Aus der

Kinderklinik und Kinderpoliklinik im Dr. von Haunerschen Kinderspital

Klinikum der Ludwig-Maximilians-Universität München

The proteome of newborns with maternal obesity

Dissertation

zum Erwerb des Doktorgrades der Medizin

an der Medizinischen Fakultät

der Ludwig-Maximilians-Universität München

vorgelegt von

Nadja van Hagen

aus

Den Haag, Niederlande

Jahr

2025

Mit Genehmigung der Medizinischen Fakultät der

Ludwig-Maximilians-Universität zu München

Erstes Gutachten:	Prof. Dr. Orsolya Genzel-Boroviczény
Zweites Gutachten:	Prof. Dr. med. Regina Ensenauer
Drittes Gutachten:	Prof. Dr. Susanna Hofmann
Dekan:	Prof. Dr. med. Thomas Gudermann
Tag der mündlichen Prüfung:	30.07.2025

Inhaltsverzeichnis

Zusammenfassung:

Hintergrund

Die Prävalenz von Übergewicht inlk. Adipositas hat in den letzten Jahren rapide zugenommen. Veränderungen in der intrauterinen Umgebung aufgrund von mütterlichem Übergewicht wirken sich nach der Geburt auf die Gesundheit der Kinder aus. Dies führt insbesondere bereits in der Kindheit zu Adipositas und einem höheren Risiko für die Entwicklung von Herz-Kreislauf-Erkrankungen und Diabetes mellitus im späteren Kinder- und Erwachsenenalter. In dieser Studie haben wir das Proteom von Neugeborenen mit mütterlicher Adipositas mit dem Proteom von Neugeborenen mit normalem mütterlichem Gewicht verglichen. Unser Ziel war es, betroffene Proteine und Veränderungen in den Signalwegen zu identifizieren, um somit zu einem besseren Verständnis der Pathophysiologie zu gelangen und zu einer anderen Perspektive beizutragen welche als Grundlage für einen neuen Ansatz für Präventionsprogramme dienen könnten.

Material und Methoden

Zwischen Februar 2017 und Juni 2019 haben wir 15 Neugeborene mit mütterlicher Adipositas, definiert als mütterlichem BMI > 30 m2/kg zu Beginn der Schwangerschaft, und 344 Neugeborene mit mütterlichem BMI < 25 m2/kg eingeschlossen. Die medizinische Vorgeschichte und die anthropometrischen Daten wurden der Patientenakte und einem Fragebogen entnommen. Die Vollblutprobe wurde auf einer Trockenblutkarte gesammelt und die proteomische Analyse wurde mittels Massenspektrometrie am Max-Planck-Institut durchgeführt. Anschließend wurden die proteomischen sowie auch die deskriptiven Patientendaten statistisch analysiert.

Ergebnisse

Die Analyse der proteomischen Daten zeigte einen höheren Proteinspiegel des Ribosomal Protein S21 und Kallistatin bei der Neugeborenen mit mütterlicher Adipositas im Vergleich zu den Neugeborenen mit normalem mütterlichem Gewicht. Dahingegen zeigte sich im Proteom der Neugeborenen mit normalem mütterlichem Gewicht einen höheren Spiegel des Prostaglandin-E2- Rezeptor EP.

Diskussion

Die Ergebnisse dieser Studie legen nahe, dass mütterliche Adipositas während der Schwangerschaft zu Veränderungen bestimmter Proteinspiegel beim Neugeborenen führen kann. Da unsere initiale Ergebnisse nach FDR Korrektur, vermutlich aufgrund der kleinen Stichprobengröße, nicht standhielten, sind weitere Studien mit einer größeren Stichprobengröße erforderlich. Darüber hinaus wäre eine Follow up Studie interessant, um zu sehen ob die Veränderungen beim Neugeborenen möglicherweise sogar bis zum Erwachsenenalter bestehen bleiben.

Abstract:

Background

The prevalence of overweight including obesity has been growing rapidly over the past years. Changes in the intrauterine environment due to maternal overweight incl. obesity impact health after birth. Leading specifically to child- and adulthood obesity and a higher risk of developing cardiovascular and metabolic diseases. In this study, we compared the proteome of full-term newborns with maternal overweight and obesity during pregnancy, with the proteome of newborns with normal maternal weight using liquid chromatography-mass spectrometry. We aimed to identify affected proteins and differently regulated signaling pathways caused by the different intrauterine environment of mothers with overweight and obesity. We aimed to provide a different perspective and potentially new target for prevention programs.

Material and methods

Between February 2017 and June 2019, we included 15 newborns with maternal obesity, defined as a maternal BMI > 30 m^2/kg at the beginning of pregnancy and 344 newborns with maternal BMI < 25 m^2/kg. Medical history and anthropometric data were taken from the maternal medical records as well as from a questionnaire. The blood sample was collected on a dried blood spot card and the proteomic analysis was performed by Mass Spectrometry at the Max Planck Institute. Afterwards, the proteomic and descriptive patient data were statistically analyzed.

Results

Analysis of the proteomic data showed a notably higher abundance of ribosomal protein S21 and Kallistatin in newborns with maternal obesity. Contrarily, Prostaglandin E2 receptor EP3 was less abundant in newborns with maternal obesity compared to newborns with normal maternal weight.

Conclusions

Based on this study we propose that maternal obesity during pregnancy may cause changes in certain protein levels in the newborn. Since our initial results did not hold up after FDR correction, presumably due to the small sample size, further research with a larger sample size is necessary. Additionally, a follow-up study would be interesting to see if the changes in the newborn persist maybe even up until adulthood.

Abbildungsverzeichnis

Tabellenverzeichnis

Abkürzungsverzeichnis

ATGL ..... **Adipose triglyceride lipase**

ATP ..... **Adenosine triphosphate**

BMI ..... **Body Mass Index**

cAMP ..... **Cyclic adenosine monophosphate**

COX-2 ..... **Cyclooxygenase-2**

CRP ..... **C-reactive protein**

CT ..... **Cytotoxic T cells**

CVD ..... **Cardiovascular disease**

DBS ..... **Dried blood spots**

EP3 ..... **E2 receptor subtype**

FDR ..... **False discovery rate**

FFA ..... **Free fatty acid**

GDM ..... **Gestational diabetes mellitus**

HDL ..... **High density lipoprotein**

HELLP ..... **Haemolysis, Elevated Liver enzymes, Low Platelet**

HFD ..... **High-fat diet**

HSL ..... **Hormone sensitve lipase**

IL ..... **Interleukin**

IQTIG ..... **Institute of Quality assurance and Transparency in Healthcare**

JNK ..... **C-jun-N-terminal kinase**

KS-TG ..... **Kallistatin-transgenic**

LC-MS ..... **Liquid chormatography-mass spectrometry**

LEF ..... **Lymphoid enhancer factor**

MAPK ..... **Mitogen-activated protein kinase**

MGL ..... **Monoglyceride lipase**

MODY ..... **Maturity-onset diabetes of the young**

MS ..... **Mass spectrometry**

NK ..... **Natural killer cells**

OR ..... **Odds ratio**

PGE2 ..... **Prostaglandin E2**

PKA ..... **Protein kinase A**

PPARy.....**Peroxisome proliferator-activated receptor γ**

PTGER3 .....**Prostaglandin E2 receptor subtype 3**

Raf.....**Rapidly accelerated fibrosarcoma**

RPS21 ..... **Ribosomal Protein S21**

RR.....**Relative ratio**

SD..... **Standard deviation**

TCF..... **T-cell factor**

TNF-α.....**Tumor necrosis factor alpha**

UNC13D ..... **Protein unic-13 homolog**

VEGF ..... **Vascular endothelial growth factor**

WHO.....**World health organisation**

1. Introduction

1.1 Obesity

In the past three decades, the prevalence of obesity has risen tremendously in countries of all income levels [1, 2]. The World Health Organization (WHO) estimates that 59% of adults are living with overweight including obesity, most of them from the WHO European and American Region. The Mediterranean and eastern European countries have the highest numbers of overweight and obesity. Germany ranks 24th out of 53 member states in the European Region in number of adults living with obesity [3].

This increase of obesity is not just noticeable in adulthood. The prevalence of childhood obesity is also rising alarmingly fast. The WHO reported that in 2016 more than 340 million children and adolescents were overweight or obese. This number has risen drastically from a global prevalence of 4% in 1975 to just over 18% in 2016. The numbers are even more alarming when we just look at the Western Pacific (24.9% children with obesity), Europe (26.2%), and the Americas (33.6%). The prevalence of overweight including obesity in the European Region is highest among the age group 5-9 years, with 29.5% [3, 4].

Obesity is linked to an increased risk for many noncommunicable diseases and the fast rise of both child- and adulthood obesity naturally also led to an increase in comorbidities [5-7]. The main comorbidities include type 2 diabetes, different types of cancer, chronic kidney disease, and most importantly cardiovascular diseases [4, 7].

1.2 Cardiovascular disease

According to the World Health Organization, deaths due to cardiovascular disease account for almost 32% of all deaths globally, making this the most common cause of death globally. Cardiovascular disease (CVD) is the universal term for all conditions involving the heart and blood vessels, including coronary artery disease, cerebrovascular disease, peripheral arterial disease as well as deep vein thrombosis but also rheumatic and congenital heart diseases. Most deaths related to cardiovascular disease are due to arterial thrombotic events leading to, coronary artery disease, myocardial infarction, or ischemic stroke [8].

The main pathophysiological process behind arterial thrombotic events is atherosclerosis. Atherosclerosis is a progressive disease in which atheromatous plaques, consisting of lipids, calcium, and fibrous elements, accumulate in the large arteries [9]. Atherosclerosis is a long process, developing over several years, with the first lesions in the tunica intima being visible in the aortas and coronary arteries of children as young as 7-9 years old [10]. The first change in the arterial wall is the subendothelial accumulation of oxidized lipoproteins, triggering an inflammatory response. Monocytes adhere to the surface of the endothelium, after which they transmigrate into the tunica intima. There, the monocytes differentiate into macrophages, proliferate, and take up lipoproteins, forming ‘foam cells’ [11]. In time, the foam cells die, further stimulating the inflammatory process.

Additionally, smooth muscle cells migrate from the tunica media to the tunica intima, in response to cytokines and growth factors secreted by macrophages and T-Cells. Fibrous plaques develop with the secretion of fibrous elements by the smooth muscle cells. Furthermore, atherosclerosis and its clinical manifestation are also affected by vascular calcification. Arterial calcification can occur in both the tunica intima and tunica media. The first step appears to be the transformation of vascular smooth muscle cells to an osteoblast/chondrocytic phenotype. These cells then stimulate mineralization by secreting matrix proteins and vesicles as well as through apoptosis [12]. Medial or circumferential calcification can lead to reduced compliance due to arterial stiffening, resulting in impaired vasodilation during ischemia. Medial calcification of the aorta will lead to increased pulse wave velocity, high pulse pressure, and systolic hypertension.

Even though the progressive enlargement of the atheroma into the lumen of the artery combined with increasing intimal vascular calcification can lead to arterial stenosis, most acute ischemic events occur after ulceration of the atheroma. When the fibrous cap covering the atheroma ruptures, collagen and tissue factor are exposed, activating platelets and the coagulation cascade causing accelerated coagulation and the formation of a thrombus.

Multiple risk factors for developing arterial thrombosis and consequently cardiovascular diseases have been identified. Obesity was found to be one of the main risk factors, along with well-known obesity-related comorbidities like arterial hypertension, raised glucose levels, and dyslipidemia [13-17].

1.3 Measuring obesity

Adult obesity is commonly measured through body height- and weight and expressed as BMI in kg/m^2. As height and weight measurements are noninvasive and easily performed, BMI remains recognized as the most practical approach, even though it does not provide a direct measure of adiposity as it does not distinguish between fat and other tissue. BMI does however correlate with total body fat [18], ill health [19] and total abdominal adipose tissue [20]. Waist-to-hip ratio and waist circumference are less commonly used to measure obesity, although they have shown a similar association with morbidity and mortality later in life as BMI [21, 22]. In children and adolescents, BMI is also the most used way of measuring obesity. Due to changes in growth during this period in life, different BMI thresholds for sex and age must be used. These thresholds are visualized in sex specific child growth reference curves and the weight status is defined by deviance from the mean. The WHO classifies overweight as +1 SD above mean and obesity as +2 SD above mean, as that is respectively equivalent to 25 kg/m^2 and 30 kg/m^2 at age 19 [23].

1.4 Adipose tissue

Adipose tissue consists of several different cells. The majority are adipocytes, but adipose tissue contains preadipocytes, macrophages, endothelial cells, blood cells and fibroblasts as well [24, 25]. Adipose tissue is usually classified into white, brown and beige (or ‘brown in white’) adipose tissue [26]. Brown adipose tissue is composed of thermogenic adipocytes with

high mitochondrial content, that have a high capacity of liquid oxidation [27]. Beige adipose tissue describes tissue composed of thermogenic adipocytes located within white adipose tissue. These 'brown adipocytes' appearing in white adipose tissue, derive from a cell line closer to the white adipocyte cell lineage, i.e. may have undergone transdifferentiation from white adipocytes to brown [28]. Lastly, the white adipose tissue specializes in fat storage and release. Additionally, white adipocytes secrete paracrine and endocrine regulators, called adipokines, regulating appetite, energy substrate homeostasis and cardiovascular function [29].

Adipose tissue mass can be increased by adipocyte hyperplasia and/or adipocyte hypertrophy, leading to obesity [29, 30]. Adipocyte hypertrophy is linked to obesity associated pathology like dyslipidemia, inflammation, and impaired glucose homeostasis, possibly leading to insulin resistance, cardiovascular disease, and other obesity-associated pathology[31, 32]. The 'adipocyte expendability hypothesis' states that adipocytes have an individually defined limit of expansion. When this limit is reached, it leads to ectopic lipid deposition, macrophage infiltration, systemic inflammation, altered adipokine profiles, hypoxia, and fibrosis [29, 33]. Some individuals with obesity can be metabolically 'healthy', i.e. show no dyslipidemia, insulin resistance, non-alcoholic fatty liver disease or metabolic syndrome [34]. Klöting et al. set out to determine the role of adipose tissue (dys-)function in the development of either metabolically healthy or unhealthy obesity. They found that metabolically healthy individuals with obesity showed smaller adipocyte size, less immune cell infiltration into visceral fat depots and higher serum adiponectin levels [32]. This might give some insight into the role of adipose tissue dysfunction in the development of metabolically unhealthy obesity. However, it remains not fully understood how to predict whether the adipose tissue will act towards a metabolically healthy or unhealthy obese phenotype.

