXA_2 and PGH_2 (Maddipati et al., 2014). 12-HHTrE has been linked to PGI_2 synthesis, and the primary downstream metabolite of 12-HHTrE, 12-oxoheptadeca-5(Z)-8(E)-10(E)-trienoic acid, is an antagonist of TXA_2 receptors (Csanyi et al., 2007). Therefore, 12-HHTrE related pathways could potentially increase PGI_2 modulated vasodilation (Csanyi et al., 2007). Adrenic acid is a polyunsaturated 22-carbon fatty acid which serves as a substrate for eicosanoid production, and adrenic acid-derived metabolites have been linked to modulate adrenal cortical artery relaxation (Kopf et al., 2010).

Our results demonstrate that eicosanoids are strongly associated with BP: both positive and negative associations were observed, and we were able to replicate the main findings using an independent cohort. However, laborious analyte identification forced us to focus more detailed analyses in small subset of eicosanoids. High correlation between analytes unavoidably introduces arbitrariness in metabolite selection. In the future, improved methodology could allow general analyte identification and utilization of metabolic databases to find novel pathways between eicosanoids and BP even using the current data.

6.2.2 Amino acids

In our cross-sectional sample, we observed a positive association for leucine, isoleucine, and alanine and a negative association for glutamine and glycine with BP (Study IV). Leucine and isoleucine belong to branched-chain amino acids, a group of essential amino acids that may have a role in the cell signaling of impaired insulin action and aggravated oxidative stress (Z.-Y. Zhang et al., 2018). In our longitudinal multivariable model, glycine, leucine, phenylalanine, and histidine were among the

top 15 model covariates (Study IV). Repeated measurements of circulating amino acid levels, finer dietary information, or both may be required to accurately assess the role of amino acids in cardiovascular health and BP.

6.2.3 Energy metabolism-related measures

Insulin resistance, diabetes, hypertension, and CAD are related comorbidities (Hall et al., 2015). We observed positive cross-sectional associations for glucose, pyruvate, lactate, glycerol, acetoacetate, and beta-hydroxybutyrate with hypertension (Study IV). These metabolites were also among the top 15 covariates in multivariable models. Therefore, our results highlight the importance of energy metabolism abnormalities in the development of hypertension.

6.2.4 Fluid balance-related measures

We observed a positive cross-sectional association between albumin and BP. Albumin was also among the top covariates in our cross-sectional multivariable model. A partial dependence plot revealed a positive linear relationship between albumin and systolic BP. In the cross-sectional Oslo Health Study (N = 5,171) and the Neuroprotective Model for Healthy Longevity among the Malaysian Elderly study (N = 2,322), albumin was positively associated with BP (Eshkoor et al., 2016; Høstmark et al., 2005). However, a normotensive Japanese (N = 2,240) observed a negative longitudinal association between albumin and hypertension onset.However, a normotensive Japanese (N = 2,240) observed negative longitudinal association between albumin and hypertension onset (Oda, 2014). Therefore, the association between albumin and BP may be multifaceted or non-causal.

6.2.5 Inflammation markers

Numerous studies have reported associations between markers of inflammatory activity such as cytokines and acute phase reactants (Harrison et al., 2011; Barrows et al., 2019). Downstream acute phase reactants have been favored in clinical research, because they are comparatively stable relative to propagators of inflammation cascade, such as IL-6 with a circulating half-life <2 h (Danesh et al., 2008). We observed positive cross-sectional association between BP and a novel low-grade inflammation biomarker, glycoprotein acetyl (Study IV). Eicosanoids have been reported to modulate inflammatory- and anti-inflammatory responses (Dennis & Norris, 2015; Harizi et al., 2008). We observed that eicosanoids and related oxylipins demonstrated strong overall association with BP (Study III).

6.3 Limitations of the study

6.3.1 Study I

Our literature review was performed according to an established protocol and a university library informatician was consulted with the search terms. However, our results must be interpreted in the context of their limitations. First, we performed single author screening of the included manuscripts. Second, the choice to focus on specific intervention types was artificial. Third, we did not provide numerical summary statistics. These limitations could potentially result in false exclusion of valid publications and mistakes in summary tables.

6.3.2 Study II

Our study was able to improve prior study designs using objective BP measurement, register based information about medication use, and shotgun metagenomics. However, some limitations remain. First, fecal sampling is proxy for the gut microbiota. Second, the samples were stored for prolonged time before analysis. Third, shotgun metagenomics has method specific limitations (Thomas & Segata, 2019). Fourth, our 24-hour urine sodium subsample had limited size (11.9%) and urine collection offers only limited information about dietary habits (Rakova et al., 2017). Fifth, hypertensive individuals may have received guidance to limit their dietary sodium intake. Sixth, different analysis methods and the use of different databases can produce heterogenous results (Nearing et al., 2021). Microbes observed in stool do not accurately represent all sites of the gastrointestinal tract and, in particular, the microbes in small intestine and mucosal layer of colon may not be detected in accurate proportions in stool. The methodological limitations reduce reproducibility between different metabolomic studies and could induce both type I and type II errors in results. Limited size of the 24-hour urinary sodium subsample reduces the power of the statistical analysis. Lifestyle guidance provided to hypertensive individuals produces confounding bias to the statistical models that can potentially reduce the observed effect sizes.

6.3.3 Study III

Our study has several strengths, including a large, unselected population sample, external replication of our results, and assays of a large number of eicosanoids. However, our study also has its limitations. First, LC–MS is highly sensitive, and we were unable to find all the metabolites observed in discovery cohort in replication cohort. Second, metabolite identification was laborious and therefore performed

only for six metabolites. Third, many eicosanoids have short half-lives imposing chemical instability in LC–MS analysis. Fourth, not all identified metabolites were eicosanoids. These limitations reduce the reproducibility of results between different studies and make difficult to utilize human metabolome databases to find potential functional pathways behind the observed associations.

6.3.4 Study IV

Our study has several strengths, including large cross-sectional and moderate longitudinal population sample sizes, access to repeated measurements, and consistent biomarker quantification. However, several limitations exist. First, NMR provided only limited window to serum high abundance metabolic measures. Second, our longitudinal sample size may have been insufficient to capture all potential associations. Third, baseline examinations ranged over a period of 15 years imposing differences in freezing times between studies. Fourth, the effect sizes observed were modest. These methodological limitations reduce the potential to capture novel biomarkers that are associated with BP and the limited prospective data reduces the power of performed statistical analyses.

7 Summary

The overall motivation for this study was to integrate modern metabolomics and metagenomics into hypertension research using the large and well-phenotyped Finnish cohort studies. The specific aims were to 1) estimate the relation between the gut microbiota and hypertension, 2) study the metabolic profile of hypertension, and to 3) establish if a family of small molecule activators and suppressors of systemic inflammation, eicosanoids, are associated with BP.

The four large human microbiota studies published to date demonstrate that gut microbiota is associated with human hypertension (Jackson et al., 2018; Sun et al., 2019; Verhaar, Collard, et al., 2020). The results of Study II, together with findings from prior studies, also suggest that dietary sodium may affect the gut microbial species, depleting potentially beneficial species. As our study is mainly observational, we can only speculate on the underlying causes of these findings. First, gut microbial end products may influence the gastrointestinal permeability and induce inflammation in the gut wall. Second, some metabolites, including SCFAs, have the potential to be absorbed in circulation where they can interact with host receptors. Third, gut microbiota may also modulate the sympathetic response of the host. However, the true significance of these effects on population level BP and health remains unclear. Third-generation sequencing, using a multiomics approach, and having access to additional longitudinal data could enable future epidemiological studies to better answer this question. Additionally, our growing understanding of microbiota and BP enables us to better plan human interventions that investigate the effect of dietary challenges to gut microbiota, circulating metabolites, and BP.

Eicosanoids and related oxylipins demonstrate a strong association with BP (Study III). As eicosanoid compounds affect numerous physiological processes that are central to BP regulation, they may offer new insights about the pathogenesis of hypertension, as well as serve as potential targets for therapeutic intervention. The largest challenge to fully utilize these data, however, is the non-trivial metabolite identification. However, the growing databases of human metabolites could allow better metabolite identification in the future, which, in turn, may be used to improve risk prediction and find potential therapeutic targets.

We also used high abundance metabolic measures to identify a serum signature associated with BP and BP change in follow-up using conventional statistics and machine learning approaches (Study IV). Our results suggest that serum lipids, and particularly LDL-derived and VLDL-derived cholesterol measures, and glucose metabolism abnormalities are associated with hypertension onset. Use of serum metabolite determination could be used to identify individuals at high risk of developing hypertension in time range of 7–11 years.

Our studies improve the current knowledge of the associations of gut microbiota and circulating metabolites with BP. Metabolomics and metagenomics offer novel approaches to improve hypertension risk prediction and to discover potential targets for therapeutic intervention of elevated BP.

