From the Institute of Endocrinology and Diabetes of the University Hospital Schleswig-Holstein in Lübeck

Director:

Prof. Dr. med. Sebastian Meyhöfer

Prof. Dr. rer. nat. Jens Mittag

Association Between Angiopoietin-2 and Obese-related Human Metabolic Homeostasis

Thesis

For the acquisition of doctorate

at University of Lübeck

-Medical Section-

presented by

Xueqi Cheng

from Hubei, China

Lübeck 2021

1. Referee: Prof. Dr. med. Sebastian Meyhöfer

2. Referee: Prof. B. Gibbs PhD

Day of oral examination: September 8th, 2022

Granted for print. Lübeck, September 8th, 2022

TABLE OF CONTENTS

ZUSAMMENFASSUNGEN

Hintergrund: Die Prävalenz von Übergewicht und Adipositas nimmt weltweit zu. Übergewicht und Adipositas sind mit einer Beeinträchtigung der Endothelfunktion assoziiert und diese beginnt bereits in einer sehr frühen Phase der Adipositas und trägt zu der hohen Morbiditäts- und Mortalitätsrate bei Adipositas bei. Zirkulierende Biomarker einer beeinträchtigten Endothelfunktion sind jedoch noch nicht vollständig bekannt. Das Cytokin Angiopoietin-2 spielt im Angiopoietin/Tie-Signalweg eine Rolle beim Überleben von Endothelzellen, und es Evidenz dafür, dass Ang-2 eine Beeinträchtigung der Endothelzellen induzieren könnte. Zudem ist bekannt, das Angiopoietin mit dem Körpergewicht korreliert ist. Daher könnte zirkulierendes Angiopoietin-2 das Verständnis adipositasbedingter Gefäßschäden von Bedeutung sein Es gibt jedoch nur wenige Studien zum zirkulierenden Angiopoietin-2 bei Adipositas und den damit verbundenen Risikofaktoren beim Menschen.

Methoden: Diese Arbeit bestand aus zwei Teilen. Zunächst wurde eine systematische Literaturrecherche zu Angiopoietin-2 bei Adipositas durchgeführt. Anschliessend wurde auf Basis vorab durchgeführter Studien der Arbeitsgruppe Angiopoietin-2 im Plasma unter vier Gesichtspunkten untersucht: 1) ob Angiopoietin-2 bei gesunden Männern eine zirkadiane Rhythmik aufweist; 2) ob Angiopoietin- durch eine fettreiche Diät über 24 Stunden ansteigt; 3) ob Angiopoietin-2 durch ein Nacht mit Schlafrestriktion auf 4 Stunden ansteigt; 4) ob Angiopoietin-2 nach 1 jähriger Therapie der obstruktiven Schlafapnoe abnimmt.

Ergebnisse: In der narrativen Übersichtsarbeit haben wir gezeigt, dass Angiopoietin-2 im Blut nur schwach und im subkutanen Fettgewebe stärker als in anderen Stoffwechselorganen von Mäusen und Menschen exprimiert wird. Es wurde festgestellt, dass Angiopoietin-2 durch das Körpergewicht, das Alter und verschiedene Zytokine erhöht wird. Eine anhaltend hohe Angiopoietin-2-Konzentration verschlechtert die endotheliale Entzündung und beeinträchtigt die Gefäßbildungskapazität und -funktionen in Organen. Angiopoietin-2 ist jedoch für die Lipidakkumulation in Fettgeweben notwendig. Darüber hinaus wurden erhöhte Angiopoietin-2-Spiegel bei Fettleibigkeit und verschiedenen mit Fettleibigkeit zusammenhängenden Krankheiten beim Menschen festgestellt, und die erhöhten Angiopoietin-2-Spiegel wurden mit einer schlechten Prognose bei einer Vielzahl dieser Krankheiten in Verbindung gebracht. In Studien am Menschen fanden wir Folgendes heraus: i) Bei gesunden jungen Männern ist keiner diurnaler Rhythmus von Angiopoietin-2 im Plasma festzustellen. ii) Eine eintägige fettreiche Diät wirkte sich im Vergleich zu einer Kontrolldiät bei gesunden jungen Männern nicht auf den Angiopoietin-2-Plasmaspiegel aus. iii) Eine Nacht Schlafrestriktion bei gesunden jungen Männern konnte den Angiopoietin-2-Plasmaspiegel im Vergleich zu regelmäßigem Schlaf nicht verändern. iv) Nach einer 12-monatigen Behandlung der obstruktiven Schlafapnoe veränderte sich der Angiopoietin-2-Plasmaspiegel der Personen nicht.

Diskussion: Die in dieser Arbeit erbrachten Nachweise werden zu einem besseren Verständnis der Rolle von Angiopoietin-2 bei Adipositas und des Musters von Angiopoietin-2 im Blut bei verschiedenen mit Adipositas verbundenen Risikofaktoren beitragen. Die Ergebnisse gehen jedoch weit auseinander. In unseren experimentellen Studien stellten wir fest, dass der Angiopoietin-2-Spiegel im Blut sich nicht veränderten. Um die Rolle von Angiopoietin bei fettleibigkeitsbedingten Krankheiten weiter zu erforschen, sind jedoch Humanstudien mit längerfristiger Intervention und mehr Teilnehmern erforderlich.

ABBREVIATIONS LIST

Ang-1	Angiopoietin-1
Ang-2	Angiopoietin-2
AT	Adipose tissue
AHI	Apnea hypopnea index
BMI	Body mass index
FOXC2	Forkhead box protein C2
HFD	High fat diet
IGT	Impaired glucose tolerance
IQGAP1	IQ motif-containing GTPase activating protein 1
MetS	Metabolic syndrome
NAFLD	Non-alcoholic fatty liver disease
NF-κB	Nuclear factor κB cell
OSA	Obstructive sleep apnea
PI3K	Phosphatidyl-inositol 3-kinase
SAT	Subcutaneous adipose tissue
SEM	Standard error of means
T2D	Type 2 diabetes
Tie-2	Tyrosine kinase-2
TNF-α	Tumor necrosis factor-alpha
UAS	Upper airway stimulation
VAT	Visceral adipose tissue
VE	Vascular endothelial
VEGF	Vascular endothelial growth factor
WAT	White adipose tissue

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

1.1 General outline of the thesis

Obesity has undoubtedly become a worldwide epidemic and increased the total number of deaths dramatically [1]. Lifestyle in our 24h societies such as unhealthy eating habits and short or disrupted sleep are associated with obesity which is closely related to many metabolic diseases such as type 2 diabetes (T2D), cardiovascular diseases, or obstructive sleep apnea (OSA) syndrome. This cluster of metabolic disorders triggers endothelial vascular damage [2]. In turn, these endothelial impairment-related diseases contributed to the major mortalities in obesity [2]. Notably, the impaired endothelial function can be present in a very early phase of the progress of obesity [3]. Thus, an early prediction of endothelial impairment becomes essential, as a target to prevent or postpone obesity-related metabolic diseases [3]. However, the circulating biomarkers related to vascular wall biology have not yet been fully studied [4].

