PAULA SINGMANN

METABOLOMICS AND AGING

Helmholtz Zentrum München
Institute of Epidemiology 2
Research Unit Molecular Epidemiology
Head: Prof. Dr. rer. biol. hum. Annette Peters

METABOLOMICS AND AGING

Thesis
Submitted for a Doctoral Degree in Human Biology

at the
Faculty of Medicine
Ludwig-Maximilians-Universität München

Submitted by
PAULA SINGMANN
from
Dresden, Germany

2015

WITH APPROVAL OF THE FACULTY OF MEDICINE
LUDWIG-MAXIMILIANS UNIVERSITY MUNICH, GERMANY

SUPERVISOR:
apl. Prof. Dr. rer. nat. Thomas Illig
CO-EXAMINERS:
apl. Prof. Dr. Jörg Schirra
Priv. Doz. Dr. Irmgard Bumeder
DEAN: Prof. Dr. med. Dr. h.c. M. Reiser, FACR, FRCR
DATE OF THE ORAL EXAMINATION: June 19th 2015

Dedicated to Michi and Ole.

ABSTRACT

As life expectancy has risen steadily over the last years and disease-free aging is more and more challenging, understanding the complexity of age and aging is of great importance. Metabolomics is one of the novel approaches in systems biology with high potential to deliver answers to these questions. However, only a few metabolic studies based on large samples are available so far.
In this thesis, I present results from two population-based studies, the German KORA Follow-Up 4 (KORA F4) study as a discovery cohort with a sample of 1,038 female and 1,124 male healthy participants (32–81 years) and 724 healthy females from UK Adult Twin Registry (TwinsUK) as replication. Targeted metabolomics of fasting serum samples with flow injection analysis coupled with tandem mass spectrometry (FIA-MS/MS) positively quantified 131 metabolites after stringent quality control. Among these, 71 and 34 metabolites were significantly associated with age in females and males, respectively , after adjustment for body mass index (BMI), which is highly correlated (r=0.9) with age.
These results indicate that metabolic profiles are age dependent and sex specific. Then, a set of the 12 most age-discriminative, independent metabolites was identified in women with an approach based on random forest and stepwise variable selection. This set showed highly significant differences between subjects aged 32–51 years and 52–77 (p-values range 1.3E-09 – 1.9E-46, significance threshold p=0.004). Ten out of these 12 metabolites replicated in unrelated females from the TwinsUK study, including five metabolites the concentrations of which increased with age (C12:1, C18:1, sphingomyelin (SM) C16:1, SM C18:1 and phosphatidylcholine (PC) aa C28:1), while histidine decreased gradually. Three glycerophospholipids (PC ae C42:4, PC ae C42:5, PC ae C44:4) showed declines around the age of 51 years. Meta-analysis of both studies gave virtually the same results as KORA alone.
These observations might reflect many different processes of aging such as incomplete mitochondrial fatty acid oxidation, counteracting oxidative stress, and changes in vascular functions. The identification of these ten age-related metabolites should help better understand aging pathways and networks and with —more discoveries in the future— eventually help enhance healthy aging and longevity.

ZUSAMMENFASSUNG

Die Lebenserwartung nimmt in den letzten Jahren stetig zu und gesundes Altern wird dadurch zu einer immer größeren Herausforderung. Daher ist das Verständnis der Komplexität des Alterungsprozesses von großer Bedeutung. Metabolomics ist einer der neuartigen Methoden in der Systembiologie, die ein großes Potential mitbringt, um diese Probleme zu bewerkstelligen. Bisher sind jedoch kaum populationsbasierte Metabolomics-Studien veröffentlicht, die auf einer großen Fallzahl beruhen.

In dieser Doktorarbeit stelle ich die Ergebnisse zweier populationsbasierten Studien vor, zum einen aus der deutschen KORA F_4-Studie, die mit 1.038 gesunden Frauen und 1.124 gesunden Männern im Alter von 32 bis 81 Jahren als Untersuchungskohorte diente, und zum anderen aus der englischen TwinsUK-Studie, deren 724 gesunde Frauen zur Replikation meiner Ergebnisse dienten. Die Nüchtern-Serum-Proben wurden mittels gezielter Metabolomics-Messungen (FIA-MS/MS) analysiert und nach strenger Qualitätskontrolle konnten 131 Metaboliten quantifiziert werden. Darunter waren 71 bzw. 34 Metabolite signifikant mit dem Alter in Frauen bzw. Männern assoziiert, nachdem für den BMI adjustiert worden war. Der BMI ist stark mit dem Alter korreliert (r=0,9).

Diese Ergebnisse deuten darauf hin, dass die metabolischen Profile sowohl altersabhängig als auch geschlechtsspezifisch sind. Daraufhin konnte ein Set aus 12 Metaboliten in Frauen ermittelt werden, das hochsignifikante Unterschiede zwischen Frauen in der Altergruppe 32–51 und in der Altergruppe 52–77 zeigte (p-Wert-Spanne 1,3E-09 – 1,9E-46, korrigiertes α of 0,004). Zehn dieser 12 Metaboliten konnten in den nicht-verwandten Frauen der TwinsUK-Studie repliziert werden. Darunter waren fünf Metabolitenkonzentrationen, die mit dem Alter anstiegen (C12:1, C18:1, SM C16:1, SM C18:1 und PC aa C28:1), die Histidinkonzentration dahingegen fiel allmählich ab. Drei Glycerophospholipide (PC ae C42:4, PC ae C42:5, PC ae C44:4) wiesen Konzentrationsabfälle um ein Alter von 51 Jahren auf. Eine Metaanalyse beider Studien erbrachte mit den KORA F_4-Ergebnissen vergleichbare Resultate.

Diese Beobachtungen spiegeln möglicherweise viele verschiedene Alterungsprozesse wider, wie zum Beispiel eine unvollständige Oxidation der Fettsäuren, das Entgegenwirken von oxidativem Stress, und Veränderungen in den Gefäßfunktionen. Die Identifikation dieser altersassoziierten Metabolite kann helfen, alterungsbetroffene Stoffwechselwege und Netzwerke besser zu verstehen und zusammen mit weiteren zukünftigen Forschungsergebnissen letztlich dazu beitragen, gesundes Altern und gesunde Langlebigkeit zu fördern.

PUBLICATIONS

Publication underlying the present thesis:

- Yu Z*, Zhai G*, **Singmann P***, He Y, Xu T, Prehn C, Roemisch-Margl W, Lattka E, Gieger C, Soranzo N, Heinrich J, Standl M, Thiering E, Mittelstrass K, Wichmann HE, Peters A, Suhre K, Li Y, Adamski J, Spector TD, Illig T, Wang-Sattler R: Human serum metabolic profiles are age dependent. Aging Cell. 2012 Dec;11(6):960-7.
  *These authors contributed equally.

Further work in the field of METABOLOMICS

- Foecker M, Timmesfeld N, Scherag S, Knoll N, **Singmann P**, Wang-Sattler R, Buehren K, Schwarte R, Egberts K, Fleischhaker C, Adamski J, Illig T, Suhre K, Albayrak O, Hinney A, Herpertz-Dahlmann B, Hebebrand J: Comparison of metabolic profiles of acutely ill and short-term weight recovered patients with anorexia nervosa reveals alterations of 33 out of 163 metabolites. J Psychiatr Res. 2012 Dec;46(12):1600-9.

- Wahl S, Yu Z, Kleber M, **Singmann P**, Holzapfel C, He Y, Mittelstrass K, Polonikov A, Prehn C, Roemisch-Margl W, Adamski J, Suhre K, Grallert H, Illig T, Wang-Sattler R, Reinehr T. Childhood obesity is associated with changes in the serum metabolite profile. Obes Facts. 2012;5(5):660-70.

- Jaremek M, Yu Z, Mangino M, Mittelstrass K, Prehn C, **Singmann P**, Xu T, Dahmen N, Weinberger KM, Suhre K, Peters A, Doering A, Hauner H, Adamski J, Illig T, Spector TD, Wang-Sattler R: Alcohol-induced metabolomic differences in humans. Transl Psychiatry. 2013 Jul 2;3:e276.

- Wahl S, Holzapfel C, Yu Z, Breier M, Kondofersky I, Fuchs C, **Singmann P**, Prehn C, Adamski J, Grallert H, Illig T, Wang-Sattler R, Reinehr R: Metabolomics reveals determinants of overweight reduction during lifestyle intervention in obese children (submitted)

Further work in the field of GENETICS

- Rzehak P, Thijs C, Standl M, Mommers M, Glaser C, Jansen E, Klopp N, Koppelman GH, **Singmann P**, Postma DS, Sausenthaler S, Dagnelie PC, van den Brandt PA, Koletzko B, Heinrich J; KOALA study group; LISA study group: Variants of

the FADS1 FADS2 gene cluster, blood levels of polyunsaturated fatty acids and eczema in children within the first 2 years of life. PLoS One. 2010 Oct 11;5(10):e13261.

- **Singmann P**, Rzehak P, Berdel D, Wichmann HE, Heinrich J: No association between FADS polymorphisms and atopic diseases in children from the GINI and LISA birth cohorts. Allergy. 2010 Dec;65(12):1627-9.

- **Singmann P**, Baumert J, Herder C, Meisinger C, Holzapfel C, Klopp N, Wichmann HE, Klingenspor M, Rathmann W, Illig T, Grallert H: Gene-gene interaction between APOA5 and USF1: two candidate genes for the metabolic syndrome. Obes Facts. 2009;2(4):235-42.

Aging can be fun if you lay back and enjoy it.

— Clint Eastwood

ACKNOWLEDGMENTS

This thesis would not have been possible without help and support of many kind people, to whom I want to express my gratitude. Prof. Thomas Illig, Rui Wang-Sattler, Prof. H.-Erich Wichmann, and Prof. Annette Peters made it possible to do my doctoral thesis at the Institute of Epidemiology 2/Research Unit of Molecular Epidemiology. I am very thankful for all their support and good advice given during that time.

I acknowlegde Eva Reischl and Prof. Karsten Suhre for the cooperation in the very first beginning of the project and Prof. Jerzy Adamski for sharing his incredible biochemical knowledge with me.

Without Zhonghao Yu and his statistical work for the paper and thus my thesis. All the numbers would probably still be just numbers not telling their scientific secrets. Ying He kindly shared her knowledge on drawing networks with me. Many thanks to both of you!

I am very greatful to Guangju Zhai and Tim Spector, because they offered the fruitful cooperation by replicating our results in the TwinsUK study and pushing the publication forth with me and my co-authors.

Marie Standl, Carla Tiesler, and Elisabeth Thiering were great and patient teachers in statistics and the art of programming in R. Peter Rzehak worked with me on a great project in the GINI/LISA cohorts on allergic outcomes. Joachim Heinrich was a wonderful mentor in statistical, epidemiological lessons and proper scientific working. My deepest appreciation goes to these people and the whole Heinrich group for being great colleagues during my year with them and afterwards.

Many thanks to all my co-authors for doing good science.

I don't want to forget all members of AME for a very pleasant atmosphere for working. Particularly Simone Wahl and Harald Grallert scientific and mental support has been invaluable to me.

I thank my proof-readers Simone Wahl and Michaela Breier for their time on giving my thesis the final touch.

Of course, I thank my family for their patience, encouraging words and their unstinting support.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS

AA amino acid

AC	acylcarnitine
AGE	advanced glycation end-product
AIC	Akaike information criterion
AMP	adenosine monophosphate
AMPK	AMP kinase
ATP	adenosine triphosphate
BMI	body mass index
BMR	basal metabolic rate
cer	ceramide
CoA	coenzyme A
CR	caloric restriction
CV	coefficient of variation
CVD	cardiovascular disease
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DNA	deoxyribonucleic acid
fa	fatty acid
FADS1	fatty acid desaturase 1
ffa	free fatty acid
FFM	fat free mass
FIA-MS/MS	flow injection analysis coupled with tandem mass spectrometry
GC	gas chromatography
GenAge	GenAge Database of Ageing-Related Genes
GH	growth hormone
H1	sum of hexoses
HC	healthy centenarian
HDL	high density lipoproteins
HMDB	Human Metabolome Database

IGF1	insulin-like growth factor 1
IIS	insulin/insulin-like growth factor signalling
KEGG	Kyoto Encyclopedia of Genes and Genomes
KORA	Cooperative Health Research in the Region of Augsburg
KORA F4	KORA Follow-Up 4
KORA S4	KORA Survey 4
LC	liquid chromatography
LDL	low density lipoprotein
LOD	limit of detection
loess	locally weighted scatterplot smoothing
lyso PC	lyso phosphatidylcholine
lyso PC a	lyso phosphatidylcholine acyl
miRNA	microRNA
MS	mass spectrometry
mTOR	mammalian target of rapamycin
µM	micromolar
NMR	nuclear magnetic resonance
NO	nitric oxide
OAT	ornithine aminotransferase
ODC	ornithine decarboxylase
OR	odds ratio
PC	phosphatidylcholine
PC aa	diacyl phosphatidylcholine
PC ae	acyl-alkyl phosphatidylcholine
PE	phosphatidylethanolamine
QC	quality control
ROS	reactive oxygen species
SD	standard deviation
SE	standard error

SM	sphingomyelin
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
TCA cycle	tricarboxylic acid cycle (also citric acid cycle or Krebs cycle)
TG	triglyceride
TOR	target of rapamycin
TwinsUK	UK Adult Twin Registry
VLDL	very low density protein

1 INTRODUCTION

1.1 AGING - DEFINITION, DEMOGRAPHICS, AND MOLECULAR BIOLOGY

1.1.1 *Definition and demography*

Aging describes a biological process that every human being encounters. The term 'aging' itself is on the one hand very familiar to everyone but on the other hand lacks in a common scientific definition. Many suggestions are made, the common idea of a complex, time-dependent process with various changes and associated risks might be well described by Simm et al. in 2008 [70] though it is quite general[1]:

Different definitions

> [T]he ageing process [...] can be defined as a time-dependent decline of functional capacity and stress resistance associated with increased risk of morbidity and mortality. Ageing is a complex process and affects most if not all tissues and organs of the body.

Following this definition, aging is an all-embracing transformation of an organism. The generality of this definition might mirror the gaps in capturing and understanding the aging process in its entirety. Nevertheless there are, of course, also more uncommon points of view. Famous gerontologist Leonard Hayflick, for example, provided a very physico-chemical sight on the process in 2007 [32]:

> Aging is an increase in molecular disorder. It is a stochastic process that occurs systemically after reproductive maturity in animals that reach a fixed size in adulthood. This escalating loss of molecular fidelity ultimately exceeds repair and turnover capacity and increases vulnerability to pathology or age-associated diseases. The fundamental cause of molecular disorder is rooted in the intrinsic thermodynamic instability of most complex biological molecules [...].

More details on his ideas on a indispensable entropic cause of aging are given in section 1.1.2. The difficulties of definition also seem to reflect that underlying processes have not been fully understood and disentangled, yet, although Hayflick in his forecited article even states

1 Similar definition, for example, by Henry et al. [33].

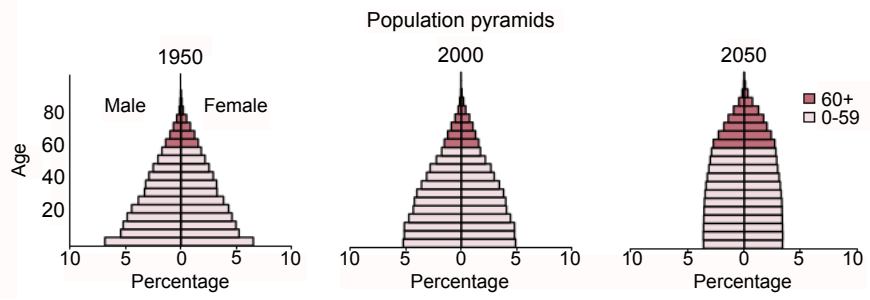

Figure 1: **Development of world's age structure.**
Age structure of the world population has been changing remarkably over the last 50 years and is predicted to continue in this way[81]. Reprinted with the permission of the United Nations.

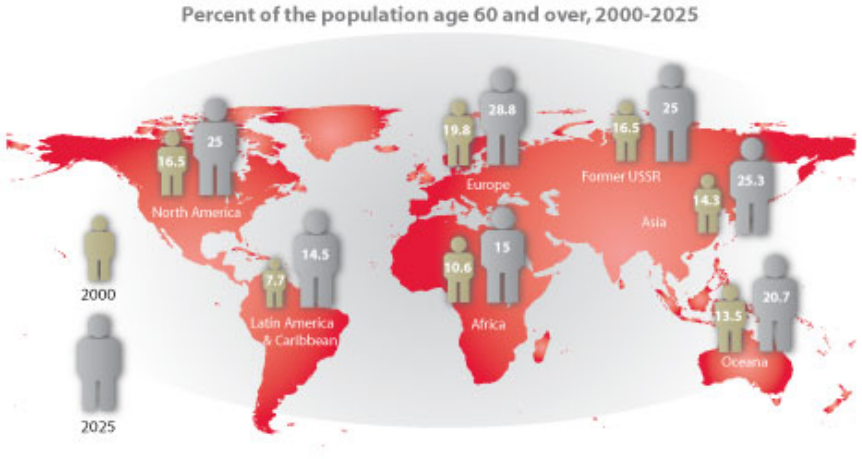

Figure 2: **Development of world's proportion of elderly.**
Proportion of the elderly (>60 yrs.) is predicted to remarkably grow in all parts of the world in a quarter of a century [51]. Reprinted with the permission of the MIT age lab.

that it *is* understood[2]. However, understanding aging's biochemistry in detail will gain importance as life expectancy of humans and hence percentage of elderly people has permanently risen throughout the world during the past century [25, 81]. Figures 1 and 2 show that this process is expected to go on in the future. The proportion of persons aged 60+ years of total world population is predicted to grow from 11% in 2012 to 22% in 2050 [82]. By 2050, older people (60+ years) will outnumber the population of children.

Demography

The aging population entails great challenges for world population in health, economics, and brings up social issues [10, 83]. Particularly health care systems will be challenged in the future as aging is often accompanied by disabilities (e. g., vision and hearing impairment, cognitive decline, physical limitations in standing, reaching, grasping) and diseases (e. g., cancer, cardiovascular disease (CVD), chronic lower

2 The pursuit of entropy of all molecules also causes aging.

respiratory tract disease, Alzheimer's disease, chronic joint symptoms, arthritis, and diabetes)[25]. Also, co-morbidity is common along with old age. Therefore, understanding the physiology and biochemistry of aging is of tremendous importance to detect and combat pathological deviations and eventually permit a preferably disease-free senescence with a good quality of life.

1.1.2 *Overview of main aging theories*

As it has become apparent in the definitions above, many transformations happen in the aging process to the human organism that affect all levels from organ systems to cell organelles and lead to a wide variety of altered functions. Genetics and environmental influences seem to be involved in the process, but insights from different points of view are imperatively desired [41, 42, 64]. Popular theories of aging include oxidative stress theory (refinement of the free radical theory from 1956), theory of accumulation of glycated proteins (advanced glycation end-products (AGEs)), involvement of chronic low-grade inflammation, altered impact of several hormones, and chromosome telomere shortening [70, 76, 92, 28, 62, 40]. Still the largest part of knowledge stems from cell culture studies and studies in C. elegans, Drosophila, rat, and mice, but not from human studies. However, for the wide scientific field of aging one common consensus theory is not found so far [7].The mentioned theories should be explained in a short, simplified manner subsequently.

OXIDATIVE STRESS THEORY [70, 53]: An unpaired electron characterizes reactive oxygen species (ROS) making them highly reactive and thus liable to strip electrons from other molecules, which in turn become radicals, and so forth, resulting in damaged proteins, membranes and nucleic acids. A prominent product is 8-hydroxy-29-deoxyguanosine that may lead to altered gene expression via mutation of the deoxyribonucleic acid (DNA) and is employed as a marker for oxidative stress. ROS are mainly produced in the mitochondrial respiratory chain. Besides ROS, other forms of activated oxygen like peroxides and aldehydes contribute to total oxidative stress.

THEORY OF ACCUMULATION OF GLYCATED PROTEINS [70, 92, 69]: In a non-enzymatic reaction, a sugar molecule is bonded to a protein or lipid molecule forming, e. g., carboxymethyl lysine and pentosine, which both are employed as markers of AGE accumulation. This reaction creates protease-resistant cross-links of proteins. AGEs can activate cells and thereby lead to ROS generation. Inversely, ROS may also induce formation of AGEs.

CHRONIC LOW-GRADE INFLAMMATION [70, 28]: Both the innate and the adaptive immune system are activated with older age together with increasing levels of proinflammatory cytokines (e. g., TNFα

and IL-6). Thereby, changes in innate immunity result in a chronic low-grade inflammation and alterations in adaptive immunity lead to accumulation of megaclones of memory cells, occupy immunological space and thus lead to decreased t-cell repertoire.

ALTERED IMPACT OF SEVERAL HORMONES [70, 62]: Secretion and tissue responsiveness of *growth hormone (GH)* (esp. via release of insulin-like growth factor 1 (IGF1)) decline with aging, which is associated with obesity, loss of muscle mass, and with higher antioxidative capacity. Decreased levels of *insulin* during aging are associated with caloric restriction (CR) in mammals and with normal to low glucose levels resulting in improved insulin sensitivity and longevity. The *dehydroepiandrosterone (DHEA)* is the precursor of sex hormones in peripheral tissues (others than ovary and testes). Its levels decrease with age, but supplementation has neither positive nor negative effects on different health outcomes, whereas declining levels of its sulfate (dehydroepiandrosterone sulfate (DHEAS)) are associated with death in general and death from CVD. *Estrogen* levels decrease after menopause accompanied by increased risk of cardiovascular events, loss of skeletal mass, vasomotoric instability, psychological symptoms and atrophy of estrogen-responsive tissue. *Testosterone* decrease is associated with an increase in fat mass, loss of muscle and bone mass, fatigue, depression, anemia, poor libido, erectile deficiency, insulin resistance and higher cardiovascular risk.

CHROMOSOME TELOMERE SHORTENING [70, 40]: *Telomere shortening* is the main cause of cellular senescence[3] and is itself caused by inability of DNA polymerases to prime DNA synthesis in this region during cell division in somatic cells[4] and also by oxidative stress. As telomeres have a protective effect on the DNA it is supposed that telomere shortening has effects on morbidity and mortality but results are inconsistent.

Hayflick's aging theory

Following the forecited Hayflick paper [32], there are two major classes of aging theories, according to his considerations in the paper, only one of the two is tenable. He argues that there is no direct evidence for a gene-driven age change within the meaning of a predetermined program, but evidence exists for aging as a stochastic process that is characterized by a 'loss of molecular fidelity'. The author brings forward the argument that all molecules follow their thermodynamic features by loosing energy (idea of entropy) and thus constantly change. Definition of biological aging (in contrast to aging of non-living things) is extended by the repair systems, that are themselves prone to aging processes, resulting in the aging definition cited at the beginning of this chapter.

Processes that can be assigned to this theory of aging are glycation

3 Arrest of cell cycle and growth after a certain time in culture, altered gene expression, allocation of the phenomenon to the whole organism is speculated.

4 Stem cells possess telomerase, which is able to maintain telomere length. Embryonic stem cells have full telomerase function whereas it is limited in adult stem cells.

processes (formation of AGEs), changes in three-dimensional structure of molecules, different aggregations, amyloid formations, alterations in metabolic processes like protein degradation and synthesis rates, as well as damages and modifications on nuclear and mitochondrial DNA (e. g., telomere shortening, damages due to ROS). However, genetic influence comes into the discussion again with determinants of longevity. Hayflick explains that this is not a stochastic process like aging, but gene-governed levels of physiological capacity, repair, and turnover indirectly determine potential longevity. Although Hayflick argues that the cause of aging is disentangled, I hold the view that consequences of these stochastic processes in terms of biochemical pathways and others need to be explored. Especially, there is room for studies in humans although other models are easier to follow through their lifespan.

1.1.3 *Current knowledge of the metabolism of the elderly*

As early as 1908, Max Rubner suggested a link between metabolic rate/oxygen consumption and lifespan in eutherian mammals, which was refined by Pearl to the *rate of living* theory in 1928 [35, 53]. Although the *rate of living* theory is no longer accepted, it can be seen as a start point of research into the aging metabolism. Today's state of knowledge is outlined in this subsection.

Global changes

Body composition and anthropometry of aging individuals experience changes. Body fat content rises most notably in abdominal regions, lean mass declines (both muscles and organs loose weight with differing extent between organ systems), and all results in higher BMI [80, 33, 68]. Together with changed endocrine functions, anthropometric remodeling evokes that alterations accompanying older age are also observable on metabolic levels. The basal metabolic rate (BMR) is described to decline with aging though with large intra- and inter-individual ranges, and diseases may increase BMR. Underlying reasons might derive from lower muscle and organ masses. In a systems biology approach aiming the question of the interconnection between development and aging, aerobic metabolism appears as a major regulator, besides epigenetics and the immune system [24]. Glucose metabolism enzymes (glycerol-3-phosphate dehydrogenase 1 and 2), factors for glucose homeostasis (IGF1, mammalian target of rapamycin (mTOR)) and citric acid cycle enzymes (succinate dehydrogenase complex, aconitase) were highlighted.