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References

Abraham, G., Havulinna, A. S., Bhalala, O. G., Byars, S. G., De Livera, A. M., Yetukuri, L., Tikkanen, E., Perola, M., Schunkert, H., Sijbrands, E. J., Palotie, A., Samani, N. J., Salomaa, V., Ripatti, S., & Inouye, M. (2016). Genomic prediction of coronary heart disease. *European Heart Journal*, *37*(43), 3267–3278. https://doi.org/10.1093/eurheartj/ehw450

Adnan, S., Nelson, J. W., Ajami, N. J., Venna, V. R., Petrosino, J. F., Bryan, R. M., & Durgan, D. J. (2016). Alterations in the gut microbiota can elicit hypertension in rats. *Physiological Genomics*, *49*(2), 96–104. https://doi.org/10.1152/physiolgenomics.00081.2016

Al Khodor, S., Reichert, B., & Shatat, I. F. (2017). The Microbiome and Blood Pressure: Can Microbes Regulate Our Blood Pressure? *Frontiers in Pediatrics*, *5*, 138. https://doi.org/10.3389/fped.2017.00138

Ali, M. A., Rizvi, S., & Syed, B. A. (2017). Trends in the market for antihypertensive drugs. *Nature Reviews Drug Discovery*, *16*(5), 309–310. https://doi.org/10.1038/nrd.2016.262

Alsaleh, M., Barbera, T. A., Andrews, R. H., Sithithaworn, P., Khuntikeo, N., Loilome, W., Yongvanit, P., Cox, I. J., Syms, R. R. A., Holmes, E., & Taylor-Robinson, S. D. (2019). Mass Spectrometry: A Guide for the Clinician. *Journal of Clinical and Experimental Hepatology*, *9*(5), 597–606. https://doi.org/10.1016/j.jceh.2019.04.053

Anderson, E. L., Li, W., Klitgord, N., Highlander, S. K., Dayrit, M., Seguritan, V., Yooseph, S., Biggs, W., Venter, J. C., Nelson, K. E., & Jones, M. B. (2016). A robust ambient temperature collection and stabilization strategy: Enabling worldwide functional studies of the human microbiome. *Scientific Reports*, *6*(1), 31731. https://doi.org/10.1038/srep31731

Barrows, I. R., Ramezani, A., & Raj, D. S. (2019). Inflammation, Immunity, and Oxidative Stress in Hypertension-Partners in Crime? *Advances in Chronic Kidney Disease*, *26*(2), 122–130. https://doi.org/10.1053/j.ackd.2019.03.001

Baxter, N. T., Schmidt, A. W., Venkataraman, A., Kim, K. S., Waldron, C., & Schmidt, T. M. (2019). Dynamics of Human Gut Microbiota and Short-Chain Fatty Acids in Response to Dietary Interventions with Three Fermentable Fibers. *MBio*, *10*(1), e02566-18. https://doi.org/10.1128/mBio.02566-18

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., … Schloter, M. (2020). Microbiome definition re-visited: Old concepts and new challenges. *Microbiome*, *8*(1), 103. https://doi.org/10.1186/s40168-020-00875-0

Bier, A., Braun, T., Khasbab, R., Di Segni, A., Grossman, E., Haberman, Y., & Leibowitz, A. (2018). A High Salt Diet Modulates the Gut Microbiota and Short Chain Fatty Acids Production in a Salt-Sensitive Hypertension Rat Model. *Nutrients*, *10*(9), 1154. https://doi.org/10.3390/nu10091154

Bischl, B., Richter, J., Bossek, J., Horn, D., Thomas, J., & Lang, M. (2017). *mlrMBO: A Modular Framework for Model-Based Optimization of Expensive Black-Box Functions*. https://arxiv.org/abs/1703.03373v3

Borodulin, K., & Sääksjärvi, K. (2019). *FinHealth 2017 Study: Methods*. THL. http://www.julkari.fi/handle/10024/139084

Borodulin, K., Tolonen, H., Jousilahti, P., Jula, A., Juolevi, A., Koskinen, S., Kuulasmaa, K., Laatikainen, T., Männistö, S., Peltonen, M., Perola, M., Puska, P., Salomaa, V., Sundvall, J., Virtanen, S. M., & Vartiainen, E. (2018). Cohort Profile: The National FINRISK Study. *International Journal of Epidemiology*, *47*(3), 696–696i. https://doi.org/10.1093/ije/dyx239

Buczynski, M. W., Dumlao, D. S., & Dennis, E. A. (2009). Thematic Review Series: Proteomics. An integrated omics analysis of eicosanoid biology1[S]. *Journal of Lipid Research*, *50*(6), 1015–1038. https://doi.org/10.1194/jlr.R900004-JLR200

Calderón-Pérez, L., Gosalbes, M. J., Yuste, S., Valls, R. M., Pedret, A., Llauradó, E., Jimenez-Hernandez, N., Artacho, A., Pla-Pagà, L., Companys, J., Ludwig, I., Romero, M.-P., Rubió, L., & Solà, R. (2020). Gut metagenomic and short chain fatty acids signature in hypertension: A cross-sectional study. *Scientific Reports*, *10*(1), 6436. https://doi.org/10.1038/s41598-020-63475-w

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Capra, V., Rovati, G. E., Mangano, P., Buccellati, C., Murphy, R. C., & Sala, A. (2015). Transcellular biosynthesis of eicosanoid lipid mediators. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1851*(4), 377–382. https://doi.org/10.1016/j.bbalip.2014.09.002

Chae, C. U., Lee, R. T., Rifai, N., & Ridker, P. M. (2001). Blood pressure and inflammation in apparently healthy men. *Hypertension (Dallas, Tex.: 1979)*, *38*(3), 399–403. https://doi.org/10.1161/01.hyp.38.3.399

Chakraborty, S., Mandal, J., Yang, T., Cheng, X., Yeo, J.-Y., McCarthy, C. G., Wenceslau, C. F., Koch, L. G., Hill, J. W., Vijay-Kumar, M., & Joe, B. (2020). Metabolites and Hypertension: Insights into Hypertension as a Metabolic Disorder: 2019 Harriet Dustan Award. *Hypertension (Dallas, Tex. : 1979)*, *75*(6), 1386–1396. https://doi.org/10.1161/HYPERTENSIONAHA.120.13896

Chen, L., He, F. J., Dong, Y., Huang, Y., Wang, C., Harshfield, G. A., & Zhu, H. (2020). Modest Sodium Reduction Increases Circulating Short-Chain Fatty Acids in Untreated Hypertensives: A Randomized, Double-Blind, Placebo-Controlled Trial. *Hypertension (Dallas, Tex. : 1979)*, *76*(1), 73–79. https://doi.org/10.1161/HYPERTENSIONAHA.120.14800

Chen, T., & Guestrin, C. (2016). XGBoost: A Scalable Tree Boosting System. *Proceedings of the 22nd ACM SIGKDD International Conference on Knowledge Discovery and Data Mining*, 785–794. https://doi.org/10.1145/2939672.2939785

Chen, Y., Lun, A. T. L., & Smyth, G. K. (2014). Differential Expression Analysis of Complex RNA-seq Experiments Using edgeR. In S. Datta & D. Nettleton (Eds.), *Statistical Analysis of Next Generation Sequencing Data* (pp. 51–74). Springer International Publishing. https://doi.org/10.1007/978-3-319-07212-8_3

Cicero, A. F. G., Rosticci, M., Baronio, C., Morbini, M., Parini, A., Grandi, E., D'Addato, S., & Borghi, C. (2014). Serum LDL cholesterol levels and new onset of arterial hypertension: An 8-year follow-up. *European Journal of Clinical Investigation*, *44*(10), 926–932. https://doi.org/10.1111/eci.12325

Claesson, M. J., Clooney, A. G., & O'Toole, P. W. (2017). A clinician's guide to microbiome analysis. *Nature Reviews. Gastroenterology & Hepatology*, *14*(10), 585–595. https://doi.org/10.1038/nrgastro.2017.97

Collaboration (CCGC), C. R. P. C. H. D. G. (2011). Association between C reactive protein and coronary heart disease: Mendelian randomisation analysis based on individual participant data. *BMJ*, *342*, d548. https://doi.org/10.1136/bmj.d548

Crescente, M., Menke, L., Chan, M. V., Armstrong, P. C., & Warner, T. D. (2019). Eicosanoids in platelets and the effect of their modulation by aspirin in the cardiovascular system (and beyond). *British Journal of Pharmacology*, *176*(8), 988–999. https://doi.org/10.1111/bph.14196

Csanyi, G., Lepran, I., Flesch, T., Telegdy, G., Szabo, G., & Mezei, Z. (2007). Lack of endothelium-derived hyperpolarizing factor (EDHF) up-regulation in endothelial dysfunction in aorta in diabetic rats. *Pharmacological Reports: PR*, *59*(4), 447–455.