Most individuals with obesity fall into the category of metabolically unhealthy obesity, although exact prevalence numbers are difficult to interpret due to the lack of a standardized definition. Based on comparing different studies, Blüher et al. estimated a prevalence ranging from 80-90% [35].

Generally speaking, metabolically unhealthy individuals with obesity show elevated blood glucose levels, higher triglyceride levels, higher HDL-cholesterol levels, lower levels of leptin and resistin, higher levels of adiponectin and elevated levels of inflammatory cytokines like, tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP) and multiple interleukins[30, 36, 37].

1.5 Obesity prevention

Most of the risk factors for obesity seem preventable by changes in lifestyle. Health care providers across the globe have tried to stop the global increase of obesity with extensive prevention programs targeting adult lifestyle factors [15] but unfortunately appear to be insufficient [38].

The prevalence of metabolic syndrome, defined as the association of insulin resistance, glucose intolerance, hypertension, and characteristic dyslipidemia [39], is already high among children and adolescents with obesity, and it increases directly with the degree of obesity [40].

Biomarkers associated with increased risk of cardiovascular disease, like low levels of adiponectin, high c-reactive protein, and elevated levels of interleukin-6 (IL-6), are already present in childhood overweight and obesity [40].

Therefore, it seems that the prevention programs targeting adolescent and adult lifestyle may not be targeting the right audience. Prevention programs directed at younger children are already a great way to reduce the risk of comorbidities and complications later in life, but since the prevalence of obesity is still rising, it seems that a better insight into the development of childhood obesity is necessary.

1.6 Fetal origin of disease

Several studies show a connection between a hyperglycemic intrauterine environment, or the intrauterine exposition to nonglucose metabolic abnormalities of obesity, and an increased risk of obesity and/or diabetes in both early and late childhood as well as adult life [41-46]. This forces us to also look at the perinatal period as a highly influential time in the etiology of obesity and the metabolic syndrome in children and adolescents and consequently in the etiology of adult obesity as well as metabolic and cardiovascular disease [47, 48].

Changes in the intrauterine environment cause a mechanism known as fetal programming, which can have a long-term impact on health after birth [49]. This makes the ‘fetal origin of disease hypothesis’ an interesting framework for further understanding the development of obesity, diabetes, and consequently cardiovascular diseases in child- as well as adulthood, but also for creating or improving prevention programs targeting these non-communicable diseases.

About thirty years ago, Barker et al. first published their papers proposing the 'Barker Hypothesis'. This ‘fetal origin of disease hypothesis’ links pregnancy complications or fetal development deficits with an infant's risk of developing metabolic and cardiovascular diseases in adult life [50-53]. In the years that followed, a growing number of studies confirmed and expanded this hypothesis [41, 53]. For example, low birth weight has repeatedly been linked to raised blood pressure and type 2 diabetes later in life [55-57]. Later studies revealed that both low and high birthweight increased the risk of type 2 diabetes[57, 58]. It is important to note, that it is not the birthweight per se that increases the risk of obesity, metabolic syndrome, and cardiometabolic diseases in later life, but that the different intrauterine conditions caused fetal programming resulting in high or low birth- weight.

1.7 Maternal obesity

According to data from the Institute of Quality Assurance and Transparency in Healthcare (IQTIG) the prevalence of overweight and obesity among pregnant women in Germany has continuously grown over the past decades. In 2021 43% of pregnant women were overweight or obese (BMI > 25 kg/m2). Of those women, 18.55 % had a Body Mass Index > 30 kg/m2, whereas in 2005 the percentage of pregnant women with a BMI > 30 kg/m2 was only 12%. [59, 60].

Obesity during pregnancy comes with an elevated risk of morbidity for both mother and child. Mothers with obesity are more likely to develop cardiovascular and metabolic complications such as thromboembolisms or gestational diabetes[61, 62]. Newborns of women with obesity are more likely to be large-for-gestational age, develop congenital abnormalities and even insulin resistance in utero and consequently hyperinsulinemia postpartum [61, 63-65].

Interestingly, maternal obesity does not only seem to have a direct effect on their newborns, but the effect seems to extend into child- and adulthood of the offspring. The connection between maternal obesity and their children's risk of obesity, diabetes, and cardiovascular diseases later in life, has been of growing interest. Research shows an association between maternal obesity and a higher risk of developing childhood obesity in the offspring[66, 67]. For example, Gaillard et al. found that the adolescents of mothers with a higher prepregnancy BMI showed a bigger waist circumference and a higher hip-to-waist-ratio [68].

But it seems like the offspring of mothers with obesity are not only at a higher risk of developing obesity, but also of negative cardiovascular and metabolic outcomes. In the years that followed, multiple studies found recurrent evidence towards the association that maternal obesity as well as excessive gestational weight gain, especially in early pregnancy, leads to higher blood pressure, dyslipidemia and insulin resistance in childhood [68-71]. Reynolds et al. described a 1.3 times higher risk of hospital admissions due to cardiovascular events in adult offspring of mothers with obesity[72]. Similarly, Eriksson et al. described that adult offspring of mothers with obesity were significantly more likely to develop cardiovascular disease, coronary heart disease, type 2 diabetes, and stroke[73].

1.8 Maternal diabetes

Interestingly, not only overweight and obesity itself has been associated with changes and adverse outcomes in the offspring. Several studies suggest that exposure to a hyperglycemic uterine environment due to maternal diabetes, either type 1, 2 or gestational, seems to influence the offspring as well.

Overweight including obesity during pregnancy comes with a higher risk of developing gestational diabetes. A meta-analysis of 20 studies calculated ORs of 2.14, 3.56 and 8.56 for developing gestational diabetes mellitus among pregnant women with overweight, obesity and severe obesity respectively[74]. In 2017 5.9% of pregnant women delivering their babies in German hospitals were diagnosed with gestational diabetes mellitus. Since 2002, this number has continuously grown[75].

Overweight and obesity in the non-pregnant population has extensively been linked to the development of type 2 diabetes. A meta-analysis of 18 prospective cohort studies calculated overall RRs of 2.99 and 7.19 for developing diabetes as individuals with a BMI over 25 and over 30, respectively [76]. Naturally, chances are higher that a woman with a BMI over 25 has already developed type 2 diabetes, even before becoming pregnant.

Clausen et al. compared 597 children of either diet-treated gestational diabetes mellitus (GDM) or type 1 diabetes during pregnancy at 22 years of age and found an increased risk of type 2 diabetes and impaired glucose tolerance in young adults. They found an eight times higher risk in children with maternal GDM, while maternal type 1 diabetes increased the risk four times [77]. In Scotland, Lindsay et al. showed a significant effect of maternal type 1 diabetes on waist circumference, overweight, and obesity at 7 years of age [78]. Furthermore, exposure to a hyperglycemic intrauterine environment was shown to increase the penetrance of maturity-onset diabetes of the young (MODY) in HNF-1α gene mutation carriers [79]. Gillman et al. studied the effect of intensive treatment of mild GDM on the BMI of 4- to 5-year-olds. Interestingly, they did observe a reduced number of macrosomic neonates but no change in BMI at age 4-5 [80]. Pirkola et al. found that maternal gestational hyperglycemia by itself had only a small effect, whereas the combination of GDM with maternal pre-pregnancy obesity had a significant effect on overweight and obesity in their offspring[81]. Furthermore, Boney et al. demonstrated that not only did the macrosomic offspring of diabetic mothers have a higher risk of developing metabolic syndrome in childhood; children exposed to maternal obesity had an increased risk of developing metabolic syndrome as well [82].

This adds weight to the hypothesis suggesting a role for the periconceptional environment in fetal programming and indicates that nonglucose metabolic abnormalities of obesity like lipids and fatty acids may also play a part.

By finding critical influences in fetal programming, we will find new opportunities for targeted pre- or periconceptional and gestational prevention strategies, that hopefully will decrease the risk of obesity and metabolic syndrome in childhood and adolescence, thereby reducing the risk of cardiovascular disease in adult life.

1.9 Proteomics and Mass Spectrometry

The molecular process associated with the long-term effects of being exposed to a hyperglycemic intrauterine environment is believed to be based in the proteome composition of the infant.

The human blood consists of cellular components and a liquid portion. The cellular components are lymphocytes, thrombocytes, and erythrocytes. The liquid portion is called plasma and consists of electrolytes, small molecules, lipids, and proteins. The plasma proteins have different levels of abundance, and as such can be divided into three groups [83]. The first group consists of functional plasma proteins. These proteins have a high level of abundance. Functional plasma proteins include human serum albumin, acute phase proteins of the innate immune response like C-reactive Protein and ferritin, apolipoproteins, and coagulation proteins. The second protein group is tissue leakage proteins, such as alanine aminotransferase, aspartate aminotransferase, or cardiac troponins. The last protein group, with the lowest level of abundance, are signaling proteins like cytokines and small protein hormones (Figure 1). The proteome from whole blood samples, such as from a dried blood spot, will also contain proteins from erythrocytes (like hemoglobin), thrombocytes and leukocytes [101].

In clinical practice, the quantitative analysis of individual plasma proteins is usually done by immunoassays or enzymatic essays against a single target. These techniques have inherent limitations, for example, their specificity for protein isoforms and consequently their incompatibility with hypothesis-free investigations. In contrast, mass spectrometry (MS)-based proteomics analyzes all proteins in the sample – the proteome – and is, therefore, hypothesis-free and unbiased. Proteome analysis by mass spectrometry allows the identification of all protein proteoforms, including relative ratio determination and subsequentially systemic signaling pathways, without necessary prior linkage of a specific protein to a specific disease making it a promising tool for discovering biomarkers in plasma [84, 85].

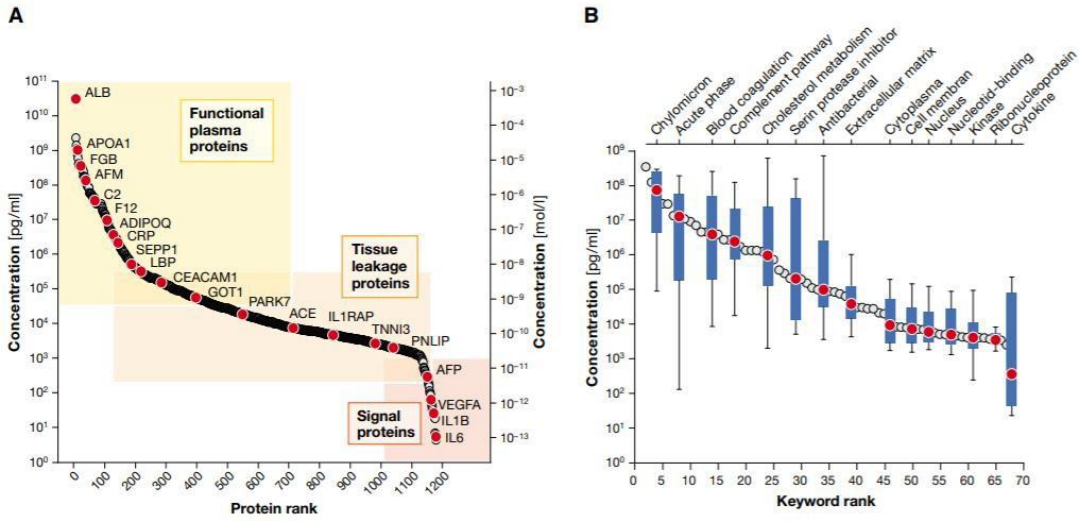

Figure 1. Blood-based laboratory testing in a clinical setting.

(A) Concentration range of plasma proteins with the gene names of several illustrative blood proteins (red dots). Concentrations are in serum or plasma and measured with diverse methods as retrieved from the plasma proteome database in May 2017 (http://www.plasmaproteomedatabase.org/) (Nanjappa et al, 2014). (B) Bioinformatic keyword annotation of the plasma proteome database. The blue boxplots with the 10–90% whiskers visualize the range of diverse proteins contributing to distinct functions. *Graph and Text from: Geyer et al. (2017) Creative Commons Licence. Copywright Geyer* [82].

Nonetheless, MS-based proteomics is challenging in its own way for several reasons, but mostly due to the high dynamic range of protein abundance and lack of reproducible, high-throughput proteomic workflows. The wide dynamic range presents a barrier to the detection of low and medium abundant proteins in mass spectrometry [86]. To deal with this problem, two strategies are available: depletion and fractionation. Depletion works through columns containing immobilized antibodies, removing some of the most abundant plasma proteins. These antibodies are unfortunately not entirely specific and some proteins may already be bound to carrier proteins such as human serum albumin [87, 88]. This means that the plasma sample after depletions cannot be considered a quantitative representation of the original. Additionally, mass spectrometry has been combined with extensive peptide fractionation methods to increase resolving power and further discriminate between different protein isoforms. While fractionation can be performed in various ways, strong cation exchange with a salt gradient or salt plug is the most frequently used fractionation technique in proteomics [89, 90]. On the downside, fractionation decreases throughput and thus prolongs the analysis, which is undesirable in clinical practice.

As our main analytical tool, we used liquid-chromatography-mass spectrometry. Liquid chromatography-mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry (MS). We used a previously designed LC-MS workflow for shotgun plasma proteomics [91]. This workflow consists of three phases. The first phase is sample preparation, which takes less than 2 hours including protein digestion. Partly because depletion was omitted and partly because experiments had shown adequate protein digestion had occurred after as soon as just one hour. During the second phase, liquid mass spectrometry is performed. It turned out, that the number of identified proteins only increased very little after 20 minutes of analysis, thus setting the standard single-run gradient type at 20 minutes. The final phase of this workflow is quantitative data analysis, taking about half an hour per sample [91].

Although this workflow as described above, was originally designed for plasma analysis, it is also applicable for dried blood spot analytics.