Fat metabolism

Mechanistic insight into alterations in fat metabolism is given by an article on lipid metabolism of the elderly by Toth and Tchernof [80]. Higher levels of free fatty acids (ffas) observed in elder subjects are thought to contribute to age-associated body fat accumulation via two different mechanisms: altered recruitment of fat substrates and

altered fat oxidation.
Study results from both aged rats and humans showed a reduced stimulation of lipolysis by catecholamines (inhibition of lipolysis, however, was not affected by age). Release of ffas was not impaired, but their release from adipose tissue exceeds energy needs of the elderly. As the inhibitory effect of insulin on lipolysis declines with age and ffa release is regulated by insulin (and others), a causal relationship suggests itself. However, ffa rise may also simply result from larger adipose tissue.
In elderly, fat oxidation decreases postabsorbtively, which might be simply explained by reduced resting energy expenditure. But the article describes findings that showed impaired ability to increase fat oxidation after a rise in ffa availability with older ages, e. g., in postprandial state. This impairment might come from a decline in fat free mass (FFM)[5], which decreases with age. As this only explains the descent of resting energy expenditure, alterations in oxidative capacity of FFM could also clarify the changes postprandial and during exercise. Glycolytic capacity of skeletal muscle, which represents FFM during exercise, is found to be maintained with age, but activity of enzymes of oxidative metabolism and β-oxidation of fatty acids are reduced. The reduction in size of skeletal muscle with age add to these declines.
Taken together, the age-related changes in lipid metabolism could generate increased glucose production, impaired insulin-dependent glucose uptake, and diminished hepatic insulin extraction. In turn, these effects promote hyperinsulinemia and insulin resistance. Atherosclerotic processes would be enhanced by incorporation of not oxidized ffa into very low density protein (VLDL). In their article from 2009, Dennis et al. report that homeostatic set points of metabolic feedback mechanisms for glucose and fatty acid metabolism are subjects to age-dependent changes. This statement supports the mechanisms described by Toth and Tchernoff [80]. Concerning amino acids concentrations, both increases and decreases were reported in different studies [46], what points to inconsistent data by the current state.

Healthy centenarians

Further insights into aging metabolism were drawn from studies on healthy centenarians (HCs). Paolisso and his group interpreted HCs as subjects with a successful remodeling process during aging with effective mechanisms against age-related diseases [60, 4]. The reasons for their success is unknown for the most part. Observations suggest that significant improvement in glucose handling, especially ameliorated insulin sensitivity and deteriorated IGF1 levels, plays a prominent role. There is a number of factors likely involved:

5 Fat free mass is a proxy of mass of metabolically active tissue.

- CR[6]: leads to lower body temperature, improved insulin sensitivity via lowered fasting plasma glucose and insulin levels and improved insulin action, lowers fasting plasma free IGF1 and DHEA[7] levels, improves thyroid function;
  all resulting in lowered energy production in the mitochondrial complex (ad libitum diet is associated with higher ROS production that activates different mechanisms that then impair enzyme activity, CR acts oppositely: less ROS slow cellular aging and reduce danger of DNA damage) and minimized DNA damage, i. e., genomic stability

- CHANGES IN GLUCOSE METABOLISM: impaired glucose metabolism due to a decline in insulin action, HCs have a very low prevalence of insulin resistance

- ANTHROPOMETRIC CHANGES: HCs do not show the association between elevated waist-hip ratio and insulin resistance or dyslipidemia

- ENDOCRINE ALTERATIONS: higher levels of *free plasma IGF1* in HCs than in control elderly subjects had positive effects on anthropometric and metabolic parameters; lower levels of *leptin* in HC associated with lower body fat content; lower degree of *oxidative stress* in HC along with higher plasma antioxidant defense levels than elderly control subjects (mainly vitamin E)

Another interesting group for studying aging is the Ashkenazi Jews, who live very isolated in the US (genetic homogeneity). Barzilai et al. investigated a group of 213 healthy Ashkenazi Jews with exceptional longevity aged between 95 and 107 years and one first-degree offspring of each (216 individuals, 51–89years) [5] and additionally one Ashkenazi control group with "normal" longevity and one population-based control group from the Framingham Offspring Study. A focus of the study was lipoprotein levels in blood. The long-living individuals showed clearly larger high density lipoproteins (HDL) and low density lipoprotein (LDL) particles independently from absolute levels of lipoproteins and apolipoproteins resulting in lower prevalences of hypertension, CVD, and metabolic syndrome. This observation is suggested to contribute to the longevity. The mechanism behind might be that large LDL particles move more slowly into arterial tissue, bind less tightly to arterial surface molecules, are less prone to oxidation compared to small LDL and thus might protect against endothelial dysfunction and CVD.

Pathways involved in aging

Upon findings from genetic studies in animal models, there are

6 The description in the papers is not completely clear, but I don't think HCs have a CR, but the corresponding effects and pathways might play a role in their successful aging.

7 endogenous steroid hormone, precursor of sex steroids, advertized as anti-aging hormone, further information in 1.1.2

two nutrient-sensing pathways known to be involved in aging mechanisms across different species [61]. The *target of rapamycin (TOR) pathway* regulates numerous processes in cell function, metabolism, transcription and others and is attributed to have an evolutionary conserved role in aging. Both TOR and its mammalian equivalent mTOR are kinases inhibited through rapamycin[8]. In more detail, mTOR controls cellular functions in response to growth factors and nutrients, in particular amino acids (AAs) and among these especially leucine. An interplay between mitochondrial dysfunction (through mitochondrial inhibitors, adenosine triphosphate (ATP) depletion) and mTOR is possible via AMP kinase (AMPK)[9] activation and subsequent mTOR-p70α[10] inhibition [79]. Modulation of mTOR in mice extended lifespan through delayed death from cancer, slowed aging mechanisms, or both [31]. Another evolutionary conserved pathway is the *insulin/insulin-like growth factor signalling (IIS) pathway*, that has been described to balance nutrient consuming processes (e.g. growth, metabolism) and nutrient availability [61] and is further involved in reproduction, stress resistance, and diabetes development [63]. This pathway also prolongs lifespan by delaying effects of aging.

Due to their central roles in nutrient-sensing pathways, mTOR and IIS signaling might be the mechanisms behind the life-extending effect of CR [72]. CR inactivates one or more pathways partially that would usually be activated by nutrients [27]. These inactivations may lead to extended life-span. The existence of these mechanisms might evolutionary be explained by a stop in cell division and reproduction to save energy for maintenance systems during starvation.

1.2 METABOLOMICS

1.2.1 *Definitions*

Metabolomics is a key technology of modern systems biology and is targeted on an integral depiction of the current, i. e., time-related, metabolic status of an organism, associated with physiological and pathophysiological processes [65, 50]. The ideal is to measure all metabolites of a sample, i. e., the so-called metabolome [1]. Different technologies are in use, until now none capable of measuring the whole metabolome. Hence, metabolomics divides into two different approaches. Untargeted metabolomics is applied in hypothesis-free studies that focus on the comparison of two groups, e. g., ill versus healthy probands, mostly with help of nuclear magnetic res-

targeted versus untargeted

8 Rapamycin is a drug that inhibits TOR and has anti-neoplastic effects.

9 AMPK senses the level of adenosine monophosphate (AMP) as a measure of cellular energy status and activates energy conserving and producing pathways in response to high levels.

10 p70α stands for the ribosomal protein S6 kinase from the family of serine/threonine kinases and is activated by mTOR.

onance (NMR). Targeted metabolomics is used in studies aiming at specific pathways or known molecules, most often by application of mass spectrometry (MS) with the restriction to a limited subset of the metabolome [1, 75].

metabolomics versus metabonomics

There is furthermore a subtle differentiation between the terms metabo*lomics* and metabo*nomics*, albeit usage in the literature is not that distinct [50]. In older definitions metabo*nomics* was used for plant studies and metabo*lomics* for animal studies; a later definition connects them to NMR and MS technologies, respectively. Although Mishur and Rea write that the latter definition is outdated [50], many others still apply it, e. g., Suhre and Gieger describe metabo*nomics* as a common synonym for NMR-based approaches [75]. Theodoridis et al. even point out that the terms are often used interchangeably and recommend terms like metabolite profiling instead [78]. As a current definition Mishur and Rea specify metabo*nomics* as the studies of *dynamic changes* in the metabolome caused by perturbations by disease, drugs, or toxins and metabo*lomics* as a *metabolic snapshot* of the momentary metabolites in the sample. Obviously, these descriptions overlay with those for targeted and untargeted approaches given above. Summarizing this section so far, metabo*nomics* is often connected to NMR application in untargeted comparison of the dynamic metabolic changes of two study groups whereas metabo*lomics* often applies MS for targeting a selected subset of the metabolome. All definitions I read also circumvent a number of analytes that is necessary to justify the usage of the term metabolomics. Evans et al. probably give the gist of the matter by saying that metabolomics should aim at a greater understanding of the perturbation-prone processes investigated [23].

In fact, following these definitions studying human aging metabolome refers to 'metabo*nomics*', but as the term 'metabo*lomics*' has become much more prominent in literature and also a substitute of 'metabo*nomics*' or a general term for both, 'metabo*lomics*' is used here. One could also argue that the present thesis deals with age but not with ag*ing* as it employs a cross-sectional population study (details in 2.1.1) and thus is metabo*lomics*.

metabolite, metabolome

Some additional definitions are helpful in the metabolomics field. The numerous *metabolites* measured are referred as small molecules with a molecular weight below 1500 Dalton [1] and can be both endogenous and exogenous, intermediate and end products of biochemical pathways. They ideally represent the whole range of intermediate metabolic pathways, and may serve as biomarkers indicating distinct physiological or pathophysiological states of an organism. Metabolomics is therefore a valuable tool to investigate all these influences on the metabolism together in one approach and link them to the phenotypic outcome of interest. Metabolites in their entirety in a specimen are named *metabolome* according to the established terms

genome, transcriptome, and proteome (appendant to the methods of genomics, transcriptomics, and proteomics, respectively). However, the aim of analyzing the whole metabolome is ambitious as numbers from Human Metabolome Database (HMDB) show. The HMDB, a freely accessible collection of vast metabolic information, records 40,278 metabolites identified from human organism[11], of which 20,146 from blood ([91, 36] HMDB version 3.0, Jan 2013).

Metabolites have various tasks and particularly fulfill critical roles in an organism as they are involved in energetic processes, regulation of enzyme activities, structure build-up, and signaling [84]. Notably, metabolites are not coded in the genome, but products of metabolism, which, in turn, is the whole of biochemical pathways of the cells. Concentrations of metabolites are functional end points of metabolic pathways and reflect individual affectors like nutrition, physical activity, and medication [88].

1.2.2 *Technical aspects*

Metabolomics is described as a technique easy to measure compared to other omics [45]. After preparation of the sample the general analytical steps are separation and identification of the compounds of interest. Two main methods for identification are in use: NMR and MS. NMR spectroscopy applies as basic principle of identification the specific pattern in the chemical shift of specific atoms[12] [75]. MS-methods are based on an initial ion formation —various methods of ionization exist— followed by ion separation according to the mass-charge ratio resulting in the measurement of the specific molecular mass of a metabolite and, if a tandem mass spectrometry is used, also on the specific fractionation pattern of the molecule. If combined with liquid chromatography (LC) or gas chromatography (GC) for separation (so-called hyphenated methods), retention time is another parameter of characterization [75]. The actual identification of the compounds is done by computer programs after the experiments. MS-based methods are assigned to have higher sensitivity than NMR-based ones but need more extensive sample preparation and measurement procedure, which is accompanied by higher risk of experimental errors [75]. MS needs metabolites to be extracted from the samples and thus destroys the sample whereas NMR employs the sample as a whole

11 According to the release note for version 3.0, there are now both detected and expected metabolites included in the database. However, the number of detected is 20,911.

12 Molecules with spin in a strong magnetic field can accept energy and thus generate local magnetic fields, which are dependent on local electronic and magnetic environment in the molecule. The resonance frequency applied to the molecule shifts the frequency of the nucleus in dependence of the local surroundings, i. e., the chemical shift which can then be measured. As ^{12}C and ^{16}O do not have spin, ^{1}H is well suitable for identification of diverse metabolites. [47]

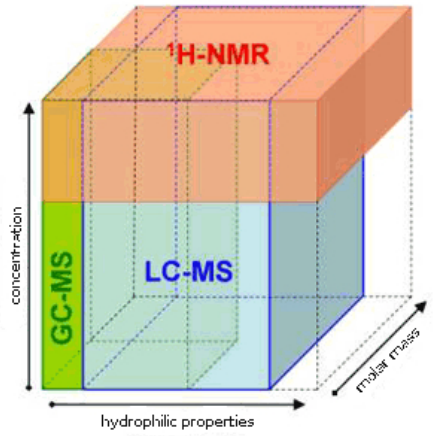

Figure 3: **Analytical performance of NMR and MS.**
Comparison of metabolomics methods: hydrophilic properties and concentration range of the metabolites differ between the methods [3].

and leaves it intact for further use. Furthermore, the number of reference samples differs considerably between the methods: one to two for NMR but almost as many references as metabolites measured for MS. Reproducibility is clearly higher for NMR than for MS. Figure 3 displays analytical differences of NMR and MS depending on metabolite properties.

The technical section should be finished with a few general remarks about the application of metabolomics. Concerning the comparability of metabolomics studies, it should be noted that small differences in the experimental assembly may induce significant effects on the analytical results. The biochemical identification of the metabolites turns out to be still the bottleneck as many of them remain unidentified [75]. Particularly, the vast differences in chemical properties of the metabolites challenge the measurement methods [84].

*Biocrates Absolute**IDQ**™p150 kit*

The experimental setup used in the Cooperative Health Research in the Region of Augsburg (KORA) study comprises an additional metabolomic setup: an assay kit was applied that comes with specially designed plates, all standards and quality control samples as well as software for managing the workflow and concentration calculation (Biocrates Absolute***IDQ***™[13] p150 together with Met***IDQ*** software). This kit was designed to target 163 common metabolites in human samples, i. e., a wide range of lipid species (PCs, lyso phosphatidylcholines (lyso PCs), SMs, and acylcarnitines (ACs)) complemented by the sum of hexoses (H1) and AAs (see table 1). The focus on lipids also suits well to the topic of aging as these metabolites were assigned to play a key role in the process by many studies [93]. Ma-

13 IDQ stands for *id*entification and *q*uantification

Table 1: Metabolite classes in the Biocrates kit, adapted from Biocrates product information sheet and [95]

metabolite class	N	biological relevance (examples)
acylcarnitines	41	energy metabolism, FA transport, β-oxidation, oxidative stress
amino acids	14	AA metabolism, urea cycle, insulin sensitivity/resistance
sum of hexosess	01	carbohydrate metabolism
phosphatidylcholines	77	membrane composition and damage, FA profile, activity of desaturases
lyso phosphatidylcholines	15	phospholipase activity, membrane damage, FA profile
sphingomyelins	15	signaling cascades, membrane damage

chine facility used in this study was a FIA-MS/MS. The methods section expatiates on experimental details (2.1).

A major disadvantage of the Biocrates kit is the lack of exact determination of side chains in PCs and SMs, only empirical formulas denominating the number of C atoms and sum of double bonds are determined but length of each side chain with its exact positions of the double bonds is not available. More information on the identified compounds is given in chapter 2.1.2

1.2.3 *Challenges of metabolomics*

For investigation of complex diseases/traits with multiple determining parameters in one approach, metabolomics is a well-suited tool that provides more robust data than mono-parametric approaches by reduction of false-positive results and is also more cost-effective [88]. It is already used routinely in newborn screenings for inborn errors of metabolism. Despite all the praise for it, metabolomics also holds many challenges. Metabolomics platforms shall produce data with low variability, detect still small concentrations of a metabolite, distinguish molecules with similar chemical properties and at the same time capture the whole range of very different molecules of the metabolome [23]. The researcher has to find ways to handle the huge data-sets generated by metabolomics experiments, to deal with multiple testing and with correlations between metabolites as they are interlinked by biochemical reactions in metabolic networks [75]. Thereupon, complex results need to be displayed in a clear and plain manner and at last to be interpreted. Interpretation requires manual integration of biochemical information from databases like HMDB and the Kyoto Encyclopedia of Genes and Genomes (KEGG) which encounters limits when it comes to less known or unknown metabolites. Furthermore, one has to take into account the influence on metabo-

lite concentrations of parameters like sampling time[14], fasting status, co-morbidity, physical activity, diet, gender.

Attention needs to be drawn to the fact that altered levels of a metabolite can derive from both anabolic and catabolic processes [46]. Lawton and co-authors give the example of 3-methylhistidine whose blood levels can correspond to a high protein diet or to an acute increase in protein breakdown. Constant levels however may point to adaptive processes in other functions like expression and function of a related transporter.

1.3 METABOLOMICS STUDIES ON AGING

For capturing the complexity of the aging process in a large cohort study, metabolomics is a very promising tool and according to Partridge et al. in 2011 [61], systemic approaches into aging have just begun. Following genomics, transcriptomics, and proteomics in aging research, metabolomics is assigned to deliver information about several tissues at once concerning their short-term regulation [45]. As described by Kristal and Shurubor in 2005 [45], genomics helped identifying potential genes involved in longevity and age-associated diseases, but does not help understanding the aging process better[15]. Transcriptomics reveals translational changes of the genome with older age, but is limited to single tissues and gives no insight into post-translational processes. Proteomics could give some insight into post-translational products and into accumulated proteins, a common age-phenomenon, however, its limits are short-term regulation and analytical challenges with serum samples. Metabolomics seems to be the logical next step in the aging field. The article also raises several questions that metabolomics may answer uniquely; two of them of interest for this thesis: Is there a qualitative or quantitative effect of aging to the metabolism and will metabolomics help identifying biomarkers of aging? A more integral and comprehensive approach to the complexity of aging that includes dynamics of metabolic networks combined with genomic networks is also claimed by West and Berman [89]. Soltow et al. even name metabolomic changes the best representation of age-associated declines of function [73] and point out that an overall process like aging requires a systems approach as the traits studies so far as stand-alone are likely to be involved together.

To my best knowledge, only few aging studies with metabolomics analyses have been conducted in animal models yet. There are very few published results from human adult studies that concentrate on aging so far, derived from low participant numbers [46]. Orešič men-

14 Many metabolites are know to be subjects of circadian rhythm [49].

15 Which fits well to the Hayflick theory in section 1.1.2 that genetics do not play a role in aging.

tioned that human metabolome has been shown to be sensitive to age [59], but the given references for that review supply only one comparable publication. Nikkilä and colleagues performed a metabolomics study in early childhood with 59 children followed up from birth to an age of four years and identified previously unknown metabolic changes with age [56]. A study of 269 individuals of both male and female gender analyzed the human plasma metabolome, but with NMR technique and thus a very different set of metabolites than used for this thesis [46]. Nevertheless, comparing the 3 age groups revealed more than 100 metabolites associated with age, some associations were sex affected, but no race effects were found. Most concentrations were highest in the oldest group compared to the youngest group (unless indicated otherwise): From AA metabolism there were serine, alanine, glutamine, glycine, glutamate, histidine, isoleucine, valine, leucine, lysine, hippurate, p-cresolsulfate[16], 3-methylhistidine, ornithine. From carbohydrate, lipid, and energy metabolism myo-inositol, lactate, mannose (lower), a number of fa, β-hydroxybutyrate, cholesterol, DHEA-sulfate (lower) showed up, and furthermore citric acid intermediates and metabolites associated with nucleotide metabolism were identified. The latter study illustrates two main challenges occurring with metabolomics in epidemiological context. Number of subjects is often very low compared to the high number of variables that comes along with metabolomics analyses and that is often even split up into groups (e. g., age, sex). Comparability is another problem, because of different platforms as discussed in 1.2.3 and also different sample sources (e. g., serum, urine).

An overview of studies on aging with help of metabolomics techniques is given in tables 2 and 3. For this, a Pubmed search with "metabolomics and age" was done, similar searches did not yield more results (using terms like metabonomics, metabolic profile, aging). Only studies more or less "pure" into age/aging were included, however, some with an additional intervention group related to the aging field, e. g., CR, but studies on age-related diseases were excluded.

16 p-cresol sulfate is a microbial metabolite that is found in urine and likely derives from secondary metabolism of p-cresol. from HMDB entry HMDB11635; p-cresol (4-methylphenol) is an end-product of protein breakdown. HMDB01858

Table 2: Studies on aging with metabolomics – cells and animals

subjects	n[1] sex[2]	age[3]	design	method[4]	U or T[5]	material	results regarding aging	ref.	remarks
human mesenchymal stem cells (hMSC)	1.5E6 cells per sample	cultured 10–14d, +2d after H_2O_2 exposure	H_2O_2 induced early senescent vs. CTL cells	NMR	U (26)	cell content	gly and pro ↓, choline, leu, NAD$^+$, and UDP-Glc ↑ in senescent cells	[44]	
oregon-R wild-type Drosophila	5 x 20m, half old/ half young	3d, 40d	3 conditions for each sample: 4h room air, 4h hypoxia, 4h hypoxia + 5min recovery	NMR	T (37)	muscle tissue	young and old flies had very similar profiles in control and hypoxic conditions	[15]	description of samples confusing
ERCC1D/-mice (accelerated/ premature aging) + WT controls	10 mutants/ 10 WT (serum); 13/13 (urine)	8–20W	serum sampling at 8, 12, 16, 20W; urine sampling unclear	NMR	U	serum + urine	serum: differences in lipids (↑ and ↓ ones), Glc, lactate ↓ in mutants; urine: lactate, citrate, succinate, 2-oxoglutarate ↑ in mutants, presence of 3-hydroxybutyrate in mutant (i.e., ketone body, energy metabolism altered)) – similar age-related patterns but differences in lipid and energy metabolism, transition to ketosis	[55]	
C57BL/6 mice	?f	3–26mo	3 groups: 3mo, 26mo, 26mo+ CR (10% at 14W, 25% at 15W, 40% at 16W+)	LC/MS	U + T (3)	serum	changes in byproducts of lipid metabolism, fatty acid metabolism, and bile acid biosynthesis; ↓ lyso PCs 16:1 and 18:4 restored by CR (in total, 15 metabolite levels restored totally/completely by CR)	[18]	n not specified
Alderley Park (Wistar-derived) rats	6m	4–20W	sampling every 2 weeks	NMR, LC/MS	U	urine	creatinine, taurine, hippurate and AAs/fas ↑, citrate and Glc/myo-inositol ↓, some compounds only present in urine at 4W, carnitine and others ↑	[90]	short observation time, low n
Sprague-Dawley rats	18m/30f	1–24mo	3 age groups: 1–2mo (g1), 5–6mo (g2), 22–24mo (g3)	NMR	U (30)	brain (3 regions)	old rats showed myo-inositol and lactate ↑ in all 3 brain regions, N-acetylaspartate ↓ in temporal and frontal cortex, Glutamate-GABA level became imbalanced in temporal cortex of old rats, sex-differences observed	[96]	
Sprague-Dawley rats	75m	4–24mo	5 groups: 4mo, 10mo, 18mo, 24mo, 24mo +epimedium	LC/MS	U	serum	30 age-related endogenous metabolites, 25 identified	[93]	epimedium - Chinese medicine
Labrador dogs	48 (from 7 litters)	9W, 1.5Y, 9Y	2 groups: CTL and CR of 75%	NMR	U	urine	urinary excretion of creatinine ↑ with maximum between 5Y and 9Y and ↓ thereafter, excretion of mixed glycoproteins at earlier ages (may be a reflection of growth patterns)	[86]	

Table 3: Studies on aging with metabolomics – humans

subjects	n[1] sex[2]	age[3]	design	method[4]	U or T[5]	material	results regarding aging	ref.	remarks
healthy (self-identified)	30m/30f	19–69y	2 age groups, cut at 40 (mean age), 8 samples per proband	NMR	T	urine	Metabolites from mitochondrial energy metabolism differed in gender and age; carnitine, 3-hydroxyisovalerate, creatinine, Ala, and trigonelline were significantly different between young and old, similar results for m and f separately	[71]	low n for large age range, health status self-reported
healthy	131m/138f	20–65y	3 age groups: 20–35y, 36–50y, 51–65y	LC/MS + GC/MS	U	plasma	nearly 100 compounds altered with age; changes with protein, energy and lipid metabolism, oxidative stress with older age, TCA cycle intermediates, creatine, essential and non-essential AA, urea, ornithine, polyamines, oxidative stress markers ↑, DHEAS ↓	[46]	
children, no chronic diseases	27m/32f	3mo–4y	ca. 11 samples per kid taken around every 3mo	LC/MS	T (lipids) (64)	serum	most changes in phospholipid profiles (e. g., lyso PCs, SMs) and short- and medium-chain TGs occurred at 1y, later changes dominated by longer chain TGs – major developmental state differences between girls and boys attributed to sphingolipids	[56]	
children, healthy	55m + f	0–12y	Cross-sectional	NMR	U	urine	age correlation of creatinine, creatine, Gly, betaine/TMAO, citrate, succinate, acetone; creatinine ↑, all others ↓	[30]	no sex-separation
children, healthy at least at birth	9m/31f	9mo vs. 17y	retrospective, metabolomics at 17y compared to 9mo levels of TGs, HDL, LDL, VLDL, cholesterol	NMR	U	plasma	relationships between parameters measured early in life and metabolic status at a later stage: medium correlations between 17y metabolome and TG, VLDL, and cholesterol level at 9mo	[8]	
two different isolated Italian populations (somewhat fixed genetic background), healthy	387/184	<12–>65y	Cross-sectional	NMR	T (AAs)	urine	age-related U-shape pattern for most AAs, 3 clusters: His/Thr/Ala, Gly/Phe, larger group – impact of transporters/altered tubular handling of AA	[17]	

Comments for tables 2 and 3

NOTES

[1]'n' refers to the number of subjects given in the table refers to the n used for metabolomics analyses, not necessarily equals the whole study group.

[2]m. . . male, f. . . female

[3]d. . . days, w. . . weeks, mo. . . months, y. . . years

[4]Same metabolomics method does not necessarily mean that it was the same experimental procedures as there are lots of parameters that can vary. For the purpose here, it was simplified to NMR (which was in all listed studies ^{1}H NMR) and LC-MS (which often was combined with a second MS measurement and predominantly was HPLC-MS).