Daly, A. J., Baetens, J. M., & De Baets, B. (2018). Ecological Diversity: Measuring the Unmeasurable. *Mathematics*, *6*(7), 119. https://doi.org/10.3390/math6070119

Dan, X., Mushi, Z., Baili, W., Han, L., Enqi, W., Huanhu, Z., & Shuchun, L. (2019). Differential Analysis of Hypertension-Associated Intestinal Microbiota. *International Journal of Medical Sciences*, *16*(6), 872–881. https://doi.org/10.7150/ijms.29322

Danesh, J., Kaptoge, S., Mann, A. G., Sarwar, N., Wood, A., Angleman, S. B., Wensley, F., Higgins, J. P. T., Lennon, L., Eiriksdottir, G., Rumley, A., Whincup, P. H., Lowe, G. D. O., & Gudnason, V. (2008). Long-term interleukin-6 levels and subsequent risk of coronary heart disease: Two new prospective studies and a systematic review. *PLoS Medicine*, *5*(4), e78. https://doi.org/10.1371/journal.pmed.0050078

Davies, N. M., Holmes, M. V., & Davey Smith, G. (2018). Reading Mendelian randomisation studies: A guide, glossary, and checklist for clinicians. *BMJ (Clinical Research Ed.)*, *362*, k601. https://doi.org/10.1136/bmj.k601

de la Cuesta-Zuluaga, J., Mueller, N. T., Álvarez-Quintero, R., Velásquez-Mejía, E. P., Sierra, J. A., Corrales-Agudelo, V., Carmona, J. A., Abad, J. M., & Escobar, J. S. (2018). Higher Fecal Short-Chain Fatty Acid Levels Are Associated with Gut Microbiome Dysbiosis, Obesity, Hypertension and Cardiometabolic Disease Risk Factors. *Nutrients*, *11*(1). https://doi.org/10.3390/nu11010051

DeFilippis, A. P., Oloyede, O. S., Andrikopoulou, E., Saenger, A. K., Palachuvattil, J. M., Fasoro, Y. A., Guallar, E., Blumenthal, R. S., Kickler, T. S., Jaffe, A. S., Gerstenblith, G., Schulman, S. P., & Rade, J. J. (2013). Thromboxane A(2) generation, in the absence of platelet COX-1 activity, in patients with and without atherothrombotic myocardial infarction. *Circulation Journal: Official Journal of the Japanese Circulation Society*, *77*(11), 2786–2792. https://doi.org/10.1253/circj.cj-12-1421

Dennis, E. A., & Norris, P. C. (2015). Eicosanoid storm in infection and inflammation. *Nature Reviews Immunology*, *15*(8), 511–523. https://doi.org/10.1038/nri3859

Elshenawy, O., Shoieb, S., Mohamed, A., & El-Kadi, A. (2017). Clinical Implications of 20-Hydroxyeicosatetraenoic Acid in the Kidney, Liver, Lung and Brain: An Emerging Therapeutic Target. *Pharmaceutics*, *9*(4), 9. https://doi.org/10.3390/pharmaceutics9010009

Eshkoor, S. A., Hamid, T. A., Shahar, S., Ng, C. K., & Mun, C. Y. (2016). Factors Affecting Hypertension among the Malaysian Elderly. *Journal of Cardiovascular Development and Disease*, *3*(1), 8. https://doi.org/10.3390/jcdd3010008

Fan, F., Muroya, Y., & Roman, R. J. (2015). Cytochrome P450 eicosanoids in hypertension and renal disease. *Current Opinion in Nephrology and Hypertension*, *24*(1), 37–46. https://doi.org/10.1097/MNH.0000000000000088

Fändriks, L. (2017). Roles of the gut in the metabolic syndrome: An overview. *Journal of Internal Medicine*, *281*(4), 319–336. https://doi.org/10.1111/joim.12584

Fendt, S.-M., & Lunt, S. Y. (Eds.). (2019). *Metabolic Signaling: Methods and Protocols* (Vol. 1862). Humana Press. https://doi.org/10.1007/978-1-4939-8769-6

Ferguson, J. F., Aden, L. A., Barbaro, N. R., Van Beusecum, J. P., Xiao, L., Simmons, A. J., Warden, C., Pasic, L., Himmel, L. E., Washington, M. K., Revetta, F. L., Zhao, S., Kumaresan, S., Scholz, M. B., Tang, Z., Chen, G., Reilly, M. P., & Kirabo, A. (2019). High dietary salt-induced dendritic cell activation underlies microbial dysbiosis-associated hypertension. *JCI Insight*, *5*(13). https://doi.org/10.1172/jci.insight.126241

Finnish Institute for Health and Welfare. (2021). *Care Register for Health Care*. Finnish Institute for Health and Welfare (THL), Finland. https://thl.fi/en/web/thlfi-en/statistics-and-data/data-and-services/register-descriptions/care-register-for-health-care

Flores-Guerrero, J. L., Groothof, D., Connelly, M. A., Otvos, J. D., Bakker, S. J. L., & Dullaart, R. P. F. (2019). Concentration of Branched-Chain Amino Acids Is a Strong Risk Marker for Incident Hypertension. *Hypertension*, *74*(6), 1428–1435. https://doi.org/10.1161/HYPERTENSIONAHA.119.13735

Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Le Chatelier, E., Sunagawa, S., Prifti, E., Vieira-Silva, S., Gudmundsdottir, V., Pedersen, H. K., Arumugam, M., Kristiansen, K., Voigt, A. Y., Vestergaard, H., Hercog, R., Costea, P. I., Kultima, J. R., Li, J., Jørgensen, T., … Pedersen, O. (2015). Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*, *528*(7581), 262–266. https://doi.org/10.1038/nature15766

Franke, T., & Deppenmeier, U. (2018). Physiology and central carbon metabolism of the gut bacterium Prevotella copri. *Molecular Microbiology*, *109*(4), 528–540. https://doi.org/10.1111/mmi.14058

GBD 2017 Risk Factor Collaborators. (2018). Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet (London, England)*, *392*(10159), 1923–1994. https://doi.org/10.1016/S0140-6736(18)32225-6

Ge, X., Zheng, L., Zhuang, R., Yu, P., Xu, Z., Liu, G., Xi, X., Zhou, X., & Fan, H. (2020). The Gut Microbial Metabolite Trimethylamine N-Oxide and Hypertension Risk: A Systematic Review and Dose–Response Meta-analysis. *Advances in Nutrition*, *11*(1), 66–76. https://doi.org/10.1093/advances/nmz064

Glenn, T. C., Nilsen, R. A., Kieran, T. J., Sanders, J. G., Bayona-Vásquez, N. J., Finger, J. W., Pierson, T. W., Bentley, K. E., Hoffberg, S. L., Louha, S., Leon, F. J. G.-D., Portilla, M. A. del R., Reed, K. D., Anderson, J. L., Meece, J. K., Aggrey, S. E., Rekaya, R., Alabady, M., Belanger, M., … Faircloth, B. C. (2019). Adapterama I: Universal stubs and primers for 384 unique dual-indexed or 147,456 combinatorially-indexed Illumina libraries (iTru & iNext). *PeerJ*, *7*, e7755. https://doi.org/10.7717/peerj.7755

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome Datasets Are Compositional: And This Is Not Optional. *Frontiers in Microbiology*, *8*, 2224. https://doi.org/10.3389/fmicb.2017.02224

Greenwell, B., M. (2017). pdp: An R Package for Constructing Partial Dependence Plots. *The R Journal*, *9*(1), 421. https://doi.org/10.32614/RJ-2017-016

Guzik, T. J., Hoch, N. E., Brown, K. A., McCann, L. A., Rahman, A., Dikalov, S., Goronzy, J., Weyand, C., & Harrison, D. G. (2007). Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *The Journal of Experimental Medicine*, *204*(10), 2449–2460. https://doi.org/10.1084/jem.20070657

Hall, J. E., do Carmo, J. M., da Silva, A. A., Wang, Z., & Hall, M. E. (2015). Obesity-induced hypertension: Interaction of neurohumoral and renal mechanisms. *Circulation Research*, *116*(6), 991–1006. https://doi.org/10.1161/CIRCRESAHA.116.305697

Harizi, H., Corcuff, J.-B., & Gualde, N. (2008). Arachidonic-acid-derived eicosanoids: Roles in biology and immunopathology. *Trends in Molecular Medicine*, *14*(10), 461–469. https://doi.org/10.1016/j.molmed.2008.08.005

Harrison, D. G., Guzik, T. J., Lob, H. E., Madhur, M. S., Marvar, P. J., Thabet, S. R., Vinh, A., & Weyand, C. M. (2011). Inflammation, Immunity, and Hypertension. *Hypertension*, *57*(2), 132–140. https://doi.org/10.1161/HYPERTENSIONAHA.110.163576

He, W. J., Li, C., Mi, X., Shi, M., Gu, X., Bazzano, L. A., Razavi, A. C., Nierenberg, J. L., Dorans, K., He, H., & Kelly, T. N. (2020). An untargeted metabolomics study of blood pressure: Findings from the Bogalusa Heart Study. *Journal of Hypertension*, *38*(7), 1302–1311. https://doi.org/10.1097/HJH.0000000000002363

Heistaro, S. (2008). *Methodology report: Health 2000 survey*. http://www.julkari.fi/handle/10024/78185