The analysis of dried blood spots (DBS) has become increasingly routine in pharmaceutical and clinical communities. The first biomedical application of DBS on filter paper dates back to 1963 when Professor Robert Guthrie introduced this alternative sampling method for the detection of phenylketonuria in the newborn population [92]. Today, the most famous usage of dried blood spot analytics is probably in the newborn heel prick screening.

There are multiple advantages of dried blood spot analytics compared to blood plasma analytics. For example, dried blood spot sampling is less invasive, since a finger or heel prick collecting about 20 µL on the filter paper will suffice [93, 94]. Also, analytes preserved in dried blood spots can be stable for years if stored adequately [95-97] Lastly, since most pathogenic agents become deactivated during drying the risk of infection is reduced [97-99]. Dried blood spot mass spectrometry was initially almost entirely limited to the analysis of small metabolites, but in the past years, an increasing number of studies have focused on protein analysis by mass spectrometry in dried blood spots [93, 101]. Preparing the DBS for mass spectrometry can be done by stenciling out a small area of the DBS and extracting the proteins with an extraction solvent [101].

1.10 Aim of the study

In this study, we aim to systematically compare proteome compositions in full-term newborns of mothers with obesity, with the proteome composition of newborns of mothers without obesity using a predesigned workflow for liquid chromatography-mass spectrometry. With this case-control study design, we aim to identify differences in proteome composition in newborns of affected mothers and thus identifying the affected proteins and the possibly differently regulated signaling pathways.

2. Materials und Methods

The work was approved by the Ludwig Maximillian University Munich Ethical Committee. Written informed consent was obtained from at least one custodial parent after birth, before blood sampling.

2.1 Study population

From February 2017 through June 2019, we included newborns born at the LMU Frauenklinik Maistraße of the Ludwig Maximillian Universität München. The inclusion phase consisted of three separate phases. First, from February 2017 until May 2018, the focus was exclusively on recruiting preterm newborns. Secondly, from May 2018 until December 2018 we aimed to recruit all newborns (term as well as preterm) that were born in that period at the LMU Frauenklinik Maistraße. Because of the small number of cases, from December 2018 until June 2019 we aimed to include additional cases. During this last phase, we did not include all newborns but included only newborns that were either preterm with a gestational age < 30 weeks, or term newborns with neonatal sepsis, perinatal asphyxia and/or maternal diabetes, or term newborns that were small for gestation with a birthweight < 10. percentile (Table 1).

Recruitment phase	**Inclusion criteria**
February 2017 until May 2018	Gestational age < 37 weeks
May 2018 until December 2018	All births, regardless of gestational age
December 2018 until June 2019	1. Preterm (gestational age < 30 weeks) 2. Neonatal sepsis 3. Perinatal asphyxia 4. Maternal diabetes 5. Small for gestational age

Table 1. Inlcusion criteria

Because the focus of this dissertation was the proteome of newborns with maternal obesity, I determined specific exclusion criteria catering to this focus.

For the analysis that is the focus of this dissertation, we excluded preterm newborns, newborns with a clinically diagnosed and/or genetically confirmed syndromal disorder, newborns with a major congenital deformity or heart disease, as well as newborns with clinical and/or laboratory signs for neonatal sepsis, and newborns with perinatal asphyxia. There were no other restrictions such as birth weight, mode of delivery, or gender. Furthermore, we excluded newborns of mothers who had been diagnosed with infectious, inflammatory, or metabolic disease (Table 2).

Exclusion criteria	**Definition**
Preterm Birth	Gestational Age < 37+0 weeks
Syndromal disorder	Clinically diagnosed by an experienced physician and/or confirmed by genetic testing i.e. karyotype testing, FISH, or whole-exome analysis.
Congenital heart disease	Diagnosed prenatally or by a pediatric cardiologist through echocardiography
Neonatal Sepsis	Clinically diagnosed by an experienced physician and/or elevated infectious disease markers
Perinatal Asphyxia	pH < 7.0, Base-Excess < -16 mmol/l; 5-Minute-Apgar-Score < 6
Maternal diabetes	Specifically maternal diabetes Type I
Other maternal disease	Infectious, inflammatory, or metabolic disease.
Maternal weight	Mothers with a BMI between 25 and 30 kg/m^2 were excluded.

Table 2. Exclusion criteria

Therefore, our case group consisted of full-term, by all appearances healthy, newborns of otherwise healthy mothers with maternal obesity. Maternal obesity was defined as a BMI > 30 kg/m^2. The control group consisted of full-term, healthy, newborns without maternal overweight including obesity (i.e., maternal BMI < 25 kg/m^2).

2.2 Data collection

2.2.1 Patient data and medical history

Medical data regarding the pregnancy, maternal health status, maternal height and weight, as well as the delivery and anthropometric data of the newborn, was taken from the maternal and newborn medical records.

Additionally, the parents were given a questionnaire regarding their medical history, focusing on cardiovascular, metabolic, endocrinological, inflammatory, hereditary diseases and allergies, as well as the mother's vaccination status and use of medication and/or vitamins, before and/or during the pregnancy. Furthermore, the questions covered the medical history of direct family and siblings of the newborn, focusing on allergies, metabolic, endocrinological, inflammatory, and hereditary disorders (Attachment 1).

2.2.2 Blood Samples

The blood samples were always collected simultaneously with a clinically necessary drawing of blood to avoid unnecessary additional stress for the newborn. For most term newborns (98%) this resulted in sample collection simultaneously with the newborn blood screening between 36 and 72 hours of life. In total, 10% of blood sample collections occurred in the first 36 hours of life, 86% between 36 and 72 hours of life, and 4% shortly before being discharged from the hospital. For the analysis concerning this dissertation, I focused on samples collected between 36 and 72 hours of life.

The sample consisted of a couple of drops of capillary (48%), venous (38%), or arterial (2%) blood on a blood spot card. For 12% of samples the collection method c.q. the type of blood sample was unknown. Before proceeding with these three different collection methods, the comparability of the samples was examined and confirmed.

The blood spot card was left to dry at room temperature for a maximum of 24 hours before being transferred to a -80 °C freezer. The blood spot cards were collected and sent to the Max Planck Institute for biochemical research for analysis by mass spectrometry.

2.3 Analysis

To analyze and interpret the protein quantifications and post-translational modification data we used the Perseus computational platform from MaxQuant [102]. The proteomic data was analyzed with a T-test and presented in Volcano Plots. We added a false discovery rate (FDR) threshold of 0.05 to correct for random events that falsely appear significant.

Additionally, we used IBM SPSS Statistics to analyze descriptive statistics. A p-value of ≤0.05 will be considered significant.

3. Results

3.1 Study population

Between February 2017 and June 2019, we included 662 newborns in total. Of those 662 newborns, 359 met the inclusion criteria for this study, i.e. full term, without perinatal asphyxia, perinatal infection, and/or congenital heart disease, as well as no maternal diabetes mellitus Type 1. For our main analysis, we identified 15 newborns with maternal obesity, defined as a maternal BMI > 30 m^2/kg at the beginning of pregnancy. As controls, we identified 344 newborns with maternal BMI < 25 m^2/kg (Figure 2).

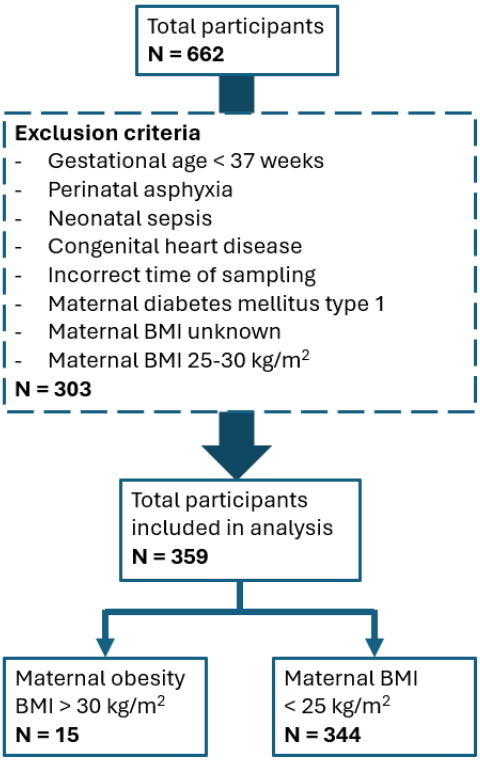

Figure 2. Study Flow-chart

We observed no differences between the groups in most descriptive characteristics like gestational age, birth weight or maternal age. We did however observe a significant difference between the groups in occurrence of maternal diabetes, either gestational or diabetes mellitus type 2, as well as the occurrence of hypertensive gestational diseases, i.e. pre-eclampsia, hypertension, and/or HELLP. These differences between the groups were as expected since obesity is a well-known risk factor for gestational diabetes, diabetes mellitus type 2, and hypertensive gestational diseases (Table 3).

	Maternal BMI > 30 (n=15)	Maternal BMI < 25 (n=344)	p-Value
Infant Characteristics			
Gestational age, mean (SD), d	277 (7.8)	278 (8.3)	0.741
Birth weight, mean (SD), g	3395 (450)	3385 (474)	0.939
Female (%)	5 (33.3 %)	163 (47.4%)	0.429
Small for gestational age, < P.10 (%)	1 (6.7%)	38 (11%)	0.716
Large for gestational age, > P. 90 (%)	-	17 (4.9%)	0.630
Postnatal hypoglycemia	-	28 (8.1%)	0.618
Maternal Characteristics			
Maternal age at birth (SD), y	32.5 (5.1)	34.1 (4.2)	0.136
Maternal BMI, weighed at first prenatal visit (SD)	32.4 (2.1)	21.3 (1.7)	0.000
Maternal BMI, reported (SD)	30.9 (2.5)	21.1 (1.8)	0.000
Maternal Diabetes, gestational or Type 2 (%)	3 (20.0%)	16 (4.7%)	0.009
(Pre-)Eclampsia, hypertension and/or HELLP (%)	2 (13.3%)	4 (1.2%)	0.020
Other			
Time of sampling (SD), h	43.2 (7.5)	45.7 (7.4)	0.196

Table 3. Infant and maternal characteristics. * Significant if p<0.05

When visualizing the concentration range of plasma proteins of our population in a scatter plot, the expected pattern appeared (Figure 3). The scatter plot shows a small group of high-abundant functional plasma proteins such as albumin, hemoglobin, gamma globulin, and apolipoprotein A1, a large middle group of different proteins ranging from nucleotide-binding proteins like rab-2A, signaling proteins like interleukin-18, and enzymes like NADH-cytochrome b5 reductase 3, and lastly a smaller group of low-abundant proteins like FMC1 that plays a role in the ATP synthase in mitochondria, YIPF6 which is important for intestinal epithelial cell development or SERPINB1 which is a leucocyte elastase inhibitor with the primary function to protect the cell from proteases released in the cytoplasm during stress or infection.

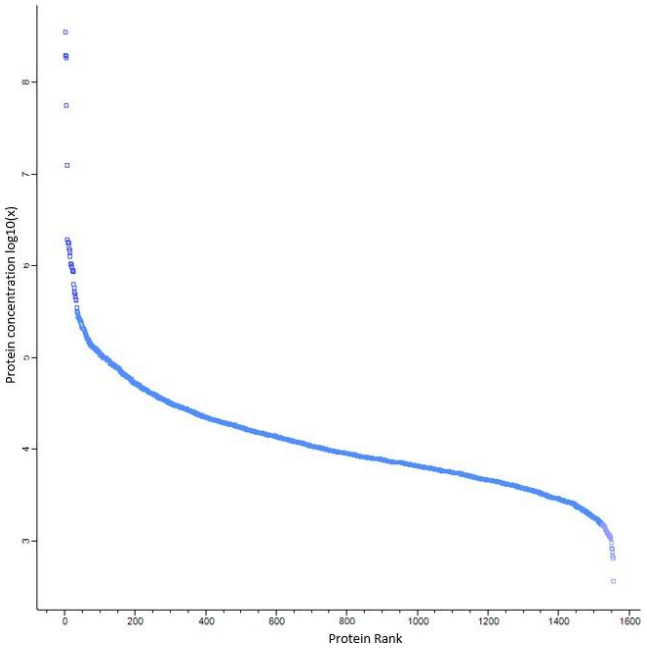

Figure 3. Concentration range of plasma proteins

3.2 T-test analysis

The results of the t-test analysis comparing the proteome of the newborns with maternal obesity with the proteome of newborns with normal maternal weight were visualized in a volcano plot (Figure 4). Four proteins stood out; Ribosomal protein S21 (RPS21), UNC13D, Kallistatin (SERPINA4) and Prostaglandin E2 receptor EP (PTGER3). The first three appeared to be more abundant in newborns with maternal obesity, the latter appeared to be less abundant in newborns with maternal obesity. Unfortunately, when introducing an FDR threshold of 0.05, one notices that we did not detect a big enough difference in abundance and/or a small enough p-value for any of the proteins.

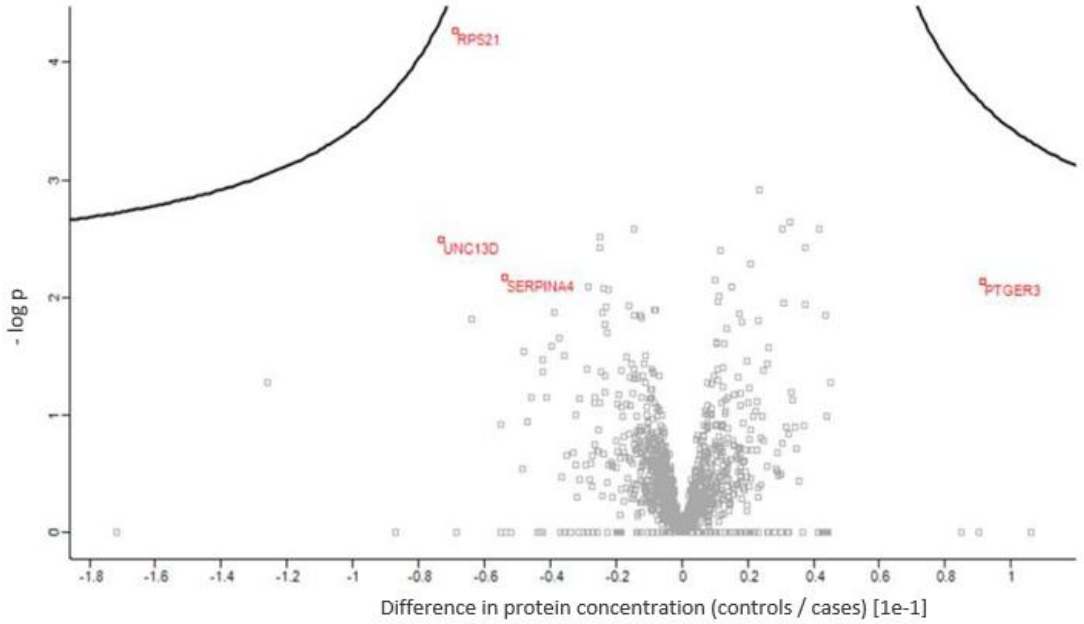

Figure 4. T-Test Volcano Plot. Cases = maternal BMI > 30 kg/m2, Controls = maternal BMI < 25 kg/m2. Highlighted proteins showed an at least 4 fold difference in abundance in the controls with an p-value < 0.01.