[5](*u*n-)*t*argeted; in brackets no. of compounds identified

1.4 HYPOTHESES AND AIMS OF THE THESIS

Many papers on aging investigate special details of aging processes. However, I applied a novel method to capture more of the complexity of the matter. The aim was to characterize the metabolic profile of a large and healthy group of subjects from KORA with a wide age range (n=1038, 32–81 years) to provide a first solid basis for further metabolomics studies and for further studies on age-related diseases with metabolomics technology and prolongation of the phase of healthy aging. It was also sought to possibly connect the age-associated profile to some extent to common aging theories as the oxidative damage in mitochondria or aging phenomena as vascular lesions and altered cell functions to better understand aging process with regard to the metabolism.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL PROCEDURES

2.1.1 *Population Source*

Cooperative Health Research in the Region of Augsburg (KORA) is a population-based research platform with subsequent follow-up studies in the fields of epidemiology, health economics and health care research [67]. It is based on interviews in combination with medical and laboratory examinations, as well as the collection of biological samples. Four surveys were conducted with 18,079 participants living in the city of Augsburg and 16 surrounding towns and villages. KORA Survey 4 (KORA S_4) consists of representative samples from 4261 individuals. The dataset comprises individuals aged 25–74 years resident in the region of Augsburg, Southern Germany, examined in 1999–2001. During the years 2006–2008, 3,080 participants took part in a follow-up (KORA F_4) survey of the one conducted 7 years ago. For the purpose, a total of 918 subjects from KORA F_4 were excluded for subsequent analyses resulting in a number of 2,401 subjects aged 32–81 years. Among the excluded were 20 experimental failures, 18 non-fasting subjects, 332 type 2 diabetics, 80 subjects without fasting glucose or 2h glucose measurement, 77 subjects with systolic blood pressure >160 mmHg, and 153 subjects with BMI $> 35 kg/m^2$. Further removals followed during statistical analyses (n=239, see section 2.2).

2.1.2 *Sampling and metabolite measurements*

Blood was drawn into serum gel tubes in the morning between 8:00 and 10:30 a.m. after a fasting period of at least 8 hours and the tubes were gently shaken on a horizontal shaker for 5 minutes, followed by 30 minutes resting at room temperature to obtain complete coagulation. For serum collection, centrifugation of blood was performed at 2750 g and 15 °C for 10 minutes. Serum was frozen at −80 °C until execution of metabolic analyses.

The targeted metabolomics approach was based on measurements with the Absolute***IDQ***™p150 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) allowing simultaneous quantification of 163 metabolites. The method has been proven to be in conformance with FDA-Guidelines "Guidance for Industry - Bioanalytical Method Validation (May 2001)", which implies proof of reproducibility within a given error range. All liquid handling was done with a Hamilton Star robot

(Hamilton Bonaduz AG, Switzerland), 100 µl serum sample were used per participant. Technical equipment for metabolite measurements was an API 4000 Q TRAP LC-MS/MS System (Applied Biosystems) equipped with a Schimadzu Prominence LC20AD pump and a SIL-20AC autosampler applying multiple reaction monitoring, neutral loss and precursor-ion scans. Internal standard served as references for quantification. The Met***IQ*** software of the Absolute***IDQ*** kit was applied to supervise the whole analytical process.
All metabolite concentrations are reported in micromolar (µM). Some more background on the method is given in the introduction (section 1.2.2) and analytical details have been published in [38].

The Biocrates kit quantifies 14 AAs, H1, 41 ACs including free carnitine, 15 SMs, 77 PCs and 15 lyso PCs. Additional chemical groups that can be identified are hydroxyl groups denoted by OH and dicarboxyl groups denoted by DC. Lipid side chain composition is abbreviated as Cx:y, where x stands for the number of carbons in the side chain(s) and y the number of double bonds. Chemical bonds are indicated as aa for diacyl and ae for acyl-alkyl bonds. Full biochemical names with their respective short form are listed in table 9 on page 54ff. As already discussed in section1.2.2, exact conformations in terms of length of the side chains and distribution and position of double bonds cannot be distinguished with this experimental setup. Alternative metabolite names are given for cases of e. g., stereoisomeres and isobaric fragments that are not discernible.

A substantial part of the metabolites determined by the Biocrates kit shows values below limit of detection (LOD) or coefficient of variation (CV) close to 25% [38]. However, it is important to measure those as well, as their concentrations may increase drastically in disease or upon environmental challenge [59]. These metabolites could represent a valid trend to be evaluated in more detail. Metabolite measurements for the 3080 samples were performed in three batches, with two and three months time lapse in between, respectively. Within each kit, there are three different quality controls (QCs) provided by the manufacturer and representing mixed human plasma samples. In accordance with the kit instructions, concentration of each metabolite was adjusted based on the three QCs to minimize potential batch effects. To ensure data quality, each metabolite had to meet three criteria:

1. Average value of the CV for the metabolite in the three QCs should be smaller than 25

2. 90% of all measured sample concentrations for the metabolite should be above the LOD.

3. The correlation coefficient between two duplicate measurements of the metabolite in 144 re-measured samples should be above 0.5.

In total, 131 metabolites passed the three quality control steps, and the final metabolomics dataset contained the H1, 14 AAs, 24 ACs, 13 SMs, 34 diacyl phosphatidylcholine (PC aa), 37 acyl-alkyl phosphatidylcholine (PC ae) and 8 lyso PCs. Table S1 in the appendix summarizes QC approaches. Concentrations of all analyzed metabolites are reported in μM.

2.2 STATISTICS

2.2.1 *Quality control*

For the detection of outliers, concentrations obtained for the remaining 131 metabolites were first scaled to have a mean of zero and a standard deviation (SD) of one, and were projected onto the unit sphere; Mahalanobis distances for each individual were then calculated using the Robust principal components algorithm [26]. Calculations were done separately for males and females. For each group, the mean Mahalanobis distance plus three times variance were defined as cut-off. 239 individuals, whose distances were greater than these cut offs, were identified as outliers and removed subsequently.

2.2.2 *Clustering and heat map*

For participants with the same age, mean values were calculated for the concentrations of each metabolite. Spearman correlation coefficients were computed to determine the distances matrix of ages for clustering purpose. A dendrogram was generated based on hierarchical clustering algorithm using average agglomeration method. Standardized metabolite concentrations were used to draw a heat map showing the changes of the metabolite concentrations with the increase of age; the color change in the heat map represents the concentration deviation from the mean value. R-packages *bioDist* and *gplot* were used.

2.2.3 *Linear and logistic regression analyses*

Linear regression was applied to model the relationship between age and the concentration of each metabolite with BMI used as covariate. Metabolite concentrations were log-normalized. The regressions were done for males and females separately. Smoother plots were drawn for each metabolite of the set of metabolites with the R function 'qplot' (package 'ggplot2') using the options geom=smooth, method=loess, span=0.5 producing smoother plots with locally weighted regression (locally weighted scatterplot smoothing (loess)) applying a smoothing span of 0.5, which results in medium smoothing. loess computes outlier robust locally weighted regression fitted values by fitting local

polynomials using the weights and results in the loess curve as shown in our smoother plots. Further information about the method is available in ref. [14]. For better visualization the plots were truncated to observations between first and 99th percentile. The odds ratio (OR) for single metabolites were calculated between two groups with normalized metabolite concentrations and BMI as covariate. The obtained OR value stands for whether the probability of an increase in metabolite concentration of one SD was the same for both age groups. To handle false discovery rate from multiple comparisons, the cut point for significance was calculated according to Bonferroni revealing a significance level of 3.8E-4 (correcting for 131 metabolites).

2.2.4 *Criteria for metabolite selection*

Multivariate logistic regression, random forest, and a stepwise selection of logistic regression methods were applied: The metabolites were chosen if they were significant in both logistic regression for single metabolite with adjustment for BMI and also in the top 30 most important variables obtained using the random forest method, where the 131 metabolites and BMI were included as variables. The resulting set of metabolites was further reduced through a stepwise variable selection approach using the Akaike information criterion (AIC) as criterion and with BMI as covariate. This selection procedure resulted in sets of metabolites thought to characterize age in females and males, respectively.

All calculations were done with the R statistical platform, version 2.12 [66].

2.3 REPLICATION AND META-ANALYSIS

TwinsUK is a UK-wide twin registry sample of 11,000 adults founded in 1993 with the aim to explore the genetic epidemiology of common adult diseases [74]. The cohort has been tested to be generalizable to UK population singletons with no population stratification for a wide variety of musculoskeletal, CVD, and metabolic traits [2]. Over 7,000 twins have attended detailed clinical examinations with a wide range of phenotypes over last 18 years. Blood samples were taken after at least 6 hours fasting at each visit. The samples were immediately inverted three times, followed by 40 minutes resting at 4°C to obtain complete coagulation. The samples were then centrifuged for 10 minutes at 3,000 revolutions per minute. Serum was removed from the centrifuged brown-topped tubes as the top, yellow, clear layer of liquid. Aliquot in 4x1.5mls skirted micro-centrifuge tubes was then stored in a −45°C freezer until sampling. 1,237 twins were selected for the targeted metabolomics profiling for either osteoarthritis or genetic studies. Metabolite measurements were performed using the

same metabolomics platform and following an identical protocol as for the KORA study. For the purpose of the replication for the KORA F_4 study, the data on the 12 age-related metabolites identified in the KORA F_4 aging study were retrieved, and the association between age and these 12 serum metabolites were analyzed by robust regression modeling which takes into account of twin relatedness. A total of 1,237 individual with metabolomics data were available, among them, 44 males were excluded. Following KORA's exclusion criteria, 64 individuals with systolic blood pressure >160mmHg, 14 individuals with type 2 diabetes, 45 individuals with BMI >35 kg/m^2, and 328 individuals without either blood pressure, fasting serum glucose levels, or diabetes diagnosis data available were excluded . A total of 742 healthy female individuals were included in the final analysis. For the meta-analysis of KORA and TwinsUK females, a fixed effects model was used.

3 RESULTS

3.1 STUDY POPULATION

The KORA F4 study sample comprised 1,038 females and 1,124 males with a wide age range of 32–81 years. Females and males of the KORA F4 study have about the same average age, but females exhibit a larger average BMI than males (1.2 kg/m^2 difference). Comparing KORA females with the replication sample from TwinsUK, KORA females have a lower mean age than TwinsUK females (4.1 years difference) and an overall somewhat smaller age range. The BMI of the females in KORA is 1.5 kg/m^2 larger on average than in the females in the TwinsUK. As expected, BMI was significantly correlated with age in KORA F4 (Pearson's ρ=0.26, p=2.2E-16). Serum concentrations of 163 metabolites were measured in all participants. A total number of 131 metabolites passed the quality criteria levels (see chapter 2) and entered the subsequent statistical analyses. The final set of 131 metabolites with full nomenclature is summarized in table 9 in the appendix[1]. Characteristics of both the discovery and replication population are depicted in table 4.

3.2 DISCOVERY OF AGE DISCRIMINATING METABOLITES – GENERAL PROCEDURE

Due to prior results from KORA F4 showing strong metabolic differences between females and males [52], I conducted strictly sex-separated analyses. Two different approaches were applied in parallel to unravel the associations between metabolites and age. On the one hand continuous analyses were conducive to characterize age-associated changes regarding metabolite concentrations using linear regression

1 The table also includes those metabolites which failed the quality control.

Table 4: Population characteristics of KORA F4, subdivided by gender, and TwinsUK. Values given as mean ± SD.

	KORA F4		TwinsUK
	Males	Females	Females
n	1124	1038	742
Age (years)	53.6 ± 12.5	54.1 ± 13.1	57.7 ± 10.6
BMI (kg/m^2)	25.9 ± 3.9	27.1 ± 3.2	25.6 ± 3.7

Table 5: Top ten of BMI-adjusted linear regression between age and log of metabolite concentrations in KORA F4 females; ⋆ below significance level

metabolite	estimate	SE	p-value
C12:1	0.009	0.001	1.58E-40 ⋆
C10:1	0.01	0.001	2.70E-37 ⋆
PC ae C36:1	0.007	0.001	3.76E-37 ⋆
C18	0.008	0.001	1.21E-36 ⋆
C16:2	0.01	0.001	2.06E-35 ⋆
PC aa C28:1	0.007	0.001	3.83E-33 ⋆
C18:1	0.007	0.001	4.24E-33 ⋆
C16:1	0.007	0.001	1.78E-32 ⋆
C16	0.006	0.001	1.42E-29 ⋆
C14:1-OH	0.007	0.001	2.78E-29 ⋆

models, smoother plots and heat maps. On the other hand cluster analysis for age led to an approach of group comparison with two groups (32 – 51 years versus 52 – 77 years) and helped to identify a set of 12 markers differentiating age in women. As a combination of both analytical approaches, most illustrations were restricted to these 12 markers.

3.2.1 *Characterization of Age-Metabolite Associations*

An initial, BMI-adjusted *linear regression* analysis between metabolite concentrations (logarihtmized for normality) and age revealed first indications for strong associations as a number of 73 metabolites in women (top ten shown in table 5, full table in the appendix, table 10, page 62) and of 39 metabolites in men (top ten shown in table 6, full table in the appendix, table 11, page 64) reached significance level of 10^{-7}. Subsequent analyses were conducted to unravel details.

In order to broaden the initial results from linear regressions, a cluster analysis for the 131 metabolites was done across all ages separately for females and males. The resulting *heat map* for females displays metabolites with a clear decrease over age, in particular with most acylcarnitines (ACs), others showed a gradual increase with age, e. g., several PCs (Figure 4). Major metabolite groups are represented by gray boxes in the figure.

Details on the abbreviated metabolites names are listed in table 9 (see appendix, page 54), which also contains mean concentration, SD and ranges for all 131 metabolites for both females and males. The heat map for males did not show such strong and consistent changes and clusters (see appendix figure 7, 48), therefore I concentrated on

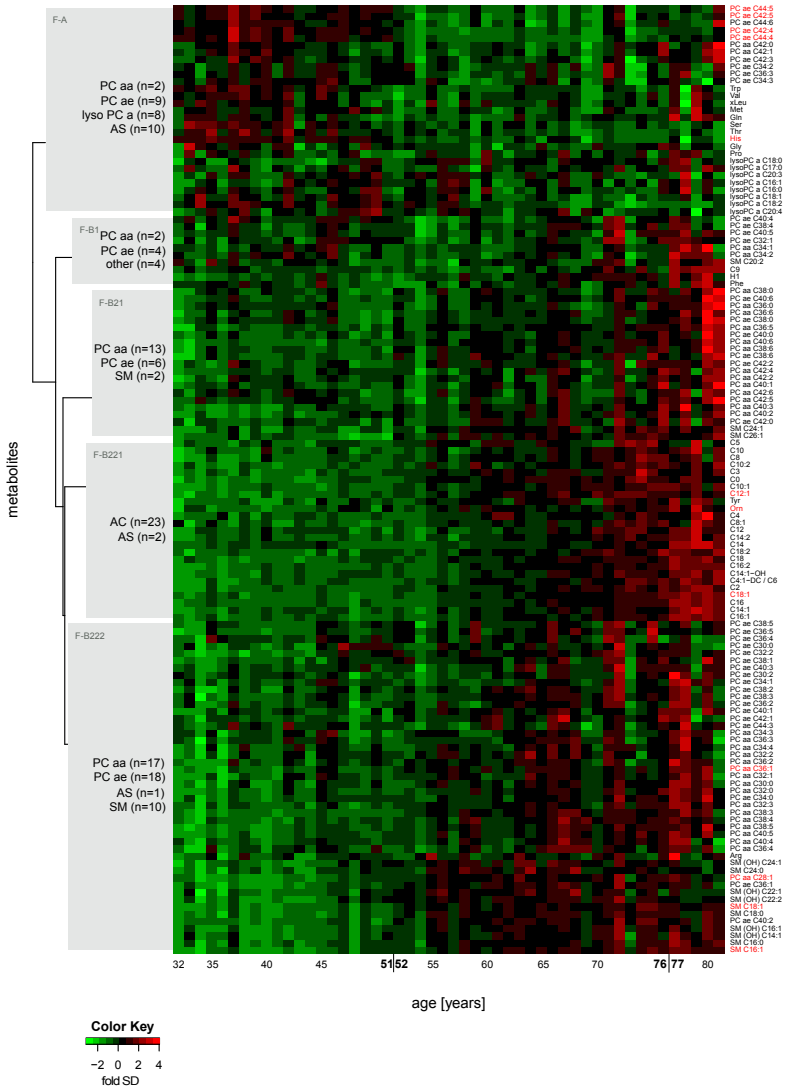

Figure 4: **Heat map of KORA F4 females.**
The heat map shows changes of fold standard deviation from the overall mean concentration for each year in a color-coded way, and clustering of these changes. Green squares represent a decrease, and red squares an increase. Gray boxes represent groups of metabolites with similar changes with number of metabolites in parentheses. Metabolite names in red indicate the set of 12 metabolites (see 3.2.2). amino acid (AA); acylcarnitine (AC); diacyl phosphatidylcholine (PC aa); acyl-alkyl phosphatidylcholine (PC ae); and lyso phosphatidylcholine acyl (lyso PC a).

Table 6: Top ten of BMI-adjusted linear regression between age and log of metabolite concentration in KORA F4 males; ⋆ below significance level

metabolite	estimate	SE	p-value
Trp	-0.003	2.78E-04	3.67E-33 ⋆
Val	-0.005	4.00E-04	6.61E-31 ⋆
C16:1	0.007	0.001	1.60E-26 ⋆
His	-0.004	3.36E-04	2.67E-26 ⋆
C12	0.008	0.001	3.50E-26 ⋆
C16:2	0.009	0.001	3.65E-26 ⋆
xLeu	-0.004	4.03E-04	7.83E-25 ⋆
lysoPC a C20:3	-0.006	0.001	4.54E-22 ⋆
C18:1	0.006	0.001	3.09E-20 ⋆
C10:1	0.007	0.001	2.38E-19 ⋆

females in all subsequent analyses. Nonetheless, results for males are provided in the appendix.

Smoother plots were drawn to characterize the trends and courses of metabolite concentration changes with age in females (figure 5) (see figure 8 for females from the TwinsUK study (replication sample) on page 49, figure 9 for males on page 50 in the appendix). The smoothing method was loess, which is a locally weighted regression robust against a small fraction of outliers. As already stated for the heat map, some metabolite concentrations showed increasing, and some showed decreasing trends with age, but smoother plots provide a deeper insight. Concentrating on the 12 markers identified in females in steps described in section 3.2.2, the larger proportion of metabolites showed clear linear associations or proximate linear trends with age (C12:1, C18:1, His, Orn, PC aa C28:1, PC ae C36:1, SM C18:1, SM C16:1). The remaining four metabolites (PC ae C42:4, PC ae 42:5, PC ae C44:4, PC ae 44:5) exhibited a decrease around age of 51 with approximate constancy prior and after this age period.

3.2.2 *Identification of a discriminating metabolite set for age in females: comparison of two age groups 32 – 51 vs. 52 – 77 yrs.*

Vertical patterns in the heat map of females and curve shapes in four of the smoother plots (PC ae C42:4, PC ae C42:5, PC ae C44:4, PC ae C44:5) prefigured a point of inflexion for age around 50 years. Subsequent *cluster analysis* for age revealed that all females of 51 years and younger grouped together due to their metabolite concentrations of the 131 metabolites and the subjects from age 52 to age 77 arranged together as well (figure 10 on page 51). A third group was formed by the 78 to 81 years old subjects, but because of its low per-

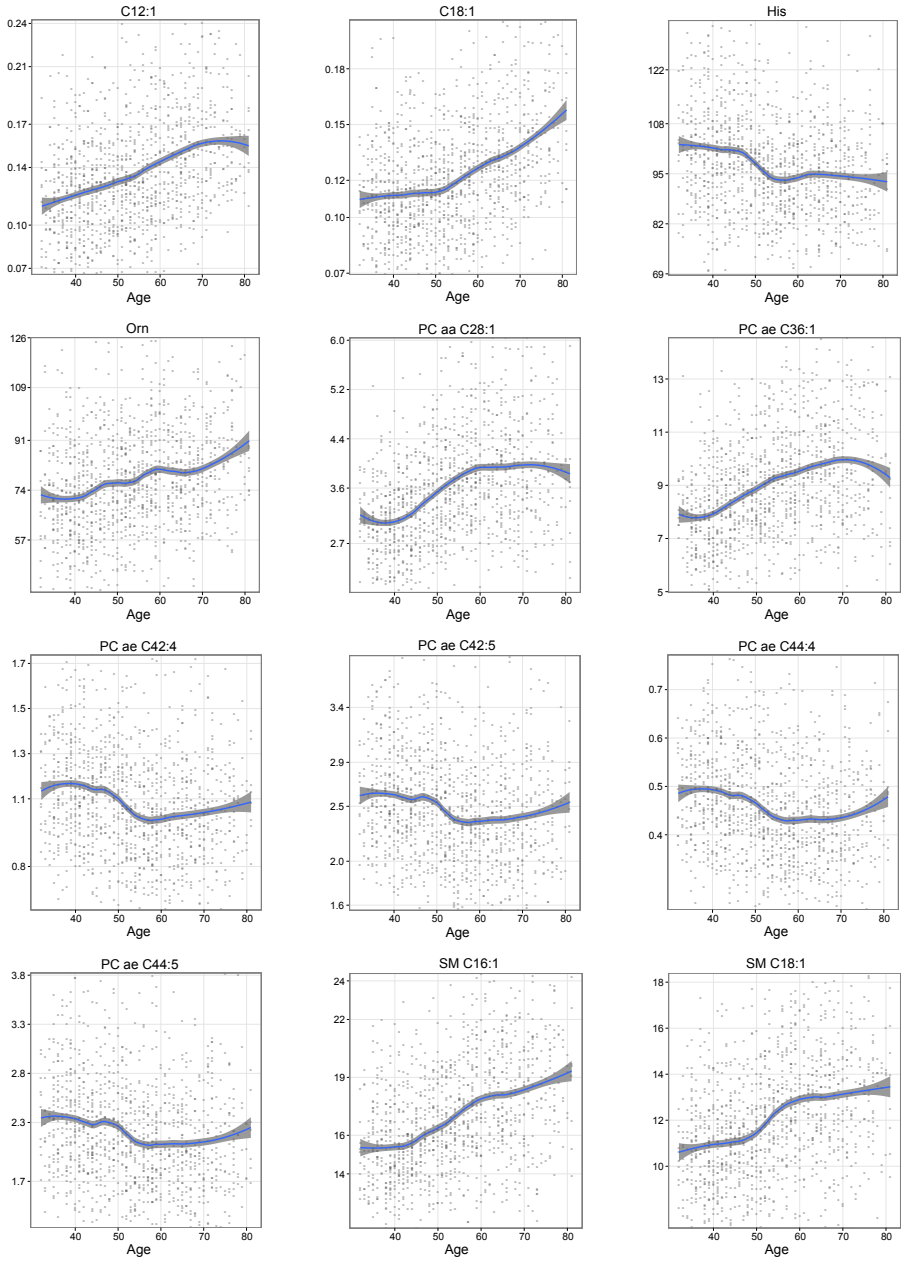

Figure 5: **Smoother plots for 12 markers in females.**
The plots show concentration trends of the 12 identified markers across age in KORA F4females with age on the x-axis and concentration on the y-axis, respectively.

son number (n=41) this group was excluded from further analyses. As consequence, two groups were used for the subsequent identification of metabolites differing in both groups: 32 – 51 years versus 52 – 77 years. Applying several statistical methods (see chapter 2), 12 metabolites could be denominated that clearly differed in their concentration between females up to an age of 51 and females above 51. These potential markers behaved differently between the two groups as some showed higher and some lower levels with higher age. Table 7 (page 31) shows the set of markers with the corresponding parameters from the linear analysis. In males 15 significantly changing metabolites could be identified as differentiating markers over the age groups (table 8 on page 34), only 3 of them were similar to females' markers (His, C12:1 and C18:1), but overall the associations between metabolites and age were not as strong as in females (i. e., higher p-values in general).

Table 7: Marker set in the discovery sample of KORA F4 females, the replication sample of TwinsUK females, and meta-analysis of both. Mean concentration in μM from serum; beta estimates represent changes per year of age, adjusted for BMI; ⋆ below significance level

	KORA F4 Females			TwinsUK			Meta-analysis	
marker	mean ± SD	beta (SE)	p-value	mean ± SD	beta (SE)	p-value	beta (SE)	p-value
C12:1	0.14 ± 0.05	81.15 (6.41)	1.18E-34 ⋆	0.17 ± 0.05	28.64 (8.60)	0.001 ⋆	62.41 (5.14)	5.80E-34 ⋆
C18:1	0.12 ± 0.04	131.79 (9.81)	1.72E-38 ⋆	0.19 ± 0.05	33.09 (7.51)	5.40E-06 ⋆	69.57 (5.96)	1.90E-31 ⋆
His	97.58 ± 14.18	-0.21 (0.02)	3.29E-17 ⋆	98.22 ± 28.93	-0.10 (0.02)	2.90E-06 ⋆	-0.14 (0.02)	1.28E-20 ⋆
Orn	77.82 ± 18.07	0.16 (0.02)	4.37E-18 ⋆	90.62 ± 25.60	0.02 (0.02)	0.160	0.10 (0.01)	7.03E-13 ⋆
PC aa C28:1	3.60 ± 0.72	5.19 (0.36)	4.23E-44 ⋆	4.17 ± 1.27	1.54 (0.33)	2.10E-06 ⋆	3.22 (0.24)	4.02E-40 ⋆
PC ae C36:1	9.03 ± 1.67	2.02 (0.16)	3.47E-36 ⋆	12.14 ± 5.23	-0.31 (0.16)	0.002 ⋆	0.89 (0.11)	1.94E-15 ⋆
PC ae C42:4	1.09 ± 0.25	-9.01 (1.33)	1.98E-11 ⋆	1.18 ± 0.46	-5.84 (1.13)	1.20E-07 ⋆	-7.17 (0.86)	8.67E-17 ⋆
PC ae C42:5	2.51 ± 0.52	-4.09 (0.67)	1.26E-09 ⋆	2.71± 0.98	-2.57 (0.52)	3.20E-07 ⋆	-3.14 (0.41)	1.88E-14 ⋆
PC ae C44:4	0.46 ± 0.12	-19.81 (2.94)	2.48E-11 ⋆	0.51 ± 0.18	-10.56 (2.52)	1.00E-05 ⋆	-14.48 (1.91)	3.86E-14 ⋆
PC ae C44:5	2.23 ± 0.60	-3.84 (0.60)	1.72E-10 ⋆	2.33 ± 0.78	-1.20 (0.59)	0.04	-2.51 (0.42)	2.28E-09 ⋆
SM C16:1	17.06 ± 2.47	1.56 (0.10)	1.93E-46 ⋆	19.58 ± 4.79	0.38 (0.09)	1.00E-05 ⋆	0.88 (0.07)	2.84E-38 ⋆
SM C18:1	12.13 ± 2.08	1.68 (0.12)	3.56E-39 ⋆	12.38 ± 3.50	0.42 (0.12)	0.001 ⋆	1.03 (0.09)	5.51E-33 ⋆

3.3 REPLICATION OF THE METABOLITE SET AND META-ANALYSIS

For replication purpose, I got support from the TwinsUK cohort with an independent sample of 742 healthy female subjects with metabolomics data available. The metabolites showed comparable mean concentrations in both studies. Among the 12 age-related metabolites found in the KORA study, 10 metabolites were well replicated in the TwinsUK sample with all p<0.004 which was the significance level after adjustment for multiple testing with Bonferroni method (α of 0.05/12 metabolites=0.004) (Table 7), and the effect direction was the same as in the discovery sample except for one metabolite, PC ae C36:1, with borderline significance in the TwinsUK sample (p=0.002). When the sample was divided into two groups (people with age $\leqslant$ 51 and people with >51 as in the KORA study, data not shown), 8 metabolites replicated. I also draw the smoother plots for the TwinsUK sample and obtained similar curve shapes for all but PC ae C36:1 (Figure 8 in the appendix, page 49). Furthermore, both study samples were analyzed in a meta-analysis which gave similar results as in the KORA females sample alone (i. e., virtually the same magnitudes of beta estimates and p-values). All 12 metabolites of the marker set reached significance with p-values range from 2.28E-09 to 4.02E-40.