Hillmann, B., Al-Ghalith, G. A., Shields-Cutler, R. R., Zhu, Q., Gohl, D. M., Beckman, K. B., Knight, R., & Knights, D. (2018). Evaluating the Information Content of Shallow Shotgun Metagenomics. *MSystems*, *3*(6), e00069-18. https://doi.org/10.1128/mSystems.00069-18

Høstmark, A. T., Tomten, S. E., & Berg, J. E. (2005). Serum albumin and blood pressure: A population-based, cross-sectional study. *Journal of Hypertension*, *23*(4), 725–730. https://doi.org/10.1097/01.hjh.0000163139.44094.1d

Huart, J., Leenders, J., Taminiau, B., Descy, J., Saint-Remy, A., Daube, G., Krzesinski, J.-M., Melin, P., de Tullio, P., & Jouret, F. (2019). Gut Microbiota and Fecal Levels of Short-Chain Fatty Acids Differ Upon 24-Hour Blood Pressure Levels in Men. *Hypertension (Dallas, Tex. : 1979)*, *74*(4), 1005–1013. https://doi.org/10.1161/HYPERTENSIONAHA.118.12588

IL6R Genetics Consortium Emerging Risk Factors Collaboration, Sarwar, N., Butterworth, A. S., Freitag, D. F., Gregson, J., Willeit, P., Gorman, D. N., Gao, P., Saleheen, D., Rendon, A., Nelson, C. P., Braund, P. S., Hall, A. S., Chasman, D. I., Tybjærg-Hansen, A., Chambers, J. C., Benjamin, E. J., Franks, P. W., Clarke, R., … Danesh, J. (2012). Interleukin-6 receptor pathways in coronary heart disease: A collaborative meta-analysis of 82 studies. *Lancet (London, England)*, *379*(9822), 1205–1213. https://doi.org/10.1016/S0140-6736(11)61931-4

Imig, J. D. (2015). Epoxyeicosatrienoic Acids, Hypertension, and Kidney Injury. *Hypertension*, *65*(3), 476–482. https://doi.org/10.1161/HYPERTENSIONAHA.114.03585

Islam, S. (2017). *Hypertension: From basic research to clinical practice*. Springer Berlin Heidelberg.

Jackson, M. A., Verdi, S., Maxan, M.-E., Shin, C. M., Zierer, J., Bowyer, R. C. E., Martin, T., Williams, F. M. K., Menni, C., Bell, J. T., Spector, T. D., & Steves, C. J. (2018). Gut microbiota associations with common diseases and prescription medications in a population-based cohort. *Nature Communications*, *9*(1), 2655. https://doi.org/10.1038/s41467-018-05184-7

Jama, H., Kaye, D. M., & Marques, F. Z. (2018). Population-Based Gut Microbiome Associations With Hypertension. *Circulation Research*, *123*(11), 1185–1187. https://doi.org/10.1161/CIRCRESAHA.118.313792

Jaworska, K., Bielinska, K., Gawrys-Kopczynska, M., & Ufnal, M. (2019). TMA (trimethylamine), but not its oxide TMAO (trimethylamine-oxide), exerts haemodynamic effects: Implications for interpretation of cardiovascular actions of gut microbiome. *Cardiovascular Research*, *115*(14), 1948–1949. https://doi.org/10.1093/cvr/cvz231

Johnson, J. S., Spakowicz, D. J., Hong, B.-Y., Petersen, L. M., Demkowicz, P., Chen, L., Leopold, S. R., Hanson, B. M., Agresta, H. O., Gerstein, M., Sodergren, E., & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*, *10*(1), 5029. https://doi.org/10.1038/s41467-019-13036-1

Juraschek. (2015). Plasma Lactate and Incident Hypertension in the Atherosclerosis Risk in Communities Study. *American Journal of Hypertension*. https://doi.org/10.1093/ajh/hpu117

Kannel, W. B., Feinleib, M., McNamara, P. M., Garrison, R. J., & Castelli, W. P. (1979). An investigation of coronary heart disease in families. The Framingham offspring study. *American Journal of Epidemiology*, *110*(3), 281–290.

Khanapure, S. P., Garvey, D. S., Janero, D. R., & Letts, L. G. (2007). Eicosanoids in inflammation: Biosynthesis, pharmacology, and therapeutic frontiers. *Current Topics in Medicinal Chemistry*, *7*(3), 311–340. https://doi.org/10.2174/156802607779941314

Kim, S., Goel, R., Kumar, A., Qi, Y., Lobaton, G., Hosaka, K., Mohammed, M., Handberg, E. M., Richards, E. M., Pepine, C. J., & Raizada, M. K. (2018). Imbalance of gut microbiome and intestinal epithelial barrier dysfunction in patients with high blood pressure. *Clinical Science*, *132*(6), 701–718. https://doi.org/10.1042/CS20180087

Knight, R., Vrbanac, A., Taylor, B. C., Aksenov, A., Callewaert, C., Debelius, J., Gonzalez, A., Kosciolek, T., McCall, L.-I., McDonald, D., Melnik, A. V., Morton, J. T., Navas, J., Quinn, R. A., Sanders, J. G., Swafford, A. D., Thompson, L. R., Tripathi, A., Xu, Z. Z., … Dorrestein, P. C.

(2018). Best practices for analysing microbiomes. *Nature Reviews Microbiology*, *16*(7), 410–422. https://doi.org/10.1038/s41579-018-0029-9

Konttinen, H., Llewellyn, C., Silventoinen, K., Joensuu, A., Männistö, S., Salomaa, V., Jousilahti, P., Kaprio, J., Perola, M., & Haukkala, A. (2018). Genetic predisposition to obesity, restrained eating and changes in body weight: A population-based prospective study. *International Journal of Obesity*, *42*(4), 858–865. https://doi.org/10.1038/ijo.2017.278

Kopf, P. G., Zhang, D. X., Gauthier, K. M., Nithipatikom, K., Yi, X.-Y., Falck, J. R., & Campbell, W. B. (2010). Adrenic acid metabolites as endogenous endothelium-derived and zona glomerulosa-derived hyperpolarizing factors. *Hypertension (Dallas, Tex.: 1979)*, *55*(2), 547–554. https://doi.org/10.1161/HYPERTENSIONAHA.109.144147

Lääkintöhallitus. (1986). *Tautiluokitus* [D5_Oppikirja, ammatillinen käsi- tai opaskirja taikka sanakirja]. Lääkintöhallitus. https://www.julkari.fi/handle/10024/131850

Laatikainen, T., Pietinen, P., Valsta, L., Sundvall, J., Reinivuo, H., & Tuomilehto, J. (2006). Sodium in the Finnish diet: 20-year trends in urinary sodium excretion among the adult population. *European Journal of Clinical Nutrition*, *60*(8), 965–970. https://doi.org/10.1038/sj.ejcn.1602406

Laffer, C. L., Laniado-Schwartzman, M., Wang, M.-H., Nasjletti, A., & Elijovich, F. (2003). Differential Regulation of Natriuresis by 20-Hydroxyeicosatetraenoic Acid in Human Salt-Sensitive Versus Salt-Resistant Hypertension. *Circulation*, *107*(4), 574–578. https://doi.org/10.1161/01.CIR.0000046269.52392.14

Legendre, P., & De Cáceres, M. (2013). Beta diversity as the variance of community data: Dissimilarity coefficients and partitioning. *Ecology Letters*, *16*(8), 951–963. https://doi.org/10.1111/ele.12141

Li, J., Zhao, F., Wang, Y., Chen, J., Tao, J., Tian, G., Wu, S., Liu, W., Cui, Q., Geng, B., Zhang, W., Weldon, R., Auguste, K., Yang, L., Liu, X., Chen, L., Yang, X., Zhu, B., & Cai, J. (2017). Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome*, *5*(1), 14. https://doi.org/10.1186/s40168-016-0222-x

Liang, C.-F., Liu, J. T., Wang, Y., Xu, A., & Vanhoutte, P. M. (2013). Toll-Like Receptor 4 Mutation Protects Obese Mice Against Endothelial Dysfunction by Decreasing NADPH Oxidase Isoforms 1 and 4. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *33*(4), 777–784. https://doi.org/10.1161/ATVBAHA.112.301087

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A., Clarke, M., Devereaux, P. J., Kleijnen, J., & Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. *BMJ*, *339*. https://doi.org/10.1136/bmj.b2700

Louis, P., & Flint, H. J. (2017). Formation of propionate and butyrate by the human colonic microbiota. *Environmental Microbiology*, *19*(1), 29–41. https://doi.org/10.1111/1462-2920.13589

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Lundqvist, A., & Mäki-Opas, T. (2016). *Health 2011 Survey – Methods*. National Public Health Institute. http://www.julkari.fi/bitstream/handle/10024/130780/URN_ISBN_978-952-302-669-8.pdf?sequence=1

Lüscher, T. F., Landmesser, U., von Eckardstein, A., & Fogelman, A. M. (2014). High-Density Lipoprotein. *Circulation Research*, *114*(1), 171–182. https://doi.org/10.1161/CIRCRESAHA.114.300935