After consideration, I excluded newborns with maternal diabetes mellitus type 2 and gestational diabetes (n=3 in the case group, n=16 in the control group) and performed another t-test analysis, the results of which were visualized in a volcano plot (Figure 5). RPS21, SERPINA4 and PTGER3 continued to appear at the outskirts of the plot. Unfortunately, again none of the proteins showed a big enough difference in abundance or small enough p-value to pass the FDR threshold.

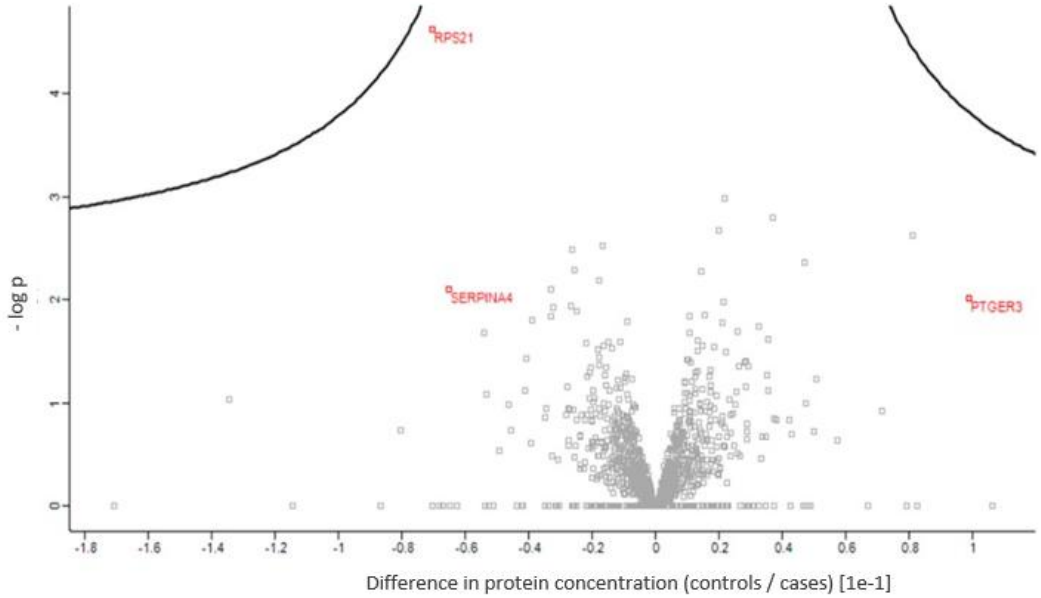

Figure 5. T-Test Volcano Plot, after excluding newborns with maternal diabetes. Cases = maternal BMI > 30 kg/m2. Controls = maternal BMI < 25 kg/m2. Highlighted proteins showed an at least 4 fold difference in abundance with a p-value < 0.01.

Nevertheless, even without having passed the FDR threshold, the three proteins that continued to emerge as outliers, might be worth looking further into. RPS21, SERPINA4 and PTGER3 showed an at least 4-fold difference in protein levels between the groups, with a p-value of <0.01 (Table 4). RPS21 and SERPINA4 showed a higher abundance in newborns with maternal obesity. The other protein, Prostaglandin E2 receptor EP, showed a lower abundance in newborns with maternal obesity.

Protein	Difference in protein levels between groups (log10)	p-value (-log p)
SERPINA4	4.48 x (- 0.651) higher abundance in newborns with maternal obesity	0.0079 (2.1069)
RPS21	5.01 x (-0.704) higher abundance in newborns with maternal obesity	0.0001 (4.6277)
PTGER3	9.71 x (0.978) lower abundance in newborns with maternal obesity	0.0095 (2.0215)

Table 4. Comparing the protein levels in the blood of newborns with maternal obesity with the protein levels in the blood of newborns without maternal obesity, after exclusion of newborns with maternal diabetes. Shown are the three proteins showing an at least 4-fold difference in abundance with a p-value < 0.01.

4. Discussion

Unfortunately, the differences in protein concentration that initially seemed to have appeared in our analysis of the proteomic data, did not pass the FDR threshold. This is presumably due to the small sample size of our case group. Therefore, it is not possible to confidently determine whether these differences are random or true.

Upon consideration, we decided to consider the proteins RPS21, SERPINA4 and PTGER3 to warrant further investigation, as they continue to appear as outliers, even though the results are not to be considered significant.

SERPINA4 and RPSS21 showed a higher abundance in newborns with maternal obesity. Whereas PTGER3 on the other hand showed a higher abundance in newborns with normal maternal weight.

UNC13D initially appeared as an outlier as well, suggesting a higher abundance in newborns with maternal obesity. This suggestion attenuated after correcting for maternal diabetes type 2 and gestational diabetes.

Here, we will be looking further into these individual proteins, their subsequent pathways, and their potential role in obesity, metabolic syndrome, and/or cardiovascular disease.

4.1 Kallistatin (SERPINA4)

Kallistatin is a serine proteinase inhibitor, which was first identified as a tissue kallikrein binding protein [103, 104]. Tissue kallikreins are expressed throughout the human body and are involved in various processes, including inflammation, coagulation, vasodilation, and blood pressure regulation [105]. The Kallikrein-kinin pathway acts to lower systemic blood pressure and reduces reactive oxygen species [106], which means it acts as a counterbalance to the renin-angiotensin-aldosterone system [107] and plays a protective role in organ damage in the heart and kidney. As a tissue kallikrein binding protein, Kallistatin directly interferes with the processes listed above.

Additionally, kallistatin has been reported to play a role in blood pressure regulation [108], vasculature relaxation [109], and neointima hyperplasia [110], independently of the tissue kallikrein-kinin system.

Furthermore, kallistatin is also involved in the canonical Wnt-pathway [111]. The canonical Wnt-pathway is activated when Wnt binds to the coreceptor complex of Frizzled and low-density lipoprotein receptor-related protein 6 (LRP6). Subsequent events lead to the prevention of phosphorylation of β-catenin. If phosphorylation of β-catenin is prevented, it accumulates and translocates to the nucleus where it binds to T-cell factor/lymphoid enhancer factor (TCF4/LEF), which in turn promotes the expression of Wnt target genes like VEGF, a known proangiogenic agent.

An older study from 1996 found that kallistatin levels were significantly reduced in the eyes of

patients with diabetic retinopathy, which might suggest a protective effect of kallistatins antiangiogenic properties on microvasculature [112]. Later, Liu et al. showed antiangiogenic and antineuroinflammatory effects of kallistatin through inhibition of the Wnt Pathway specifically in diabetic retinopathy mouse models [111]. Kallistatin inhibits the Wnt Pathway by binding to the extracellular domain of LRP6, thus inhibiting the phosphorylation of β-catenin and preventing the translocation to the nucleus [113].

In 2014, McBride et al. hypothesized that kallistatin could also be involved in wound healing through interactions with the canonical Wnt pathway since Wnt signaling modulates cell proliferation, angiogenesis, and hair follicle growth [114]. To further investigate this theory, they generated kallistatin-transgenic (KS-TG) mice that overexpressed and secreted human kallistatin. KS- TG mice showed reduced microvascular density and hair follicle density, as well as thinning of the panniculus adiposus layer; features also found in human lower limb skin in patients with diabetes and/or peripheral vascular disease [115]. KS-TG mice also showed an impaired skin hyperemic response upon local ischemia. Most interestingly wound closure in KS-TG mice was slower compared to wild-type mice. KS-TG mice had reduced vascular density in the wound area and the expression of VEGF was significantly lower in KS-TG mice compared to wild-type mice. In addition to their research in mouse models, McBride et al. also analyzed kallistatin levels in type 2 diabetic patients with and without vascular complications and compared them to the levels of healthy controls. Kallistatin levels were significantly higher in diabetic patients with vascular complications compared to non-diabetic individuals as well as diabetic patients without vascular complications. In diabetic patients, kallistatin showed correlations to several parameters related to vascular complications such as HbA1c, large and small artery elasticity, and albumin-to-creatinine ratio. To summarize, the data of their study show that high levels of kallistatin seem to affect the structure of the skin, with reduced skin microvascularisation, hair follicle, and thinning of the panniculus adiposus layer, as well as cause a delay in the wound healing process. Human type 2 diabetic patients with vascular complications showed significantly higher levels of kallistatin, though it is not yet known whether this is due to increased secretion or decreased reuptake by the liver.

Earlier, Jenkins and McBride et al. already found higher kallistatin levels in type 1 diabetic patients with renal dysfunction, as well as type 1 diabetic patients with hypertension and other microvascular complications such as proliferative retinopathy [116]. El-Asrar et. al. found positive correlations between kallistatin and fasting blood glucose, urine albumin-to-creatinine ratio, triglycerides, total cholesterol, hs-CRP, HbA1c, and carotid intima media thickness [117].

A possible explanation as to why kallistatin seems to be elevated in patients with microvascular complications, renal dysfunction, hypertension, and vascular dysfunction, even though it is known to have anti-inflammatory, anti-angiogenic, and antioxidant effects as well as positive effects on blood pressure regulation, might be that the elevation is compensatory. This theory was previously proposed by Jenkins and McBride [116]. They suggested, that whilst some of the effects of kallistatin may be beneficial, they may simultaneously delay wound healing and interfere with the delicate balance between pro- and anti-angiogenic factors in vessel formation

and repair, causing a higher risk of microvascular complications.

Unfortunately, to our knowledge, there is little to no data about kallistatin in patients with obesity. Gateva et al. compared kallistatin levels of patients with obesity with and without prediabetes or metabolic syndrome but did not include a control group without overweight or obesity in their study. They did find higher levels of kallistatin in obese patients with prediabetes as well as in patients with metabolic syndrome, compared to controls with normal glucose tolerance or no metabolic syndrome [118].

Our t-test analysis showed a 4.5-fold higher abundance of kallistatin in newborns with maternal obesity, with a p-value of 0.008. Building on the hypothesis of Jenkins and McBride, we could also hypothesize that the observed elevation of kallistatin levels in these newborns is compensatory as a reaction to the different intrauterine environment to which they were exposed. As listed above, kallistatin has a variety of different effects and it is, therefore, possible that it might be partly responsible for the newborn's predisposition to metabolic syndrome, obesity, or cardiovascular disease later in life. Since we didn't collect any follow-up proteomic data, it is unclear if this possibly compensatory elevation of kallistatin levels is permanent or temporary. It would be interesting to see, whether this elevation of kallistatin levels is still detectable at the age of onset of metabolic syndrome, cardiovascular disease, or obesity.

4.2 Prostaglandin E2 receptor subtype 3 (PTGER3)

The obese, insulin-resistant state comes with several physiological changes such as dyslipidemia, hyperinsulinemia, hyperglycemia, and systemic inflammation. These changes induce beta-cell stress and increase beta-cell workload. This forces the beta cell to compensate in order to survive and keep up with the demand, causing significant ER and mitochondrial oxidative stress [119, 120]. In a compensating beta cell, this mitochondrial oxidative and ER stress is im- proved by responses like the unfolded protein response (UPR) and autophagy. This proposes the hypothesis, that as long as the beta-cell is able to adapt, type 2 diabetes will not occur. Unfortunately, some compensatory mechanisms are also thought to contribute to the pathophysiology of type 2 diabetes itself.

The role and function of prostaglandin and its receptors, more specifically the E2 receptor subtype 3 (EP3), have been of growing interest regarding obesity and diabetes.

Activation of the EP3 receptor by prostaglandin E2 (PGE2) results in activation of a G protein, specifically Gαz [121], which decreases the intracellular cAMP concentration by inhibiting adenyl cyclase [122, 123]. cAMP plays a part in glucose stimulated insulin secretion (Figure 6). Mouse models for diabetes type 2 have a decreased ability to upregulate their cAMP production [124]. Pancreatic islets from both mice and humans with type 2 diabetes have a higher expression of EP3 and produce more PGE2, which leads to a decrease in cAMP levels, which in turn results in a weaker insulin secretory response to glucose [124].

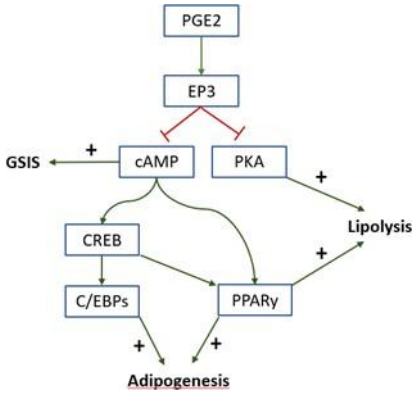

Figure 6. cAMP/PKA/PPARy pathway of the EP3 receptor.