3.4 FURTHER APPROACHES

Some further methods were applied to unravel the nature of age-metabolite associations. As from earlier publications [87, 95, 38, 29], ratios of metabolite levels can provide hints to enzyme activities affected in the outcome of interest. If the ratio consists of an enzymatic product-substrate pair, it may proxy for the reaction rate of the appendant enzyme. Gieger et al. found the ratio [PC aa C36:4]/[PC aa C36:3], product/substrate pair of the delta-5 desaturation, to be a strong proxy for the fatty acid desaturase 1 (FADS1) activity [29]. Use of metabolite ratios reduces the variance and renders statistically robust results [38]. In my analysis, no such hints for altered enzyme activities could be found as only ratios of metabolites not directly linked by an enzyme in biochemical pathways were significantly associated with age (i. e., , no product-substrate pairs). It seems, that no pair out of 131 metabolites analyzed in KORA F_4 is significantly linked by an age-associated enzyme.

A promising tool for understanding relationship between metabolites associated with age is the genetic network analysis with help of the databases GenAge Database of Ageing-Related Genes (GenAge) [19] and Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) [39]. The resulting network contained only links not withstanding a deeper check of real association (i. e., many links were created only on simultaneous occurrence of these metabolites anywhere

in a publication, but no actual known association). The failure of this approach may be due to a lack of research results in aging concerning the metabolites from the metabolomics kit used in KORA as many of these were not included in databases at that time (in particular, the lipid species). Another explanation follows up the Hayflick definition of aging from section 1.1.2. Hayflick states that the genomes does not contain instructions for the organism to age [32] and thus, networking on the basis of genes cannot work for this study.

Table 8: Potential biomarkers for age in KORA F4 males. Mean concentration in μM from serum; beta estimates represent changes per year of age, adjusted for BMI; ⋆ below significance level

	KORA F4 Males		
marker	mean ± SD	beta (SE)	p-value
C10:1	0.15 ± 0.05	53.66 (6.12)	7.62E-18 ⋆
C12:1	0.14 ± 0.04	73.10 (6.97)	1.63E-24 ⋆
C16:1	0.04 ± 0.01	389.31 (35.98)	6.26E-26 ⋆
C18:1	0.12 ± 0.03	92.61 (10.15)	3.67E-19 ⋆
Gln	603.76 ± 89.22	-0.03 (0.00)	5.87E-13 ⋆
His	97.29 ± 13.72	-0.29 (0.03)	6.15E-26 ⋆
Trp	80.23 ± 8.87	-0.46 (0.04)	3.67E-33 ⋆
Val	247.58 ± 43.66	-0.09 (0.01)	8.48E-31 ⋆
lyso PC a C18:2	25.86 ± 8.33	-0.36 (0.04)	3.69E-18 ⋆
lyso PC a C20:3	2.22 ± 0.61	-5.07 (0.55)	1.36E-19 ⋆
PC aa C32:0	15.10 ± 3.00	0.71 (0.13)	8.03E-08 ⋆
PC ae C30:2	0.17 ± 0.04	-32.6 (11.44)	0.004
PC ae C36:1	8.96 ± 2.06	1.53 (0.24)	1.56E-10 ⋆
PC ae C42:0	0.52 ± 0.10	29.29 (4.05)	9.05E-13 ⋆
SM C20:2	0.43 ± 0.11	-7.97 (4.60)	0.084

NOTES

[1]'n' refers to the number of subjects given in the table refers to the n used for metabolomics analyses, not necessarily equals the whole study group.

[2]m... male, f... female

[3]d... days, w... weeks, mo... months, y... years

[4]Same metabolomics method does not necessarily mean that it was the same experimental procedures as there are lots of parameters that can vary. For the purpose here, it was simplified to NMR (which was in all listed studies ^{1}H NMR) and LC-MS (which often was combined with a second MS measurement and predominantly was HPLC-MS).

[5](*u*n-)*t*argeted; in brackets no. of compounds identified

4 DISCUSSION

4.1 GENERAL ASPECTS

In this thesis, I present strong associations between age and the human metabolome and identified 12 metabolites from a metabolomics screen of 1,038 healthy women with potential to serve as a set of age differentiating markers in women. These results from the KORA study could be replicated for 10 out of the 12 markers in 742 women participating in the TwinsUK study. Findings in males were less definite, but also led as well to the identification of an age-discriminating set of 15 metabolites[1]. I consider these results as helpful for understanding the metabolic alterations during aging in more detail.

Gender differences

The extent of sexual dimorphism regarding the metabolome has already been shown in a recent paper by Mittelstrass et al. [52]. Hence, analyzing the study group in a sex stratified manner was a corollary. The cause of less significance of results from the male stratus was unknowable, I think of lifestyle issues and different life experiences as prominent. Nonetheless, three metabolites showed up in both in females and males thus considered as potential age differentiating metabolites without gender influence (i. e., C12:1, His, and PC ae C36:1).

Evolutionary conservation of nutrient-sensing pathways

Thereupon, I sought for biological explanations via metabolic pathways, whose alteration might have led to the observed changes in metabolite concentrations and might additionally link the observations to common aging theories. However, greatest part of the cited results stems from animal models, which requires a further comment about comparability. Fontana et al. particularly emphasized the evolutionary conservation of anti-aging pathways in terms of composition and function across different species from yeast over mice to humans [27]. Notably, nutrient-sensing pathways seem to play important roles in aging in many species. Another publication describes three major age-regulating processes as evolutionary conserved between human and mice [24], i. e., the immune system, epigenetics, and aerobic metabolism, which also plays a major role in this discussion (see paragraph on ACs below).

As apparently diseased participants had been excluded[2], I assume that the study population has aged largely healthy so far and thus exhibits the regular mechanisms to combat aging issues like the occurrence of oxidative stress and impaired neuroendocrine-immune

1 Results from males are, again, not the main object of interest here and thus, not discussed in detail.

2 I further assume that heavily ill persons do not participate in a study at all.

functions (also termed as successful aging [60, 4]). Overall, I consider that the metabolites of the marker set tag for alterations in parts or their whole substance class with age.

In general, the concentration courses illustrated in smoother plots deviate from my expectation, insofar as I hadn't anticipated to see both almost strait lines and curves with clear inflection points. One might argue that the sudden changes are related to hormonal changes in women, but several male metabolites also showed inflections (see appendix figure 9 on page 50). Reasons thus might derive from the cross-sectional design or any factors not measured. Heat maps also didn't show the expected hints to groups of metabolites that change conversely and might have indicated links to affected enzymes or pathways[3]. Instead, clusters in the heat map were a smorgasbord of metabolite classes (shown as gray boxes in figure 4 on page 27 and in appendix figure 7 on page 48). This might reflect the complexity of aging with pathways not affected in isolation but in a greater system.

Following up the state of knowledge on aging metabolism as summarized in section 1.1.3, my results clearly support the observations on altered lipids as I found different lipid classes to be affected: ACs, PCs, and SMs. Particularly the ACs are thought to reflect changes in ffas as seen in [80] and provide a link to the aging theory of mitochondrial dysfunction (discussed in more detail below). However, a comparison is difficult as my analysis is based on a much deeper look on distinct lipid species than the cited studies, which offers a much more detailed interpretation. In the following paragraphs, I discuss the metabolites on their substance class level with regard to aging mechanisms.

4.2 METABOLITE CLASSES

phosphatidylcholines (PCs)

Phospholipids are major components of cell membranes. It is known that enzyme activities of the deacetylation-acetylation cycle in the glycerophospholipid metabolism change with age. This is underlined by the findings of DeGuzman et al. of decreased levels of lyso PCs 16:1 and 18:4 in aged mice [18]. The cycle helps adjusting and regulating membrane fluidity via the fatty acid composition of phospholipids in cell membranes. A high unsaturation degree of membrane phospholipids of old rats has been observed. Highly unsaturated fas are especially prone to lipid peroxidation, which commonly occurs with higher age. Products contribute to low-grade inflammation. Interestingly, long-lived animals indeed show low degrees of unsaturated membrane fas compared to short-lived ones. Besides deacetylation-acetylation cycle enzymes, desaturase from fa desaturation pathways is also considered to contribute to the described observations. It is

3 It is furthermore conceivable that the corresponding counterparts were not identified by the metabolomics kit.

hard to say whether my marker set reflects these changes in glycerophospholipid metabolism. Possibly the different saturation degrees of the FA side chains of the PCs, which ranges from one to five double bonds, reflects the process described.

Changes in the cellular membrane that include a G protein-coupled receptor seem to coincidence with aging, which at least affects G protein activity, cell morphology and cell homeostasis [54]. A common feature of cellular senescence is an increased cell surface. Naru et al. could observe in cell experiments that senescent cells revealed higher uptake of PC species with long chain FA residues [54]. Alongside, the number of special lipid rafts named calveolae increased with senescence. Presumably, PC species were integrated into calveolae, which contain calveolin-1 as a crucial component capable of cell cycle suppression at G0/G1 phase[4].

I found several species of PCs with decreased concentrations with higher age and one PC species with elevated level. Thus, I consider an altered consumption of phospholipids due to specific membranaceous demands[5] associated with senescence. The underlying mechanisms might work as described by Engelmann and Wiedmann by the scavenger receptor SR-BI as major mediator of selective phospholipids (PC, SM and phosphatidylethanolamine (PE)) uptake from particles like HDL and LDL [22]. Phospholipids can induce fast and dynamic changes in the cell membrane composition after incorporation upon demands of distinct cell functions. Three of the four replicated PCs from the marker set showed a decrease starting around age of 51. This leads to the assumption that these metabolites might be less associated with age but rather with menopause, which is also known to influence the metabolism by hormonal changes. Unfortunately, information about menopausal status of the female subjects was not available at the time of analysis. All other metabolites of the marker set changed in an approximately linear manner implying the absence of menopausal influences.

sphingomyelins (SMs)

Further important components of cell membranes are sphingomyelins (SMs), especially of neuronal cells, as they influence membrane fluidity by their degree of unsaturation and can act as part of signal transduction. Both Ichi et al. and Corre et al. reported the conjunction between oxidative stress and SM metabolism, but in their studies, oxidative stress accelerated the degradation of SMs to ceramide (cer), what would be inconsistent with elevated SM levels in the elder subjects in my thesis [37, 16]. However, another study identified cells that had adapted to chronic oxidative stress with altered SM metabolism and major changes in membrane composition leading to stabilization [13]. The SM level elevations I observed might

4 At least senescent cells don't divide anymore [11].

5 A double bond leads to a bend in molecular 3D-structure. A higher number of double bonds thus makes the molecule bulkier than more saturated species and eventually results in higher membrane fluidity.

indicate that healthily aging humans have effective mechanisms to protect cells from oxidative stress accompanied by changes in SM metabolism and incorporation of SMs into cell membranes, although this deduction from cell culture should be considered with extra caution. Noteworthy results were described in [77]. Sphingolipid species can have completely opposite effects: some mediate anti-apoptotic effects and some are involved in apoptosis signaling. Additionally, it was observed that cer (SM derivative without the phosphocholine head group) accumulates under many forms of stress[6]. The observations about apoptotic/anti-apoptotic properties of sphingolipids and cer accumulation might also play a role in the SM elevations with higher age observed in KORA[7]. The fact that SM synthesis is situated in the mitochondria [77] could provide a possible link to ACs as their changes are distinctly connected to mitochondrial processes (discussed in the AC paragraph below). In this context the proposed link between SM cycle and Kennedy pathway brings my results in relation [9]. According to the Bieberich paper, SMs and PCs build a cycle together with cer and PE ('CDP-choline/SM cycle'). The paper describes the cycle as a link between major metabolic pathways and cell-signaling function. Hence, changes in PC and SM levels[8] with older age might also be coherent.

acylcarnitines (ACs)

Elevated serum levels of ACs may have different underlying reasons. AC species are found as a consequence of incompletely oxidized fatty acids due to an exceedance of β oxidation capacity and related pathways (i. e., higher rate of substrate use than energy demand with accumulated acyl-coenzyme A (CoA) that are converted to ACs that exit cells and tissues), but also as a consequence of oxidative stress [57]. Acylcarnitines in turn show up in the blood and can thus function as a mirror of fas metabolism. A recent rodent study by Noland et al. gave rise to an age-associated interpretation of the increased AC levels in blood as a health-beneficial emergence [57]. The studied Wistar rats under high fat diet exhibited diminished carnitine and increased AC levels in skeletal muscle cells upon perturbations in mitochondrial fuel utilization, e. g., incomplete fatty acid oxidation. Supplemented carnitine led to AC efflux, which in turn showed up in blood accompanied by meliorated fuel metabolism and improved glucose tolerance. Thus, the carnitine shuttle system is considered as prominent factor for maintenance of mitochondrial performance and glucose homeostasis. In the context of my thesis, the observed higher levels of AC with advanced age might indicate a relation of aging processes with counteraction against oxidative damage in the mitochondria via action of the carnitine-acylcarnitine shuttle.

6 cer is a pro-apoptotic second messenger with complex metabolism and further potent relatives like cer-1-phosphate have impact on cell growth, differentiation, and inflammation [12].

7 At least oncogene-induced cellular senescence results in an apoptosis resistance [11].

8 and presumably AC levels as well

ACs'capability to reflect the intracellular fa metabolism needs to be seized. Many studies support the key role of lipid metabolism in aging with lipid metabolites serving important functions therein [93]. Yan et al. support the evidence of decreased capacity in fa metabolism in aged mammals by their study on rats. They found fas to be the most important metabolites of aging (60% of all age-related metabolites) with 8 unsaturated fas decreased (linoleic acid C18:2, oleic acid C18:1, palmitoleic acid C16:1, arachidonic acid C20:4, linolenic acid C18:3, docosahexaenoic acid C22:6, eicosapentaenoic acid C20:5, and eicosatrienonic acid C20:3 – consistent to my results) and 4 saturated fas increased (palmitic acid C16:0, stearic acid C18:0, myristic acid C14:0, and nonacosanoic acid C29:0). The decrease of polyunsaturated fas might be the result of an age-associated increase in oxidation by free radicals.

The consequences of altered mitochondrial function in aging on the energy metabolism was demonstrated in another publication. Coquin et al. [15] refute the often made statement aging was a general functional decline by showing that certain metabolic pathways were concerned but others not. They challenged aged flies with hypoxia and investigated their ability to recover. Pathways detected thereby were downstream of TCA cycle and respiration (aerobic) but not in anaerobic glycolysis. This fits to the common perception that mitochondrial function decline contributes substantially to overall function decline both in Drosophila and mammals. Activity of certain enzymes in the electron transport chain decreases with age (enzyme complexes I, II, and IV) and other experiments revealed a strong down-regulation of TCA cycle and respiratory enzymes with age with resulting higher utilization of glucose and glycogen for ATP production[9]. Lawton and colleagues also saw elevations in all but one measured metabolites from energy metabolism [46].

histidine

Considering the metabolic fate of the amino acid histidine, there are two known contiguous possibilities. Its descent in blood with higher age could indicate an advanced demand in tissues. The first histidine consuming pathway is its metabolisation to the biogenic amine histamine by a decarboxylation step. Histamine is known to be involved in local immune responses and can act as neurotransmitter. However, to my knowledge an association between histidine or histamine and immunosenescence has not been reported so far. The second metabolic pathway that consumes histidine produces carnosine. This dipeptide from β-alanine and L-histidine is found in virtually all tissues, particularly in skeletal muscle cells and different brain cells [20]. Due to its antioxidant characteristics, carnosine is considered to be a naturally occuring anti-aging substance capable of suppressing oxidative damage, glycation of proteins, and scavenging

9 As all other catabolic pathways are less efficiently producing ATP, glucose and glycogen consumption is necessarily elevated

toxic molecules [34] that are all known to occur with aging process. For instance, carnosine was shown to accomplish capture of lipoxidation products and prevention of protein cross-linking [97]. Assuming lower levels of histidine due to its consumption by carnosine biosynthesis with advancing age, the observation of decreased histidine in this thesis might reflect successful counteracting of the important aging implication of oxidative stress and its consequences and thus a healthful aging process. However, Yan et al. report that antioxidants carnosine and ergothioneine decrease with higher age and would thus lead to a weakened antioxidant defense system [93]. Contrary to the decrease in serum histidine concentrations observed here, Lawton et al. found elevated levels of both histidine and histamine in urine. These results together could also point to an altered renal excretion of histidine [46].

ornithine

An obvious link from ornithine to aging would be alterations in the urea cycle, but there seems to be no such reports in the literature. However, Durante et al. published interesting results on arginase enzyme, which metabolizes arginine to ornithine, linked to nitric oxide (NO) synthesis and vascular function [21]. The known age-dependent up-regulation of arginase could produce the elevated ornithine levels observed in my study (and in the Lawton study in urine [46]). Also, the downstream enzymes that catabolize ornithine have been associated with aging features. Polyamines are the products of ornithine decarboxylase (ODC) and were associated with endothelial growth and subsequent vascular lesions. Vascular lesions and endothelial dysfunction are considered as central features of many age-associated vascular disorders. Ames dwarf mice show a prominent delay in onset of aging and were studied by Bates et al. [6]. The authors reported a post-transcriptional control of the ODC gene by microRNA (miRNA) that led to suppressed ODC activity and subsequent lower polyamine synthesis in Ames dwarf compared to wild type mice. An up-regulation of ODC activity and elevated ornithine levels have been detected in normally aging rats [85]. Furthermore, a role for miRNA in the aging process of humans has been unraveled recently [58]. I surmise from these and my findings that a factor of healthy aging in humans could arise from the arginase-ODC metabolic pathway, and that a knockdown of ODC gene product with subsequently decreased production of potentially harmful polyamines could possibly explain the effects. Besides ODC, ornithine aminotransferase (OAT) catalyzes the degradation of ornithine to the amino acid proline, which is an essential component of collagen. Excessive collagen deposition in endothelial extracellular matrix is also suggested to contribute to vascular lesions. Up-regulated OAT gene has been associated with aging in a mouse model of accelerated senescence (SAMP8 mice)[48]. Healthy aging thus could involve mechanisms for down-regulation of OAT gene or enzyme activity. Significantly diminished proline levels

in our elder subjects support the assumption of OAT down-regulation (females $\leqslant$51 versus >51 years, p-value 9.7E-10).
These pathways originating from arginase with the consequence of smooth muscle cell proliferation via increased polyamine levels could be further intensified by the arginase mediated NO suppression, which arises from competition between arginase and NO synthase for arginine [21]. The lack of NO action would further exacerbate endothelial damage.

4.3 SUMMARY

Aging is understood as a continuous and dynamic remodeling process of the human organism accompanied by numerous losses and gains on different levels including intermediate metabolism and cell function [4]. My results of a large number of age-associated metabolites and the replicated set of ten age discriminating metabolites, that showed both increase and decrease with age, might reflect these processes and help getting a more integral picture of aging organism. Overall, I observed age-dependent differences in PC, SM, AC and AA levels that might link to altered cell membrane composition, mitochondrial metabolism, and counteraction to oxidative stress, respectively. Mitochondrial dysfunction probably plays the most important role as I found hints in the literature to a large proportion of the marker set (10 of 12 ACs/SMs/PCs in KORA or 9 of 10 in the replication/meta-analysis). Having in mind that anti-aging mechanisms was proposed to be successful and effective in centenarians [4], I audaciously speculate that the observed alterations in the healthy older study subjects indicate organismal reactions to deleterious modifications.
In the introduction I cited an article that raised two questions on aging that metabolomics may answer [45]. After presenting and discussing my results, the answers from my point of view are that aging has probably both qualitative (i. e., metabolites that show altered but constant levels after a certain age, e. g., PC ae C42:4) and quantitative effects (i. e., metabolites that show constantly changing levels with age, e. g., SM C16:1). Whether the identified metabolites may serve as biomarkers is probably not to be answered at this stage, because identification of biomarkers needs several stages of proof. This study might serve as a starting point to the whole proof procedure.

Figure 6 attempts on bringing the discussed age-associated effects in my metabolite set into context to each other and should bring the results into a systems biology view. It is thus based on my findings and on the described results from the literature cited in the discussion.

In summary, I identified a set of twelve potentially differentiating markers for age in females by comparison of two age groups of the

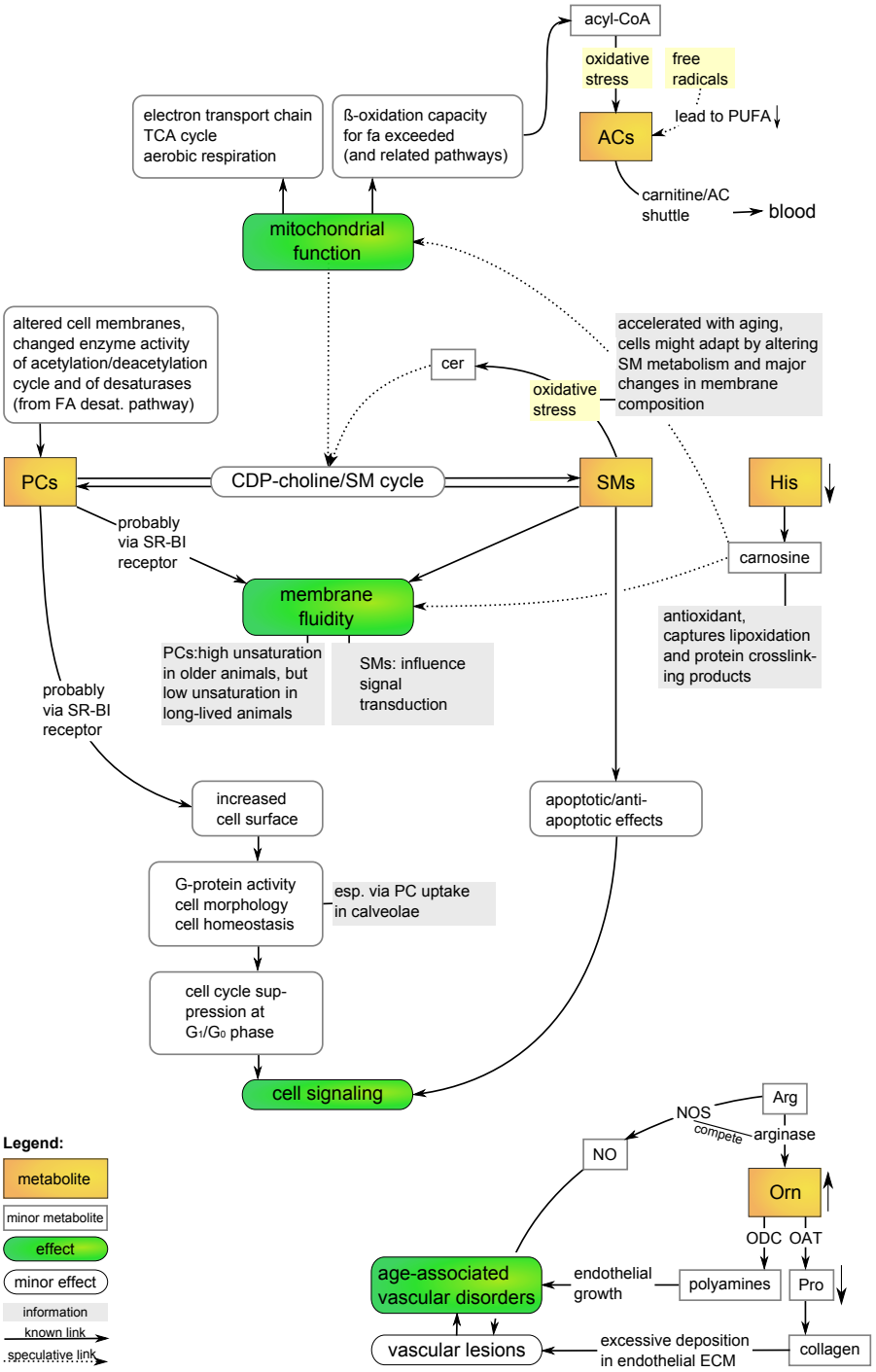

Figure 6: **Depicting the results in a systemic view.**
As metabolomics aims at an integral and systemic depiction of the outcome, this figure attempts on summarizing my results and bring it in a common context.