Maddipati, K. R., Romero, R., Chaiworapongsa, T., Zhou, S.-L., Xu, Z., Tarca, A. L., Kusanovic, J. P., Munoz, H., & Honn, K. V. (2014). Eicosanomic profiling reveals dominance of the epoxygenase pathway in human amniotic fluid at term in spontaneous labor. *The FASEB Journal*, *28*(11), 4835–4846. https://doi.org/10.1096/fj.14-254383

Mahbub, M. H., Yamaguchi, N., Hase, R., Takahashi, H., Ishimaru, Y., Watanabe, R., Saito, H., Shimokawa, J., Yamamoto, H., Kikuchi, S., & Tanabe, T. (2020). Plasma Branched-Chain and

Aromatic Amino Acids in Relation to Hypertension. *Nutrients*, *12*(12), 3791. https://doi.org/10.3390/nu12123791

Mahmud, A., & Feely, J. (2005). Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension (Dallas, Tex.: 1979)*, *46*(5), 1118–1122. https://doi.org/10.1161/01.HYP.0000185463.27209.b0

Marques, F. Z., Nelson, E., Chu, P.-Y., Horlock, D., Fiedler, A., Ziemann, M., Tan, J. K., Kuruppu, S., Rajapakse, N. W., El-Osta, A., Mackay, C. R., & Kaye, D. M. (2017). High-Fiber Diet and Acetate Supplementation Change the Gut Microbiota and Prevent the Development of Hypertension and Heart Failure in Hypertensive Mice. *Circulation*, *135*(10), 964–977. https://doi.org/10.1161/CIRCULATIONAHA.116.024545

Martinez-Porchas, M., Villalpando-Canchola, E., Ortiz Suarez, L. E., & Vargas-Albores, F. (2017). How conserved are the conserved 16S-rRNA regions? *PeerJ*, *5*. https://doi.org/10.7717/peerj.3036

Mell, B., Jala, V. R., Mathew, A. V., Byun, J., Waghulde, H., Zhang, Y., Haribabu, B., Vijay-Kumar, M., Pennathur, S., & Joe, B. (2015). Evidence for a link between gut microbiota and hypertension in the Dahl rat. *Physiological Genomics*, *47*(6), 187–197. https://doi.org/10.1152/physiolgenomics.00136.2014

Minuz, P., Jiang, H., Fava, C., Turolo, L., Tacconelli, S., Ricci, M., Patrignani, P., Morganti, A., Lechi, A., & McGiff, J. C. (2008). Altered release of cytochrome p450 metabolites of arachidonic acid in renovascular disease. *Hypertension (Dallas, Tex.: 1979)*, *51*(5), 1379–1385. https://doi.org/10.1161/HYPERTENSIONAHA.107.105395

Mitchell, J. A., & Kirkby, N. S. (2019). Eicosanoids, prostacyclin and cyclooxygenase in the cardiovascular system. *British Journal of Pharmacology*, *176*(8), 1038–1050. https://doi.org/10.1111/bph.14167

Mitchell, J. A., Kirkby, N. S., Ahmetaj-Shala, B., Armstrong, P. C., Crescente, M., Ferreira, P., Lopes Pires, M. E., Vaja, R., & Warner, T. D. (2021). Cyclooxygenases and the cardiovascular system. *Pharmacology & Therapeutics*, *217*, 107624. https://doi.org/10.1016/j.pharmthera.2020.107624

Moens, F., Verce, M., & De Vuyst, L. (2017). Lactate- and acetate-based cross-feeding interactions between selected strains of lactobacilli, bifidobacteria and colon bacteria in the presence of inulin-type fructans. *International Journal of Food Microbiology*, *241*, 225–236. https://doi.org/10.1016/j.ijfoodmicro.2016.10.019

Morton, J. T., Marotz, C., Washburne, A., Silverman, J., Zaramela, L. S., Edlund, A., Zengler, K., & Knight, R. (2019). Establishing microbial composition measurement standards with reference frames. *Nature Communications*, *10*(1), 2719. https://doi.org/10.1038/s41467-019-10656-5

Natarajan, N., Hori, D., Flavahan, S., Steppan, J., Flavahan, N. A., Berkowitz, D. E., & Pluznick, J. L. (2016). Microbial short chain fatty acid metabolites lower blood pressure via endothelial G protein-coupled receptor 41. *Physiological Genomics*, *48*(11), 826–834. https://doi.org/10.1152/physiolgenomics.00089.2016

Nearing, J. T., Douglas, G. M., Hayes, M., MacDonald, J., Desai, D., Allward, N., Jones, C. M. A., Wright, R., Dhanani, A., Comeau, A. M., & Langille, M. G. I. (2021). *Microbiome differential abundance methods produce disturbingly different results across 38 datasets* [Preprint]. Bioinformatics. https://doi.org/10.1101/2021.05.10.443486

Nie, J., Xie, L., Zhao, B.-X., Li, Y., Qiu, B., Zhu, F., Li, G.-F., He, M., Wang, Y., Wang, B., Liu, S., Zhang, H., Guo, H., Cai, Y., Huo, Y., Hou, F. F., Xu, X., & Qin, X. (2018). Serum Trimethylamine N-Oxide Concentration Is Positively Associated With First Stroke in Hypertensive Patients. *Stroke*, *49*(9), 2021–2028. https://doi.org/10.1161/STROKEAHA.118.021997

Nikolic, S. B., Sharman, J. E., Adams, M. J., & Edwards, L. M. (2014). Metabolomics in hypertension: *Journal of Hypertension*, *32*(6), 1159–1169. https://doi.org/10.1097/HJH.0000000000000168

Noverr, M. C., Erb-Downward, J. R., & Huffnagle, G. B. (2003). Production of Eicosanoids and Other Oxylipins by Pathogenic Eukaryotic Microbes. *Clinical Microbiology Reviews*, *16*(3), 517–533. https://doi.org/10.1128/CMR.16.3.517-533.2003

Oda, E. (2014). Decreased serum albumin predicts hypertension in a Japanese health screening population. *Internal Medicine (Tokyo, Japan)*, *53*(7), 655–660. https://doi.org/10.2169/internalmedicine.53.1894

Oda, E., & Kawai, R. (2011). High-density lipoprotein cholesterol is positively associated with hypertension in apparently healthy Japanese men and women. *British Journal of Biomedical Science*, *68*(1), 29–33. https://doi.org/10.1080/09674845.2011.11732838

O'Leary, N. A., Wright, M. W., Brister, J. R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., Astashyn, A., Badretdin, A., Bao, Y., Blinkova, O., Brover, V., Chetvernin, V., Choi, J., Cox, E., Ermolaeva, O., … Pruitt, K. D. (2016). Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Research*, *44*(D1), D733-745. https://doi.org/10.1093/nar/gkv1189

Otsuka, T., Takada, H., Nishiyama, Y., Kodani, E., Saiki, Y., Kato, K., & Kawada, T. (2016). Dyslipidemia and the Risk of Developing Hypertension in a Working-Age Male Population. *Journal of the American Heart Association*, *5*(3), e003053. https://doi.org/10.1161/JAHA.115.003053

Overby, H. B., & Ferguson, J. F. (2021). Gut Microbiota-Derived Short-Chain Fatty Acids Facilitate Microbiota:Host Cross talk and Modulate Obesity and Hypertension. *Current Hypertension Reports*, *23*(2), 8. https://doi.org/10.1007/s11906-020-01125-2

Palmu, J. (2021). *Tikz figures for LC-MS and NMR* (Version v2) [Computer software]. https://doi.org/10.5281/zenodo.5112024

Palmu, J., Tikkanen, E., Havulinna, A. S., Vartiainen, E., Lundqvist, A., Ruuskanen, M. O., Perola, M., Ala-Korpela, M., Jousilahti, P., Würtz, P., Salomaa, V., Lahti, L., & Niiranen, T. (2021). Comprehensive biomarker profiling of hypertension in 36 985 Finnish individuals. *Journal of Hypertension*. https://doi.org/10.1097/HJH.0000000000003051

Parada Venegas, D., De la Fuente, M. K., Landskron, G., González, M. J., Quera, R., Dijkstra, G., Harmsen, H. J. M., Faber, K. N., & Hermoso, M. A. (2019). Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Frontiers in Immunology*, *10*, 277. https://doi.org/10.3389/fimmu.2019.00277

Pärn, K., Fontarnau, J. N., Isokallio, M. A., Sipilä, T., Kilpelainen, E., Palotie, A., Ripatti, S., & Palta, P. (2018, April 13). *Genotyping chip data lift-over to reference genome build GRCh38/hg38*. Protocols.Io. https://www.protocols.io/view/genotyping-chip-data-lift-over-to-reference-genome-nqtddwn

Pärn, K., Isokallio, M. A., Fontarnau, J. N., Palotie, A., Ripatti, S., & Palta, P. (2018, May 10). *Genotype imputation workflow v3.0*. Protocols.Io. https://www.protocols.io/view/genotype-imputation-workflow-v3-0-nmndc5e