High glucose, pro-inflammatory cytokines like IL-1β and COX-2, and/or free fatty acids have been shown to upregulate the production of PGE2 and consequently enhance EP3 receptor activation [125-127]. As described above, the role of PGE2 and the EP3 receptor in glucose stimulated insulin secretion has been studied [124, 128]. However, the role of PGE2 and EP3 in beta-cell proliferation and beta-cell mass dynamics is also of interest. For example, EP3 knock out mice (EP3-/-) show increased beta-cell proliferation when fed a high-fat diet (HFD) [129]. Moreover, global loss of Gαz also results in increased beta-cell proliferation in mice during both normal as well as HFD feeding regimes [130]. Carboneau et al. showed that blocking EP3 signaling resulted in an increase in beta-cell proliferation in humans, whereas activating EP3 signaling increased cytokine-induced beta-cell death. Interestingly, they also found an increase in PTGER3 expression during aging mouse pancreatic islets, which may lead to increased PGE2 signaling via EP3 and therefore impaired beta-cell proliferation and increased beta-cell death [131].

Two other processes in which Prostaglandin E2 and its successive pathway are thought to play a part, are adipogenesis and lipolysis [132-134]. Xu et al. specifically studied the effect of activation or genetic deletion of EP3 in mice on adipogenesis and lipolysis via the cAMP/PKA/PPARy pathway. They showed that deletion of EP3 in mice resulted in increased lipolysis[135]. Lipolysis is the process of breaking down triglycerides into glycerol and free fatty acids, facilitated by adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL) (Figure 7) [136].

Increased lipolysis results in excessive free fatty acids (FFAs) in circulation, which may lead to ectopic lipid deposition, impaired insulin sensitivity, and hyperlipidemia [137]. Xu et al showed that deletion of EP3 in mice will result in higher levels of PKA and PPARy[134], which reportedly leads to enhanced expression of ATGL and HSL[137-139] and phosphorylation of HSL [136], resulting in increased lipolysis.

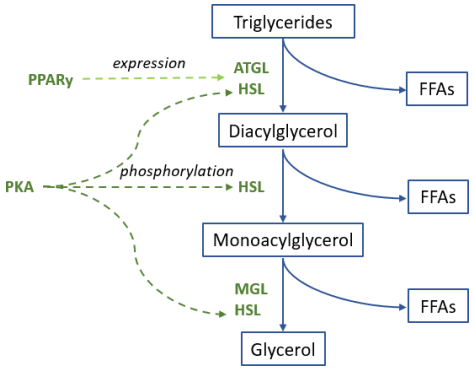

Figure 7. Lipolysis.

Additionally, Xu et al. described evidence that supports the involvement of the EP3 receptor in adipogenesis. Adipogenesis is the differentiation of preadipocytes into adipocytes and the accumulation of triglycerides in lipid droplets. This process is controlled by PPARy and CCAAT-enhancer-binding proteins (C/EBPs). The expression of PPARy and C/EBPs are promoted by cAMP response element-binding proteins (CREB), which are dependent on cAMP [141] (Figure 6). The activation of the EP3 receptor led to suppression of the cAMP axis and consequently decreased the differentiation of preadipocytes to adipocytes. Furthermore, they found that EP3-/- mice had more food intake, displayed less motor activity, and developed an obese phenotype [135]. These findings were consistent with previous research. For example, Ceddia et al. also found that EP3-/- mice were less active, developed an obese phenotype, displayed increased lipolysis, had increased ectopic lipid accumulation, and were insulin resistant [129]. Sanchez Alavez et al. described insulin resistance and increased body weight in EP3-/- mice as well. Further, their EP3-/- mice displayed increased feeding throughout the day, with additional feeding during the night [142].

Our t-test analysis showed a 9.7-fold lower abundance of EP3 receptors in newborns with maternal obesity, with a p-value of <0.01. Theoretically, fewer EP3 receptors could mean less inhibition of the cAMP/PKA pathway, and therefore more glucose stimulated insulin secretion. Since EP3 knock out mice display increased beta-cell proliferation, the downregulation of EP3 in newborns after intrauterine exposure to an adipose state accompanied by mild hyperglycemia, increased free fatty acids, and/or pro-inflammatory cytokines, may also result in increased beta-cell proliferation as a compensatory mechanism. On the other hand, these compensatory mechanisms potentially might also increase the risk of developing an obese phenotype, increased lipolysis, and ectopic lipid accumulation, since that is what has been described in EP3-/- mice. In summary, increasing research has shown the EP3 receptor to play a relevant part in adipogenesis, lipolysis, and insulin metabolism. Our research shows that newborns of mothers with obesity show a lower abundance of this receptor, although not significantly, proposing the question of the exact mechanism in which this will affect the newborn now and later in life. Further research is necessary to shed more light on this interesting phenomenon.

4.3 Ribosomal protein S21 (RPS21)

Unfortunately, there is not a great amount of research available involving the ribosomal protein S21 (RPS21) and its function, especially regarding obesity, diabetes and/or metabolic syndrome in humans.

The ribosomal protein S21 is a translation initiation factor and binds the 40S subunit of the ribosome [143]. Van Duin et al. showed a vital role for RPS21 in the binding and subsequent translation of natural mRNA in E. Coli [144]. Wang et al. found that the up-regulation of RPS21 resulted in the activation of the MAPK pathway in human osteosarcoma cells, leading to enhanced cell proliferation, differentiation, and development and subsequently to a poorer prognosis [145].

MAPKs regulate a large amount of complex cellular programs like cell proliferation, differentiation, angiogenesis, and apoptosis[146-149] (Figure 8). Earlier, it was determined that cAMP will inhibit the growth of fibroblasts, smooth muscle cells, and adipocytes, by blocking the Raf/MAPK pathway [150].

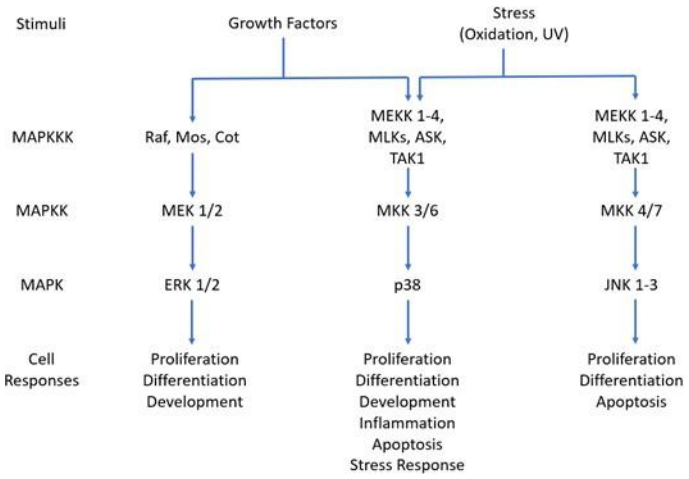

Figure 8. The MAPK signaling pathways in mammalian cells.

Other studies have linked enhanced activation of the JNK pathway to obesity and type 2 diabetes [151-154]. For example, JNK1 knockout mice show reduced improved insulin activity and are less prone to develop obesity when on a high-fat diet[151]. Also, JNK1 phosphorylates insulin receptor substrate, thus interfering with insulin signaling and playing a part in the development of insulin resistance[152- 155]. Research showed RPS21 as a regulator of the Ras/Raf/MAPK pathway in Drosophila[155], but whether the JNK/MAPK pathway is also regulated by RPS21 has not been investigated.

Our t-test analysis showed a 4.9-fold higher abundance of RPS21 in newborns with maternal obesity, with a p-value of <0.0001. Unfortunately, to this day, the exact mechanism between RPS21, subsequent enhanced cell proliferation, and differentiation in adiposity and/or insulin resistance remains largely unexplained, but a topic that warrants further investigation.

4.4 Protein unc-13 homolog D (UNC13D)

At this point in time, the unc-13 homolog D protein (UNC13D) has not yet been extensively studied. The possibility of involvement in obesity and/or diabetes is not studied at all. Current evidence shows that UNC13D is essential for the priming and maturation of granules in several hematopoietic cells[159, 160], the regulation of endocytic function[161], as well as for the regulation of exocytosis of granules in neutrophils, natural killer cells (NK cells) and cytotoxic T cells (CTs)[162- 164]. Additionally, mutations in the gene encoding for unc-13 homolog D protein is linked to the development of familial hemophagocytic lymphohistiocytosis)[165].

Before excluding the newborns with maternal diabetes type 2 and gestational diabetes, the t-test analysis showed a 5.4-fold higher abundance of UNC13D in newborns with maternal obesity, with a p-value of <0.01. These results did not pass the FDR threshold. After excluding newborns of mothers with gestational or type 2 diabetes, this suggested correlation was no longer measurable. These changes in results after exclusion could, of course, be due to the small sample size of our case group. An analysis with bigger group sizes would be necessary to confirm or disprove these results.

Nevertheless, it could be plausible that the higher abundance of this protein involved in the function of inflammatory response cells (i.e. neutrophils, CTs, and NK cells), could be a direct response to the intrauterine exposure to the high inflammatory state that is adiposity. Naturally, because of a lack of research and evidence in this direction, this remains only a theory that should be investigated more in the future.

4.5 Review

The inclusion process was designed to recruit a birth cohort. After the birth cohort was established, study cohorts were defined by individual exclusion criteria catering to the specific purpose of each study. In this study, we succeeded in establishing a birth cohort only in the second phase of the recruitment period, from May 2018 until December 2018. In the first phase of the recruitment, newborns with a gestational age > 37 weeks were already excluded and in the third phase, the focus was on including newborns with an infection, maternal diabetes, perinatal asphyxia and/or were born before 30 weeks of gestation. The healthy newborns born in the period December 2018 until June 2019 were not included. As the focus of the study discussed in this dissertation was on healthy newborns, it would have been beneficial to have extended the recruitment of the birth cohort to minimize bias while expanding our study cohort.

Some bias was presumably introduced simply because the study was conducted at a university hospital located in a central part of Munich. Adding to that, the questionnaire that was handed out to the parents was only available in German or English. Consequently, we found that most of the participating families categorized themselves as being of ‘Western’ ethnicity (93% for both mothers and fathers), reported an urban living environment (86%), and had a higher educational level (76% and 74% for mothers and fathers respectively) [166]. If the questionnaire had been available in additional languages, or if the recruitment was not limited to the centrally located university hospital the study cohort may have been more representative of the population.

Moreover, because of the observational design of the study, we experienced some difficulties that were inherent to this study design. Some women were not weighed at their first prenatal visit, some women only had their first prenatal visit way further along in their pregnancy, which would lead to missing values and uncertainties in the reliability of the documented weight.

As already mentioned in the introduction, we used BMI as a measuring and classification tool for obesity, since it is the most practical tool in an observational study design. BMI has, as discussed before, its limitations as it does not distinguish between fat and other tissues. Also, we could not differentiate between metabolically healthy or unhealthy women with obesity, as we had no data regarding levels of blood glucose, cholesterol, adipokines etc. For a follow-up study, we would recommend collecting additional data on these laboratory parameters as well as measurements like waist-to-hip ratio and waist circumference. These measurements are only meaningful if measured in the very early stages of pregnancy.

The prenatal screening method for gestational diabetes is, like all screening tools, not flawless [167]. Some women were never prenatally screened due to a multitude of reasons, or the screening could have shown a false negative outcome. Both situations would lead to the woman being considered non-diabetic, whereas she would have benefitted from dietary care or insulin therapy, possibly resulting in a higher HbA1c during their pregnancy. On the other hand, women who were considered diabetic, receive treatment and additional check-ups during the entire pregnancy, possibly resulting in a lower HbA1c during their pregnancy. Since we had no data regarding the prenatal glucose- or HbA1c-levels of women of either group, we have no way of knowing their exact metabolic state during pregnancy.

In this study, we decided to analyze blood from dried blood spots instead of blood plasma. Although dried blood spot analytics has many advantages compared to blood plasma analytics, such as easy and less invasive sampling, it also has some disadvantages. The proteome from whole blood samples will also contain proteins from erythrocytes, thrombocytes and leukocytes. These high abundant proteins like hemoglobin may overshadow smaller and minimal abundant proteins.

The blood samples were taken simultaneously with the heel prick screening to avoid unnecessary stress for the newborn. Because the study aimed to investigate differences in the proteome brought on by changes in the intrauterine environment, it would have been more suitable to take the sample immediately after birth.

Using mass spectrometry to perform the proteome analyses allows the identification of all protein proteoforms and is because of the hypothesis-free approach a fitting tool for identifying new biomarkers. With the greatly increased number of proteins analyzed, the likelihood of finding false positives increases as well. Introducing a false discovery rate aids in limiting the chances of false positives. Unfortunately, the proteins that initially seemed to show a notable difference in abundance between our groups (i.e. newborns with and without maternal obesity), did not pass the FDR threshold. It is therefore not with a certainty distinguishable whether these differences in the proteome are true or merely random. This is most probably due to the small sample size of only fifteen mothers with a BMI > 30 kg/m^2.

Even though our findings did not pass the FDR threshold and therefore none of the differences

in protein levels could be considered significant, they do pose an interesting starting point for further research, possibly with a larger sample size. Especially interesting are Kallistatin and the Prostaglandin E2 receptor, as they are known to work in pathways that could be related to, or have an interaction with, the pathogenesis of obesity, cardiovascular comorbidities and/or metabolic syndrome.

4.6 Conclusion

In summary, our study suggests but does not prove, that there are some changes in the proteome of newborns with maternal obesity. We propose these changes to be a result of intrauterine exposure to metabolic abnormalities of obesity.

We initially found four proteins that seemed to be of interest. Kallistatin and Ribosomal Protein S21 showed a higher abundance in newborns with maternal obesity compared to the newborns of mothers with normal weight, whereas Prostaglandin E2 receptor subtype 3 was less abundant in newborns with maternal obesity. The initial suggestion of a higher abundance of protein unc-13 homolog D was no longer detectable after correcting for maternal diabetes.

Unfortunately, due to our small sample size, after adding a false discovery rate threshold of 0.05, the significance of the changes in abundance of these proteins was not enough.

Even though none of these proteins showed a significantly higher or lower abundance after FDR correction, the results are not less interesting. Especially both Kallistatin and the Prostaglandin E2 receptor are known to work in pathways that could in some way be related to the pathophysiology of obesity and metabolic or cardiovascular diseases.

The next step would be to reevaluate and replicate our research with a larger sample size and more extensive data as mentioned before. If future studies could prove SERPINA4, PTGER3 or other proteins as biomarkers for predisposition to obesity, metabolic and cardiovascular diseases, it could lead to a better understanding of the pathomeganisms behind them. This could lay the groundwork for new and/or different preventative approaches to eliminating overweight and obesity.