KORA F_4 study with respect to their metabolic profile. Ten out of the twelve markers were successfully replicated in the TwinsUK study. Literature gives indications that these markers might be linked to aging processes as the action of oxidative stress, alterations in cell morphology, beta oxidation capacity, and vascular function. Thus, I think that the set will help to better understand aging pathways and their systemic concurrence and eventually help to enhance the understanding of healthy aging and longevity.

4.4 STRENGTHS AND LIMITATIONS OF THE STUDY

Although the present study was large in terms of sample size and phenotypically and metabolically well characterized, it should be noted that this was a cross-sectional study, that does not allow irrevocable conclusions of cause and consequence. Additionally, the presence of cohort effects should be taken into consideration. The year of birth might be linked to variations in risk of the outcome of interest, in particular health outcomes, due to shifts in population exposure to risk factors over time [43]. However, I consider the cohort effect as a potential factor that possibly modified my results, but did not entirely generate the associations between metabolites and age. Issues with comparison to other metabolomics studies were noticeable, because different analytical settings result in different sets of metabolites. Also, the biological specimen, study size, and proband inclusion criteria deviate largely and hence hamper comparability and placement in the current knowledge. Another critical point again arises from the cross-sectional design. I cannot clearly say why a certain concentration changes with higher age as no conclusion about the flux of the certain metabolites is possible. Whether the concentration is e. g., elevated because of higher demands with greater supply to tissues or because of less uptake and consumption of the tissue and thus an accumulation in the blood is not clear for the majority of metabolites. The Biocrates kit bears the challenge that the functions of many of the identified molecules are not well known so far. Therefore, their localization in the cell might be unknown and particularly the reasons why these occur in blood are unclear. For instance, SMs are synthesized in the cell and then incorporated in membranes, but no known scenario of secretion into the blood is described yet. Altogether, this exacerbates interpretation of the results. However, knowledge bases like HMDB [36] are growing quickly and provide ameliorating possibilities.

To cope with the complexity of aging process on the level of small molecules, metabolomics technology was used capable of the detection of a large number of molecules simultaneously. I consider small molecules as very suitable age markers as they are easily obtained from blood samples and analyzed in a relatively inexpensive and

quick manner. The kit has been successfully used in several previous studies (e. g., in [38, 94]). However, taking together complex results to an integrative picture requires good bioinformatics tools and patient literature search.

5 PERSPECTIVES

Further research on the metabolic surrounding of the metabolite set could eventually lead to the possibility to early determine a person's potential for healthy aging at the beginning of the remodeling process. However, it seems not strongly confirmed yet that effects of cellular senescence also occur in vivo [54]. Nevertheless, I suspect the set of twelve markers to have the potential to reflect different age-associated metabolic alterations of females at once. To ensure the absence of cohort effects and other disadvantages of the cross-sectional study design, I plan to analyze metabolomics results from a subset of KORA S_4, the previous examination to KORA F_4. Thereby, I will be able to analyze the concentration course of these metabolites from two time points with seven years in between and get results of real ag*ing*. Additionally, menopause status of the KORA F_4 women is available meanwhile and will eventually clarify whether the inflection point around age 51 is due to hormonal changes. An already ongoing attempt regards epigenetic changes, i. e., cytosine methylation differences, with age in KORA F_4.

It might be very promising (but also challenging) to combine metabolomics measurements from different platforms with other -omics data, analyze them with the continuously newly launched bioinformatics tools and interpret the results with constantly growing knowledge on human metabolism.

Interesting insights are expected from a closer look onto the males and the reason why they differ distinctly from women in their metabolic changes. However, as Berdasco and colleagues pointed out, the greatest barriers in conducting studies on age are the long time periods, that would be necessary to really examine the aging process in humans, and myriad of environmental influences [7].

APPENDIX

A.1 FIGURE APPENDIX

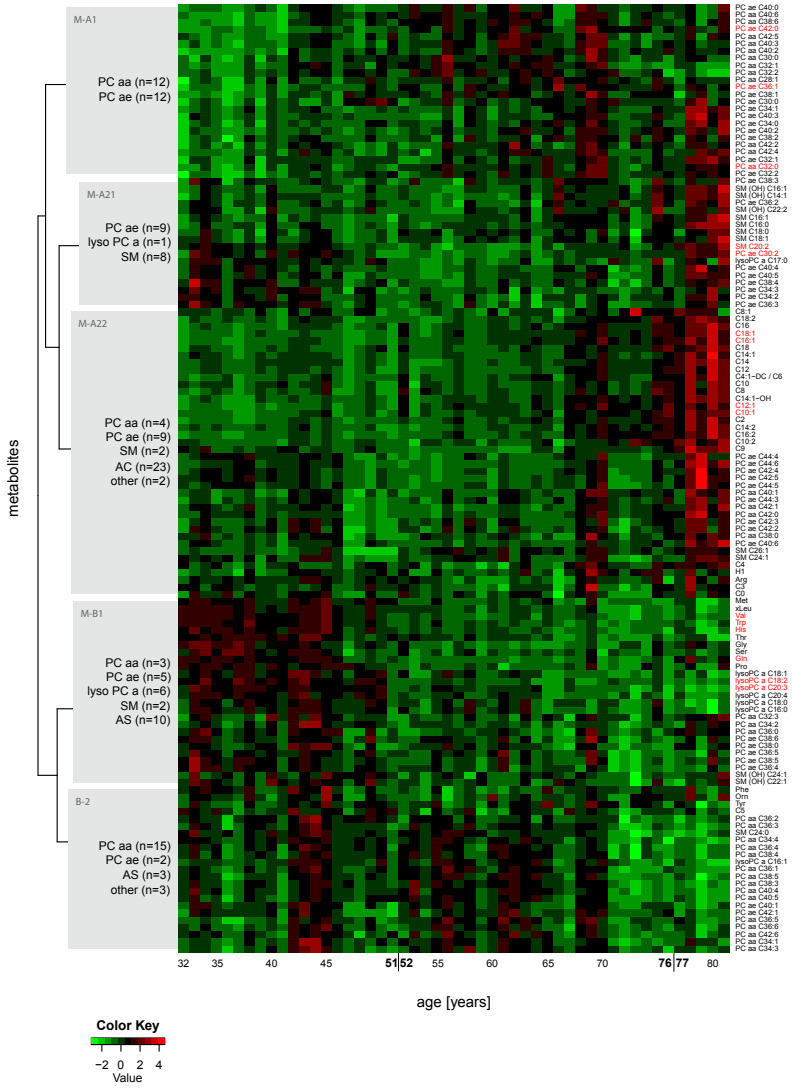

Figure 7: **Heat map of KORA F4 males.**The heat map shows changes of fold standard deviation from the overall mean concentration for each year in a color-coded way, and clustering of these changes. Green squares represent a decrease, and red squares an increase. Gray boxes represent groups of metabolites with similar changes with number of metabolites in parentheses. Metabolite names in red indicate the set of 12 metabolites (see 3.2.2). amino acid (AA); acylcarnitine (AC); diacyl phosphatidylcholine (PC aa); acyl-alkyl phosphatidylcholine (PC ae); and lyso phosphatidylcholine acyl (lyso PC a).

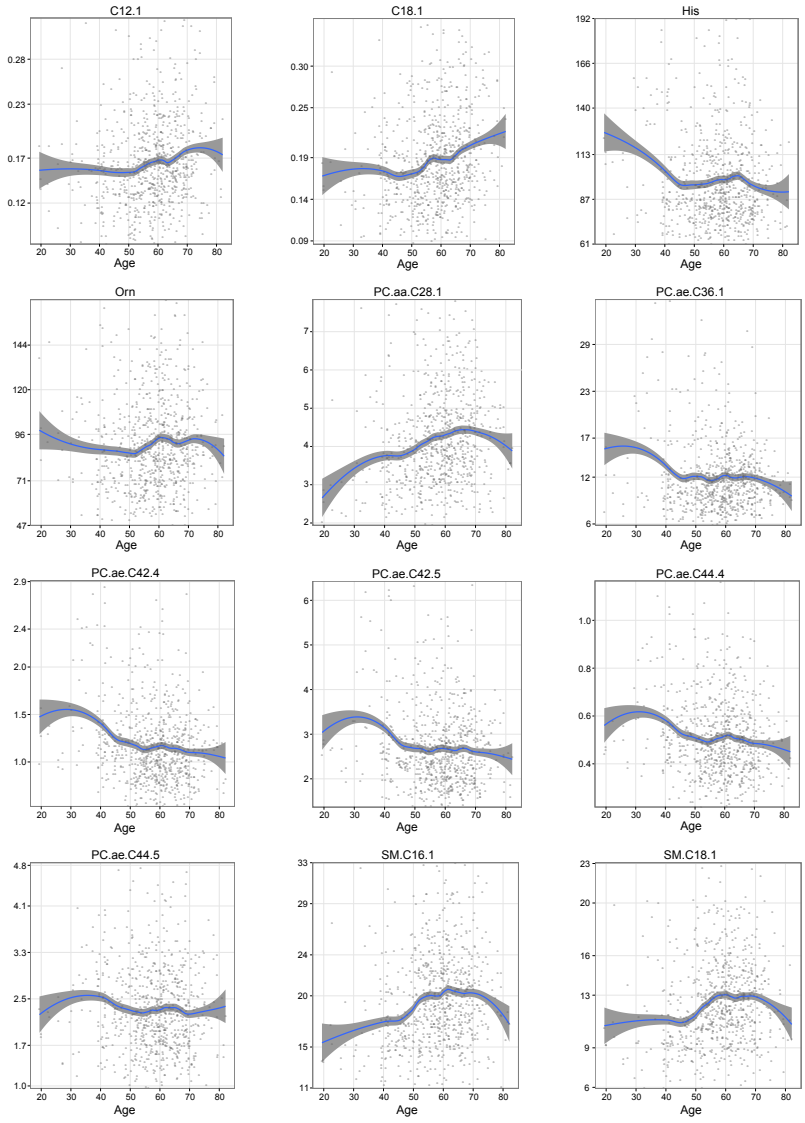

Figure 8: **Smoother plots for TwinsUK females (replication).** Concentration courses of the 12 metabolites in TwinsUK females were largely comparable to those from the KORA women.

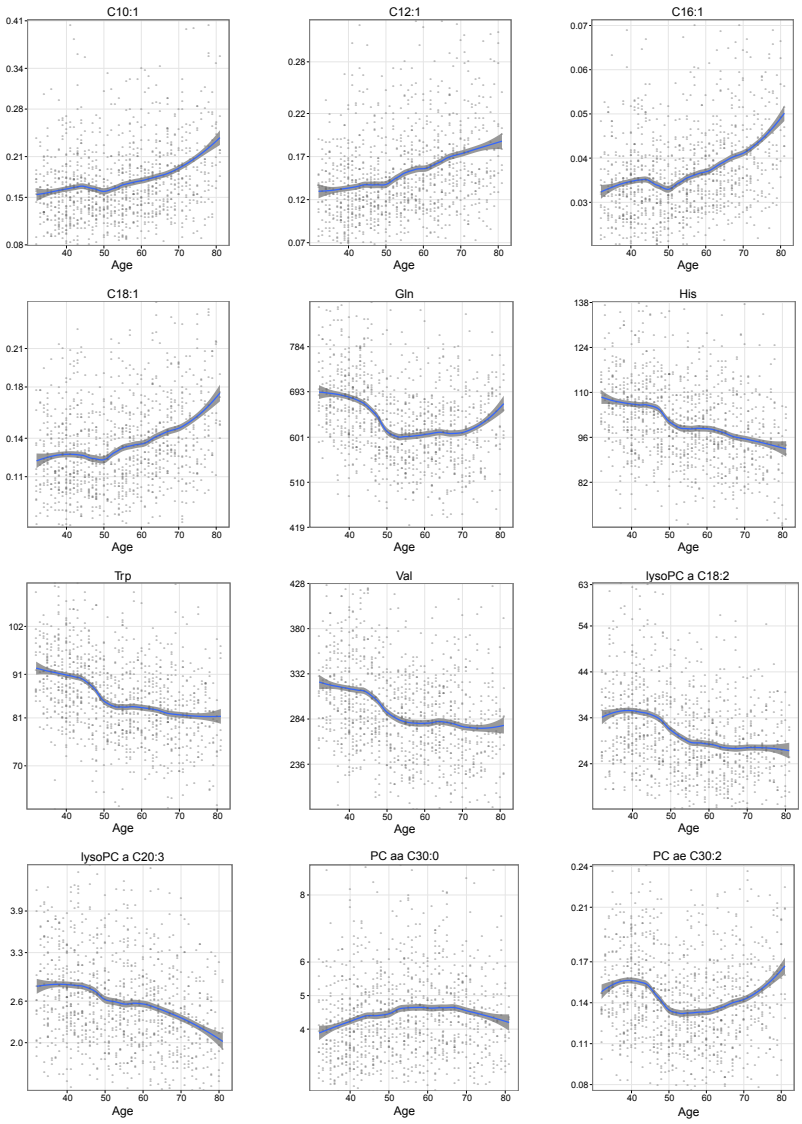

Figure 9: Smoother plots for KORA F4 males – concentration courses of the 15 metabolites for KORA F4 males

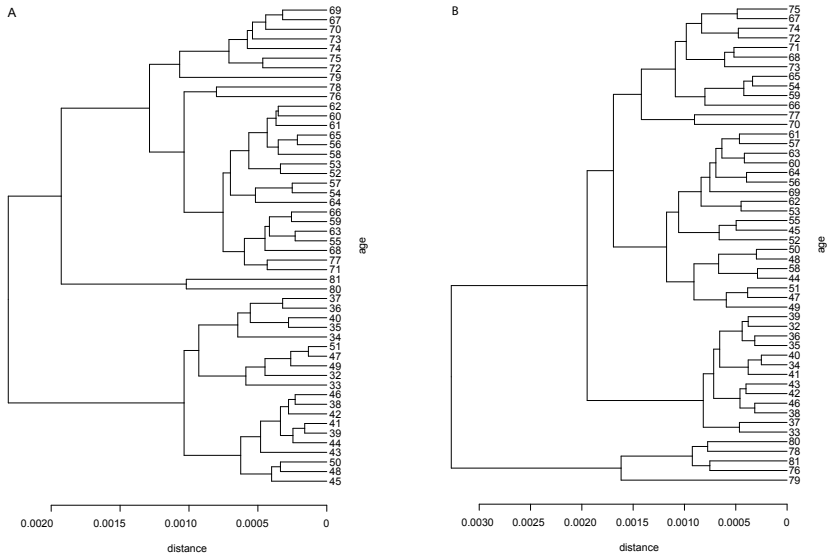

Figure 10: Cluster plots for age for KORA F4 females (A) and males (B)

A.2 TABLE APPENDIX

Table 9: Overview of all metabolites measured with Biocrates Absolute***IDQ***™p150 kit

Abbreviation	Biochemical name	coeff.	above LOD (%)	Mean (µM)	CV (%)	Appl.	mean ± SD (µM)	min – max conc.	mean ± SD (µM)	min – max conc.
C0	Carnitine	0.88	100	35.70	6.69	✓	33.03 ± 6.83	6.71 – 65.25	38.18 ± 7.38	16.08 – 67.54
C2	Acetylcarnitine	0.94	100	8.22	9.37	✓	7.81 ± 2.41	2.11 – 20.45	8.04 ± 2.78	2.66 – 20.51
C3	Propionylcarnitine	0.86	100	0.40	8.01	✓	0.35 ± 0.10	0.08 – 1.06	0.43 ± 0.12	0.17 – 1.56
C3-OH	Hydroxypropionylcarnitine	0.05	0.36	0.03	76.64					
C3:1	Propenonylcarnitine	−0.11	0.10	0.01	37.53					
C4	Butyrylcarnitine	0.89	100	0.23	8.82	✓	0.21 ± 0.09	0.05 – 0.85	0.24 ± 0.12	0.08 – 1.60
C4-OH (C3-DC)	Hydroxybutyrylcarnitine	0.47	8.40	0.10	35.49					
C4:1	Butenylcarnitine	0.04	5.65	0.02	34.69					
C5	Valerylcarnitine	0.81	95.56	0.12	14.16	✓	0.10 ± 0.03	0.04 – 0.26	0.13 ± 0.04	0.05 – 0.53
C5-DC (C6-OH)	Glutarylcarnitine (Hydroxyhexanoylcarnitine)	0.15	27.06	0.04	20.99					
C5-M-DC	Methylglutarylcarnitine	0.18	0.95	0.03	42.85					
C5-OH (C3-DC-M)	Hydroxyvalerylcarnitine (Methylmalonylcarnitine)	0.25	55.10	0.03	28.74					
C5:1	Tiglylcarnitine	0.37	0.75	0.03	26.13					
C5:1-DC	Glutaconylcarnitine	0.13	12.48	0.02	42.38					
C6 (C4:1-DC)	Hexanoylcarnitine (Fumarylcarnitine)	0.85	76.67	0.07	13.59	✓	0.07 ± 0.02	0.02 – 0.18	0.07 ± 0.03	0.03 – 0.31
C6:1	Hexenoylcarnitine	0.07	0.33	0.02	32.36					
C7-DC	Pimelylcarnitine	0.79	61.34	0.05	34.36					
C8	Octanoylcarnitine	0.89	51.54	0.22	16.33	✓	0.20 ± 0.07	0.07 – 0.57	0.23 ± 0.11	0.08 – 1.51
C8:1	Octenoylcarnitine	0.92	96.01	0.09	8.35	✓	0.08 ± 0.04	0.02 – 0.39	0.09 ± 0.05	0.02 – 0.38

Table 9: Overview of all metabolites (cont.)

Abbreviation	Biochemical name	coeff.	above LOD (%)	Mean (µM)	CV (%)	Appl.	mean ± SD (µM)	min – max conc.	mean ± SD (µM)	min – max conc.
C9	Nonaylcarnitine	0.84	83.73	0.05	20.83	✓	0.05 ± 0.02	0.01 – 0.18	0.05 ± 0.03	0.01 – 0.23
C10	Decanoylcarnitine	0.93	94.08	0.36	11.44	✓	0.33 ± 0.12	0.10 – 0.86	0.38 ± 0.20	0.11 – 2.27
C10:1	Decenoylcarnitine	0.83	48.66	0.17	10.39	✓	0.15 ± 0.05	0.05 – 0.37	0.17 ± 0.06	0.05 – 0.59
C10:2	Decadienylcarnitine	0.51	50.49	0.04	14.55	✓	0.04 ± 0.01	0.02 – 0.16	0.04 ± 0.01	0.02 – 0.12
C12	Dodecanoylcarnitine	0.86	87.35	0.13	10.35	✓	0.12 ± 0.04	0.05 – 0.31	0.14 ± 0.05	0.04 – 0.42
C12-DC	Dodecanedioylcarnitine	0.05	0.00	0.06	12.19					
C12:1	Dodecenoylcarnitine	0.73	13.69	0.15	12.99	✓	0.14 ± 0.04	0.05 – 0.35	0.15 ± 0.05	0.05 – 0.47
C14	Tetradecanoylcarnitine	0.54	51.67	0.05	12.63	✓	0.04 ± 0.01	0.02 – 0.09	0.05 ± 0.01	0.02 – 0.12
C14:1	Tetradecenoylcarnitine	0.81	100	0.15	16.92	✓	0.14 ± 0.03	0.04 – 0.31	0.15 ± 0.04	0.05 – 0.37
C14:1-OH	Hydroxytetradecenoylcarnitine	0.70	67.35	0.01	16.44	✓	0.01 ± 0.00	0.01 – 0.03	0.02 ± 0.00	0.00 – 0.04
C14:2	Tetradecadienylcarnitine	0.87	98.82	0.03	11.59	✓	0.03 ± 0.01	0.01 – 0.08	0.03 ± 0.01	0.01 – 0.10
C14:2-OH	Hydroxytetradecadienylcarnitine	0.27	38.04	0.01	17.45					
C16	Hexadecanoylcarnitine	0.84	100	0.12	8.89	✓	0.11 ± 0.02	0.03 – 0.22	0.12 ± 0.03	0.05 – 0.40
C16-OH	Hydroxyhexadecanoylcarnitine	0.20	3.33	0.01	24.05					
C16:1	Hexadecenoylcarnitine	0.71	2.78	0.04	10.17	✓	0.04 ± 0.01	0.02 – 0.12	0.04 ± 0.01	0.02 – 0.09
C16:1-OH	Hydroxyhexadecenoylcarnitine	0.38	2.25	0.01	17.49					
C16:2	Hexadecadienylcarnitine	0.57	70.69	0.01	19.37	✓	0.01 ± 0.00	0.00 – 0.02	0.01 ± 0.00	0.00 – 0.02
C16:2-OH	Hydroxyhexadecadienylcarnitine	0.32	4.67	0.01	16.57					
C18	Octadecanoylcarnitine	0.69	99.80	0.05	13.66	✓	0.05 ± 0.01	0.01 – 0.10	0.05 ± 0.01	0.02 – 0.17
C18:1	Octadecenoylcarnitine	0.87	98.33	0.13	10.19	✓	0.12 ± 0.03	0.03 – 0.35	0.13 ± 0.04	0.05 – 0.49
C18:1-OH	Hydroxyoctadecenoylcarnitine	0.06	0.95	0.01	33.44					

Table 9: Overview of all metabolites (cont.)

Abbreviation	Biochemical name	coeff.	above LOD (%)	Mean (µM)	CV (%)	Appl.	mean ± SD (µM)	min – max conc.	mean ± SD (µM)	min – max conc.
C18:2	Octadecadienylcarnitine	0.81	100	0.05	9.35	✓	0.04 ± 0.01	0.01 – 0.08	0.05 ± 0.01	0.02 – 0.12
Arg	Arginine	0.59	100	116.02	8.19	✓	114.62 ± 18.68	24.20 – 176.74	116.90 ± 19.23	16.70 – 187.64
Gln	Glutamine	0.62	100	621.15	9.88	✓	603.76 ± 89.22	153.35 – 926.08	636.48 ± 94.87	119.78 – 1152.15
Gly	Glycine	0.89	100	307.65	7.92	✓	338.26 ± 93.83	82.66 – 775.64	289.70 ± 59.72	66 – 702.11
His	Histidine	0.69	100	98.13	8.26	✓	97.29 ± 13.72	19.99 – 148.95	100.83 ± 14.18	31.07 – 156.26
Met	Methionine	0.53	100	32.21	9.72	✓	29.90 ± 5.15	5.38 – 63.86	33.34 ± 5.63	11.39 – 54.34
Orn	Ornithine	0.75	100	81.89	9.39	✓	77.26 ± 18.36	12.34 – 161.36	83.85 ± 18.07	8.48 – 199.75
Phe	Phenylalanine	0.62	100	62.27	8.42	✓	58.99 ± 9.17	9.96 – 126.54	63.38 ± 10.09	23.23 – 115.8
Pro	Proline	0.89	100	176.44	7.37	✓	155.88 ± 48.32	22.58 – 442.27	190.99 ± 50.38	78.04 – 439.81
Ser	Serine	0.62	100	128.34	9.56	✓	133.06 ± 25.78	34.25 – 227.16	125.55 ± 22.24	25.22 – 219.82
Thr	Threonine	0.71	100	105.94	12.14	✓	105.74 ± 26.18	17.19 – 215.57	107.92 ± 22.51	21.26 – 262.94
Trp	Tryptophan	0.51	100	82.66	7.49	✓	80.23 ± 8.87	15.78 – 114.25	85.76 ± 10.23	33.73 – 126.23
Tyr	Tyrosine	0.66	100	85.55	8.63	✓	79.93 ± 16.7	18.33 – 187.63	87.41 ± 16.77	26.48 – 182.99
Val	Valine	0.69	100	278.40	19.64	✓	247.58 ± 43.66	52.29 – 456.76	293.31 ± 51.56	78.47 – 536.11
xLeu	Leucine/Isoleucine	0.74	100	214.25	8.17	✓	186.94 ± 31.88	40.99 – 340.62	234.26 ± 41.15	63.06 – 451.22
PC aa C24:0	Phosphatidylcholine diacyl C24:0	0.11	72.55	0.15	26.47					
PC aa C26:0	Phosphatidylcholine diacyl C26:0	0.09	11.54	1.05	32.89					
PC aa C30:0	Phosphatidylcholine diacyl C30:0	0.89	100	4.72	7.82	✓	4.94 ± 1.48	1.16 – 11.59	4.43 ± 1.38	1.72 – 11.93
PC aa C30:2	Phosphatidylcholine diacyl C30:2	0.12	4.22	0.00	81.55					
PC aa C32:0	Phosphatidylcholine diacyl C32:0	0.83	100	15.14	7.13	✓	15.10 ± 3.00	3.32 – 46.45	14.72 ± 3.04	7.19 – 27.84
PC aa C32:1	Phosphatidylcholine diacyl C32:1	0.96	100	21.84	7.37	✓	21.76 ± 9.91	3.90 – 79.69	20.47 ± 11.71	4.31 – 103.9

Table 9: Overview of all metabolites (cont.)