Paynter, N. P., Sesso, H. D., Conen, D., Otvos, J. D., & Mora, S. (2011). Lipoprotein subclass abnormalities and incident hypertension in initially healthy women. *Clinical Chemistry*, *57*(8), 1178–1187. https://doi.org/10.1373/clinchem.2011.167544

Perry, R. J., Peng, L., Barry, N. A., Cline, G. W., Zhang, D., Cardone, R. L., Petersen, K. F., Kibbey, R. G., Goodman, A. L., & Shulman, G. I. (2016). Acetate mediates a microbiome-brain-β cell axis promoting metabolic syndrome. *Nature*, *534*(7606), 213–217. https://doi.org/10.1038/nature18309

Persson, S., de Boer, R. F., Kooistra-Smid, A. M. D., & Olsen, K. E. P. (2011). Five commercial DNA extraction systems tested and compared on a stool sample collection. *Diagnostic Microbiology and Infectious Disease*, *69*(3), 240–244. https://doi.org/10.1016/j.diagmicrobio.2010.09.023

Petriz. (2014). Exercise induction of gut microbiota modifications in obese, non-obese and hypertensive rats. *BMC Genomics*. https://doi.org/10.1186/1471-2164-15-511

Pitt, J. J. (2009). Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry. *The Clinical Biochemist Reviews*, *30*(1), 19–34.

Pluznick, J. L. (2017). Microbial Short-Chain Fatty Acids and Blood Pressure Regulation. *Current Hypertension Reports*, *19*(4), 25. https://doi.org/10.1007/s11906-017-0722-5

Pluznick, J. L., Protzko, R. J., Gevorgyan, H., Peterlin, Z., Sipos, A., Han, J., Brunet, I., Wan, L.-X., Rey, F., Wang, T., Firestein, S. J., Yanagisawa, M., Gordon, J. I., Eichmann, A., Peti-Peterdi, J., & Caplan, M. J. (2013). Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proceedings of the National Academy of Sciences*, *110*(11), 4410–4415. https://doi.org/10.1073/pnas.1215927110

Pound, P., & Ram, R. (2020). Are researchers moving away from animal models as a result of poor clinical translation in the field of stroke? An analysis of opinion papers. *BMJ Open Science*, *4*(1), e100041. https://doi.org/10.1136/bmjos-2019-100041

Qian, X.-B., Chen, T., Xu, Y.-P., Chen, L., Sun, F.-X., Lu, M.-P., & Liu, Y.-X. (2020). A guide to human microbiome research: Study design, sample collection, and bioinformatics analysis. *Chinese Medical Journal*, *133*(15), 1844–1855. https://doi.org/10.1097/CM9.0000000000000871

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., Liang, S., Zhang, W., Guan, Y., Shen, D., Peng, Y., Zhang, D., Jie, Z., Wu, W., Qin, Y., Xue, W., Li, J., Han, L., Lu, D., … Wang, J. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*, *490*(7418), 55–60. https://doi.org/10.1038/nature11450

Quehenberger, O., & Dennis, E. A. (2011). The Human Plasma Lipidome. *New England Journal of Medicine*, *365*(19), 1812–1823. https://doi.org/10.1056/NEJMra1104901

Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology*, *35*(9), 833–844. https://doi.org/10.1038/nbt.3935

R. Muralitharan, R., & Marques, F. Z. (2021). Diet-related gut microbial metabolites and sensing in hypertension. *Journal of Human Hypertension*, *35*(2), 162–169. https://doi.org/10.1038/s41371-020-0388-3

Rakova, N., Kitada, K., Lerchl, K., Dahlmann, A., Birukov, A., Daub, S., Kopp, C., Pedchenko, T., Zhang, Y., Beck, L., Johannes, B., Marton, A., Müller, D. N., Rauh, M., Luft, F. C., & Titze, J. (2017). Increased salt consumption induces body water conservation and decreases fluid intake. *The Journal of Clinical Investigation*, *127*(5), 1932–1943. https://doi.org/10.1172/JCI88530

Revez, J. A., Bain, L., Chapman, B., Powell, J. E., Jansen, R., Duffy, D. L., Tung, J. Y., Penninx, B. W., Visscher, P. M., De Geus, E. J. C., Boomsma, D. I., Hinds, D. A., Martin, N. G., Montgomery, G. W., & Ferreira, M. a. R. (2013). A new regulatory variant in the interleukin-6 receptor gene associates with asthma risk. *Genes & Immunity*, *14*(7), 441–446. https://doi.org/10.1038/gene.2013.38

Robles-Vera, I., Toral, M., de la Visitación, N., Sánchez, M., Gómez-Guzmán, M., Romero, M., Yang, T., Izquierdo-Garcia, J. L., Jiménez, R., Ruiz-Cabello, J., Guerra-Hernández, E., Raizada, M. K., Pérez-Vizcaíno, F., & Duarte, J. (2020). Probiotics Prevent Dysbiosis and the Rise in Blood Pressure in Genetic Hypertension: Role of Short-Chain Fatty Acids. *Molecular Nutrition & Food Research*, *64*(6), e1900616. https://doi.org/10.1002/mnfr.201900616

Robles-Vera, I., Toral, M., Visitación, N. de la, Sánchez, M., Romero, M., Olivares, M., Jiménez, R., & Duarte, J. (2018). The Probiotic Lactobacillus fermentum Prevents Dysbiosis and Vascular Oxidative Stress in Rats with Hypertension Induced by Chronic Nitric Oxide Blockade. *Molecular Nutrition & Food Research*, *62*(19), 1800298. https://doi.org/10.1002/mnfr.201800298

Sachdeva, A., Cannon, C. P., Deedwania, P. C., Labresh, K. A., Smith, S. C., Dai, D., Hernandez, A., & Fonarow, G. C. (2009). Lipid levels in patients hospitalized with coronary artery disease: An analysis of 136,905 hospitalizations in Get With The Guidelines. *American Heart Journal*, *157*(1), 111-117.e2. https://doi.org/10.1016/j.ahj.2008.08.010

Saklayen, M. G., & Deshpande, N. V. (2016). Timeline of History of Hypertension Treatment. *Frontiers in Cardiovascular Medicine*, *3*, 3. https://doi.org/10.3389/fcvm.2016.00003

Salosensaari, A., Laitinen, V., Havulinna, A. S., Meric, G., Cheng, S., Perola, M., Valsta, L., Alfthan, G., Inouye, M., Watrous, J. D., Long, T., Salido, R. A., Sanders, K., Brennan, C., Humphrey, G. C., Sanders, J. G., Jain, M., Jousilahti, P., Salomaa, V., … Niiranen, T. (2021). Taxonomic

signatures of cause-specific mortality risk in human gut microbiome. *Nature Communications*, *12*(1), 2671. https://doi.org/10.1038/s41467-021-22962-y

Samuelsson, B., & Hammarström, S. (1980). Nomenclature for leukotrienes. *Prostaglandins*, *19*(5), 645–648. https://doi.org/10.1016/0090-6980(80)90099-4

Santisteban, M. M., Ahmari, N., Carvajal, J. M., Zingler, M. B., Qi, Y., Kim, S., Joseph, J., Garcia-Pereira, F., Johnson, R. D., Shenoy, V., Raizada, M. K., & Zubcevic, J. (2015). Involvement of bone marrow cells and neuroinflammation in hypertension. *Circulation Research*, *117*(2), 178–191. https://doi.org/10.1161/CIRCRESAHA.117.305853

Santisteban, M. M., Qi, Y., Zubcevic, J., Kim, S., Yang, T., Shenoy, V., Cole-Jeffrey, C. T., Lobaton, G. O., Stewart, D. C., Rubiano, A., Simmons, C. S., Garcia-Pereira, F., Johnson, R. D., Pepine, C. J., & Raizada, M. K. (2017). Hypertension-Linked Pathophysiological Alterations in the Gut. *Circulation Research*, *120*(2), 312–323. https://doi.org/10.1161/CIRCRESAHA.116.309006

Schurch, N. J., Schofield, P., Gierliński, M., Cole, C., Sherstnev, A., Singh, V., Wrobel, N., Gharbi, K., Simpson, G. G., Owen-Hughes, T., Blaxter, M., & Barton, G. J. (2016). How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use? *RNA*, *22*(6), 839–851. https://doi.org/10.1261/rna.053959.115

Sekula, P., M, F. D. G., Pattaro, C., & Köttgen, A. (2016). Mendelian Randomization as an Approach to Assess Causality Using Observational Data. *Journal of the American Society of Nephrology*, *27*(11), 3253–3265. https://doi.org/10.1681/ASN.2016010098

Sesso, H. D., Buring, J. E., Rifai, N., Blake, G. J., Gaziano, J. M., & Ridker, P. M. (2003). C-reactive protein and the risk of developing hypertension. *JAMA*, *290*(22), 2945–2951. https://doi.org/10.1001/jama.290.22.2945