The core cytokine Angiopoietin-2 (Ang-2) in the Angiopoietin/Tie pathway has been shown to play a role in endothelial cells survival [5,6]. It was found that Ang-2 could induce endothelial cells impairment, which is achieved by evoking the pathological angiogenic in cancer [7,8] and sensitizing the inflammatory response [6,9,10]. Likewise, in obesity, adipose tissue (AT) angiogenesis is regulated by the same mechanism as tumor neovascularization [11,12], and systemic inflammation consists of one of the main pathologic mechanisms in obesity [13]. As a neoplastic and inflammatory-related molecule, Ang-2 may have been involved in the process of obesity.

Based on previous research, some evidence has indicated a close association between Ang-2 and obesity. Ang-2 was found to express the highest in AT in comparison to other metabolic organs such as liver and muscle [14–17]. The AT expression and blood levels of Ang-2 increased in humans with obesity [17–21], and the increased Ang-2 was associated with AT pathological angiogenesis and metabolic status [17]. In addition, in several obesity-related metabolic diseases, circulating Ang-2 increased and was associated with vascular impairment, such as cardiovascular diseases [22–26], metabolic syndrome (MetS) [27], as well as diabetes in humans [28–30]. Thus, there is evidence for a link between Ang-2, obesity, and obesity-related metabolic disorders.

In the context of obese related risk factors, animal and *in vitro* studies had shed light on the association between Ang-2 and obesity related risk factors such as high fat intake, sleep loss, and hypoxia states. Animal studies had shown that high fat diet (HFD) upregulated Ang-2 expression in AT of wild-type mice [16]. It has been found that hyperinsulinemia can increase Ang-2 expression to elicit endothelial inflammation in *vitro* [31]. In addition, circulating Ang-2 in patients with hyperinsulinemia has increased compared with normal-insulinemic subjects [31]. This is of relevance

since it has been shown that short sleep loss reduces insulin sensitivity in healthy men [32]. Thus, an indirect association between short sleep and increased Ang-2 can be hypothesized. Furthermore, Ang-2 was found up-regulated in response to hypoxia in the isolated mesangial cells [33].

Overall, studies pointed out the link between Ang-2 and the progress of obesity. However, data on Ang-2 metabolic regulation and its association to obesity-related metabolic dysfunctions in humans are scarce to date. Besides, it is currently lack of a review related to Ang-2 and obesity and AT so far. Therefore, the project aims to attempt to explore the association between Ang-2 and obese-related human metabolic homeostasis.

This doctoral thesis consisted of two parts. Firstly, the first narrative review mainly focusing on the association between Ang-2 and obesity was carried out. Based on the narrative review, studies of Ang-2 in the context of obesity have been performed in order to answer unsolved questions: i) whether circulating Ang-2 has a circadian rhythmicity in healthy men; ii) whether blood Ang-2 can be impacted by short HFD; iii) whether blood Ang-2 is altered by short sleep deprivation; iv) whether blood levels of Ang-2 could be decreased by OSA management.

To answer these questions, Ang-2 blood levels under the aforementioned four approaches based on studies previously were taken. To this end, evidence provided in this thesis will help to a better understanding of the association between Ang-2 and obesity, and the pattern of blood levels of Ang-2 in the context of obese related risk factors.

From our data, we found the short higher fat intake, sleep loss and 50 % of OSA improvement are not strong enough to trigger the changes of circulating Ang-2. For this, we might not be able to answer whether there is a link between Ang-2 and endothelial cell impairment in these obesity risk factors. However, in our review, several in *vitro* and animal evidence has raised a link between Ang-2 and obese related risk factors via endothelial cells impairment. Thus, further human studies still need for a longer intervention including females, older age, with and without metabolic disorders, in which to explore the correlation and mechanisms between Ang-2 and obese related risk factors in humans.

1.2 Angiopoietin-2— One of the core cytokines in regulating endothelial cells survival

The vascular endothelium consists of a single layer of endothelial cells covering the luminal side of blood vessels, which forms an essential barrier to control the passage of plasma and cells from the bloodstream into the underlying tissues [34]. Healthy angiogenesis requires a balance between pro-angiogenic and anti-angiogenic molecules. Imbalanced angiogenic molecule expression breaks the quiescence of endothelial cells status, which results in inadequate blood vessel formation [35]. It has been shown that except for the female reproductive organs, e.g., ovary, placenta, and uterus, the angiogenesis induced by microenvironmental factors were almost exclusively associated with pathology, e.g. hypoxia or inflammation [36]. In line with this, a broad array of diseases that are associated with the activation of endothelial cells is as diverse as cancer, inflammatory and infections, heart diseases, as well as diabetes and obesity [37]. However, studies in the prediction of endothelial cells status are scarce. Ang-2 as one of the core componence of the Angiopoietin/Tie pathway has been shown to play a role in endothelial cells survival [5,6]. Ang-2 might be a link to diseases that are associated with endothelial cells impairment.

1.2.1 Angiopoietin/Tie pathway

The Angiopoietin/Tie pathway was discovered in the 1990s and is one of the main systems regulating vascular function other than the vascular endothelial growth factor (VEGF) /VEGF-receptor system [38,39]. The ligands Angiopoietin-1 (Ang-1), Ang-2, and the endothelial cells-specific tyrosine kinase-2 (Tie-2) receptor are the core components of the Angiopoietin/Tie pathway [40]. Both ligands Ang-1 and Ang-2 are ~75-kD secreted proteins with a similar molecular structure which share about 60% amino acid homology with each other [38]. It has been shown that the balance of the Angiopoietin/Tie system is essential for normal vascular development in adult mice and humans [40]. The homeostasis of the Angiopoietin/Tie system appears to promote endothelial cells' survival and vascular assembly, stability, and maturation [5,6] (**Figure 1**).