Abbreviation	Biochemical name	coeff.	above LOD (%)	Mean (µM)	CV (%)	Appl.	mean ± SD (µM)	min – max conc.	mean ± SD (µM)	min – max conc.
PC aa C32:2	Phosphatidylcholine diacyl C32:2	0.91	99.93	3.89	11.14	✓	4.21 ± 1.60	0.40 – 10.21	3.53 ± 1.44	0.22 – 12.05
PC aa C32:3	Phosphatidylcholine diacyl C32:3	0.79	100	0.48	8.94	✓	0.52 ± 0.12	0.14 – 0.92	0.44 ± 0.09	0.18 – 0.83
PC aa C34:1	Phosphatidylcholine diacyl C34:1	0.83	100	240.27	7.19	✓	238.52 ± 51.07	51.41 – 542.05	237.49 ± 55.58	105.69 – 571.95
PC aa C34:2	Phosphatidylcholine diacyl C34:2	0.75	100	391.39	7.74	✓	393.26 ± 59.69	111.82 – 685.45	381.85 ± 64.88	184.11 – 697.17
PC aa C34:3	Phosphatidylcholine diacyl C34:3	0.91	100	17.89	8.6	✓	19.18 ± 5.09	4.02 – 45.08	16.69 ± 4.89	6.93 – 51.42
PC aa C34:4	Phosphatidylcholine diacyl C34:4	0.92	100	2.26	7.97	✓	2.37 ± 0.83	0.61 – 6.49	2.13 ± 0.73	0.54 – 5.58
PC aa C36:0	Phosphatidylcholine diacyl C36:0	0.74	100	2.69	17.35	✓	2.73 ± 0.75	0.81 – 6.30	2.67 ± 0.77	0.98 – 6.19
PC aa C36:1	Phosphatidylcholine diacyl C36:1	0.84	100	53.83	8.48	✓	53.98 ± 12.22	9.48 – 115.82	52.69 ± 13.64	21.31 – 166.06
PC aa C36:2	Phosphatidylcholine diacyl C36:2	0.80	100	231.93	6.73	✓	236.47 ± 39.51	55.14 – 406.19	226.50 ± 42.86	98.84 – 492.07
PC aa C36:3	Phosphatidylcholine diacyl C36:3	0.86	100	149.60	7.51	✓	152.43 ± 29.67	32.68 – 288.10	144.83 ± 29.90	68.19 – 284.26
PC aa C36:4	Phosphatidylcholine diacyl C36:4	0.87	100	219.58	7.76	✓	216.96 ± 48.45	61.12 – 431.79	216.32 ± 51.28	85.16 – 570.96
PC aa C36:5	Phosphatidylcholine diacyl C36:5	0.82	100	29.30	8.64	✓	28.55 ± 12.89	6.46 – 125.23	29.23 ± 14.04	6.38 – 137.73
PC aa C36:6	Phosphatidylcholine diacyl C36:6	0.89	100	1.12	11.06	✓	1.18 ± 0.43	0.25 – 4.61	1.06 ± 0.41	0.24 – 3.09
PC aa C38:0	Phosphatidylcholine diacyl C38:0	0.86	100	3.26	13.79	✓	3.38 ± 0.89	0.79 – 8.06	3.20 ± 0.84	1.38 – 7.05
PC aa C38:1	Phosphatidylcholine diacyl C38:1	0.34	99.84	0.86	18.08					
PC aa C38:3	Phosphatidylcholine diacyl C38:3	0.86	100	53.99	7.56	✓	54.06 ± 12.59	12.08 – 111.43	51.54 ± 12.76	23.69 – 103.10
PC aa C38:4	Phosphatidylcholine diacyl C38:4	0.88	100	119.65	7.29	✓	117.82 ± 28.03	29.46 – 264.97	116.33 ± 31.00	42.27 – 312.74
PC aa C38:5	Phosphatidylcholine diacyl C38:5	0.83	100	62.17	7.95	✓	62.11 ± 14.07	13.84 – 160.46	61.25 ± 15.12	23.01 – 133.86
PC aa C38:6	Phosphatidylcholine diacyl C38:6	0.93	100	90.24	8.06	✓	90.79 ± 25.93	15.59 – 215.69	87.98 ± 25.49	30.73 – 207.83
PC aa C40:1	Phosphatidylcholine diacyl C40:1	0.51	8.66	0.47	13.48	✓	0.48 ± 0.09	0.24 – 0.93	0.46 ± 0.09	0.25 – 0.94
PC aa C40:2	Phosphatidylcholine diacyl C40:2	0.51	100	0.36	11.70	✓	0.36 ± 0.09	0.14 – 1.02	0.35 ± 0.09	0.11 – 0.85

Table 9: Overview of all metabolites (cont.)

Abbreviation	Biochemical name	coeff.	above LOD (%)	Mean (µM)	CV (%)	Appl.	mean ± SD (µM)	min – max conc.	mean ± SD (µM)	min – max conc.
PC aa C40:3	Phosphatidylcholine diacyl C40:3	0.60	100	0.65	11.18	✓	0.66 ± 0.14	0.17 – 1.54	0.64 ± 0.15	0.26 – 1.48
PC aa C40:4	Phosphatidylcholine diacyl C40:4	0.86	100	4.16	7.58	✓	4.04 ± 1.05	0.80 – 11.41	4.11 ± 1.24	1.60 – 10.80
PC aa C40:5	Phosphatidylcholine diacyl C40:5	0.89	100	11.50	6.98	✓	11.20 ± 2.89	1.86 – 31.32	11.41 ± 3.26	4.97 – 27.89
PC aa C40:6	Phosphatidylcholine diacyl C40:6	0.93	100	28.75	7.10	✓	28.20 ± 9.06	4.27 – 75.68	27.70 ± 9.14	9.32 – 72.88
PC aa C42:0	Phosphatidylcholine diacyl C42:0	0.85	99.97	0.59	12.33	✓	0.64 ± 0.18	0.14 – 1.44	0.57 ± 0.18	0.18 – 2.15
PC aa C42:1	Phosphatidylcholine diacyl C42:1	0.72	100	0.30	14.84	✓	0.32 ± 0.08	0.08 – 0.74	0.29 ± 0.08	0.10 – 0.77
PC aa C42:2	Phosphatidylcholine diacyl C42:2	0.56	100	0.21	14.60	✓	0.22 ± 0.06	0.05 – 0.42	0.21 ± 0.06	0.06 – 0.56
PC aa C42:4	Phosphatidylcholine diacyl C42:4	0.51	100	0.22	11.68	✓	0.22 ± 0.04	0.06 – 0.46	0.21 ± 0.04	0.08 – 0.36
PC aa C42:5	Phosphatidylcholine diacyl C42:5	0.75	100	0.43	10.64	✓	0.43 ± 0.11	0.08 – 1.15	0.41 ± 0.12	0.18 – 1.18
PC aa C42:6	Phosphatidylcholine diacyl C42:6	0.62	60.16	0.63	12.53	✓	0.64 ± 0.13	0.28 – 1.18	0.61 ± 0.13	0.30 – 1.24
PC ae C30:0	Phosphatidylcholine acyl-alkyl C30:0	0.76	98.86	0.46	18.07	✓	0.51 ± 0.14	0.08 – 1.09	0.44 ± 0.13	0.08 – 1.07
PC ae C30:1	Phosphatidylcholine acyl-alkyl C30:1	0.18	94.12	0.22	41.66					
PC ae C30:2	Phosphatidylcholine acyl-alkyl C30:2	0.65	86.34	0.16	17.54	✓	0.17 ± 0.04	0.05 – 0.34	0.14 ± 0.04	0.06 – 0.29
PC ae C32:1	Phosphatidylcholine acyl-alkyl C32:1	0.83	100	2.85	7.97	✓	3.02 ± 0.58	0.68 – 6.22	2.71 ± 0.53	1.25 – 5.75
PC ae C32:2	Phosphatidylcholine acyl-alkyl C32:2	0.77	100	0.75	11.65	✓	0.81 ± 0.18	0.23 – 1.92	0.68 ± 0.14	0.29 – 1.42
PC ae C34:0	Phosphatidylcholine acyl-alkyl C34:0	0.82	100	1.72	7.87	✓	1.82 ± 0.44	0.41 – 3.53	1.63 ± 0.38	0.65 – 3.16
PC ae C34:1	Phosphatidylcholine acyl-alkyl C34:1	0.87	100	10.52	7.56	✓	11.29 ± 2.19	2.52 – 20.89	9.82 ± 1.91	4.56 – 17.96
PC ae C34:2	Phosphatidylcholine acyl-alkyl C34:2	0.90	100	12.61	7.65	✓	13.71 ± 3.11	3.07 – 27.80	12.06 ± 2.99	4.72 – 26.68
PC ae C34:3	Phosphatidylcholine acyl-alkyl C34:3	0.91	100	8.34	7.90	✓	9.14 ± 2.42	2.13 – 21.81	8.05 ± 2.20	3.28 – 17.96
PC ae C36:0	Phosphatidylcholine acyl-alkyl C36:0	0.35	100	1.06	35.57					
PC ae C36:1	Phosphatidylcholine acyl-alkyl C36:1	0.85	100	8.30	9.81	✓	8.96 ± 2.06	2.41 – 18.17	7.87 ± 1.67	2.86 – 14.42

Table 9: Overview of all metabolites (cont.)

Abbreviation	Biochemical name	coeff.	above LOD (%)	Mean (µM)	CV (%)	Appl.	mean ± SD (µM)	min – max conc.	mean ± SD (µM)	min – max conc.
PC ae C36:2	Phosphatidylcholine acyl-alkyl C36:2	0.92	100	15.06	8.28	✓	16.64 ± 3.64	3.71 – 29.99	14.14 ± 3.36	5.40 – 29.51
PC ae C36:3	Phosphatidylcholine acyl-alkyl C36:3	0.86	100	8.55	8.09	✓	9.12 ± 1.95	1.95 – 17.09	8.28 ± 1.90	3.43 – 16.47
PC ae C36:4	Phosphatidylcholine acyl-alkyl C36:4	0.87	100	20.77	7.94	✓	20.54 ± 4.89	5.75 – 40.19	21.22 ± 5.47	8.93 – 42.10
PC ae C36:5	Phosphatidylcholine acyl-alkyl C36:5	0.89	100	13.81	7.96	✓	13.68 ± 3.42	3.82 – 33.15	14.03 ± 3.49	5.57 – 27.37
PC ae C38:0	Phosphatidylcholine acyl-alkyl C38:0	0.81	100	2.46	10.84	✓	2.60 ± 0.72	0.59 – 8.73	2.36 ± 0.70	0.88 – 5.25
PC ae C38:1	Phosphatidylcholine acyl-alkyl C38:1	0.48	100	0.81	12.37	✓	0.84 ± 0.27	0.19 – 2.91	0.79 ± 0.24	0.15 – 2.13
PC ae C38:2	Phosphatidylcholine acyl-alkyl C38:2	0.73	100	2.12	10.28	✓	2.30 ± 0.48	0.53 – 4.41	2.03 ± 0.44	0.71 – 3.91
PC ae C38:3	Phosphatidylcholine acyl-alkyl C38:3	0.85	100	4.29	9.17	✓	4.66 ± 0.99	1.22 – 8.84	4.03 ± 0.84	1.50 – 6.83
PC ae C38:4	Phosphatidylcholine acyl-alkyl C38:4	0.82	100	15.63	8.62	✓	16.18 ± 3.08	4.10 – 29.36	15.29 ± 2.99	6.51 – 28.23
PC ae C38:5	Phosphatidylcholine acyl-alkyl C38:5	0.82	100	19.86	8.31	✓	19.75 ± 4.04	4.96 – 37.15	20.04 ± 4.26	9.70 – 35.91
PC ae C38:6	Phosphatidylcholine acyl-alkyl C38:6	0.85	100	8.68	8.09	✓	8.82 ± 2.09	2.19 – 16.63	8.57 ± 2.05	3.49 – 18.07
PC ae C40:0	Phosphatidylcholine acyl-alkyl C40:0	0.87	1.05	10.20	4.82	✓	10.45 ± 1.48	5.95 – 16.36	9.99 ± 1.46	6.88 – 17.72
PC ae C40:1	Phosphatidylcholine acyl-alkyl C40:1	0.68	100	1.66	10.53	✓	1.70 ± 0.37	0.50 – 3.17	1.68 ± 0.39	0.63 – 3.09
PC ae C40:2	Phosphatidylcholine acyl-alkyl C40:2	0.85	100	2.08	9.51	✓	2.21 ± 0.51	0.76 – 4.66	2.00 ± 0.45	0.80 – 3.70
PC ae C40:3	Phosphatidylcholine acyl-alkyl C40:3	0.73	100	1.13	9.52	✓	1.24 ± 0.23	0.35 – 3.18	1.05 ± 0.21	0.40 – 1.92
PC ae C40:4	Phosphatidylcholine acyl-alkyl C40:4	0.82	100	2.57	9.57	✓	2.72 ± 0.51	0.62 – 4.92	2.50 ± 0.50	1.08 – 7.42
PC ae C40:5	Phosphatidylcholine acyl-alkyl C40:5	0.78	100	3.55	8.32	✓	3.69 ± 0.66	0.81 – 7.66	3.48 ± 0.65	1.55 – 7.47
PC ae C40:6	Phosphatidylcholine acyl-alkyl C40:6	0.88	100	5.04	8.65	✓	5.30 ± 1.23	1.02 – 10.81	4.86 ± 1.14	2.15 – 9.18
PC ae C42:0	Phosphatidylcholine acyl-alkyl C42:0	0.60	14.87	0.51	15.66	✓	0.52 ± 0.10	0.26 – 1.04	0.50 ± 0.10	0.25 – 1.15
PC ae C42:1	Phosphatidylcholine acyl-alkyl C42:1	0.51	100	0.37	11.47	✓	0.38 ± 0.09	0.14 – 0.86	0.37 ± 0.09	0.13 – 0.76
PC ae C42:2	Phosphatidylcholine acyl-alkyl C42:2	0.69	100	0.66	12.85	✓	0.70 ± 0.15	0.20 – 1.38	0.65 ± 0.14	0.22 – 1.32

Table 9: Overview of all metabolites (cont.)

Abbreviation	Biochemical name	coeff.	above LOD (%)	Mean (µM)	CV (%)	Appl.	mean ± SD (µM)	min – max conc.	mean ± SD (µM)	min – max conc.
PC ae C42:3	Phosphatidylcholine acyl-alkyl C42:3	0.80	100	0.86	10.82	✓	0.92 ± 0.19	0.21 – 1.57	0.84 ± 0.19	0.33 – 1.65
PC ae C42:4	Phosphatidylcholine acyl-alkyl C42:4	0.78	100	1.01	9.17	✓	1.08 ± 0.25	0.18 – 2.00	0.97 ± 0.25	0.38 – 4.35
PC ae C42:5	Phosphatidylcholine acyl-alkyl C42:5	0.86	99.97	2.35	7.44	✓	2.49 ± 0.50	0.68 – 4.42	2.29 ± 0.52	1.07 – 8.61
PC ae C44:3	Phosphatidylcholine acyl-alkyl C44:3	0.50	100	0.11	12.52	✓	0.12 ± 0.03	0.04 – 0.22	0.11 ± 0.03	0.03 – 0.25
PC ae C44:4	Phosphatidylcholine acyl-alkyl C44:4	0.71	100	0.43	11.42	✓	0.46 ± 0.11	0.09 – 1.05	0.41 ± 0.12	0.16 – 1.55
PC ae C44:5	Phosphatidylcholine acyl-alkyl C44:5	0.86	100	2.11	7.96	✓	2.21 ± 0.56	0.34 – 4.58	2.09 ± 0.60	0.78 – 9.19
PC ae C44:6	Phosphatidylcholine acyl-alkyl C44:6	0.89	100	1.37	7.73	✓	1.45 ± 0.38	0.26 – 3.68	1.34 ± 0.38	0.56 – 4.92
lysoPC a C14:0	Lysophosphatidylcholine acyl C14:0	0.45	21.24	3.21	23.83					
lysoPC a C16:0	Lysophosphatidylcholine acyl C16:0	0.75	100	94.41	8.8	✓	89.99 ± 18.13	11.57 – 181.22	99.79 ± 19.28	53.36 – 179.47
lysoPC a C16:1	Lysophosphatidylcholine acyl C16:1	0.84	100	2.91	8.58	✓	2.83 ± 0.86	0.43 – 7.18	2.96 ± 1.08	1.23 – 13.09
lysoPC a C17:0	Lysophosphatidylcholine acyl C17:0	0.84	100	1.73	12.65	✓	1.77 ± 0.49	0.26 – 3.97	1.74 ± 0.49	0.58 – 3.55
lysoPC a C18:0	Lysophosphatidylcholine acyl C18:0	0.80	100	26.07	9.74	✓	25.35 ± 6.10	3.31 – 47.17	27.23 ± 5.68	12.16 – 52.13
lysoPC a C18:1	Lysophosphatidylcholine acyl C18:1	0.84	100	19.28	9.20	✓	18.30 ± 5.05	2.72 – 46.44	21 ± 5.91	8.96 – 68.97
lysoPC a C18:2	Lysophosphatidylcholine acyl C18:2	0.93	100	27.24	8.82	✓	25.86 ± 8.33	3.98 – 64.4	30.93 ± 10.18	10.01 – 88.15
lysoPC a C20:3	Lysophosphatidylcholine acyl C20:3	0.77	100	2.39	8.98	✓	2.22 ± 0.61	0.50 – 5.30	2.60 ± 0.71	0.89 – 6.36
lysoPC a C20:4	Lysophosphatidylcholine acyl C20:4	0.87	100	6.80	9.04	✓	6.16 ± 1.80	1.41 – 16.59	7.53 ± 2.27	2.42 – 21.03
lysoPC a C24:0	Lysophosphatidylcholine acyl C24:0	0.09	12.45	0.36	21.06					
lysoPC a C26:0	Lysophosphatidylcholine acyl C26:0	0.09	59.58	0.55	30.96					
lysoPC a C26:1	Lysophosphatidylcholine acyl C26:1	−0.04	0.00	2.03	7.90					
lysoPC a C28:0	Lysophosphatidylcholine acyl C28:0	0.17	49.61	0.49	29.10					
lysoPC a C28:1	Lysophosphatidylcholine acyl C28:1	0.29	99.84	0.62	22.64					

Table 9: Overview of all metabolites (cont.)

Abbreviation	Biochemical name	coeff.	above LOD (%)	Mean (µM)	CV (%)	Appl.	mean ± SD (µM)	min – max conc.	mean ± SD (µM)	min – max conc.
lysoPC a C6:0	Lysophosphatidylcholine acyl C6:0	−0.14	33.33	0.02	62.55					
SM (OH) C14:1	Hydroxysphingomyeline C14:1	0.91	100	6.22	7.75	✓	6.72 ± 1.60	1.48 – 13.83	5.61 ± 1.37	2.19 – 11.17
SM (OH) C16:1	Hydroxysphingomyeline C16:1	0.86	100	3.35	8.78	✓	3.63 ± 0.85	0.78 – 6.52	3.05 ± 0.70	1.19 – 5.54
SM (OH) C22:1	Hydroxysphingomyeline C22:1	0.82	100	13.50	11.24	✓	14.25 ± 2.95	3.41 – 25.09	12.55 ± 2.65	5.23 – 23.11
SM (OH) C22:2	Hydroxysphingomyeline C22:2	0.87	100	11.41	10.32	✓	12.68 ± 2.68	3.15 – 24.67	10.17 ± 2.2	4.51 – 18.29
SM (OH) C24:1	Hydroxysphingomyeline C24:1	0.75	100	1.34	15.08	✓	1.40 ± 0.34	0.34 – 2.99	1.28 ± 0.30	0.56 – 2.30
SM C16:0	Sphingomyeline C16:0	0.73	100	106.65	7.97	✓	108.87 ± 16.07	23.98 – 184.20	101.33 ± 16.00	55.93 – 158.48
SM C16:1	Sphingomyeline C16:1	0.84	100	16.06	7.54	✓	16.88 ± 2.99	4.08 – 30.23	14.54 ± 2.47	8.03 – 25.28
SM C18:0	Sphingomyeline C18:0	0.79	100	23.22	8.99	✓	23.98 ± 4.68	5.14 – 44.81	21.73 ± 4.23	10.61 – 38.26
SM C18:1	Sphingomyeline C18:1	0.84	100	11.30	8.20	✓	12.01 ± 2.56	2.71 – 21.51	10.06 ± 2.08	4.83 – 17.94
SM C20:2	Sphingomyeline C20:2	0.61	99.93	0.38	12.65	✓	0.43 ± 0.11	0.06 – 1.02	0.33 ± 0.09	0.03 – 0.68
SM C22:3	Sphingomyeline C22:3	−0.04	55.85	0.11	57.64					
SM C24:0	Sphingomyeline C24:0	0.78	100	21.79	10.68	✓	21.58 ± 4.13	4.71 – 43.26	21.56 ± 4.24	10.04 – 38.24
SM C24:1	Sphingomyeline C24:1	0.75	100	52.57	9.96	✓	52.47 ± 9.36	14.02 – 97.92	51.00 ± 9.42	25.33 – 83.66
SM C26:0	Sphingomyeline C26:0	0.46	100	0.18	67.78					
SM C26:1	Sphingomyeline C26:1	0.69	100	0.42	20.79	✓	0.41 ± 0.13	0.09 – 1.18	0.41 ± 0.12	0.14 – 0.98
H1 (mM)	Sum of Hexoses	0.69	100	5.19	6.30E11	✓	4.80 ± 0.59	0.91 – 9.96	5.10 ± 0.60	2.43 – 8.32

Table 10: Results from BMI-adjusted linear regression in KORA F4 females; ⋆ below significance level

metabolite	estimate	SE	p-value
C12:1	0.009	0.001	1.58E-40 ⋆
C10:1	0.010	0.001	2.70E-37 ⋆
PC ae C36:1	0.007	0.001	3.76E-37 ⋆
C18	0.008	0.001	1.21E-36 ⋆
C16:2	0.010	0.001	2.06E-35 ⋆
PC aa C28:1	0.007	0.001	3.83E-33 ⋆
C18:1	0.007	0.001	4.24E-33 ⋆
C16:1	0.007	0.001	1.78E-32 ⋆
C16	0.006	0.001	1.42E-29 ⋆
C14:1-OH	0.007	0.001	2.78E-29 ⋆
SM C16:1	0.005	4.11E-04	1.28E-28 ⋆
C5	0.008	0.001	2.89E-28 ⋆
C10:2	0.006	0.001	2.13E-27 ⋆
PC ae C40:2	0.006	0.001	5.36E-26 ⋆
C2	0.008	0.001	1.69E-24 ⋆
C8	0.008	0.001	1.21E-23 ⋆
C14:1	0.006	0.001	3.39E-23 ⋆
PC ae C34:0	0.006	0.001	6.66E-23 ⋆
C14:2	0.009	0.001	3.33E-22 ⋆
SM (OH) C16:1	0.006	0.001	6.47E-22 ⋆
C14	0.005	0.001	1.44E-21 ⋆
SM C18:1	0.005	0.001	1.14E-20 ⋆
SM (OH) C22:2	0.005	0.001	5.17E-20 ⋆
C12	0.007	0.001	1.03E-19 ⋆
C18:2	0.006	0.001	1.83E-19 ⋆
PC ae C32:2	0.005	0.001	3.28E-19 ⋆
PC aa C38:5	0.005	0.001	6.02E-19 ⋆
PC aa C40:6	0.007	0.001	7.00E-19 ⋆
C0	0.005	0.001	1.03E-18 ⋆
PC aa C32:0	0.004	4.70E-04	1.59E-18 ⋆
SM (OH) C14:1	0.005	0.001	2.64E-18 ⋆
PC aa C40:2	0.005	0.001	8.03E-18 ⋆
PC aa C40:5	0.005	0.001	1.26E-17 ⋆
SM C18:0	0.004	4.79E-04	1.62E-17 ⋆
C10	0.008	0.001	2.51E-17 ⋆
SM C16:0	0.003	3.72E-04	4.14E-17 ⋆
C3	0.006	0.001	4.53E-17 ⋆
PC aa C40:3	0.004	0.001	3.61E-14 ⋆
SM C26:1	0.006	0.001	7.96E-14 ⋆
C4	0.007	0.001	8.01E-14 ⋆
PC aa C36:1	0.004	0.001	1.01E-13 ⋆
PC aa C38:4	0.004	0.001	1.12E-13 ⋆
PC ae C38:1	0.006	0.001	1.53E-13 ⋆
SM (OH) C24:1	0.004	0.001	4.45E-13 ⋆
PC aa C30:0	0.005	0.001	8.15E-13 ⋆
SM (OH) C22:1	0.004	0.001	1.29E-12 ⋆
PC ae C38:2	0.004	0.001	1.82E-12 ⋆

Table 10: Linear regression females (cont.)

metabolite	estimate	SE	p-value
Orn	0.004	0.001	2.72E-12 ⋆
PC aa C36:5	0.007	0.001	2.78E-12 ⋆
PC aa C38:3	0.004	0.001	5.61E-12 ⋆
His	-0.002	3.61E-04	1.10E-11 ⋆
PC ae C40:0	0.002	3.48E-04	1.94E-11 ⋆
PC aa C32:3	0.004	0.001	2.28E-11 ⋆
Thr	-0.004	0.001	3.66E-11 ⋆
PC ae C40:1	0.004	0.001	4.01E-11 ⋆
PC ae C38:3	0.003	0.001	1.64E-10 ⋆
PC ae C36:2	0.003	0.001	6.42E-10 ⋆
Val	-0.003	4.32E-04	3.19E-09 ⋆
SM C24:0	0.003	4.79E-04	3.49E-09 ⋆
PC ae C36:5	0.004	0.001	4.17E-09 ⋆
SM C24:1	0.003	4.44E-04	8.80E-09 ⋆
PC aa C38:6	0.004	0.001	1.01E-08 ⋆
PC ae C42:0	0.003	4.60E-04	1.28E-08 ⋆
Tyr	0.003	0.001	1.40E-08 ⋆
PC ae C40:6	0.003	0.001	1.42E-08 ⋆
PC ae C38:6	0.003	0.001	3.76E-08 ⋆
PC ae C42:1	0.003	0.001	4.83E-08 ⋆
PC ae C34:1	0.003	4.89E-04	5.18E-08 ⋆
Trp	-0.002	2.98E-04	7.36E-08 ⋆
PC aa C36:2	0.002	4.35E-04	8.20E-08 ⋆
PC aa C32:1	0.005	0.001	1.95E-07 ⋆
PC aa C42:4	0.002	4.92E-04	5.24E-07 ⋆
C8:1	0.005	0.001	6.60E-07 ⋆
PC aa C40:4	0.003	0.001	1.89E-06 ⋆
PC ae C40:3	0.002	4.77E-04	2.38E-06 ⋆
lysoPC a C18:0	0.003	0.001	3.30E-06 ⋆
Ser	-0.002	4.91E-04	3.66E-06 ⋆
xLeu	-0.002	4.23E-04	4.08E-06 ⋆
PC ae C42:2	0.002	0.001	5.76E-06 ⋆
PC ae C38:5	0.002	0.001	5.93E-06 ⋆
PC aa C32:2	0.005	0.001	7.67E-06 ⋆
PC aa C36:6	0.004	0.001	7.90E-06 ⋆
PC aa C38:0	0.003	0.001	1.14E-05 ⋆
PC aa C34:4	0.004	0.001	1.36E-05 ⋆
PC aa C40:1	0.002	4.51E-04	1.46E-05 ⋆
PC ae C30:0	0.003	0.001	2.36E-05 ⋆
PC ae C44:4	-0.003	0.001	2.45E-05 ⋆
PC ae C42:4	-0.002	0.001	5.44E-05 ⋆
PC aa C34:3	0.003	0.001	1.14E-04 ⋆
PC ae C44:5	-0.002	0.001	1.21E-04 ⋆
PC ae C40:5	0.002	4.56E-04	1.53E-04 ⋆
PC aa C42:2	0.002	0.001	1.56E-04 ⋆
PC aa C36:4	0.002	0.001	1.57E-04 ⋆
C6(C4:1-DC)	0.003	0.001	2.31E-04 ⋆
PC ae C38:4	0.002	4.93E-04	2.78E-04 ⋆