Literaturverzeichnis

1. Balakumar, P., U.K. Maung, and G. Jagadeesh, *Prevalence and prevention of cardiovascular disease and diabetes mellitus.* Pharmacol Res, 2016. **113**(Pt A): p. 600-609.
2. Boutari C, Mantzoros CS. *A 2022 update on the epidemiology of obesity and a call to action: as its twin COVID-19 pandemic appears to be receding, the obesity and dysmetabolism pandemic continues to rage on.* Metabolism. 2022 Aug;133:155217. doi: 10.1016/j.metabol.2022.155217. Epub 2022 May 15.
3. World Health Organisation, r.o.f.E. *WHO European Regional Obesity Report 2022.* . 2022.
4. (WHO), W.H.O. *Obesity and Overweight.* https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Updated on 01.03.2024. Accessed on 24.06.2024.
5. Dietz, W.H. and T.N. Robinson, *Clinical practice. Overweight children and adolescents.* N Engl J Med, 2005. **352**(20): p. 2100-9.
6. Kelsey, M.M., et al., *Age-related consequences of childhood obesity.* Gerontology, 2014. **60**(3): p. 222-8.
7. Afshin, A., et al., *Health Effects of Overweight and Obesity in 195 Countries over 25 Years.* N Engl J Med, 2017. **377**(1): p. 13-27.
8. (WHO), W.H.O., *Global Health Estimates: Life expectancy and leading causes of death and disability.* https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-leading-causes-of-death Accessed on 24.06.2024.
9. Lusis, A.J., *Atherosclerosis.* Nature, 2000. **407**(6801): p. 233-241.
10. Gidding, S.S., et al., *Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Risk Score in Young Adults Predicts Coronary Artery and Abdominal Aorta Calcium in Middle Age: The CARDIA Study.* Circulation, 2016. **133**(2): p. 139-146.
11. Robbins, C.S., et al., *Local proliferation dominates lesional macrophage accumulation in atherosclerosis.* Nature medicine, 2013. **19**(9): p. 1166-1172.
12. Chen, N.X. and S.M. Moe, *Vascular calcification: pathophysiology and risk factors.* Current hypertension reports, 2012. **14**(3): p. 228-237.
13. Kondo, T., et al., *Effects of Tobacco Smoking on Cardiovascular Disease.* Circ J, 2019. **83**(10): p. 1980-1985.
14. Joseph, P., et al., *Reducing the Global Burden of Cardiovascular Disease, Part 1: The Epidemiology and Risk Factors.* Circ Res, 2017. **121**(6): p. 677-694.
15. Francula-Zaninovic, S. and I.A. Nola, *Management of Measurable Variable Cardiovascular Disease' Risk Factors.* Current cardiology reviews, 2018. **14**(3): p. 153-163.
16. Jokinen, E., *Obesity and cardiovascular disease.* Minerva Pediatr, 2015. **67**(1): p. 25-32.
17. von Eckardstein, A., *Risk factors for atherosclerotic vascular disease.* Handb Exp Pharmacol, 2005(170): p. 71-105.
18. Sardinha, L.B., et al., *Comparison of air displacement plethysmography with dual-energy X-ray absorptiometry and 3 field methods for estimating body composition in middle-aged men.* Am J Clin Nutr, 1998. **68**(4): p. 786-93.
19. Friedman, J.M., *Obesity: Causes and control of excess body fat.* Nature, 2009. **459**(7245): p. 340-2.
20. Browning, L.M., et al., *Measuring abdominal adipose tissue: comparison of simpler methods with MRI.* Obes Facts, 2011. **4**(1): p. 9-15.
21. Ross, R., et al., *Quantification of adipose tissue by MRI: relationship with anthropometric variables.* J Appl Physiol (1985), 1992. **72**(2): p. 787-95.
22. Pouliot, M.C., et al., *Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related*

cardiovascular risk in men and women. Am J Cardiol, 1994. **73**(7): p. 460-8.
23. Organisation, W.H. *Growth reference data for 5-19 years*. Indicators, BMI-for-age (5-19 years) 2007 [cited 2023 May].
24. Fève, B., *Adipogenesis: cellular and molecular aspects.* Best Pract Res Clin Endocrinol Metab, 2005. **19**(4): p. 483-99.
25. Ali, A.T., et al., *Adipocyte and adipogenesis.* Eur J Cell Biol, 2013. **92**(6-7): p. 229-36.
26. Cinti, S., *The adipose organ.* Prostaglandins Leukot Essent Fatty Acids, 2005. **73**(1): p. 9-15.
27. Diaz, M.B., S. Herzig, and A. Vegiopoulos, *Thermogenic adipocytes: from cells to physiology and medicine.* Metabolism, 2014. **63**(10): p. 1238-49.
28. Giralt, M. and F. Villarroya, *White, brown, beige/brite: different adipose cells for different functions?* Endocrinology, 2013. **154**(9): p. 2992-3000.
29. Vegiopoulos, A., M. Rohm, and S. Herzig, *Adipose tissue: between the extremes.* Embo j, 2017. **36**(14): p. 1999-2017.
30. Al-Mansoori, L., et al., *Role of Inflammatory Cytokines, Growth Factors and Adipokines in Adipogenesis and Insulin Resistance.* Inflammation, 2022. **45**(1): p. 31-44.
31. Hoffstedt, J., et al., *Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity.* Diabetologia, 2010. **53**(12): p. 2496-503.
32. Klöting, N., et al., *Insulin-sensitive obesity.* Am J Physiol Endocrinol Metab, 2010. **299**(3): p. E506-15.
33. Virtue, S. and A. Vidal-Puig, *Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome--an allostatic perspective.* Biochim Biophys Acta, 2010. **1801**(3): p. 338-49.
34. Smith, G.I., B. Mittendorfer, and S. Klein, *Metabolically healthy obesity: facts and fantasies.* J Clin Invest, 2019. **129**(10): p. 3978-3989.
35. Blüher, M., *Metabolically Healthy Obesity.* Endocr Rev, 2020. **41**(3).
36. Borel, A.L., et al., *Cardiometabolic risk improvement in response to a 3-yr lifestyle modification program in men: contribution of improved cardiorespiratory fitness vs. weight loss.* Am J Physiol Endocrinol Metab, 2017. **312**(4): p. E273-e281.
37. Cobos-Palacios, L., et al., *Adipokines Profile and Inflammation Biomarkers in Prepubertal Population with Obesity and Healthy Metabolic State.* Children (Basel), 2022. **9**(1).
38. *NCD Countdown 2030: worldwide trends in non-communicable disease mortality and progress towards Sustainable Development Goal target 3.4.* Lancet, 2018. **392**(10152): p. 1072-1088.
39. Hansen, B.C., *The metabolic syndrome X.* Ann N Y Acad Sci, 1999. **892**: p. 1-24.
40. Weiss, R., et al., *Obesity and the metabolic syndrome in children and adolescents.* N Engl J Med, 2004. **350**(23): p. 2362-74.
41. Dabelea, D., *The predisposition to obesity and diabetes in offspring of diabetic mothers.* Diabetes Care, 2007. **30 Suppl 2**: p. S169-74.
42. Metzger, B.E., et al., *Summary and Recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus.* Diabetes Care, 2007. **30**(Supplement 2): p. S251-S260.
43. Metzger, B.E., et al., *Hyperglycemia and adverse pregnancy outcomes.* N Engl J Med, 2008. **358**(19): p. 1991-2002.
44. Vohr, B.R. and C.M. Boney, *Gestational diabetes: the forerunner for the development of maternal and childhood obesity and metabolic syndrome?* J Matern Fetal Neonatal Med, 2008. **21**(3): p. 149-57.
45. Simeoni, U. and D.J. Barker, *Offspring of diabetic pregnancy: long-term outcomes.* Semin Fetal Neonatal Med, 2009. **14**(2): p. 119-24.
46. Patro Golab, B., et al., *Influence of maternal obesity on the association between common pregnancy complications and risk of childhood obesity: an individual participant data meta-analysis.* Lancet Child Adolesc Health, 2018. **2**(11): p. 812-821.
47. Levitt, N.S. and E.V. Lambert, *The foetal origins of the metabolic syndrome--a South African perspective.* Cardiovasc J S Afr, 2002. **13**(4): p. 179-80.
48. Ozanne, S.E. and C.N. Hales, *Early programming of glucose-insulin metabolism.* Trends

Endocrinol Metab, 2002. **13**(9): p. 368-73.
49. Fernandez-Twinn, D.S., M. Constância, and S.E. Ozanne, *Intergenerational epigenetic inheritance in models of developmental programming of adult disease.* Semin Cell Dev Biol, 2015. **43**: p. 85-95.
50. Barker, D.J., et al., *Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease.* BMJ (Clinical research ed.), 1989. **298**(6673): p. 564-567.
51. Barker, D.J., C. Osmond, and C.M. Law, *The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis.* Journal of epidemiology and community health, 1989. **43**(3): p. 237-240.
52. Barker, D.J., et al., *Weight in infancy and death from ischaemic heart disease.* Lancet, 1989. **2**(8663): p. 577-80.
53. Barker, D.J.P., et al., *Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth.* Diabetologia, 1993. **36**(1): p. 62-67.
54. Gillman, M.W., *Developmental origins of health and disease.* The New England journal of medicine, 2005. **353**(17): p. 1848-1850.
55. Huxley, R.R., A.W. Shiell, and C.M. Law, *The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature.* J Hypertens, 2000. **18**(7): p. 815-31.
56. Hales, C.N., et al., *Fetal and infant growth and impaired glucose tolerance at age 64.* Bmj, 1991. **303**(6809): p. 1019-22.
57. Whincup, P.H., et al., *Birth weight and risk of type 2 diabetes: a systematic review.* Jama, 2008. **300**(24): p. 2886-97.
58. McCance, D.R., et al., *Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genotype?* Bmj, 1994. **308**(6934): p. 942-5.
59. (IQTIG), I.f.Q.u.T.i.G., *Bundesauswertung zum Erfassungsjahr 2022 - Geburtshilfe - Qualitätsindikatoren. .* 2023.
60. Robert Koch Institut (RKI). *AdiMon Themenblatt: Schwangerschaft*, 2020. (Stand 08 Oktober 2020)
61. Baeten, J.M., E.A. Bukusi, and M. Lambe, *Pregnancy complications and outcomes among overweight and obese nulliparous women.* Am J Public Health, 2001. **91**(3): p. 436-40.
62. Sebire, N.J., et al., *Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London.* Int J Obes Relat Metab Disord, 2001. **25**(8): p. 1175-82.
63. Catalano, P.M., et al., *Fetuses of obese mothers develop insulin resistance in utero.* Diabetes Care, 2009. **32**(6): p. 1076-80.
64. Watkins, M.L. and L.D. Botto, *Maternal prepregnancy weight and congenital heart defects in offspring.* Epidemiology, 2001. **12**(4): p. 439-46.
65. Hedermann, G., et al., *Maternal obesity and metabolic disorders associate with congenital heart defects in the offspring: A systematic review.* PLoS One, 2021. **16**(5): p. e0252343.
66. Tie, H.T., et al., *Risk of childhood overweight or obesity associated with excessive weight gain during pregnancy: a meta-analysis.* Arch Gynecol Obstet, 2014. **289**(2): p. 247-57.
67. Whitaker, R.C., et al., *Predicting obesity in young adulthood from childhood and parental obesity.* N Engl J Med, 1997. **337**(13): p. 869-73.
68. Gaillard, R., et al., *Associations of maternal prepregnancy body mass index and gestational weight gain with cardio-metabolic risk factors in adolescent offspring: a prospective cohort study.* Bjog, 2016. **123**(2): p. 207-16.
69. Gaillard, R., et al., *Maternal weight gain in different periods of pregnancy and childhood cardio-metabolic outcomes. The Generation R Study.* Int J Obes (Lond), 2015. **39**(4): p. 677-85.
70. Gaillard, R., et al., *Childhood cardiometabolic outcomes of maternal obesity during*