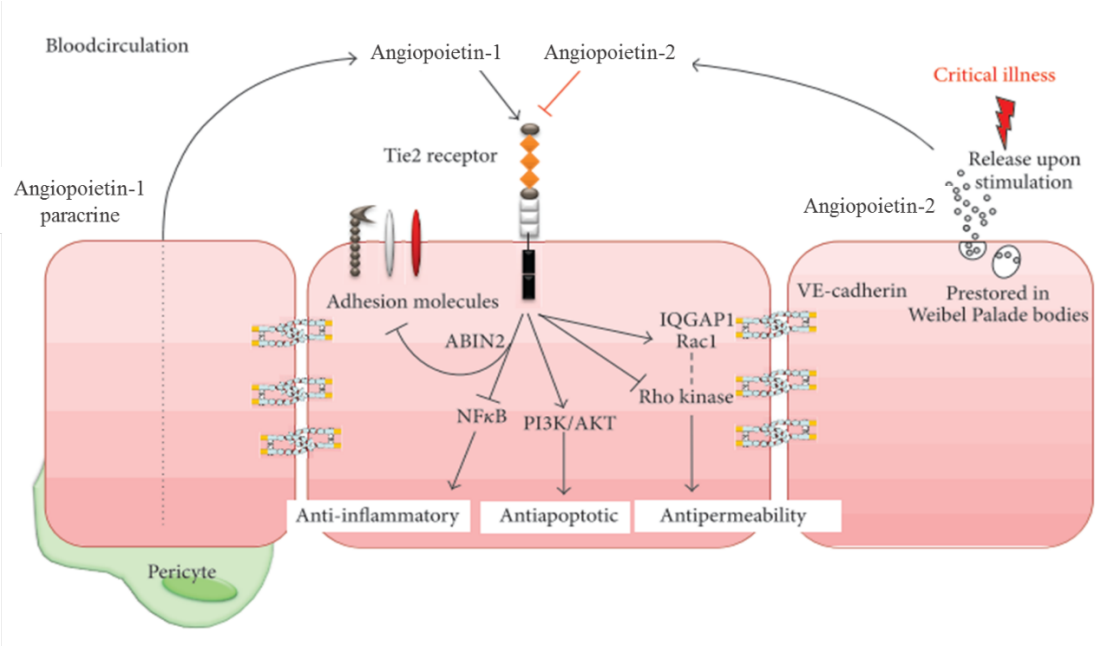

Figure 1. *Scheme of the endothelial Angiopoietin/Tie-2 system highlighting fundamental signaling pathways.* nuclear factor κB cell (NF-κB); phosphatidyl-inositol 3-kinase (PI3K)/Akt Pathway; IQ motif-containing GTPase activating protein 1 (IQGAP1); vascular endothelial (VE). Alexander Lukasz, et al. 2012, is licensed under Creative Commons Attribution 3.0 Genetic License. The terms "Angiopoietin-1" and "Angiopoietin-2" were modified from their abbreviation to full name from the original.

1.2.2 Expression of Angiopoietin-2

Regarding the expression of the ligands, Ang-1 is continuously secreted from pericyte and vascular smooth muscle cells and is widely expressed, although in small amounts, in the heart and liver [41]. By contrast, Ang- 2 is expressed slightly by the resting endothelium and is almost exclusively expressed by endothelial cells [42]. Besides, Ang-2 is pre-stored in Weibel-Palade bodies of endothelial cells but can be released rapidly in response to external stimuli [43]. Thus, compared to the continuously widely expressed Ang-1, Ang-2 as the endothelial-specific expressed factor might be a better predictor of endothelial cells impairments.

1.2.3 Function of Angiopoietin-2

Functionally, ligand Ang-1 could bind directly to Tie-2 and induce rapid activity (phosphorylation) of Tie-2 in endothelial cells [41]. Ang-1-mediated Tie-2 activation induces anti-apoptotic, anti-inflammatory, and anti-permeability effects [38,40]. Thus, Ang-1 contributes to maintaining the quiescent status of the endothelium [44]. The absence of Ang-1 or Tie-2 causes severe vascular abnormalities in the developing mouse embryo [38] (**Figure 1**).

Ang- 2 was found strongly upregulated following endothelial activation [6]. Ang-2 was initially

described as an antagonist to Tie-2 [38,40]. Compared to Ang-1, Ang-2 has a similar binding affinity to Tie-2 [45]. By competitive inhibiting the Ang-1–Tie-2 signaling, Ang-2 leads to endothelial cells inflammation, apoptosis, and permeability [38]. Subsequently, Ang-2 was found could be either antagonist or agonist to Tie-2 depending on the conditions [6,46]. Ang-2 could stimulate angiogenesis (i.e. tumor) in combining with VEGF, or the activity of Ang-2 promotes the inflammation by upregulating the response of endothelial cells to tumor necrosis factor-alpha (TNF-α) [47] (**Figure 2**), which will be introduced further in the following subsections.

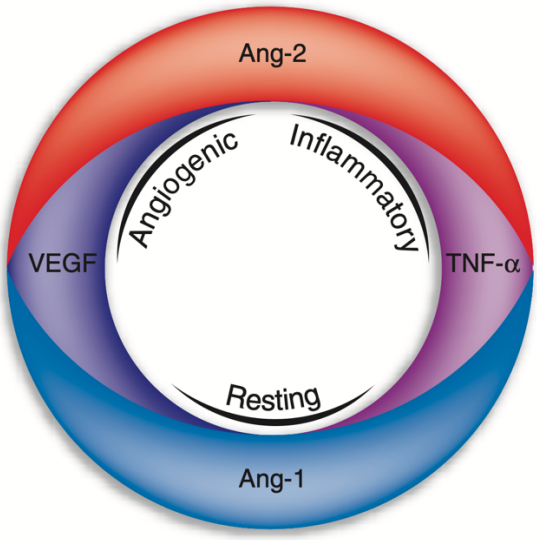

Figure 2. *The functions of Angiopoietin-2*. Ang-1, Angiopoietin-1; Ang-2, Angiopoietin-2; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor. (Reproduced with permission from Beat A Imhof et al, 2006 [47])

1.2.3.1 Angiopoietin-2 involves in pathological angiogenesis in tumors

Pathological angiogenesis plays an important role in the onset and progression of malignant tumors [48]. Overexpressed Ang-2 is broadly observed in tumor-induced angiogenesis and metastasis, which provides strong evidence for cooperation between VEGF and Ang-2 in the angiogenic switch from the avascular to the vascular phase in cancer [7]. Through blocking Ang-1, Ang-2 activates blood vessels that reverts the vessels into a more plastic state, which allows the endothelial cells to have a higher responsiveness to the sprouting signal provided by VEGF [49]. In addition, hypoxia is a further common cause of many malignant tumors. Ang-2 was observed to be highly expressed in necrotic and hypoxic regions in human malignant glioma tissues [8].