Table 10: Linear regression females (cont.)

metabolite	estimate	SE	p-value
lysoPC a C17:0	0.003	0.001	3.78E-04 ⋆
PC ae C32:1	0.002	4.92E-04	4.17E-04
PC ae C42:5	-0.002	4.97E-04	7.60E-04
PC aa C42:5	0.002	0.001	8.95E-04
H1	0.001	3.17E-04	0.001
lysoPC a C18:2	-0.002	0.001	0.001
PC aa C36:3	0.002	0.001	0.002
PC aa C42:6	0.001	4.87E-04	0.002
PC ae C38:0	0.002	0.001	0.004
Arg	0.001	4.37E-04	0.005
PC aa C34:1	0.001	0.001	0.008
C9	0.003	0.001	0.009
PC ae C44:3	0.002	0.001	0.010
PC ae C30:2	0.001	0.001	0.019
PC aa C36:0	0.002	0.001	0.025
PC ae C44:6	-0.001	0.001	0.037
Met	-0.001	4.46E-04	0.040
PC ae C34:2	0.001	0.001	0.154
Gly	0.001	0.001	0.169
lysoPC a C16:1	0.001	0.001	0.232
PC ae C36:4	0.001	0.001	0.251
SM C20:2	-0.001	0.001	0.290
Phe	-4.06E-04	3.93E-04	0.303
lysoPC a C16:0	3.99E-04	0.001	0.453
Pro	-4.65E-04	0.001	0.518
PC aa C34:2	2.42E-04	3.89E-04	0.535
PC ae C40:4	2.94E-04	4.81E-04	0.541
Gln	-2.35E-04	3.89E-04	0.547
PC ae C34:3	-3.52E-04	0.001	0.585
PC ae C42:3	2.54E-04	0.001	0.629
lysoPC a C20:4	2.50E-04	0.001	0.731
lysoPC a C18:1	1.98E-04	0.001	0.772
PC aa C42:1	-1.56E-04	0.001	0.814
lysoPC a C20:3	1.34E-04	0.001	0.848
PC ae C36:3	7.65E-05	0.001	0.888
PC aa C42:0	6.01E-05	0.001	0.930

Table 11: Results from BMI-adjusted linear regression in KORA F4 males; ⋆ below significance level

metabolite	estimate	SE	p-value
Trp	-0.003	2.78E-04	3.67E-33 ⋆
Val	-0.005	4.00E-04	6.61E-31 ⋆
C16:1	0.007	0.001	1.60E-26 ⋆
His	-0.004	3.36E-04	2.67E-26 ⋆
C12	0.008	0.001	3.50E-26 ⋆
C16:2	0.009	0.001	3.65E-26 ⋆

Table 11: Linear regression males (cont.)

metabolite	estimate	SE	p-value
xLeu	-0.004	4.03E-04	7.83E-25 ⋆
lysoPC a C20:3	-0.006	0.001	4.54E-22 ⋆
C18:1	0.006	0.001	3.09E-20 ⋆
C10:1	0.007	0.001	2.38E-19 ⋆
C2	0.007	0.001	3.54E-19 ⋆
lysoPC a C18:2	-0.006	0.001	3.90E-19 ⋆
Thr	-0.004	4.92E-04	1.29E-15 ⋆
C14:1-OH	0.005	0.001	4.99E-15 ⋆
C16	0.004	0.001	4.46E-14 ⋆
lysoPC a C20:4	-0.005	0.001	8.63E-14 ⋆
Met	-0.003	4.07E-04	3.09E-13 ⋆
Gln	-0.003	3.74E-04	1.40E-12 ⋆
PC ae C42:0	0.003	4.42E-04	2.54E-12 ⋆
C18:2	0.005	0.001	4.42E-12 ⋆
C14:2	0.007	0.001	1.35E-11 ⋆
PC ae C36:4	-0.004	0.001	2.63E-11 ⋆
PC ae C40:2	0.004	0.001	5.34E-11 ⋆
C6(C4:1-DC)	0.005	0.001	8.42E-11 ⋆
C8	0.006	0.001	1.09E-10 ⋆
PC ae C32:2	0.003	4.90E-04	1.20E-10 ⋆
C14	0.004	0.001	2.72E-10 ⋆
C18	0.004	0.001	2.56E-09 ⋆
PC aa C40:2	0.004	0.001	2.59E-09 ⋆
C12:1	0.005	0.001	4.28E-09 ⋆
Pro	-0.004	0.001	4.68E-09 ⋆
PC ae C36:1	0.003	0.001	9.88E-09 ⋆
SM C24:0	-0.003	4.64E-04	2.93E-08 ⋆
PC ae C34:0	0.003	0.001	6.53E-08 ⋆
lysoPC a C18:1	-0.003	0.001	1.16E-07 ⋆
PC aa C32:0	0.003	4.74E-04	1.37E-07 ⋆
C10	0.005	0.001	2.55E-07 ⋆
C10:2	0.003	0.001	2.90E-07 ⋆
Gly	-0.002	4.73E-04	5.20E-07 ⋆
PC aa C34:4	-0.004	0.001	1.05E-06 ⋆
Ser	-0.002	4.48E-04	1.19E-06 ⋆
PC ae C40:3	0.002	4.69E-04	3.42E-06 ⋆
C14:1	0.003	0.001	7.68E-06 ⋆
PC aa C40:6	0.003	0.001	1.34E-05 ⋆
SM (OH) C16:1	0.002	0.001	1.34E-05 ⋆
lysoPC a C18:0	-0.002	0.001	1.48E-05 ⋆
PC ae C38:1	0.003	0.001	2.16E-05 ⋆
PC aa C38:3	-0.002	0.001	2.98E-05 ⋆
PC aa C40:4	-0.003	0.001	3.40E-05 ⋆
lysoPC a C16:0	-0.002	4.58E-04	3.58E-05 ⋆
PC aa C42:4	0.002	4.78E-04	4.54E-05 ⋆
PC ae C40:0	0.001	3.39E-04	4.63E-05 ⋆
PC ae C32:1	0.002	4.71E-04	4.91E-05 ⋆
PC ae C38:5	-0.002	0.001	5.77E-05 ⋆

Table 11: Linear regression males (cont.)

metabolite	estimate	SE	p-value
SM (OH) C14:1	0.002	0.001	6.96E-05 ⋆
PC aa C36:4	-0.002	0.001	6.99E-05 ⋆
PC aa C38:6	0.003	0.001	9.41E-05 ⋆
PC ae C34:1	0.002	4.59E-04	1.41E-04 ⋆
PC aa C28:1	0.002	0.001	1.87E-04 ⋆
PC aa C40:3	0.002	0.001	2.47E-04 ⋆
PC aa C36:2	-0.002	4.46E-04	3.48E-04 ⋆
PC ae C36:5	-0.002	0.001	3.82E-04
SM C26:1	0.002	0.001	4.73E-04
C5	-0.002	0.001	0.001
PC aa C36:3	-0.002	4.87E-04	0.001
PC aa C38:4	-0.002	0.001	0.001
PC aa C40:1	0.002	4.55E-04	0.001
PC aa C42:0	0.002	0.001	0.001
PC ae C36:3	-0.002	0.001	0.001
Phe	-0.001	3.74E-04	0.001
SM C16:0	0.001	3.81E-04	0.001
PC aa C34:2	-0.001	4.06E-04	0.002
PC ae C30:0	0.002	0.001	0.002
PC ae C30:2	-0.002	0.001	0.002
PC ae C38:2	0.002	0.001	0.002
PC ae C44:3	0.002	0.001	0.002
SM (OH) C22:1	-0.002	0.001	0.002
PC aa C36:0	-0.002	0.001	0.003
PC aa C36:1	-0.002	0.001	0.003
PC aa C42:2	0.002	0.001	0.003
PC ae C38:4	-0.001	4.75E-04	0.003
PC aa C30:0	0.002	0.001	0.004
PC ae C36:2	0.002	0.001	0.006
PC ae C40:6	0.002	0.001	0.006
SM C16:1	0.001	4.07E-04	0.008
PC aa C42:5	0.002	0.001	0.010
SM C20:2	-0.002	0.001	0.010
PC ae C38:0	-0.002	0.001	0.013
lysoPC a C16:1	-0.002	0.001	0.015
PC ae C34:3	-0.001	0.001	0.022
PC aa C40:5	-0.001	0.001	0.025
PC ae C40:1	-0.001	0.001	0.026
PC aa C34:1	-0.001	0.001	0.029
PC ae C38:6	-0.001	0.001	0.029
C8:1	0.002	0.001	0.041
PC ae C42:3	0.001	0.001	0.048
PC ae C44:6	0.001	0.001	0.056
SM (OH) C24:1	-0.001	0.001	0.057
C4	0.002	0.001	0.072
Tyr	-0.001	4.41E-04	0.091
Arg	-0.001	4.53E-04	0.126
PC aa C36:6	-0.001	0.001	0.128

Table 11: Linear regression males (cont.)

metabolite	estimate	SE	p-value
PC aa C42:6	-0.001	0.001	0.132
PC aa C38:5	-0.001	0.001	0.147
SM (OH) C22:2	0.001	0.001	0.173
C3	-0.001	0.001	0.207
PC aa C42:1	0.001	0.001	0.224
Orn	-0.001	0.001	0.244
H1	3.14E-04	2.82E-04	0.265
PC ae C34:2	-0.001	0.001	0.267
PC aa C32:1	0.001	0.001	0.291
PC aa C34:3	-0.001	0.001	0.310
PC aa C32:3	-0.001	0.001	0.316
C0	-4.58E-04	4.62E-04	0.321
PC ae C38:3	4.15E-04	0.001	0.421
C9	0.001	0.001	0.422
SM C24:1	-3.59E-04	4.51E-04	0.426
lysoPC a C17:0	-4.96E-04	0.001	0.453
SM C18:0	3.44E-04	4.65E-04	0.459
SM C18:1	3.61E-04	4.93E-04	0.464
PC aa C36:5	0.001	0.001	0.516
PC aa C38:0	3.98E-04	0.001	0.525
PC ae C40:4	-2.78E-04	4.60E-04	0.546
PC ae C42:4	2.96E-04	0.001	0.594
PC ae C42:1	-2.80E-04	0.001	0.641
PC aa C32:2	-4.24E-04	0.001	0.679
PC ae C40:5	-1.49E-04	4.41E-04	0.736
PC ae C44:5	-1.91E-04	0.001	0.760
PC ae C42:5	-1.07E-04	4.89E-04	0.827
PC ae C42:2	-2.56E-05	0.001	0.961
PC ae C44:4	2.29E-05	0.001	0.970

[1] Jerzy Adamski and Karsten Suhre. Metabolomics platforms for genome wide association studies-linking the genome to the metabolome. *Curr Opin Biotechnol*, 24(1):39–47, Feb 2013. doi: 10.1016/j.copbio.2012.10.003. URL http://dx.doi.org/10.1016/j.copbio.2012.10.003. (Cited on pages 8 and 9.)

[2] T. Andrew, D. J. Hart, H. Snieder, M. de Lange, T. D. Spector, and A. J. MacGregor. Are twins and singletons comparable? a study of disease-related and lifestyle characteristics in adult women. *Twin Res*, 4(6):464–477, Dec 2001. (Cited on page 22.)

[3] Baltic Analytics, Jan 2013. URL http://www.baltic-analytics.de/index.php?id=8&L=1. (Cited on page 11.)

[4] Michelangela Barbieri, Virginia Boccardi, Michela Papa, and Giuseppe Paolisso. Metabolic journey to healthy longevity. *Horm Res*, 71 Suppl 1:24–27, Jan 2009. doi: 10.1159/000178032. URL http://dx.doi.org/10.1159/000178032. (Cited on pages 6, 36, and 41.)

[5] Nir Barzilai, Gil Atzmon, Clyde Schechter, Ernst J. Schaefer, Adrienne L. Cupples, Richard Lipton, Suzanne Cheng, and Alan R. Shuldiner. Unique lipoprotein phenotype and genotype associated with exceptional longevity. *JAMA*, 290(15):2030–2040, Oct 2003. doi: 10.1001/jama.290.15.2030. URL http://dx.doi.org/10.1001/jama.290.15.2030. (Cited on page 7.)

[6] David J. Bates, Na Li, Ruqiang Liang, Harshini Sarojini, Jin An, Michal M. Masternak, Andrzej Bartke, and Eugenia Wang. Microrna regulation in ames dwarf mouse liver may contribute to delayed aging. *Aging Cell*, 9(1):1–18, Feb 2010. doi: 10.1111/j.1474-9726.2009.00529.x. URL http://dx.doi.org/10.1111/j.1474-9726.2009.00529.x. (Cited on page 40.)

[7] María Berdasco and Manel Esteller. Hot topics in epigenetic mechanisms of aging: 2011. *Aging Cell*, 11(2):181–186, Apr 2012. doi: 10.1111/j.1474-9726.2012.00806.x. URL http://dx.doi.org/10.1111/j.1474-9726.2012.00806.x. (Cited on pages 3 and 45.)

[8] Hanne Christine Bertram, Jens Ø. Duus, Bent O. Petersen, Camilla Hoppe, Anni Larnkjaer, Lene Schack-Nielsen, Christian Mølgaard, and Kim F. Michaelsen. Nuclear magnetic resonance-based metabonomics reveals strong sex effect on plasma metabolism in 17-year-old scandinavians and correlation to retrospective infant plasma parameters. *Metabolism*, 58(7):1039–1045, Jul 2009. doi: 10.1016/j.metabol.2009.03.011. URL http://dx.doi.org/10.1016/j.metabol.2009.03.011. (Cited on page 16.)

[9] Erhard Bieberich. Ceramide signaling in cancer and stem cells. *Future Lipidol*, 3(3):273–300, Jun 2008. doi: 10.2217/17460875.3.3.273. URL http://dx.doi.org/10.2217/17460875.3.3.273. (Cited on page 38.)

[10] Robert N. Butler, Richard A. Miller, Daniel Perry, Bruce A. Carnes, T Franklin Williams, Christine Cassel, Jacob Brody, Marie A. Bernard, Linda Partridge, Thomas Kirkwood, George M. Martin, and S Jay Olshansky. New model of health promotion and disease prevention for the 21st century. *BMJ*, 337:a399, 2008. (Cited on page 2.)

[11] Judith Campisi and Fabrizio d'Adda di Fagagna. Cellular senescence: when bad things happen to good cells. *Nat Rev Mol Cell Biol*, 8(9):729–740, Sep 2007. doi: 10.1038/nrm2233. URL http://dx.doi.org/10.1038/nrm2233. (Cited on pages 37 and 38.)

[12] Hui Chen, Julie-Thu A. Tran, Richard S. Brush, Anisse Saadi, Abul K. Rahman, Man Yu, Douglas Yasumura, Michael T. Matthes, Kelly Ahern, Haidong Yang, Matthew M. LaVail, and Md Nawajes A. Mandal. Ceramide signaling in retinal degeneration. *Adv Exp Med Biol*, 723:553–558, 2012. doi: 10.1007/978-1-4614-0631-0_70. URL http://dx.doi.org/10.1007/978-1-4614-0631-0_70. (Cited on page 38.)

[13] Angela B. Clement, Martin Gamerdinger, Irfan Y. Tamboli, Dieter Lütjohann, Jochen Walter, Isabell Greeve, Gerald Gimpl, and Christian Behl. Adaptation of neuronal cells to chronic oxidative stress is associated with altered cholesterol and sphingolipid homeostasis and lysosomal function. *J Neurochem*, 111(3):669–682, Nov 2009. doi: 10.1111/j.1471-4159.2009.06360.x. URL http://dx.doi.org/10.1111/j.1471-4159.2009.06360.x. (Cited on page 37.)

[14] William S Cleveland and Susan T Devlin. Locally weighted regression: An approach to regression analysis by local fitting. *J. Am. Stat. Assoc.*, 83(403):596–610, Sep. 1988. URL http://www.jstor.org/stable/2289282. (Cited on page 22.)

[15] Laurence Coquin, Jacob D. Feala, Andrew D. McCulloch, and Giovanni Paternostro. Metabolomic and flux-balance analysis of age-related decline of hypoxia tolerance in drosophila muscle tissue. *Mol Syst Biol*, 4:233, 2008. doi: 10.1038/msb.2008.71. URL http://dx.doi.org/10.1038/msb.2008.71. (Cited on pages 15 and 39.)

[16] Isabelle Corre, Colin Niaudet, and Francois Paris. Plasma membrane signaling induced by ionizing radiation. *Mutat Res*, 704(1-3):61–67, 2010. doi: 10.1016/j.mrrev.2010.01.014. URL http://dx.doi.org/10.1016/j.mrrev.2010.01.014. (Cited on page 37.)

[17] Pio D'Adamo, Sheila Ulivi, Amerigo Beneduci, Gabriele Pontoni, Giovambattista Capasso, Carmela Lanzara, Gilberto Andrighetto, Uros Hladnik, Virginia Nunes, Manuel Palacin, and Paolo Gasparini. Metabonomics and population studies: age-related amino acids excretion and inferring networks through the study of urine samples in two italian isolated populations. *Amino Acids*, 38(1):65–73, Jan 2010. doi: 10.1007/s00726-008-0205-8. URL http://dx.doi.org/10.1007/s00726-008-0205-8. (Cited on page 16.)

[18] Jennifer M. De Guzman, Ginger Ku, Ryan Fahey, Yun-Hee Youm, Ignatius Kass, Donald K. Ingram, Vishwa Deep Dixit, and Indu Kheterpal. Chronic caloric restriction partially protects against age-related alteration in serum metabolome. *Age (Dordr)*, Jun 2012. doi: 10.1007/s11357-012-9430-x. URL http://dx.doi.org/10.1007/s11357-012-9430-x. (Cited on pages 15 and 36.)

[19] Joao Pedro de Magalhaes, Arie Budovsky, Gilad Lehmann, Joana Costa, Yang Li, Vadim Fraifeld, and George M. Church. The human ageing genomic resources: online databases and tools for biogerontologists. *Aging Cell*, 8(1):65–72, Feb 2009. doi: 10.1111/j.1474-9726.2008.00442.x. URL http://dx.doi.org/10.1111/j.1474-9726.2008.00442.x. (Cited on page 32.)

[20] Wim Derave, Inge Everaert, Sam Beeckman, and Audrey Baguet. Muscle carnosine metabolism and beta-alanine supplementation in relation to exercise and training. *Sports Med*, 40(3):247–263, Mar 2010. doi: 10.2165/11530310-000000000-00000. URL http://dx.doi.org/10.2165/11530310-000000000-00000. (Cited on page 39.)

[21] William Durante, Fruzsina K. Johnson, and Robert A. Johnson. Arginase: a critical regulator of nitric oxide synthesis and vascular function. *Clin Exp Pharmacol Physiol*, 34(9):906–911, Sep 2007. doi: 10.1111/j.1440-1681.2007.04638.x. URL http://dx.doi.org/10.1111/j.1440-1681.2007.04638.x. (Cited on pages 40 and 41.)

[22] Bernd Engelmann and Markus K H. Wiedmann. Cellular phospholipid uptake: flexible paths to coregulate the functions of intracellular lipids. *Biochim Biophys Acta*, 1801(6):609–616, Jun 2010. doi: 10.1016/j.bbalip.2010.02.013. URL http://dx.doi.org/10.1016/j.bbalip.2010.02.013. (Cited on page 37.)

[23] Anne M. Evans, Corey D. DeHaven, Tom Barrett, Matt Mitchell, and Eric Milgram. Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. *Anal Chem*, 81(16):6656–6667, Aug 2009. doi: 10.1021/ac901536h. URL http://dx.doi.org/10.1021/ac901536h. (Cited on pages 9 and 12.)

[24] Bruno César Feltes, Joice de Faria Poloni, and Diego Bonatto. The developmental aging and origins of health and disease hypotheses explained by different protein networks. *Biogerontology*, 12(4):293–308, Aug 2011. doi: 10.1007/s10522-011-9325-8. URL http://dx.doi.org/10.1007/s10522-011-9325-8. (Cited on pages 5 and 35.)

[25] Luigi Ferrucci, Francesco Giallauria, and Jack M. Guralnik. Epidemiology of aging. *Radiol Clin North Am*, 46(4):643–52, v, Jul 2008. doi: 10.1016/j.rcl.2008.07.005. URL http://dx.doi.org/10.1016/j.rcl.2008.07.005. (Cited on pages 2 and 3.)

[26] P. Filzmoser, R. Maronna, , and M. Werner. Outlier identification in high dimensions. *Computational Statistics & Data Analysis*, 52(3):1694–1711, 2008. URL ISI:000253669700032. Times Cited: 12 Article English Filzmoser, P Vienna Univ Technol, Dept Stat & Probabil Theory, Wiedner Hauptstr 8-10, A-1040 Vienna, Austria Cited References Count: 32 269SE PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS AMSTERDAM. (Cited on page 21.)

[27] Luigi Fontana, Linda Partridge, and Valter D. Longo. Extending healthy life span–from yeast to humans. *Science*, 328(5976):321–326, Apr 2010. doi: 10.1126/science.1172539. URL http://dx.doi.org/10.1126/science.1172539. (Cited on pages 8 and 35.)

[28] Claudio Franceschi, Miriam Capri, Daniela Monti, Sergio Giunta, Fabiola Olivieri, Federica Sevini, Maria Panagiota Panourgia, Laura Invidia, Laura Celani, Maria Scurti, Elisa Cevenini, Gastone C. Castellani, and Stefano Salvioli. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev*, 128(1):92–105, Jan 2007. doi: 10.1016/j.mad.2006.11.016. URL http://dx.doi.org/10.1016/j.mad.2006.11.016. (Cited on page 3.)

[29] Christian Gieger, Ludwig Geistlinger, Elisabeth Altmaier, Martin Hrabé de Angelis, Florian Kronenberg, Thomas Meitinger, Hans-Werner Mewes, H-Erich Wichmann, Klaus M. Weinberger, Jerzy Adamski, Thomas Illig, and Karsten Suhre. Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. *PLoS Genet*, 4(11):e1000282, Nov 2008. doi: 10.1371/journal.pgen.1000282. URL http://dx.doi.org/10.1371/journal.pgen.1000282. (Cited on page 32.)

[30] Haiwei Gu, Zhengzheng Pan, Bowei Xi, Bryan E. Hainline, Narasimhamurthy Shanaiah, Vincent Asiago, G A Nagana Gowda, and Daniel Raftery. 1h nmr metabolomics study of age profiling in children. *NMR Biomed*, 22(8):826–833, Oct 2009. doi: 10.1002/nbm.1395. URL http://dx.doi.org/10.1002/nbm.1395. (Cited on page 16.)

[31] David E. Harrison, Randy Strong, Zelton Dave Sharp, James F. Nelson, Clinton M. Astle, Kevin Flurkey, Nancy L. Nadon, J Erby Wilkinson, Krystyna Frenkel, Christy S. Carter, Marco Pahor, Martin A. Javors, Elizabeth Fernandez, and Richard A. Miller. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature*, 460(7253):392–395, Jul 2009. doi: 10.1038/nature08221. URL http://dx.doi.org/10.1038/nature08221. (Cited on page 8.)

[32] Leonard Hayflick. Biological aging is no longer an unsolved problem. *Ann N Y Acad Sci*, 1100:1–13, Apr 2007. doi: 10.1196/annals.1395.001. URL http://dx.doi.org/10.1196/annals.1395.001. (Cited on pages 1, 4, and 33.)

[33] C. J. Henry. Mechanisms of changes in basal metabolism during ageing. *Eur J Clin Nutr*, 54 Suppl 3:S77–S91, Jun 2000. (Cited on pages 1 and 5.)

[34] Alan R. Hipkiss. Aging, proteotoxicity, mitochondria, glycation, nad and carnosine: Possible inter-relationships and resolution of the oxygen paradox. *Front Aging Neurosci*, 2: 10, 2010. doi: 10.3389/fnagi.2010.00010. URL http://dx.doi.org/10.3389/fnagi.2010.00010. (Cited on page 40.)

[35] A. J. Hulbert. On the importance of fatty acid composition of membranes for aging. *J Theor Biol*, 234(2):277–288, May 2005. doi: 10.1016/j.jtbi.2004.11.024. URL http://dx.doi.org/10.1016/j.jtbi.2004.11.024. (Cited on page 5.)

[36] Human metabolome project, 2013. URL http://www.hmdb.ca/statistics. (Cited on pages 10 and 43.)

[37] Ikuyo Ichi, Chiaki Kamikawa, Tomoka Nakagawa, Keiko Kobayashi, Ryoko Kataoka, Eri Nagata, Yuko Kitamura, Chihiro Nakazaki, Tatsuya Matsura, and Shosuke Kojo. Neutral sphingomyelinase-induced ceramide accumulation by oxidative stress during carbon tetrachloride intoxication. *Toxicology*, 261(1-2):33–40, Jun 2009. doi: 10.1016/j.tox.2009.04.040. URL http://dx.doi.org/10.1016/j.tox.2009.04.040. (Cited on page 37.)