pregnancy: the Generation R Study. Hypertension, 2014. **63**(4): p. 683-91.
71. Yu, Z., et al., *Pre-pregnancy body mass index in relation to infant birth weight and offspring overweight/obesity: a systematic review and meta-analysis.* PLoS One, 2013. **8**(4): p. e61627.
72. Reynolds, R.M., et al., *Maternal obesity during pregnancy and premature mortality from cardiovascular event in adult offspring: follow-up of 1 323 275 person years.* Bmj, 2013. **347**: p. f4539.
73. Eriksson, J.G., et al., *Long-term consequences of maternal overweight in pregnancy on offspring later health: findings from the Helsinki Birth Cohort Study.* Ann Med, 2014. **46**(6): p. 434-8.
74. Chu, S.Y., et al., *Maternal Obesity and Risk of Gestational Diabetes Mellitus.* Diabetes Care, 2007. **30**(8): p. 2070-2076.
75. Baumert, J., Du, Y., Heidemann, K., Paprott, R., Reitzle, L., Scheidt-Nave, C., Schmidt, C., Ziese, T., *Diabetes in Deutschland. Bericht der Nationalen Diabetes-Surveillance 2019.* Robert Koch Institut, 2019.
76. Abdullah, A., et al., *The magnitude of association between overweight and obesity and the risk of diabetes: a meta-analysis of prospective cohort studies.* Diabetes Res Clin Pract, 2010. **89**(3): p. 309-19.
77. Clausen, T.D., et al., *High prevalence of type 2 diabetes and pre-diabetes in adult offspring of women with gestational diabetes mellitus or type 1 diabetes: the role of intrauterine hyperglycemia.* Diabetes Care, 2008. **31**(2): p. 340-6.
78. Lindsay, R.S., et al., *Programming of adiposity in offspring of mothers with type 1 diabetes at age 7 years.* Diabetes Care, 2010. **33**(5): p. 1080-5.
79. Stride, A., et al., *Intrauterine hyperglycemia is associated with an earlier diagnosis of diabetes in HNF-1alpha gene mutation carriers.* Diabetes Care, 2002. **25**(12): p. 2287-91.
80. Gillman, M.W., et al., *Effect of treatment of gestational diabetes mellitus on obesity in the next generation.* Diabetes care, 2010. **33**(5): p. 964-968.
81. Pirkola, J., et al., *Risks of overweight and abdominal obesity at age 16 years associated with prenatal exposures to maternal prepregnancy overweight and gestational diabetes mellitus.* Diabetes care, 2010. **33**(5): p. 1115-1121.
82. Boney CM, Verma A, Tucker R, Vohr BR. *Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus.* Pediatrics. 2005 Mar;115(3):e290-6. doi: 10.1542/peds.2004-1808.
83. Geyer, P.E., et al., *Revisiting biomarker discovery by plasma proteomics.* Molecular systems biology, 2017. **13**(9): p. 942-942.
84. Aebersold, R. and M. Mann, *Mass-spectrometric exploration of proteome structure and function.* Nature, 2016. **537**(7620): p. 347-355.
85. Anderson, L., *Six decades searching for meaning in the proteome.* J Proteomics, 2014. **107**: p. 24-30.
86. Anderson, N.L. and N.G. Anderson, *The human plasma proteome: history, character, and diagnostic prospects.* Mol Cell Proteomics, 2002. **1**(11): p. 845-67.
87. Tu, C., et al., *Depletion of abundant plasma proteins and limitations of plasma proteomics.* Journal of proteome research, 2010. **9**(10): p. 4982-4991.
88. Bellei, E., et al., *High-abundance proteins depletion for serum proteomic analysis: concomitant removal of non-targeted proteins.* Amino Acids, 2011. **40**(1): p. 145-56.
89. Essader, A.S., et al., *A comparison of immobilized pH gradient isoelectric focusing and strong-cation-exchange chromatography as a first dimension in shotgun proteomics.* Proteomics, 2005. **5**(1): p. 24-34.
90. Manadas, B., et al., *Peptide fractionation in proteomics approaches.* Expert Review of Proteomics, 2010. **7:5**: p. 655-663.
91. Geyer, P.E., et al., *Plasma Proteome Profiling to Assess Human Health and Disease.* Cell Syst, 2016. **2**(3): p. 185-95.
92. Guthrie, R. and A. Susi, *A simple phenylalinine method for detecting phenylketonuria in large populations of newborn infants.* Pediatrics, 1963. **32**: p. 338-43.

93. McDade, T.W., S. Williams, and J.J. Snodgrass, *What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research.* Demography, 2007. **44**(4): p. 899-925.
94. Mei, J.V., et al., *Use of filter paper for the collection and analysis of human whole blood specimens.* J Nutr, 2001. **131**(5): p. 1631s-6s.
95. Bowen, C.L., et al., *Utility of dried blood spot sampling and storage for increased stability of photosensitive compounds.* Bioanalysis, 2010. **2**(11): p. 1823-8.
96. van der Heijden, J., et al., *Therapeutic drug monitoring of everolimus using the dried blood spot method in combination with liquid chromatography-mass spectrometry.* J Pharm Biomed Anal, 2009. **50**(4): p. 664-70.
97. Björkesten, J., et al., *Stability of Proteins in Dried Blood Spot Biobanks.* Molecular & Cellular Proteomics, 2017. **16**(7): p. 1286.
98. Déglon, J., et al., *Direct analysis of dried blood spots coupled with mass spectrometry: concepts and biomedical applications.* Anal Bioanal Chem, 2012. **402**(8): p. 2485-98.
99. Keevil, B.G., *The analysis of dried blood spot samples using liquid chromatography tandem mass spectrometry.* Clin Biochem, 2011. **44**(1): p. 110-8.
100. Li, W. and F.L. Tse, *Dried blood spot sampling in combination with LC-MS/MS for quantitative analysis of small molecules.* Biomed Chromatogr, 2010. **24**(1): p. 49-65.
101. Martin, N.J., J. Bunch, and H.J. Cooper, *Dried blood spot proteomics: surface extraction of endogenous proteins coupled with automated sample preparation and mass spectrometry analysis.* Journal of the American Society for Mass Spectrometry, 2013. **24**(8): p. 1242-1249.
102. Tyanova, S., et al., *The Perseus computational platform for comprehensive analysis of (prote)omics data.* Nature Methods, 2016. **13**(9): p. 731-740.
103. Chao, J., et al., *Identification of a new tissue-kallikrein-binding protein.* Biochem J, 1986. **239**(2): p. 325-31.
104. Chao, J., et al., *Tissue kallikrein-binding protein is a serpin. I. Purification, characterization, and distribution in normotensive and spontaneously hypertensive rats.* J Biol Chem, 1990. **265**(27): p. 16394-401.
105. Mamenko, M., et al., *Chapter Five - Control of ENaC-Mediated Sodium Reabsorption in the Distal Nephron by Bradykinin*, in *Vitamins & Hormones*, G. Litwack, Editor. 2015, Academic Press. p. 137-154.
106. Kayashima, Y., O. Smithies, and M. Kakoki, *The kallikrein-kinin system and oxidative stress.* Curr Opin Nephrol Hypertens, 2012. **21**(1): p. 92-6.
107. Shen, B. and S.S. El-Dahr, *Cross-talk of the renin-angiotensin and kallikrein-kinin systems.* Biol Chem, 2006. **387**(2): p. 145-50.
108. Chen, L.M., et al., *Tissue kallikrein-binding protein reduces blood pressure in transgenic mice.* J Biol Chem, 1996. **271**(44): p. 27590-4.
109. Chao, J., et al., *Kallistatin is a potent new vasodilator.* J Clin Invest, 1997. **100**(1): p. 11-7.
110. Miao, R.Q., et al., *Kallistatin stimulates vascular smooth muscle cell proliferation and migration in vitro and neointima formation in balloon-injured rat artery.* Circ Res, 2000. **86**(4): p. 418-24.
111. Liu, X., et al., *Antiangiogenic and antineuroinflammatory effects of kallistatin through interactions with the canonical Wnt pathway.* Diabetes, 2013. **62**(12): p. 4228-38.
112. Ma, J.X., et al., *Kallistatin in human ocular tissues: reduced levels in vitreous fluids from patients with diabetic retinopathy.* Curr Eye Res, 1996. **15**(11): p. 1117-23.
113. Zhao, L., et al., *Antagonizing Wnt pathway in diabetic retinopathy.* Diabetes, 2013. **62**(12): p. 3993-5.
114. McBride, J.D., et al., *Elevated circulation levels of an antiangiogenic SERPIN in patients with diabetic microvascular complications impair wound healing through suppression of Wnt signaling.* J Invest Dermatol, 2014. **134**(6): p. 1725-1734.

115. Petrofsky, J.S., M. Prowse, and E. Lohman, *The influence of ageing and diabetes on skin and subcutaneous fat thickness in different regions of the body.* Journal of Applied Research, 2008. **8**: p. 55-61.
116. Jenkins, A.J., et al., *Increased serum kallistatin levels in type 1 diabetes patients with vascular complications.* J Angiogenes Res, 2010. **2**: p. 19.
117. El-Asrar, M.A., et al., *Kallistatin as a marker of microvascular complications in children and adolescents with type 1 diabetes mellitus: Relation to carotid intima media thickness.* Vasc Med, 2015. **20**(6): p. 509-17.
118. Gateva, A., et al., *Increased kallistatin levels in patients with obesity and prediabetes compared to normal glucose tolerance.* Endocr Res, 2017. **42**(2): p. 163-168.
119. Wang, J., X. Yang, and J. Zhang, *Bridges between mitochondrial oxidative stress, ER stress and mTOR signaling in pancreatic β cells.* Cell Signal, 2016. **28**(8): p. 1099-104.
120. Tabatabaie, T., et al., *Free radicals and the pathogenesis of type 1 diabetes: beta-cell cytokine-mediated free radical generation via cyclooxygenase-2.* Diabetes, 2003. **52**(8): p. 1994-9.
121. Kimple, M.E., et al., *A role for G(z) in pancreatic islet beta-cell biology.* J Biol Chem, 2005. **280**(36): p. 31708-13.
122. Sugimoto, Y. and S. Narumiya, *Prostaglandin E receptors.* J Biol Chem, 2007. **282**(16): p. 11613-7.
123. Negishi, M., et al., *Signal transductions of three isoforms of mouse prostaglandin E receptor EP3 subtype.* Adv Prostaglandin Thromboxane Leukot Res, 1995. **23**: p. 255-7.
124. Kimple, M.E., et al., *Prostaglandin E2 receptor, EP3, is induced in diabetic islets and negatively regulates glucose- and hormone-stimulated insulin secretion.* Diabetes, 2013. **62**(6): p. 1904-12.
125. Robertson, R.P., *Dominance of cyclooxygenase-2 in the regulation of pancreatic islet prostaglandin synthesis.* Diabetes, 1998. **47**(9): p. 1379-83.
126. Robertson, R.P., *The COX-2/PGE(2)/EP3/G(i/o)/cAMP/GSIS Pathway in the Islet: The Beat Goes On.* Diabetes, 2017. **66**(6): p. 1464-1466.
127. Neuman, J.C., et al., *Enriching Islet Phospholipids With Eicosapentaenoic Acid Reduces Prostaglandin E(2) Signaling and Enhances Diabetic β-Cell Function.* Diabetes, 2017. **66**(6): p. 1572-1585.
128. Robertson, R.P., et al., *Receptor-mediated adenylate cyclase-coupled mechanism for PGE2 inhibition of insulin secretion in HIT cells.* Diabetes, 1987. **36**(9): p. 1047-53.
129. Ceddia, R.P., et al., *The PGE2 EP3 Receptor Regulates Diet-Induced Adiposity in Male Mice.* Endocrinology, 2016. **157**(1): p. 220-32.
130. Kimple, M.E., et al., *Deletion of GαZ protein protects against diet-induced glucose intolerance via expansion of β-cell mass.* J Biol Chem, 2012. **287**(24): p. 20344-55.
131. Carboneau, B.A., et al., *Opposing effects of prostaglandin E(2) receptors EP3 and EP4 on mouse and human β-cell survival and proliferation.* Mol Metab, 2017. **6**(6): p. 548-559.
132. García-Alonso, V. and J. Clària, *Prostaglandin E2 signals white-to-brown adipogenic differentiation.* Adipocyte, 2014. **3**(4): p. 290-6.
133. Fain, J.N., et al., *Regulation of leptin release and lipolysis by PGE2 in rat adipose tissue.* Prostaglandins Other Lipid Mediat, 2000. **62**(4): p. 343-50.
134. Fujimori, K., et al., *Termination mechanism of CREB-dependent activation of COX-2 expression in early phase of adipogenesis.* Mol Cell Endocrinol, 2014. **384**(1-2): p. 12-22.
135. Xu, H., et al., *Prostaglandin E2 receptor EP3 regulates both adipogenesis and lipolysis in mouse white adipose tissue.* J Mol Cell Biol, 2016. **8**(6): p. 518-529.
136. Ahmadian, M., Y. Wang, and H.S. Sul, *Lipolysis in adipocytes.* Int J Biochem Cell Biol, 2010. **42**(5): p. 555-9.
137. Arner, P. and D. Langin, *Lipolysis in lipid turnover, cancer cachexia, and obesity-induced insulin resistance.* Trends Endocrinol Metab, 2014. **25**(5): p. 255-62.

138. Kershaw, E.E., et al., *PPARgamma regulates adipose triglyceride lipase in adipocytes in vitro and in vivo.* Am J Physiol Endocrinol Metab, 2007. **293**(6): p. E1736-45.
139. McTernan, P.G., et al., *Insulin and rosiglitazone regulation of lipolysis and lipogenesis in human adipose tissue in vitro.* Diabetes, 2002. **51**(5): p. 1493-8.
140. Rodriguez-Cuenca, S., et al., *Peroxisome proliferator-activated receptor γ-dependent regulation of lipolytic nodes and metabolic flexibility.* Mol Cell Biol, 2012. **32**(8): p. 1555-65.
141. Gustafson, B., et al., *Insulin resistance and impaired adipogenesis.* Trends Endocrinol Metab, 2015. **26**(4): p. 193-200.
142. Sanchez-Alavez, M., et al., *Night eating and obesity in the EP3R-deficient mouse.* Proceedings of the National Academy of Sciences, 2007. **104**(8): p. 3009-3014.
143. Török, I., et al., *Down-regulation of RpS21, a putative translation initiation factor interacting with P40, produces viable minute imagos and larval lethality with overgrown hematopoietic organs and imaginal discs.* Mol Cell Biol, 1999. **19**(3): p. 2308-21.
144. Van Duin, J. and R. Wijnands, *The function of ribosomal protein S21 in protein synthesis.* Eur J Biochem, 1981. **118**(3): p. 615-9.
145. Wang, T., et al., *Down-Regulation of Ribosomal Protein RPS21 Inhibits Invasive Behavior of Osteosarcoma Cells Through the Inactivation of MAPK Pathway.* Cancer Manag Res, 2020. **12**: p. 4949-4955.
146. Nishida, E. and Y. Gotoh, *The MAP kinase cascade is essential for diverse signal transduction pathways.* Trends Biochem Sci, 1993. **18**(4): p. 128-31.
147. Chang, L. and M. Karin, *Mammalian MAP kinase signalling cascades.* Nature, 2001. **410**(6824): p. 37-40.
148. Kuo, W.H., et al., *Induction of apoptosis in the lung tissue from rats exposed to cigarette smoke involves p38/JNK MAPK pathway.* Chem Biol Interact, 2005. **155**(1-2): p. 31-42.
149. Zhang, W. and H.T. Liu, *MAPK signal pathways in the regulation of cell proliferation in mammalian cells.* Cell Res, 2002. **12**(1): p. 9-18.
150. Yehia, G., et al., *Mitogen-activated protein kinase phosphorylates and targets inducible cAMP early repressor to ubiquitin-mediated destruction.* J Biol Chem, 2001. **276**(38): p. 35272-9.
151. Hirosumi, J., et al., *A central role for JNK in obesity and insulin resistance.* Nature, 2002. **420**(6913): p. 333-6.
152. Aguirre, V., et al., *The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307).* J Biol Chem, 2000. **275**(12): p. 9047-54.
153. Aguirre, V., et al., *Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action.* J Biol Chem, 2002. **277**(2): p. 1531-7.
154. Lee, Y.H., et al., *c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade.* J Biol Chem, 2003. **278**(5): p. 2896-902.
155. Sawyer, J.K., et al., *Exploiting codon usage identifies intensity-specific modifiers of Ras/MAPK signaling in vivo.* PLoS Genet, 2020. **16**(12): p. e1009228.
156. Liu, L., et al., *Fasting differentially alters the hypothalamic proteome of chickens from lines with the propensity to be anorexic or obese.* Nutr Diabetes, 2019. **9**(1): p. 13.
157. Henegar, C., et al., *Adipose tissue transcriptomic signature highlights the pathological relevance of extracellular matrix in human obesity.* Genome Biol, 2008. **9**(1): p. R14.
158. Agarwal, P., et al., *Gestational Diabetes Adversely Affects Pancreatic Islet Architecture and Function in the Male Rat Offspring.* Endocrinology, 2019. **160**(8): p. 1907-1925.
159. Ménager, M.M., et al., *Secretory cytotoxic granule maturation and exocytosis require the effector protein hMunc13-4.* Nat Immunol, 2007. **8**(3): p. 257-67.