Shaham, O., Wei, R., Wang, T. J., Ricciardi, C., Lewis, G. D., Vasan, R. S., Carr, S. A., Thadhani, R., Gerszten, R. E., & Mootha, V. K. (2008). Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. *Molecular Systems Biology*, *4*(1), 214. https://doi.org/10.1038/msb.2008.50

Sharma, R. K., Yang, T., Oliveira, A. C., Lobaton, G. O., Aquino, V., Kim, S., Richards, E. M., Pepine, C. J., Sumners, C., & Raizada, M. K. (2019). Microglial Cells Impact Gut Microbiota and Gut Pathology in Angiotensin II-Induced Hypertension. *Circulation Research*, *124*(5), 727–736. https://doi.org/10.1161/CIRCRESAHA.118.313882

Sheppe, A. E. F., & Edelmann, M. J. (2021). Roles of Eicosanoids in Regulating Inflammation and Neutrophil Migration as an Innate Host Response to Bacterial Infections. *Infection and Immunity*, *89*(8), e0009521. https://doi.org/10.1128/IAI.00095-21

Shimizu, Y., Sato, S., Koyamatsu, J., Yamanashi, H., Nagayoshi, M., Kadota, K., Kawashiri, S.-Y., & Maeda, T. (2017). Association between high-density lipoprotein-cholesterol and hypertension in relation to circulating CD34-positive cell levels. *Journal of Physiological Anthropology*, *36*(1), 26. https://doi.org/10.1186/s40101-017-0143-9

Soininen, P., Kangas, A. J., Würtz, P., Suna, T., & Ala-Korpela, M. (2015). Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circulation. Cardiovascular Genetics*, *8*(1), 192–206. https://doi.org/10.1161/CIRCGENETICS.114.000216

Stephens, O. W., Zhang, Q., Qu, P., Zhou, Y., Chavan, S., Tian, E., Williams, D. R., Epstein, J., Barlogie, B., & Shaughnessy, J. D. (2012). An intermediate-risk multiple myeloma subgroup is defined by sIL-6r: Levels synergistically increase with incidence of SNP rs2228145 and 1q21 amplification. *Blood*, *119*(2), 503–512. https://doi.org/10.1182/blood-2011-07-367052

Sun, S., Lulla, A., Sioda, M., Winglee, K., Wu, M. C., Jacobs, D. R., Shikany, J. M., Lloyd-Jones, D. M., Launer, L. J., Fodor, A. A., & Meyer, K. A. (2019). Gut Microbiota Composition and Blood Pressure: The CARDIA Study. *Hypertension*, *73*(5), 998–1006. https://doi.org/10.1161/HYPERTENSIONAHA.118.12109

Taddei, S., Versari, D., Cipriano, A., Ghiadoni, L., Galetta, F., Franzoni, F., Magagna, A., Virdis, A., & Salvetti, A. (2006). Identification of a cytochrome P450 2C9-derived endothelium-derived

hyperpolarizing factor in essential hypertensive patients. *Journal of the American College of Cardiology*, *48*(3), 508–515. https://doi.org/10.1016/j.jacc.2006.04.074

Tang, W. H. W., Kitai, T., & Hazen, S. L. (2017). Gut Microbiota in Cardiovascular Health and Disease. *Circulation Research*, *120*(7), 1183–1196. https://doi.org/10.1161/CIRCRESAHA.117.309715

Thanassoulis, G., Williams, K., Ye, K., Brook, R., Couture, P., Lawler, P. R., de Graaf, J., Furberg, C. D., & Sniderman, A. (2014). Relations of change in plasma levels of LDL-C, non-HDL-C and apoB with risk reduction from statin therapy: A meta-analysis of randomized trials. *Journal of the American Heart Association*, *3*(2), e000759. https://doi.org/10.1161/JAHA.113.000759

The Social Insurance Institution of Finland. (2012). *Statistics on reimbursements for prescription medicines*. https://www.kela.fi/web/en/492

Thomas, A. M., & Segata, N. (2019). Multiple levels of the unknown in microbiome research. *BMC Biology*, *17*(1), 48. https://doi.org/10.1186/s12915-019-0667-z

Tian, W., Jiang, X., Sung, Y. K., Qian, J., Yuan, K., & Nicolls, M. R. (2014). Leukotrienes in pulmonary arterial hypertension. *Immunologic Research*, *58*(2–3), 387–393. https://doi.org/10.1007/s12026-014-8492-5

Tognarelli, J. M., Dawood, M., Shariff, M. I. F., Grover, V. P. B., Crossey, M. M. E., Cox, I. J., Taylor-Robinson, S. D., & McPhail, M. J. W. (2015). Magnetic Resonance Spectroscopy: Principles and Techniques: Lessons for Clinicians. *Journal of Clinical and Experimental Hepatology*, *5*(4), 320–328. https://doi.org/10.1016/j.jceh.2015.10.006

Tolonen, H. (2016). *EHES Manual: Part B. Fieldwork procedures*. THL. https://www.julkari.fi/handle/10024/131503

Tomaszewski, M., White, C., Patel, P., Masca, N., Damani, R., Hepworth, J., Samani, N. J., Gupta, P., Madira, W., Stanley, A., & Williams, B. (2014). High rates of non-adherence to antihypertensive treatment revealed by high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) urine analysis. *Heart*, *100*(11), 855–861. https://doi.org/10.1136/heartjnl-2013-305063

Toral, M., Robles-Vera, I., de la Visitación, N., Romero, M., Sánchez, M., Gómez-Guzmán, M., Rodriguez-Nogales, A., Yang, T., Jiménez, R., Algieri, F., Gálvez, J., Raizada, M. K., & Duarte, J. (2019). Role of the immune system in vascular function and blood pressure control induced by faecal microbiota transplantation in rats. *Acta Physiologica (Oxford, England)*, *227*(1), e13285. https://doi.org/10.1111/apha.13285

Toral, M., Robles-Vera, I., de la Visitación, N., Romero, M., Yang, T., Sánchez, M., Gómez-Guzmán, M., Jiménez, R., Raizada, M. K., & Duarte, J. (2019). Critical Role of the Interaction Gut Microbiota – Sympathetic Nervous System in the Regulation of Blood Pressure. *Frontiers in Physiology*, *10*, 231. https://doi.org/10.3389/fphys.2019.00231

Trompette, A., Gollwitzer, E. S., Yadava, K., Sichelstiel, A. K., Sprenger, N., Ngom-Bru, C., Blanchard, C., Junt, T., Nicod, L. P., Harris, N. L., & Marsland, B. J. (2014). Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature Medicine*, *20*(2), 159–166. https://doi.org/10.1038/nm.3444

Tsukamoto, I., & Sugawara, S. (2018). Low levels of linoleic acid and α-linolenic acid and high levels of arachidonic acid in plasma phospholipids are associated with hypertension. *Biomedical Reports*, *8*(1), 69–76. https://doi.org/10.3892/br.2017.1015

Valsta, L. (2019). *Information about collection and storage of stool samples in FINRISK 2002 provided by adjunct professor, research manager Liisa Valsta, THL.* [Personal communication].

van Goor, H., van den Born, J. C., Hillebrands, J.-L., & Joles, J. A. (2016). Hydrogen sulfide in hypertension. *Current Opinion in Nephrology and Hypertension*, *25*(2), 107–113. https://doi.org/10.1097/MNH.0000000000000206

Varvel, S. A., Dayspring, T. D., Edmonds, Y., Thiselton, D. L., Ghaedi, L., Voros, S., McConnell, J. P., Sasinowski, M., Dall, T., & Warnick, G. R. (2015). Discordance between apolipoprotein B and low-density lipoprotein particle number is associated with insulin resistance in clinical practice. *Journal of Clinical Lipidology*, *9*(2), 247–255. https://doi.org/10.1016/j.jacl.2014.11.005

Verhaar, B. J. H., Collard, D., Prodan, A., Levels, J. H. M., Zwinderman, A. H., Bäckhed, F., Vogt, L., Peters, M. J. L., Muller, M., Nieuwdorp, M., & van den Born, B.-J. H. (2020). Associations between gut microbiota, faecal short-chain fatty acids, and blood pressure across ethnic groups: The HELIUS study. *European Heart Journal*, *41*(44), 4259–4267. https://doi.org/10.1093/eurheartj/ehaa704

Verhaar, B. J. H., Prodan, A., Nieuwdorp, M., & Muller, M. (2020). Gut Microbiota in Hypertension and Atherosclerosis: A Review. *Nutrients*, *12*(10), 2982. https://doi.org/10.3390/nu12102982

Vijay-Kumar, M., Aitken, J. D., Carvalho, F. A., Cullender, T. C., Mwangi, S., Srinivasan, S., Sitaraman, S. V., Knight, R., Ley, R. E., & Gewirtz, A. T. (2010). Metabolic Syndrome and Altered Gut Microbiota in Mice Lacking Toll-Like Receptor 5. *Science*, *328*(5975), 228–231. https://doi.org/10.1126/science.1179721