[38] Thomas Illig, Christian Gieger, Guangju Zhai, Werner Römisch-Margl, Rui Wang-Sattler, Cornelia Prehn, Elisabeth Altmaier, Gabi Kastenmüller, Bernet S. Kato, Hans-Werner Mewes, Thomas Meitinger, Martin Hrabé de Angelis, Florian Kronenberg, Nicole Soranzo, H-Erich Wichmann, Tim D. Spector, Jerzy Adamski, and Karsten Suhre. A genome-wide perspective of genetic variation in human metabolism. *Nat Genet*, 42(2):137–141, Feb 2010. doi: 10.1038/ng.507. URL http://dx.doi.org/10.1038/ng.507. (Cited on pages 20, 32, and 44.)

[39] Lars J. Jensen, Michael Kuhn, Manuel Stark, Samuel Chaffron, Chris Creevey, Jean Muller, Tobias Doerks, Philippe Julien, Alexander Roth, Milan Simonovic, Peer Bork, and Christian von Mering. String 8–a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res*, 37(Database issue):D412–D416, Jan 2009. doi: 10.1093/nar/gkn760. URL http://dx.doi.org/10.1093/nar/gkn760. (Cited on page 32.)

[40] Hong Jiang, Eric Schiffer, Zhangfa Song, Jianwei Wang, Petra Zürbig, Kathrin Thedieck, Suzette Moes, Heike Bantel, Nadja Saal, Justyna Jantos, Meiken Brecht, Paul Jenö, Michael N. Hall, Klaus Hager, Michael P. Manns, Hartmut Hecker, Arnold Ganser, Konstanze Döhner, Andrzej Bartke, Christoph Meissner, Harald Mischak, Zhenyu Ju, and K Lenhard Rudolph. Proteins induced by telomere dysfunction and dna damage represent biomarkers of human aging and disease. *Proc Natl Acad Sci U S A*, 105(32):11299–11304, Aug 2008. doi: 10.1073/pnas.0801457105. URL http://dx.doi.org/10.1073/pnas.0801457105. (Cited on pages 3 and 4.)

[41] David Karasik, Serkalem Demissie, L Adrienne Cupples, and Douglas P. Kiel. Disentangling the genetic determinants of human aging: biological age as an alternative to the use of survival measures. *J Gerontol A Biol Sci Med Sci*, 60(5):574–587, May 2005. (Cited on page 3.)

[42] Richard A. Kerber, Elizabeth O'Brien, and Richard M. Cawthon. Gene expression profiles associated with aging and mortality in humans. *Aging Cell*, 8(3):239–250, Jun 2009. doi: 10.1111/j.1474-9726.2009.00467.x. URL http://dx.doi.org/10.1111/j.1474-9726.2009.00467.x. (Cited on page 3.)

[43] Katherine M Keyes, Rebecca L Utz, Whitney Robinson, and Guohua Li. What is a cohort effect? comparison of three statistical methods for modeling cohort effects in obesity prevalence in the united states, 1971-2006. *Soc Sci Med*, 70(7):1100–1108, Apr 2010. doi: 10.1016/j.socscimed.2009.12.018. URL http://dx.doi.org/10.1016/j.socscimed.2009.12.018. (Cited on page 43.)

[44] Ji-Soo Kim, Eui-Jin Kim, Hyun-Jung Kim, Ji-Young Yang, Geum-Sook Hwang, and Chan-Wha Kim. Proteomic and metabolomic analysis of h202-induced premature senescent human mesenchymal stem cells. *Exp Gerontol*, 46(6):500–510, Jun 2011. doi: 10.1016/j.exger.2011.02.012. URL http://dx.doi.org/10.1016/j.exger.2011.02.012. (Cited on page 15.)

[45] Bruce S. Kristal and Yevgeniya I. Shurubor. Metabolomics: opening another window into aging. *Sci Aging Knowledge Environ*, 2005(26):pe19, Jun 2005. doi: 10.1126/sageke.2005.26.pe19. URL http://dx.doi.org/10.1126/sageke.2005.26.pe19. (Cited on pages 10, 13, and 41.)

[46] Kay A. Lawton, Alvin Berger, Matthew Mitchell, K Eric Milgram, Anne M. Evans, Lining Guo, Richard W. Hanson, Satish C. Kalhan, John A. Ryals, and Michael V. Milburn. Analysis of the adult human plasma metabolome. *Pharmacogenomics*, 9(4):383–397, Apr 2008. doi: 10.2217/14622416.9.4.383. URL http://dx.doi.org/10.2217/14622416.9.4.383. (Cited on pages 6, 13, 14, 16, 39, and 40.)

[47] Georg Löffler and Petro E. Petrides. *Biochemie und Pathobiochemie*, volume 7. Springer-Verlag Berlin Heidelberg New York Hongkong Mailand Paris Tokio, 2003. (Cited on page 10.)

[48] Yashu Liu, Jintang He, Shaoyi Ji, Qingsong Wang, Hai Pu, Tingting Jiang, Lingyao Meng, Xiuwei Yang, and Jianguo Ji. Comparative studies of early liver dysfunction in senescence-accelerated mouse using mitochondrial proteomics approaches. *Mol Cell Proteomics*, 7(9):1737–1747, Sep 2008. doi: 10.1074/mcp.M800109-MCP200. URL http://dx.doi.org/10.1074/mcp.M800109-MCP200. (Cited on page 40.)

[49] Yoichi Minami, Takeya Kasukawa, Yuji Kakazu, Masayuki Iigo, Masahiro Sugimoto, Satsuki Ikeda, Akira Yasui, Gijsbertus T J. van der Horst, Tomoyoshi Soga, and Hiroki R. Ueda. Measurement of internal body time by blood metabolomics. *Proc Natl Acad Sci U S A*, 106(24):9890–9895, Jun 2009. doi: 10.1073/pnas.0900617106. URL http://dx.doi.org/10.1073/pnas.0900617106. (Cited on page 13.)

[50] Robert J. Mishur and Shane L. Rea. Applications of mass spectrometry to metabolomics and metabonomics: detection of biomarkers of aging and of age-related diseases. *Mass Spectrom Rev*, 31(1):70–95, 2012. doi: 10.1002/mas.20338. URL http://dx.doi.org/10.1002/mas.20338. (Cited on pages 8 and 9.)

[51] MIT AgeLab Website, 2012. URL http://agelab.mit.edu/disruptive-demographics. (Cited on page 2.)

[52] Kirstin Mittelstrass, Janina S. Ried, Zhonghao Yu, Jan Krumsiek, Christian Gieger, Cornelia Prehn, Werner Roemisch-Margl, Alexey Polonikov, Annette Peters, Fabian J. Theis, Thomas Meitinger, Florian Kronenberg, Stephan Weidinger, Heinz Erich Wichmann, Karsten Suhre, Rui Wang-Sattler, Jerzy Adamski, and Thomas Illig. Discovery of sexual dimorphisms in metabolic and genetic biomarkers. *PLoS Genet*, 7(8):e1002215, Aug 2011. doi: 10.1371/journal.pgen.1002215. URL http://dx.doi.org/10.1371/journal.pgen.1002215. (Cited on pages 25 and 35.)

[53] Florian L. Muller, Michael S. Lustgarten, Youngmok Jang, Arlan Richardson, and Holly Van Remmen. Trends in oxidative aging theories. *Free Radic Biol Med*, 43(4):477–503, Aug 2007. doi: 10.1016/j.freeradbiomed.2007.03.034. URL http://dx.doi.org/10.1016/j.freeradbiomed.2007.03.034. (Cited on pages 3 and 5.)

[54] Eiji Naru, Yasukazu Takanezawa, Misako Kobayashi, Yuko Misaki, Kazuhiko Kaji, and Kumi Arakane. Increased levels of a particular phosphatidylcholine species in senescent human dermal fibroblasts in vitro. *Hum Cell*, 21(3):70–78, Aug 2008. doi: 10.1111/j.1749-0774.2008.00052.x. URL http://dx.doi.org/10.1111/j.1749-0774.2008.00052.x. (Cited on pages 37 and 45.)

[55] Ekaterina Nevedomskaya, Axel Meissner, Sibel Goraler, Monique de Waard, Yanto Ridwan, Gerben Zondag, Ingrid van der Pluijm, André M. Deelder, and Oleg A. Mayboroda. Metabolic profiling of accelerated aging ercc1 d/- mice. *J Proteome Res*, 9(7):3680–3687, Jul 2010. doi: 10.1021/pr100210k. URL http://dx.doi.org/10.1021/pr100210k. (Cited on page 15.)

[56] Janne Nikkilä, Marko Sysi-Aho, Andrey Ermolov, Tuulikki Seppänen-Laakso, Olli Simell, Samuel Kaski, and Matej Oresic. Gender-dependent progression of systemic metabolic states in early childhood. *Mol Syst Biol*, 4:197, 2008. doi: 10.1038/msb.2008.34. URL http://dx.doi.org/10.1038/msb.2008.34. (Cited on pages 14 and 16.)

[57] Robert C. Noland, Timothy R. Koves, Sarah E. Seiler, Helen Lum, Robert M. Lust, Olga Ilkayeva, Robert D. Stevens, Fausto G. Hegardt, and Deborah M. Muoio. Carnitine insufficiency caused by aging and overnutrition compromises mitochondrial performance and metabolic control. *J Biol Chem*, 284(34):22840–22852, Aug 2009. doi: 10.1074/jbc.M109.032888. URL http://dx.doi.org/10.1074/jbc.M109.032888. (Cited on page 38.)

[58] Nicole Noren Hooten, Kotb Abdelmohsen, Myriam Gorospe, Ngozi Ejiogu, Alan B. Zonderman, and Michele K. Evans. microrna expression patterns reveal differential expression of target genes with age. *PLoS One*, 5(5):e10724, 2010. doi: 10.1371/journal.pone.0010724. URL http://dx.doi.org/10.1371/journal.pone.0010724. (Cited on page 40.)

[59] M. Oresic. Metabolomics, a novel tool for studies of nutrition, metabolism and lipid dysfunction. *Nutr Metab Cardiovasc Dis*, 19(11):816–824, Dec 2009. doi: 10.1016/j.numecd.2009.04.018. URL http://dx.doi.org/10.1016/j.numecd.2009.04.018. (Cited on pages 14 and 20.)

[60] G. Paolisso, M. Barbieri, M. Bonafè, and C. Franceschi. Metabolic age modelling: the lesson from centenarians. *Eur J Clin Invest*, 30(10):888–894, Oct 2000. (Cited on pages 6 and 36.)

[61] Linda Partridge, Janet Thornton, and Gillian Bates. The new science of ageing. *Philos Trans R Soc Lond B Biol Sci*, 366(1561):6–8, Jan 2011. doi: 10.1098/rstb.2010.0298. URL http://dx.doi.org/10.1098/rstb.2010.0298. (Cited on pages 8 and 13.)

[62] Antti Perheentupa and Ilpo Huhtaniemi. Aging of the human ovary and testis. *Mol Cell Endocrinol*, 299(1):2–13, Feb 2009. doi: 10.1016/j.mce.2008.11.004. URL http://dx.doi.org/10.1016/j.mce.2008.11.004. (Cited on pages 3 and 4.)

[63] M D W. Piper, C. Selman, J. J. McElwee, and L. Partridge. Separating cause from effect: how does insulin/igf signalling control lifespan in worms, flies and mice? *J Intern Med*, 263(2):179–191, Feb 2008. doi: 10.1111/j.1365-2796.2007.01906.x. URL http://dx.doi.org/10.1111/j.1365-2796.2007.01906.x. (Cited on page 8.)

[64] Matthew D W. Piper and Andrzej Bartke. Diet and aging. *Cell Metab*, 8(2):99–104, Aug 2008. doi: 10.1016/j.cmet.2008.06.012. URL http://dx.doi.org/10.1016/j.cmet.2008.06.012. (Cited on page 3.)

[65] Nikolaos Psychogios, David D. Hau, Jun Peng, An Chi Guo, Rupasri Mandal, Souhaila Bouatra, Igor Sinelnikov, Ramanarayan Krishnamurthy, Roman Eisner, Bijaya Gautam, Nelson Young, Jianguo Xia, Craig Knox, Edison Dong, Paul Huang, Zsuzsanna Hollander, Theresa L. Pedersen, Steven R. Smith, Fiona Bamforth, Russ Greiner, Bruce McManus, John W. Newman, Theodore Goodfriend, and David S. Wishart. The human serum metabolome. *PLoS One*, 6(2):e16957, 2011. doi: 10.1371/journal.pone.0016957. URL http://dx.doi.org/10.1371/journal.pone.0016957. (Cited on page 8.)

[66] R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2011. URL http://www.R-project.org/. ISBN 3-900051-07-0. (Cited on page 22.)

[67] W. Rathmann, K. Strassburger, M. Heier, R. Holle, B. Thorand, G. Giani, and C. Meisinger. Incidence of type 2 diabetes in the elderly german population and the effect of clinical and lifestyle risk factors: Kora s4/f4 cohort study. *Diabet Med*, 26(12):1212–1219, Dec 2009. doi: 10.1111/j.1464-5491.2009.02863.x. URL http://dx.doi.org/10.1111/j.1464-5491.2009.02863.x. (Cited on page 19.)

[68] Colin Selman and Dominic J. Withers. Mammalian models of extended healthy lifespan. *Philos Trans R Soc Lond B Biol Sci*, 366(1561):99–107, Jan 2011. doi: 10.1098/rstb.2010.0243. URL http://dx.doi.org/10.1098/rstb.2010.0243. (Cited on page 5.)

[69] Andreas Simm and Thomas A. Rando. Tissue ageing: do insights into molecular mechanisms of ageing lead to new therapeutic strategies? *Exp Gerontol*, 43(7):603–604, Jul 2008. doi: 10.1016/j.exger.2008.05.009. URL http://dx.doi.org/10.1016/j.exger.2008.05.009. (Cited on page 3.)

[70] Andreas Simm, Norbert Nass, Babett Bartling, Britt Hofmann, Rolf-Edgar Silber, and Alexander Navarrete Santos. Potential biomarkers of ageing. *Biol Chem*, 389(3):257–265, Mar 2008. doi: 10.1515/BC.2008.034. URL http://dx.doi.org/10.1515/BC.2008.034. (Cited on pages 1, 3, and 4.)

[71] Carolyn M. Slupsky, Kathryn N. Rankin, James Wagner, Hao Fu, David Chang, Aalim M. Weljie, Erik J. Saude, Bruce Lix, Darryl J. Adamko, Sirish Shah, Russ Greiner, Brian D. Sykes, and Thomas J. Marrie. Investigations of the effects of gender, diurnal variation, and age in human urinary metabolomic profiles. *Anal Chem*, 79(18):6995–7004, Sep 2007. doi: 10.1021/ac0708588. URL http://dx.doi.org/10.1021/ac0708588. (Cited on page 16.)

[72] Erica D. Smith, Mitsuhiro Tsuchiya, Lindsay A. Fox, Nick Dang, Di Hu, Emily O. Kerr, Elijah D. Johnston, Bie N. Tchao, Diana N. Pak, K Linnea Welton, Daniel E L. Promislow, James H. Thomas, Matt Kaeberlein, and Brian K. Kennedy. Quantitative evidence for conserved longevity pathways between divergent eukaryotic species. *Genome Res*, 18(4):564–570, Apr 2008. doi: 10.1101/gr.074724.107. URL http://dx.doi.org/10.1101/gr.074724.107. (Cited on page 8.)

[73] Quinlyn A. Soltow, Dean P. Jones, and Daniel E L. Promislow. A network perspective on metabolism and aging. *Integr Comp Biol*, 50(5):844–854, Nov 2010. doi: 10.1093/icb/icq094. URL http://dx.doi.org/10.1093/icb/icq094. (Cited on page 13.)

[74] Tim D. Spector and Frances M K. Williams. The uk adult twin registry (twinsuk). *Twin Res Hum Genet*, 9(6):899–906, Dec 2006. doi: 10.1375/183242706779462462. URL http://dx.doi.org/10.1375/183242706779462462. (Cited on page 22.)

[75] Karsten Suhre and Christian Gieger. Genetic variation in metabolic phenotypes: study designs and applications. *Nat Rev Genet*, 13(11):759–769, Nov 2012. doi: 10.1038/nrg3314. URL http://dx.doi.org/10.1038/nrg3314. (Cited on pages 9, 10, 11, and 12.)

[76] M. Szibor and J. Holtz. Mitochondrial ageing. *Basic Res Cardiol*, 98(4):210–218, Jul 2003. doi: 10.1007/s00395-003-0421-z. URL http://dx.doi.org/10.1007/s00395-003-0421-z. (Cited on page 3.)

[77] Tarek A. Taha, Thomas D. Mullen, and Lina M. Obeid. A house divided: ceramide, sphingosine, and sphingosine-1-phosphate in programmed cell death. *Biochim Biophys Acta*, 1758(12):2027–2036, Dec 2006. doi: 10.1016/j.bbamem.2006.10.018. URL http://dx.doi.org/10.1016/j.bbamem.2006.10.018. (Cited on page 38.)

[78] Georgios A. Theodoridis, Helen G. Gika, Elizabeth J. Want, and Ian D. Wilson. Liquid chromatography-mass spectrometry based global metabolite profiling: a review. *Anal Chim Acta*, 711:7–16, Jan 2012. doi: 10.1016/j.aca.2011.09.042. URL http://dx.doi.org/10.1016/j.aca.2011.09.042. (Cited on page 9.)

[79] Chiharu Tokunaga, Ken-ichi Yoshino, and Kazuyoshi Yonezawa. mtor integrates amino acid- and energy-sensing pathways. *Biochem Biophys Res Commun*, 313(2):443–446, Jan 2004. (Cited on page 8.)

[80] M. J. Toth and A. Tchernof. Lipid metabolism in the elderly. *Eur J Clin Nutr*, 54 Suppl 3: S121–S125, Jun 2000. (Cited on pages 5, 6, and 36.)

[81] UN Department of Economic and Social Affairs Population Division. World population ageing: 1950 – 2050, annex iii profiles of ageing, major areas and regions, 2002. URL http://www.un.org/esa/population/publications/worldageing19502050/index.htm. (Cited on page 2.)

[82] United Nations, Department of Economic and Social Affairs, Population Division. Population ageing and development 2012, 2012. URL http://www.un.org/esa/population/publications/2012WorldPopAgeingDev_Chart/2012PopAgeingandDev_WallChart.pdf. (Cited on page 2.)

[83] J. W. Vaupel. Biodemography of human ageing. *Nature*, 464(7288):536–542, 2010. URL PM:20336136. DA - 20100325 IS - 1476-4687 (Electronic) IS - 0028-0836 (Linking) LA - eng PT - Journal Article PT - Research Support, N.I.H., Extramural PT - Research Support, Non-U.S. Gov't PT - Review SB - IM. (Cited on page 2.)

[84] Nawaporn Vinayavekhin, Edwin A. Homan, and Alan Saghatelian. Exploring disease through metabolomics. *ACS Chem Biol*, 5(1):91–103, Jan 2010. doi: 10.1021/cb900271r. URL http://dx.doi.org/10.1021/cb900271r. (Cited on pages 10 and 11.)

[85] M. Virgili, D. Necchi, E. Scherini, and A. Contestabile. Increase of the ornithine decarboxylase/polyamine system and transglutaminase upregulation in the spinal cord of aged rats. *Neurosci Lett*, 309(1):62–66, Aug 2001. (Cited on page 40.)

[86] Yulan Wang, Dennis Lawler, Brian Larson, Ziad Ramadan, Sunil Kochhar, Elaine Holmes, and Jeremy K. Nicholson. Metabonomic investigations of aging and caloric restriction in a life-long dog study. *J Proteome Res*, 6(5):1846–1854, May 2007. doi: 10.1021/pr060685n. URL http://dx.doi.org/10.1021/pr060685n. (Cited on page 15.)

[87] Rui Wang-Sattler, Yao Yu, Kirstin Mittelstrass, Eva Lattka, Elisabeth Altmaier, Christian Gieger, Karl H. Ladwig, Norbert Dahmen, Klaus M. Weinberger, Pei Hao, Lei Liu, Yixue Li, H-Erich Wichmann, Jerzy Adamski, Karsten Suhre, and Thomas Illig. Metabolic profiling reveals distinct variations linked to nicotine consumption in humans–first results from the kora study. *PLoS One*, 3(12):e3863, 2008. doi: 10.1371/journal.pone.0003863. URL http://dx.doi.org/10.1371/journal.pone.0003863. (Cited on page 32.)

[88] Klaus M. Weinberger, Armin Graber, and Jörg Katzenberger. Targeted metabolomics. *BIOspektrum*, 02:231–232, 2006. (Cited on pages 10 and 12.)

[89] Geoffrey B. West and Aviv Bergman. Toward a systems biology framework for understanding aging and health span. *J Gerontol A Biol Sci Med Sci*, 64(2):205–208, Feb 2009. doi: 10.1093/gerona/gln066. URL http://dx.doi.org/10.1093/gerona/gln066. (Cited on page 13.)

[90] R. E. Williams, E. M. Lenz, J. S. Lowden, M. Rantalainen, and I. D. Wilson. The metabonomics of aging and development in the rat: an investigation into the effect of age on the profile of endogenous metabolites in the urine of male rats using 1h nmr and hplc-tof ms. *Mol Biosyst*, 1(2):166–175, Jul 2005. doi: 10.1039/b500852b. URL http://dx.doi.org/10.1039/b500852b. (Cited on page 15.)

[91] David S. Wishart, Craig Knox, An Chi Guo, Roman Eisner, Nelson Young, Bijaya Gautam, David D. Hau, Nick Psychogios, Edison Dong, Souhaila Bouatra, Rupasri Mandal, Igor Sinelnikov, Jianguo Xia, Leslie Jia, Joseph A. Cruz, Emilia Lim, Constance A. Sobsey, Savita Shrivastava, Paul Huang, Philip Liu, Lydia Fang, Jun Peng, Ryan Fradette, Dean Cheng, Dan Tzur, Melisa Clements, Avalyn Lewis, Andrea De Souza, Azaret Zuniga, Margot Dawe, Yeping Xiong, Derrick Clive, Russ Greiner, Alsu Nazyrova, Rustem Shaykhutdinov, Liang Li, Hans J. Vogel, and Ian Forsythe. Hmdb: a knowledgebase for the human metabolome. *Nucleic Acids Res*, 37(Database issue):D603–D610, Jan 2009. doi: 10.1093/nar/gkn810. URL http://dx.doi.org/10.1093/nar/gkn810. (Cited on page 10.)

[92] Shi Fang Yan, Vivette D'Agati, Ann Marie Schmidt, and Ravichandran Ramasamy. Receptor for advanced glycation endproducts (rage): a formidable force in the pathogenesis of the cardiovascular complications of diabetes & aging. *Curr Mol Med*, 7(8):699–710, Dec 2007. (Cited on page 3.)

[93] Shikai Yan, Bin Wu, Zhongying Lin, Huizi Jin, Jianhua Huang, Yun Yang, Xinmin Zhang, Ziyin Shen, and Weidong Zhang. Metabonomic characterization of aging and investigation on the anti-aging effects of total flavones of epimedium. *Mol Biosyst*, 5(10):1204–1213, Oct 2009. doi: 10.1039/b816407j. URL http://dx.doi.org/10.1039/b816407j. (Cited on pages 11, 15, 39, and 40.)

[94] Zhonghao Yu, Gabi Kastenmüller, Ying He, Petra Belcredi, Gabriele Möller, Cornelia Prehn, Joaquim Mendes, Simone Wahl, Werner Roemisch-Margl, Uta Ceglarek, Alexey Polonikov, Norbert Dahmen, Holger Prokisch, Lu Xie, Yixue Li, H-Erich Wichmann, Annette Peters, Florian Kronenberg, Karsten Suhre, Jerzy Adamski, Thomas Illig, and Rui Wang-Sattler. Differences between human plasma and serum metabolite profiles. *PLoS One*, 6(7):e21230, 2011. doi: 10.1371/journal.pone.0021230. URL http://dx.doi.org/10.1371/journal.pone.0021230. (Cited on page 44.)

[95] Guangju Zhai, Rui Wang-Sattler, Deborah J. Hart, Nigel K. Arden, Alan J. Hakim, Thomas Illig, and Tim D. Spector. Serum branched-chain amino acid to histidine ratio: a novel metabolomic biomarker of knee osteoarthritis. *Ann Rheum Dis*, 69(6):1227–1231, Jun 2010. doi: 10.1136/ard.2009.120857. URL http://dx.doi.org/10.1136/ard.2009.120857. (Cited on pages 12 and 32.)

[96] Xianrong Zhang, Huilang Liu, Junfang Wu, Xu Zhang, Maili Liu, and Yong Wang. Metabonomic alterations in hippocampus, temporal and prefrontal cortex with age in rats. *Neurochem Int*, 54(8):481–487, Jul 2009. doi: 10.1016/j.neuint.2009.02.004. URL http://dx.doi.org/10.1016/j.neuint.2009.02.004. (Cited on page 15.)

[97] Xiaochun Zhu, Molly M. Gallogly, John J. Mieyal, Vernon E. Anderson, and Lawrence M. Sayre. Covalent cross-linking of glutathione and carnosine to proteins by 4-oxo-2-nonenal. *Chem Res Toxicol*, 22(6):1050–1059, Jun 2009. doi: 10.1021/tx9000144. URL http://dx.doi.org/10.1021/tx9000144. (Cited on page 40.)

COLOPHON

This document was typeset using the typographical look-and-feel `classicthesis` for LaTeX typesetting program developed by André Miede. The style was inspired by Robert Bringhurst's seminal book on typography *"The Elements of Typographic Style"*.

Final Version as of July 1, 2015 (`classicthesis` version 1.0).

B

DECLARATION – EIDESSTATTLICHE VERSICHERUNG

Ich erkläre an Eides statt,
dass ich die vorliegende Dissertation mit dem Thema

"Metabolomics and Aging"

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

München/Neuherberg, den

Paula Singmann