1.2.3.2 Angiopoietin-2 involves in the pro-inflammatory states

In addition to its role in angiogenesis, Ang-2 has been shown to act as an important pro-inflammatory factor [9]. It has been known that the inflammatory response occurs in a highly regulated manner [50]. The pathogen that enters the body induces the secretion of cytokines and chemokines, which can activate neighboring endothelial cells and attracts leukocytes. Leukocytes then extravasate from the blood into the tissue by leukocyte rolling, activation, firm adhesion [50]. TNF-α is one of the inflammatory cytokines, which prompt the upregulation of the adhesion molecules in endothelial cells. These upregulated adhesion molecules promote leukocytes' adhesion and extravasation. Ang-2 was able to sensitize endothelial cells to inflammatory stimuli by upregulating the TNF-α-induced monocyte adhesion in mice [9]. In turn, Ang-2-deficient mice cannot elicit an acute inflammatory response in the peritonitis mice model as a result of impairing the ability of cytokine-inducible adhesion molecule expression on the surface of their luminal cells after inflammatory activation [9]. Besides, a positive association has been observed between Ang-2 and several inflammatory factors such as high-sensitive C-reactive protein and white blood cells count in humans [10]. Further, increased Ang-2 levels are reported in pneumonia, sepsis [6], and several autoimmune diseases such as patients with lupus [51] and rheumatoid arthritis [52,53].

Tumors are an illustration of the concept that angiogenesis inhibition can serve as an effective complementary tool to limit tissue expansion [54]. Likewise, in obesity, AT angiogenesis is regulated by the same mechanism as tumor neovascularization, such as the VEGF-dependent pathway [11,12]. It is known that systemic inflammation consists of one of the main pathologic mechanisms in obesity [13]. As a neoplastic and inflammatory-related endothelial-specific stimulated molecular, Ang-2 is highly possible had involved in the progress of obesity.

1.3 Angiopoietin-2 involves in the progress of obesity

Ang-2 was found to be expressed higher in AT than other metabolic organs [14–17]. Ang-2 is not only to be increased in humans with obesity [17–21], but also involves in the AT pathological angiogenesis and metabolic status [17] as well as leptin release [14,55].

1.3.1 Obesity—A pandemic disease with endothelial impairments

AT could be broadly classified into two distinct categories: white adipose tissue (WAT) and brown adipose tissue (BAT) [56]. Obesity is a chronic disease in which a positive energy balance over a long term leads to an excessive accumulation, dysfunction, and remodeling of WAT [57]. Based on WHO definition, a body mass index (BMI) over 30.0 kg/m^2 is considered as obesity [57]. The worldwide prevalence of obesity nearly tripled between 1975 and 2016 [57]. In 2016, more than 1.9 billion adults over the age of 18 were overweight, i.e. BMI between 25.0 and 29.9 kg/m^2, and more than 650 million (13%) of the world's adult population were obese [57]. Likewise, the epidemic of obesity is quite prominent in Germany where 20 million people are obese, and over half of adults are overweight [58]. A different distribution may be diverse by race and region, but no sign of any country or region worldwide shows a downward trend in the prevalence of obesity [59].

It has been known that obesity has a close association with total deaths and disability-adjusted lifespans [1], such as the increase in vascular mortality, diabetes-related mortality, and neoplastic mortality [2]. Further, rates of musculoskeletal disease such as osteoarthritis, Alzheimer's disease, and depression can be increased by obesity. These diseases require long-term treatment and frequent medical care, which can induce unemployment, lower productivity, and social disadvantages, thereby contributing to a decline in life expectancy [59,60], and quality of life [61]. In this scenario, improving strategies for the prevention of obesity has become crucial.

In AT depots, the vascular circulatory system is essential to provide nutrition and oxygen [62]. Besides, blood vessels also transport growth factors, cytokines, and hormones between AT and other organs to maintain the homeostasis of AT microenvironment [63]. In healthy AT, the size of lipid droplets expands in adipocytes to store lipids when nutrients intake increases, and as lipid droplets size expands, the utilization of oxygen decreases. This mild hypoxic state can induce angiogenesis and extracellular matrix remodeling to reduce hypoxia [64]. In obesity, AT depots rapid and prolonged expansion [64]. The point when fat accumulation exceeds the tissue's angiogenic capacity leads to persistent hypoxia, fibrosis, cells aging, and necrotizing adipocyte death [64]. During AT structure reshaping, its function is altered leading to the imbalanced secretion of pro-inflammation factors, likely promoting systemic low-grade inflammation, i.e., macrophage infiltration and cytokines production [13]. Thus, inadequate vascular expansion in AT with systemic low-grade

inflammation consists of the basic pathologic mechanism in obesity [13]. These pathologic states ultimately lead to unhealthy AT tissue expansion, which contributes to the main cause of systemic metabolic disorders in obesity [64].

Vascular mortality is one of the main poor prognoses in obesity [2], such as MetS, cardiovascular diseases, T2D [65,66]. Each of these diseases is precipitated by the dysfunction in the vascular endothelial cells including vasodilating reduction and pro-inflammation [67]. Thus, the measurement of endothelial impairment is necessary to predict obesity-related metabolic diseases.

Given the diversity in function and heterogeneity of the endothelium, measurement of endothelial function is challenging [68]. For the assessment of pre-clinical diseases, an ideal technique should be non-invasive, accurate, reproducible, low cost, and easily to perform [69]. Some techniques that fulfill most of the criteria are close to being considered a clinical surrogate endpoint such as carotid ultrasonography, ankle-brachial index, and carotid-femoral pulse wave velocity [68]. However, reliable skilled operators are required for consistent and accurate results. New efficient methods for the diagnosis of endothelial impairment in humans would be an urgent need to reduce the burden of obesity worldwide. Notably, circulating biomarkers related to vascular wall biology could be easier measured, wider available, and methodological standardized has not yet been fully studied [4]. C-reactive protein is currently the only confirmed circulating biomarker related to vascular wall biology [4]. But the elevated C-reactive protein levels lack specificity for vascular damage [4]. It was found that blood Ang-2 was positively associated with high-sensitive C-reactive protein [10]. Thus, it can be speculated that Ang-2 as the endothelial-specific expressed factor [42] might be a proper predictor of endothelial cells impairment.