Vogtmann, E., Chen, J., Amir, A., Shi, J., Abnet, C. C., Nelson, H., Knight, R., Chia, N., & Sinha, R. (2017). Comparison of Collection Methods for Fecal Samples in Microbiome Studies. *American Journal of Epidemiology*, *185*(2), 115–123. https://doi.org/10.1093/aje/kww177

Walejko, J. M., Kim, S., Goel, R., Handberg, E. M., Richards, E. M., Pepine, C. J., & Raizada, M. K. (2018). Gut microbiota and serum metabolite differences in African Americans and White Americans with high blood pressure. *International Journal of Cardiology*, *271*, 336–339. https://doi.org/10.1016/j.ijcard.2018.04.074

Wang, Z., Klipfell, E., Bennett, B. J., Koeth, R., Levison, B. S., Dugar, B., Feldstein, A. E., Britt, E. B., Fu, X., Chung, Y.-M., Wu, Y., Schauer, P., Smith, J. D., Allayee, H., Tang, W. H. W., DiDonato, J. A., Lusis, A. J., & Hazen, S. L. (2011). Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*, *472*(7341), 57–63. https://doi.org/10.1038/nature09922

Ward, N. C., Puddey, I. B., Hodgson, J. M., Beilin, L. J., & Croft, K. D. (2005). Urinary 20-hydroxyeicosatetraenoic acid excretion is associated with oxidative stress in hypertensive subjects. *Free Radical Biology & Medicine*, *38*(8), 1032–1036. https://doi.org/10.1016/j.freeradbiomed.2004.12.024

Ward, N. C., Tsai, I.-J., Barden, A., van Bockxmeer, F. M., Puddey, I. B., Hodgson, J. M., & Croft, K. D. (2008). A single nucleotide polymorphism in the CYP4F2 but not CYP4A11 gene is associated with increased 20-HETE excretion and blood pressure. *Hypertension (Dallas, Tex.: 1979)*, *51*(5), 1393–1398. https://doi.org/10.1161/HYPERTENSIONAHA.107.104463

Watrous, J. D., Henglin, M., Claggett, B., Lehmann, K. A., Larson, M. G., Cheng, S., & Jain, M. (2017). Visualization, Quantification, and Alignment of Spectral Drift in Population Scale Untargeted Metabolomics Data. *Analytical Chemistry*, *89*(3), 1399–1404. https://doi.org/10.1021/acs.analchem.6b04337

Watrous, J. D., Niiranen, T. J., Lagerborg, K. A., Henglin, M., Xu, Y.-J., Rong, J., Sharma, S., Vasan, R. S., Larson, M. G., Armando, A., Mora, S., Quehenberger, O., Dennis, E. A., Cheng, S., & Jain, M. (2019). Directed Non-targeted Mass Spectrometry and Chemical Networking for Discovery of Eicosanoids and Related Oxylipins. *Cell Chemical Biology*, *26*(3), 433-442.e4. https://doi.org/10.1016/j.chembiol.2018.11.015

Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J. R., Vázquez-Baeza, Y., Birmingham, A., Hyde, E. R., & Knight, R. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, *5*(1), 27. https://doi.org/10.1186/s40168-017-0237-y

Wenzel, U., Turner, J. E., Krebs, C., Kurts, C., Harrison, D. G., & Ehmke, H. (2016). Immune Mechanisms in Arterial Hypertension. *Journal of the American Society of Nephrology*, *27*(3), 677–686. https://doi.org/10.1681/ASN.2015050562

Whittaker, R. H. (1960). Vegetation of the Siskiyou Mountains, Oregon and California. *Ecological Monographs*, *30*(3), 279–338. https://doi.org/10.2307/1943563

WHO Collaborating Centre for Drug Statistics. (2018). *ATC Structure and Principles*. https://www.whocc.no/atc/structure_and_principles/

Wilck, N., Matus, M. G., Kearney, S. M., Olesen, S. W., Forslund, K., Bartolomaeus, H., Haase, S., Mähler, A., Balogh, A., Markó, L., Vvedenskaya, O., Kleiner, F. H., Tsvetkov, D., Klug, L., Costea, P. I., Sunagawa, S., Maier, L., Rakova, N., Schatz, V., … Müller, D. N. (2017). Salt-responsive gut commensal modulates T H 17 axis and disease. *Nature*, *551*(7682), 585–589. https://doi.org/10.1038/nature24628

Worp, H. B. van der, Howells, D. W., Sena, E. S., Porritt, M. J., Rewell, S., O'Collins, V., & Macleod, M. R. (2010). Can Animal Models of Disease Reliably Inform Human Studies? *PLOS Medicine*, *7*(3), e1000245. https://doi.org/10.1371/journal.pmed.1000245

Wu, D., Tang, X., Ding, L., Cui, J., Wang, P., Du, X., Yin, J., Wang, W., Chen, Y., & Zhang, T. (2019). Candesartan attenuates hypertension-associated pathophysiological alterations in the gut. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *116*, 109040. https://doi.org/10.1016/j.biopha.2019.109040

Würtz, P., Kangas, A. J., Soininen, P., Lawlor, D. A., Davey Smith, G., & Ala-Korpela, M. (2017). Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Large-Scale Epidemiology: A Primer on -Omic Technologies. *American Journal of Epidemiology*, *186*(9), 1084–1096. https://doi.org/10.1093/aje/kwx016

Yamaguchi, N., Mahbub, M. H., Takahashi, H., Hase, R., Ishimaru, Y., Sunagawa, H., Amano, H., Kobayashi-Miura, M., Kanda, H., Fujita, Y., Yamamoto, H., Yamamoto, M., Kikuchi, S., Ikeda, A., Takasu, M., Kageyama, N., Nakamura, M., & Tanabe, T. (2017). Plasma free amino acid profiles evaluate risk of metabolic syndrome, diabetes, dyslipidemia, and hypertension in a large Asian population. *Environmental Health and Preventive Medicine*, *22*(1), 35. https://doi.org/10.1186/s12199-017-0642-7

Yang, B., Ding, F., Yan, J., Ye, X.-W., Xu, X.-L., Wang, F.-L., Li, D., & Yu, W. (2016). Exploratory serum fatty acid patterns associated with blood pressure in community-dwelling middle-aged and elderly Chinese. *Lipids in Health and Disease*, *15*. https://doi.org/10.1186/s12944-016-0226-3

Yang, T., Aquino, V., Lobaton, G. O., Li, H., Colon-Perez, L., Goel, R., Qi, Y., Zubcevic, J., Febo, M., Richards, E. M., Pepine, C. J., & Raizada, M. K. (2019). Sustained Captopril-Induced Reduction in Blood Pressure Is Associated With Alterations in Gut-Brain Axis in the Spontaneously Hypertensive Rat. *Journal of the American Heart Association*, *8*(4), e010721. https://doi.org/10.1161/JAHA.118.010721

Yang Tao, Santisteban Monica M., Rodriguez Vermali, Li Eric, Ahmari Niousha, Carvajal Jessica Marulanda, Zadeh Mojgan, Gong Minghao, Qi Yanfei, Zubcevic Jasenka, Sahay Bikash, Pepine Carl J., Raizada Mohan K., & Mohamadzadeh Mansour. (2015). Gut Dysbiosis Is Linked to Hypertension. *Hypertension*, *65*(6), 1331–1340. https://doi.org/10.1161/HYPERTENSIONAHA.115.05315

Zec, M. M., Schutte, A. E., Ricci, C., Baumgartner, J., Kruger, I. M., & Smuts, C. M. (2019). Long-Chain Polyunsaturated Fatty Acids Are Associated with Blood Pressure and Hypertension over 10-Years in Black South African Adults Undergoing Nutritional Transition. *Foods (Basel, Switzerland)*, *8*(9), Article 9. https://doi.org/10.3390/foods8090394

Zhang, Y. X., Cliff, W. J., Schoefl, G. I., & Higgins, G. (1999). Coronary C-reactive protein distribution: Its relation to development of atherosclerosis. *Atherosclerosis*, *145*(2), 375–379. https://doi.org/10.1016/s0021-9150(99)00105-7

Zhang, Z.-Y., Monleon, D., Verhamme, P., & Staessen, J. A. (2018). Branched-Chain Amino Acids as Critical Switches in Health and Disease. *Hypertension*, *72*(5), 1012–1022. https://doi.org/10.1161/HYPERTENSIONAHA.118.10919

Zhou, B., Carrillo-Larco, R. M., Danaei, G., Riley, L. M., Paciorek, C. J., Stevens, G. A., Gregg, E. W., Bennett, J. E., Solomon, B., Singleton, R. K., Sophiea, M. K., Iurilli, M. L., Lhoste, V. P., Cowan, M. J., Savin, S., Woodward, M., Balanova, Y., Cifkova, R., Damasceno, A., … Ezzati, M. (2021). Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: A pooled analysis of 1201 population-representative studies with 104 million participants. *The Lancet*, *398*(10304), 957–980. https://doi.org/10.1016/S0140-6736(21)01330-1

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