160. Neeft, M., et al., *Munc13-4 is an effector of rab27a and controls secretion of lysosomes in hematopoietic cells.* Mol Biol Cell, 2005. **16**(2): p. 731-41.
161. He, J., et al., *Munc13-4 interacts with syntaxin 7 and regulates late endosomal maturation, endosomal signaling, and TLR9-initiated cellular responses.* Mol Biol Cell, 2016. **27**(3): p. 572-87.
162. Pivot-Pajot, C., et al., *Munc13-4 regulates granule secretion in human neutrophils.* J Immunol, 2008. **180**(10): p. 6786-97.
163. Marcenaro, S., et al., *Analysis of natural killer-cell function in familial hemophagocytic lymphohistiocytosis (FHL): defective CD107a surface expression heralds Munc13-4 defect and discriminates between genetic subtypes of the disease.* Blood, 2006. **108**(7): p. 2316-23.
164. Feldmann, J., et al., *Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3).* Cell, 2003. **115**(4): p. 461-73.
165. Sadeghi P, Esslami GG, Rokni-Zadeh H, Changi-Ashtiani M, Mohsenipour R. *Familial Hemophagocytic Lymphohistiocytosis secondary to UNC13D mutation: a report of two cases*. BMC Pediatr. 2022 Nov 19;22(1):667. doi: 10.1186/s12887-022-03746-9.
166. Pangratz-Fuehrer, S., et al., *Cohort profile: the MUNICH Preterm and Term Clinical study (MUNICH-PreTCl), a neonatal birth cohort with focus on prenatal and postnatal determinants of infant and childhood morbidity*. BMJ Open. 2021 Jun 24;11(6):e050652. doi: 10.1136/bmjopen-2021-050652.
167. Deutsche Diabetes Gesellschaft DDG, D.G.f.G.u.G., *S3-Leitlinie Gestationsdiabetes mellitus (GDM), Diagnostik, Therapie und Nachsorge.* AWMF-Registernummer 057-008, 2018.

Anhang 1: Questionnair

LMU KLINIKUM DER UNIVERSITÄT MÜNCHEN

CAMPUS INNENSTADT
PERINATALZENTRUM
NEONATOLOGIE DER KINDERKLINIK

Foreword

Thank you very much for taking your time to answer the following questions concerning our study "proteomics of premature infants and neonats". It will only take you about **5-15 minutes**.
To ensure the exhaustiveness of our data, we kindly want to ask you, to **answer every single question**. Should one of the medical facts not apply, please answer with a **"No"** and do only leave a blank, when you can't provide any further information.
We are only going to use the acquired data in an anonymised form in our final data analysis without breaching patient confidentiality or data protection laws.
Should there be any questions please don't hesitate to ask.

Personal details (mother)

Surname, name …… Date of birth ……

State of health of mother and father

- Has the mother or father of the new born child been diagnosed with one of the following diseases?

	mother	father
- cardiovascular diseases	……	……
- diabetes	type 1 ☐ 2 ☐	type 1 ☐ 2 ☐
- thyroid disorders	……	……
- haemophiliac & coagulation disorder	……	……
- herpes simplex (lips, genitals)	yes ☐ no ☐	yes ☐ no ☐
- autoimmune disease (e.g. rheumatoid arthritis)	……	……
- intestinal disorders (e.g. Crohn's disease, ulcerative colitis)	……	……
- genetic diseases	……	……

If yes; are you related to the father of your child?

no ☐ yes ☐ degree (e.g. cousin third degree) ……

- other (e.g. endocrine, neurological)	……	……

- Have you or the father of the new born child been diagnosed with one of the following diseases?

	mother	Father
allergic asthma	yes ☐ no ☐	yes ☐ no ☐
eczema / neurodermatitis	yes ☐ no ☐	yes ☐ no ☐

hay fever	yes ☐ no ☐	yes ☐ no ☐
which one?	……	……
other allergies	yes ☐ no ☐	yes ☐ no ☐
which one?	……	……

- If <u>you</u> do suffer from an allergic disease (hay fever, allergic asthma, neurodermatitis/ eczema), have you recognized the equivalent symptoms **during your pregnancy**?

yes ☐ no ☐

Medical history

☐ Has one of **your relatives** been diagnosed with one of the following diseases (parents, siblings) ?

allergies	yes ☐	which disease?	……
		who?	……
	no ☐		
allergic asthma	yes ☐	who?	……
	no ☐		
neurodermatitis	yes ☐	who?	……
	no ☐		
diabetes	yes ☐	type 1 ☐	who? ……
	no ☐	type 2 ☐	
genetic diseases	yes ☐	which disease?	……
		who?	……
	no ☐		
others	yes ☐	which disease?	……
		who?	……
	no ☐		

☐ Has one of **your husband's relatives** been diagnosed with one of the following diseases?

allergies	yes ☐	which disease?	……
		who?	……
	no ☐		
allergic asthma	yes ☐	who?	……
	no ☐		
neurodermatitis	yes ☐	who?	……
	no ☐		

diabetes	yes	☐	type 1 ☐	who?	.....
	no	☐	type 2 ☐		
genetic diseases	yes	☐	which disease?	.....	
			who?	.....	
	no	☐			
other diseases	yes	☐	which disease?	.....	
			who?	.....	
	no	☐			

Gynaecological questions

- What was your weight **before** the pregnancy? ..... kg

- At what age did you have your menstrual bleeding for the first time? ..... years

- How many days does your menstrual cycle last on average? ..... days

- Did you take oral contraceptives (the pill)?

  yes ☐ how long? ..... years

  name of the last product: .....

  no ☐

- Did you take any other hormonal or pharmacological treatment for contraception?

  how long? ... years

  which treatment? .....

- Have you had any surgical gynaecological procedures so far ?

Year before the pregnancy

- Were you vaccinated the year before your pregnancy?

  yes ☐ which vaccination? .....

  no ☐

- Did you take any medication the year before your pregnancy?

  yes ☐ medication ……
  pain killers ……
  sleeping pills ……
  vitamins ……
  minerals ……
  no ☐

- Did you have any operations the year before your pregnancy?

  yes ☐ which one? ……
  no ☐

- Were you abroad for a longer time during the year before your pregnancy?

  yes ☐ country ……
  no ☐

Year of the pregnancy

☐ Were you vaccinated during the year of your pregnancy?

yes ☐ which vaccination? ……
no ☐

☐ Did you take any medication during the year of your pregnancy?

yes ☐ medication ……
pain killers ……
sleeping pills ……
vitamins ……
minerals ……
no ☐

- Did you have any medical interventions the year of your pregnancy (excluding pregnancy examinations)?

  yes ☐ which one? ……
  no ☐

- Was a hospital admission during your pregnancy needed?

  yes ☐ cause ……
  no ☐

- Did you live in an urban or rural environment during your pregnancy?

  urban ☐ rural ☐

  For how long have you been living in this environment? .....years

- Were you abroad for a longer time during the year of your pregnancy?

  yes ☐ country ……
  no ☐

Earlier pregnancies

- Have you given birth to twins, triplets or even quadruplets in earlier pregnancies?

yes ☐ ..... twins ..... triplets ..... quadruplets

no ☐

- How many of your children were born prematurely (before 37th pregnancy week)?

..... premature infants none ☐

- How did you give birth to your previous children?

Number of natural births ..... forceps delivery ..... caesarean section .....

- Have you already had a miscarriage, stillbirth or abdominal pregnancy?

  yes ☐ number of misscariage ..... stillbirth .....
  abdominal pregnancies .....

  no ☐

- If there are any siblings, how many? ..... girls ..... boys

- Has one of your other children been diagnosed with one of the following diseases?

allergies yes ☐ which disease? .....
who? .....
no ☐

allergic asthma yes ☐ who? .....
no ☐

neurodermatitis yes ☐ who? .....
no ☐

diabetes yes ☐ type 1 ☐ who? .....
no ☐ type 2 ☐

genetic diseases yes ☐ which disease? .....
who? .....
no ☐

others yes ☐ which disease? .....
who? .....
no ☐

Artificial insemination / reproductive medicine

- Did you make use of reproductive medical treatments for your new born child?

				Number of attempts / implanted egg cell
yes	☐	☐	only hormonal treatment	
		☐	in-vitro-fertilisation (IVF)	……/……
		☐	intra-zytoplasmatic sperm injection (ICSI)	……/……
		☐	testicular sperm extraction with ICSI (TESE)	……/……
		☐	microsurgical epididymal sperm aspiration with ICSI (MESA)	……/……
no	☐			

If you made use of any reproductive medical treatment, was your husband's sperm injected?

yes ☐ no, a donor's sperm ☐

- Did you get hormonal treatment as part of the reproductive medical treatments?

yes ☐ name of the last product ……
no ☐

- Did your husband get hormonal treatment as part of the reproductive medical treatments?

yes ☐ name of the last product ……
no ☐

Consumption patterns

- Do you or your husband smoke?

Non smoker	mother ☐	father ☐
Ex-smoker	mother ☐	father ☐
Smoker	mother ☐	father ☐

- Did you smoke before the pregnancy?

yes ☐ How many cigarettes per day? …… per day / …… per week
How many years did you smoke? …… years
When did you stop smoking? …… years ago
no ☐

- Did you smoke during your pregnancy?

yes ☐ How many cigarettes per day? …… per day / …… per week
no ☐

- Have you been smoking in your flat in the past twelve months?
Cigarettes smoked on the balcony or terrace should be excluded.

yes ☐ cigarettes per day ……per day
amount mother ……per day
amount father ……per day
amount others ……per day
no ☐

- Had there been smoking indoors before, but stopped due to your pregnancy?

No smoking ever ☐ yes ☐ no ☐

- Did you regularly drink alcohol before your pregnancy?

  yes ☐ number of alcoholic drinks …… per day / …… per week

  no ☐

- Did you drink alcohol during your pregnancy ?

  yes ☐ number of alcoholic drinks …… per day / …… per week

  no ☐

General questions

The following questions 1-4 refer to ethnical origin rather than geopraphical origin.

- Name of country you (**mother**) were born in ……

- Name of country **your parents** were born in grandmother ……

  grandfather ……

- Name of country the **father** of the child was born in ……

- Name of country the **father's parents** were born in grandmother ……

  grandfather ……

- What is the highest qualification you achieved?

	mother	**father**
No degree	☐	☐
Elementary school	☐	☐
Secondary school	☐	☐
High school	☐	☐
University, college	☐	☐
Other degree	☐	☐

Which one? …… ……

- What is your profession?

mother …… father ……

Details of the father

- Height of the father.....cm
- Weight of the father.....kg
- Age of the father .....years

Closing question

Asked to rate your pregnancy load on a scale from 1 (“no burden“) to 10 (“unbearable burden”), which number of points would you give? .....

Afterword

Thank you very much for your patience and conscientiousness in answering our questionnaire. We wish you and your newest family member only the best for the future!

Your e-mail-adress for potential questions: .....

Danksagung

An dieser Stelle möchte ich allen beteiligten Personen danken, die mich bei der Anfertigung meiner Doktorarbeit unterstützt haben.

Mein besonderer Dank gilt Prof. Dr. Orsolya Genzel-Borovicény, Univ-Prof. Dr. Regina Ensenauer und PD Dr. med. Claudia Nussbaum: meine hervorragenden Betreuerinnen.

Liebe Orsi, vielen Dank für deine großartige Betreuung, deine Anweisungen und Korrekturen aber auch Geduld und Verständnis bei den zwei Schwangerschaften. Ohne dich wäre das Ganze vielleicht sogar nicht mal fertig geworden und ich will mich auf diesem Wege dafür ganz herzlich bedanken!

Natürlich möchte ich mich an dieser Stelle bei den Wissenschaftlern des Max-Planck-Instituts bedanken für das Verarbeiten der Trockenblutproben, das Ausführen der Massenspektrometrie und das Verwalten bzw. Bereitstellen der Daten, um mir das weitere Analysieren zu ermöglichen. Insbesondere möchte ich auch Dr. Phillip Geyer und Dr. Johannes Müller danken für das Beantworten jeglicher technischen, wissenschaftlichen und statistischen Fragen.

Susanne, Janne, Wolfgang und Robin: auch bei euch möchte ich mich für eure harte Arbeit im Rahmen der Studie ganz herzlich bedanken!

Außerdem möchte ich mich bei allen Wöchnerinnen und ihren Neugeborenen bedanken, die sich bereit erklärt haben an der Studie teilzunehmen bzw. ihre Kinder haben teilnehmen lassen.

Meinen Mann, Eltern, Geschwistern und Freunden danke ich für ihre Geduld, Ermutigungen und Zusprüche während der Arbeit an dieser Doktorarbeit. Ohne euch hätte ich es nicht geschafft!

Eidesstattliche Versicherung

van Hagen, Nadja

Name, Vorname

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

The Proteome of newborns with maternal obesity

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.

Landsberg am Lech, 08.10.2025

Ort, Datum

van Hagen, Nadja

Unterschrift Nadja van Hagen