1.3.2 Angiopoietin-2 in adipose tissues, circulations under physiological conditions

In human adults, unlike the low expression in the metabolic organs such as liver, muscle, Ang-2 was found expressed highest in AT, except for the female reproductive organs [16]. The relatively high expressed Ang-2 in AT might be due to its continuous plastic character which needs more neovascularization [70]. Among AT depots, the Ang-2 expression is higher in subcutaneous adipose tissue (SAT) than visceral adipose tissue (VAT) and BAT [16].

1.3.3 Angiopoietin-2 increases in adipose tissues and circulations under obesity

In obese patients, Ang-2 in SAT is overexpressed [17]. Besides, the Ang-2 expression in SAT was found positively associated with fasting insulin concentrations [17]. In turn, the overexpressed Ang-2 in SAT was downregulated after 6 months of weight loss, with an improved lipid and insulin metabolic status [17].

In addition, a close relationship has been shown between Ang-2 and leptin [14,55]. Leptin is one of the key adipokines, which regulates energy homeostasis through appetite suppression and energy expenditure elevation [71,72]. Hyperleptinemia initiates endothelial dysfunction as it independently increases the risk of coronary artery disease [73]. Ang-2 was found involved in leptin mediated endothelial dysfunction in AT [14,55]. It was shown that leptin was able to induce upregulated Ang-2 expression in murine AT depots without the changing of VEGF in both in *vivo* [14,57], and in *vitro* [14] studies. The upregulated murine Ang-2 in AT was associated with the apoptosis of adipose endothelial cells [14]. Circulating Ang-2 was also increased in obesity [17–21] and a positive correlation between serum Ang-2 levels and BMI has been shown in a previous study [19].

These data indicate a link between Ang-2 and AT associated metabolic disorders. Moreover, strong evidence indicates that Ang-2 was involved in obesity-related metabolic diseases in humans as introduced in the following section.

1.3.4 Angiopoietin-2 in obesity-related metabolic diseases

In human studies, Ang-2 levels were found to be associated with obese-related metabolic diseases such as cardiovascular disease [22–26], MetS [27], and non-alcoholic fatty liver disease (NAFLD) [74], as well as diabetes and diabetic chronic complications [28–30].

Several lines of evidence show that Ang-2 acts as predictor and biomarker of cardiovascular diseases including heart failure [22,23,75], and myocardial infarction [24–26]. MetS as a clinical condition including a group of metabolic risk factors that appear to directly promote the development of atherosclerotic cardiovascular disease [76] and higher serum Ang-2 levels in MetS patients than those without MetS has been reported [27]. In addition, NAFLD is a group of diseases including steatosis, nonalcoholic steatohepatitis, cirrhosis, and liver failure [77] and is commonly associated with severe obesity with BMI higher than 35 kg/m^2 [78,79]. A positive correlation was found between VAT expression of Ang-2 and lobular inflammation in patients with severe obesity and NAFLD [80], and serum Ang-2 levels were increased in patients with histological nonalcoholic steatohepatitis compared with simple steatosis [74].

Further, patients with diabetes show higher blood levels of Ang-2 in comparison to controls [81–85]. The upregulation of Ang-2 in diabetes could be induced by both hyperglycemia states and advanced

glycation end products in *in vitro* study [86]. Regarding diabetic angiopathy, the expression of Ang-2 correlates with the presence of microvascular injury such as in albuminuria [87] and retinal blood vessel permeability in an animal study [88]. Accordingly, in patients with diabetes, elevated plasma Ang-2 is associated with the indexes of endothelial damage and dysfunction [89]. Notably, after one year of intensified cardiovascular risk management, the increased Ang-2 in diabetes becomes irreversible when diabetic cardiomyopathy has developed [89]. Thus, an early intervention of the increased Ang-2 becomes essential, which might be a new target to protect the vascular impairment.

1.3.5 Endothelial impairment appears in the early phase of obesity

It was found that the impaired endothelial function can initiate in a very early phase of the progress of obesity, irrespective of comorbidities [3]. Through measuring carotid intima-media thickness and wall dilation, the endothelial impairment appears even in the absence of insulin resistance in obesity [3]. Thus, monitoring of Ang-2 in the context of obesity related risk factors is relevant to understanding the potential vascular impairment. Several animals and *in vitro* studies had shed light on the association between Ang-2 and several obesity related risk factors as introduced in the following section.

1.4 Obesity related risk factors and Angiopoietin-2

The cause of obesity consists of multiple factors, including genetics [90], epigenetics [91], and acquired lifestyle such as dietary patterns, physical activity, and sleep behavior [92]. These aspects are not independent of one another, they interplay with each other closely [92]. In this section, the evidence that Ang-2 may associate with high fat intake, sleep loss, and hypoxia status will be illustrated.

1.4.1 Diurnal (a)rhythmicity as obesity related risk factor

It is known that the cell clock generates the rhythm of most metabolic functions [93]. This cell clock system locates in the suprachiasmatic nucleus of the hypothalamus generating 24-hour periodic oscillations in humans. The suprachiasmatic nucleus regulates important physiological activities such as the endocrine rhythm, fast/eating cycle, and sleep/wake cycle [94]. Clock genes coordinate the function of lipid synthesis and fat storage regulation and can regulate liver lipid metabolism [93], hepatic gluconeogenesis [95], and circadian gene expression in glycolysis [96], respectively. Asynchrony of the circadian clock ie, sleeping out of phase of habitual sleeping times—as a consequence of shift work, chronic jet lag, intentional sleep restriction, deprivation is involved in abnormal storage of adipocytes, which supports the increase in the incidence of obesity [97]. It has been aforementioned that Ang-2 is involved in dysregulating obesity-related lipid and glucose metabolism [14,17,55]. Besides, Ang-2 can be modulated by the essential adipokine leptin in the process of obesity [17] and a clear diurnal change in circulating leptin with the highest between midnight and early morning has been reported [98].

However, whether circulating Ang-2 levels fluctuate by the circadian clock is still unclear. Thus, detecting the diurnal rhythm of circulatory Ang-2 aims for the primary step of this thesis work.

1.4.2 High fat diet as obesity related risk factor

Although there are many possible causes of the epidemic of obesity, energy-dense, high fat and palatable food and finally high caloric food is discussed as the main determinant of obesity [99]. Among them, HFD and subsequently high caloric intake are prevalent in recent life [100]. It has been shown that HFD plus high calories is a potent risk factor for weight gain and obesity [101]. Hormonal, metabolic, and cardiovascular disorders are among the consequences of HFD [43,102].

The hypothalamus has known to be a key hub for the detection of hunger and the organization of eating behavior [103,104]. However, a salience network in the brain was found associated with motivation, desire, and craving for food. This network can be activated by HFD, which makes the brain more inclined to choose HFD than ordinary food [105]. In addition, even before being tasted and absorbed, HFD can affect several key brain nodes and networks through visual and olfactory stimuli [106]. Furthermore, more factors had been discovered that can contribute to the higher chance of HFD intake: exogenous factors such as socio-cultural, e.g., cultural habits, religion, medication e.g., oral anti-diabetic agents, atypical antipsychotics, corticosteroids, alcohol, and homeostatic factors such as the biomedical, e.g., leptin deficiency, melanocortin type 4 receptor mutations, cerebral trauma, insulinoma, and mental disorders, e.g., stress, mental health conditions [107]. Together, multiple factors can contribute to the increased HFD intake and energy consumptions. Positive net calories ingested will finally lead to excessive weight gain.

Pathologically, animals under HFD tend to consume more calories, have higher AT components, suffer from hyperinsulinemia and hyperleptinemia, and are insulin resistant compared with animals under low-fat diet [108]. Nevertheless, other than the over energy-consuming, dietary fat *per se* in HFD can play as the predisposing factor of obesity. The diet composition and/or energy density could affect fat deposition and insulin sensitivity under identical energy intake [108]. Wood and his colleagues studied rats that were either fed with HFD (fat: 4.62 kcal/g) or low fat-fed rats (fat: 3.85 kcal/g), but matched energy intake [108]. After 10 weeks, HFD fed rats had similar body weights but significantly more AT than the low-fat fed rats [108]. To further assess the sensitivity of their brains to insulin, the HFD fed rats administered insulin into the brain for 4 days gained their bodyweight and behaved comparably to the HFD rats given saline after back to ad libitum food administration [109]. These data provide evidence that altering the diet composition is sufficient to render rats insensitive to central insulin [109]. In line with this, in *vitro,* insulin secretory response to glucose in islets of rats was markedly impaired after 2 weeks of HFD [110].

In pancreatic β-cells, Ang-2 seems involved in the impairment of the β-cells mass under HFD [111]. In pancreatic β-cells specific Ang-2 overexpressed mice, hypervascularization was observed under normal diet, but HFD challenge led to the decreased islet vessel area with increased apoptosis and reduced β-cells mass [111]. Furthermore, the Akt pathway was activated with increased Ang-2

expression in the corpus cavernosum in male Wistar rats after HFD. Of note, Ang-2 expression increased before serum lipid changed and obesity onset, and even preceding structural atherosclerotic features [112].

In adipocytes, increased Ang-2 is essential in maintaining the lipids in SAT [16]. It was found that Ang-2 expression is increased in both mice and humans SAT adipocytes after 24 hours of saturated fatty acids treatment in *vitro* [16]. In *vivo,* animal studies showed that HFD upregulated SAT Ang-2 expression in wild-type mice after both 3-7 days [16] and 16 weeks [113].

Adiponectin is an insulin-sensitizing adipokine [114]. It is known to stimulate skeletal muscle fatty acids oxidation and to reduce lipids uptake, which can prevent the impairment of the insulin-signaling cascade [115]. In AT-specific Ang-2 overexpressed mice, blood adiponectin increased with an overall metabolic improvement after HFD challenge [116]. In turn, the adipocyte-specific Ang-2 knockout mice decreased plasma adiponectin levels and were accompanied by impaired systemic metabolism [16]. These shreds of evidence indicated that increased Ang-2 in SAT enables an increase of the SAT fat uptake that prevents lipotoxicity in other metabolic-related tissues and circulations, to avoid ectopic lipid accumulation under HFD [16].

These animal studies raised the potential link between Ang-2 and the HFD-challenge, which proposed that Ang-2 accumulation outside of AT such as in circulations might be a predictor or marker of metabolic and lipid dysregulation under HFD. However, total calory intake had not been reported in these animal studies. A human interventional study in healthy subjects demonstrated that after a single-meal HFD (with eucaloric compared to standard meal), plasma Ang-2 levels slightly decreased in between but back to baseline at 6 hours post-prandial [117]. However, data on longer high fat intervention in humans are missing. For a better understanding of this process, we aim to assess the effects of one day of HFD on blood Ang-2 levels in healthy young men compared to a control diet.

1.4.3 Sleep restriction as obesity related risk factor

In the past few years, studies have shown that the lack of sufficient sleep, i.e., short sleep duration, sleep disturbances, and circadian desynchronization are becoming more and more common [118,119]. A meta-analysis comprised 22 populations with more than 60 thousand adults worldwide indicating that short sleep (≤5 h/night) increased the likelihood of obesity compared with regular sleep [120]. Besides, a negative correlation was found between the unit of change in BMI and per hour of sleep in adults [120]. In addition, several metabolic diseases such as hypertension, hyperlipidemia, T2D are strongly linked to short sleep duration [121]. Short sleep has become one of the important obesity related risk factors [120] and adversely affects metabolic health [118,119]. Clinical experimental studies supported the hypothesized association between short and disrupted sleep and metabolism in humans.

It has been proposed that sleep loss might trigger the hypothalamus-pituitary-adrenal axis and sympathetic activity that favor food foraging [122–124]. The activated hypothalamus-pituitary-adrenal axis then contributes to glucose tolerance impairment and positive energy balance. In accordance, the cortisol levels were increased after 40 hours of total sleep deprivation in humans [125]. However, less data was shown about the impact of one day of sleep loss on cortisol. It has been also shown that cortisol concentrations were not affected by early night sleep loss compared to regular sleep which suggests that alterations in hypothalamus-pituitary-adrenal axis activity are probably not a key mechanism after one day of sleep loss [32].

A study from our group evaluated the effect of acute sleep loss during the late vs. early night vs. regular sleep on glucose homeostasis in healthy men [32]. Data revealed that short sleep reduces insulin sensitivity irrespective of its nocturnal timing, while glucose concentrations remained stable [32]. Furthermore, carbohydrate and lipid metabolism related morning to evening regulated pathways were highly sensitive after late short sleep compared to regular sleep, whereas the rhythmic of the part of core clock genes did not been impacted [126]. It has been found that circulating Ang-2 has increased in patients with hyperinsulinemia compared with normal-insulinemic subjects [31]. In addition, hyperinsulinemia could increase the Ang-2 expression to elicit endothelial inflammation in *vitro* [31]. Besides, Ang-2 has been found essential in the regulation of lipids [16]. Whether Ang-2 had taken part in the disturbed carbohydrate and lipid metabolism after short sleep is unknown so that we aim to investigate the levels of circulating Ang-2 after short late-night sleep.

1.4.4 Obstructive sleep apnea as obesity related risk factor

OSA is a common sleep disorder and is closely related to hypoxia. The main pathological mechanism of OSA is an apnea event caused by airway obstruction, resulting in decreased blood oxygen saturation and intermittent hypoxia at night [127]. Patients with OSA revealed periods of hypopnea or apnea. The severity of OSA is defined by the apnea hypopnea index (AHI). Patients with mild OSA experience 5-15 apnea-hypopnea events /h, while those with moderate or severe OSA experience 15-30 or even more than 30 events /h [128].

It has been estimated that 40% to 70% of the obese population suffers from OSA [167]. Obese-related metabolic diseases can cause or exacerbate sleep apnea through body-dependent and physiological-dependent mechanisms. In turn, sleep apnea can lead to intermittent hypoxia, and by oxidative stress, increasing inflammation, which leads to the development of obesity related metabolic disorders such as diabetes, and cardiovascular disease [130,131].

Ang-2 has been found elevated under hypoxic conditions in *vitro* [42,132]. It has been reported that Ang-2 levels are upregulated 3.1 to 5.6-fold commitment with increased VEGF in bovine microvascular endothelial cells after 15 hours of incubation in the presence of a hypoxic environment (95% N2/ 5% CO2) [42]. Similar findings were found from mouse mesangial cells, Ang-2 and VEGF mRNA increased 8 to 24 hours with hypoxia (3% O_2) compared with normoxic (21% O_2) cells [132]. Besides, Ang-2 was increased under 1% hypoxia conditions in subcutaneous adipose-derived stem cells [133]. In addition, the secretion of Ang-2 increased when the primary cultured subcutaneous adipose-derived stem cells were exposed to oxidative stress [133].

In brief, Ang-2 has been shown as a hypoxia [42,132], oxidative stress [133], and inflammatory [9] related factor, which are analogous to the mechanisms of sleep apnea [130,131]. Thus, Ang-2 has a high possibility to be involved in the OSA prognosis. To date, there is only one study on OSA and Ang-2, showing increased plasma Ang-2 and reduced Ang-2 after adenotonsillectomy treatment [134]. However, data on Ang-2 changes in adults with OSA are not available so far.

In a recent study from our workgroup, Steffen and others followed up a group of moderate-to-severe patients with OSA (AHI, 26.5 ± 2.6 events /h) and moderate obesity. After a year under upper airway stimulation (UAS) treatment [135], not only the hypoxia status but also the glucose metabolism improved [136]. This provided us a chance to further explore changes in Ang-2 in patients with OSA.

1.5 Hypothesis and objectives

So far, complex functions of Ang-2 have been discussed in reviews. However, data focusing on the role of Ang-2 in obesity and obese-related metabolic disorders are still warranted. Therefore, to explore the current knowledge, a narrative review was conducted on original studies evaluating the role of Ang-2 in obesity. Based on the publications, we were able to point out several unsolved issues and attempted to further address these issues.

This work also aims to assess whether the obesity related metabolic risk factors (HFD, acute sleep loss, and OSA) could impact Ang-2 in humans based on data sets of previously performed studies of our work group. First, plasma Ang-2 was measured in healthy men to quantify a potential Ang-2 diurnal rhythm. Next, effects of HFD as compared to the control diet, as well as the effects of short sleep loss as compared to regular sleep on Ang-2 levels were studied in healthy young men. Lastly, the impact of intermittent hypoxia and OSA treatment on Ang-2 plasma levels in patients with OSA was explored.

It has been hypothesized that:

i) circulating Ang-2 levels show a diurnal rhythm,

ii) HFD diet increases plasma Ang-2 levels as compared to control diet in the short-term,

iii) late short sleep increases plasma Ang-2 levels as compared to regular sleep in the short-term,

iv) plasma Ang-2 levels decrease one year after OSA treatment in humans.

2 METHODS

2.1 Narrative review of the association between Angiopoietin-2 and obesity

A computerized literature search for studies reporting the association between Ang-2 and obesity was performed in PubMed, containing the following search terms (last search performed on Jun 30th, 2021): *((obese OR obesity OR fat mass OR adipose tissue OR AT) AND (Ang-2 OR Ang2 OR ANGPT2 OR ANGPT-2 OR Angiopoietin-2 OR Ang-2/Ang-1 OR Ang2/Ang1 OR ANGPT2/ANGPT1 OR ANGPT-2/ANGPT-1)).* The search was restricted to English and German publications but was neither restricted to the date of publications nor type of studies.

2.2 Experimental studies of circulating Angiopoietin-2 in humans

We took advantage of three previous studies in humans, which provide the opportunity to assess the diurnal pattern of Ang-2 (A), and the changes of Ang-2 under both a short-term (B, C) and long-term (D) approach. Assessed data should allow linking blood levels of Ang-2 to obese-related factors such as short sleep or high fat diet (**Figure 3**).

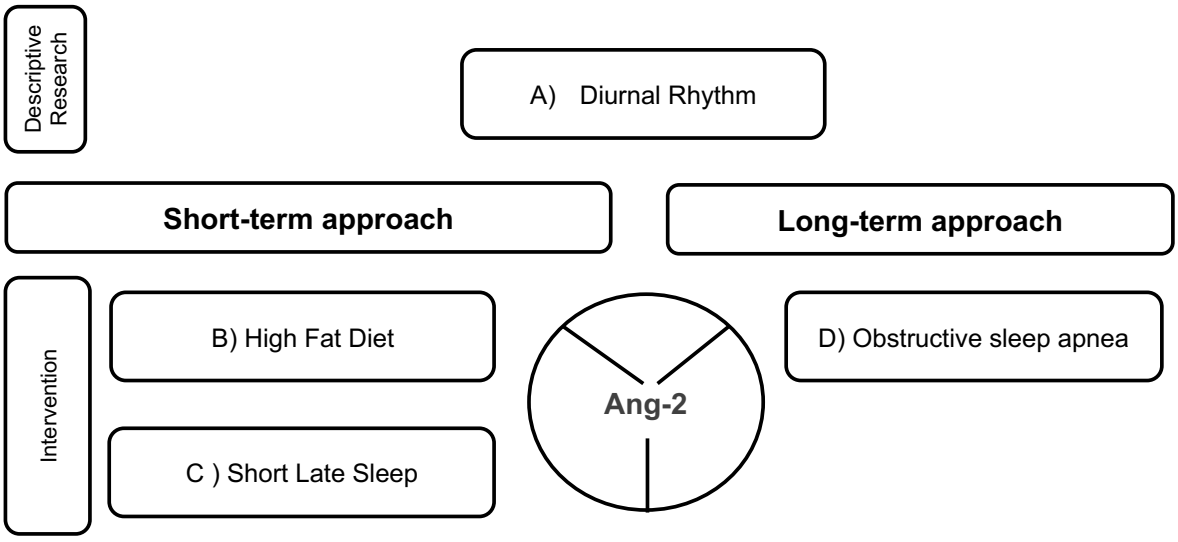

Figure 3. *Study design addressing Angiopoietin-2 metabolism using short-term and long-term approaches.*

2.2.1 Study A: Diurnal rhythm of Angiopoietin-2

In study A, the diurnal profile of Ang-2 was assessed in 19 healthy young men. This analysis is part of a comprehensive study investigating the short-term effects of eucaloric HFD on metabolism and hormone secretion patterns performed previously in our lab.

The enrollment of subjects took place via fliers at the University of Lübeck or inquiries via students' email distribution lists. Before inclusion in the study, all potential test subjects were informed in detail about the study and were examined regarding the exclusion criteria listed below. Sleeping habits and previous illnesses were asked about using an anamnesis interview.

Inclusion criteria: Healthy young men with a BMI value between 18.5 and < 25.0 kg/m² were included.

Exclusion criteria: Acute as well as chronic internal and neurological diseases, psychiatric illness (such as depression or anxiety disorder) and physical or psychological stress, positive family history of T2D. Further exclusion criteria were shifting and night work, during the past four weeks had traveled across time zones, short habitual sleep duration (< 6 hours per day), alcohol and nicotine abuse. Subjects were not allowed to be active competitive sportsmen with a high level of training. Subjects donating blood within the 3 months before or during the experiments were also excluded.

During the trial period, subjects were not allowed to participate in other medical studies. In the week before the study, subjects were asked to avoid excessive exercise and alcohol and to maintain a normal day/night rhythm, go to bed no later than 23.00 h, and not start a diet. On the day of the experiment, alcohol and the consumption of beverages containing caffeine were prohibited.

Study timeline during the trial period: Before the real test began, participants had spent an adaptation night in the sleep laboratory to get familiar with to test conditions and to rule out sleep disorders. On the test day, the subjects arrived for the respective test sessions at 18.45 h (day 1). Each subject was asked about their subjective well-being, e.g. hunger, thirst, fatigue. Furthermore, an indwelling venous catheter (Vasofix Safety® 18G von BRAUN, Melsungen, Deutschland) was inserted, which enables us to repeatedly draw blood in the following study. At 20.00 h, each subject received a controlled dinner that was individually calculated based on the subjects resting energy expenditure. Subjects went to bed at 23.00h and with 8 hours of regular sleep in our sleep laboratory.

On day 2, subjects were woken up at 7.00 h. During this day, subjects received 3 main meals in the form of breakfast (09.30 h), lunch (12.00 h), dinner (17.00 h), and a late snack (20.00 h). All subjects received a control diet, which contains 30% of fat, 55% of carbohydrate, 15% of protein, also adopted to their individual calculated total energy expenditure. Blood will be taken at regular intervals. Throughout the day until going to bed (23.00 h), subjects were encouraged to stay in the metabolic core unit of the CBBM and to be low physically active. On day 3, after 8 hours of regular sleep,

subjects were woken up at 7.00 h. The blood sample was taken at 7.30 h before starting an intravenous glucose tolerance test.

Plasma Ang-2 levels were measured repeatedly (4-time points) over 24 hours. The measurement started on day 2 from 7.30 h, then 13.00 h, 18.00 h, and the last blood taking at 07.30 h on day 3 (see **Figure 4**).

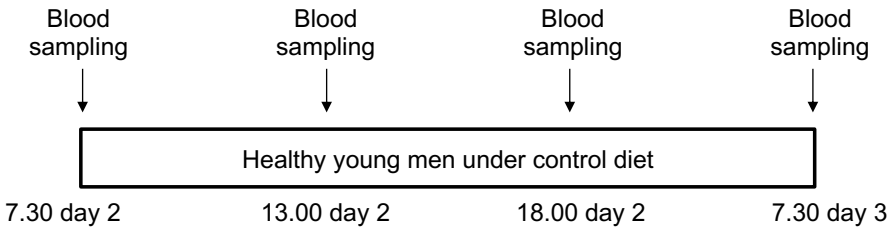

Figure 4. *Overview of Study A: Diurnal rhythm of Angiopoietin-2.*

The study was examined and approved by the ethics committee of the University of Lübeck before the start (EC 16-343). All subjects were informed both orally and in writing about the course of the study and all associated risks and gave their oral and written consent to participate in the study.

2.2.2 Study B: High fat diet and Angiopoietin-2

In study B, the effects of an eucaloric HFD on Ang-2 were assessed in the same cohort of study A. Thus, the recruiting process is described in section 2.2.1.

Study timeline during the trial period: The processes on day 1 are identical to study A, which already were mentioned above (section 2.2.1). On day 2, all subjects were examined in a total of two sessions, each with different standardized main meals manipulation:

- Control diet containing 30% of fat, 55% of carbohydrate, 15% of protein.
- HFD containing 74% of fat, 11% of carbohydrate, 15% of protein.

The total energy content of both diets was individually calculated based on subjects measured resting energy expenditure and corrected with the physical activity level of 1.3. The interval between the two sessions was at least 7 and a maximum of 21 days.

During each of the trial sessions (day 2), subjects received 3 main meals, i. e. breakfast (09.30 h), lunch (12.00 h), dinner (17.00 h), and a late snack (20.00 h). During the day, subjects were asked to behave physically inactive. At 23.00 h, subjects went to bed for 8 h regular sleep. On day 3, subjects were woken up at 7.00 h, and the last blood sample was taken at 7.30 h before breakfast. Ang-2 levels were determined at the following three timepoints over 24 hours during both sessions (**Figure 5**).

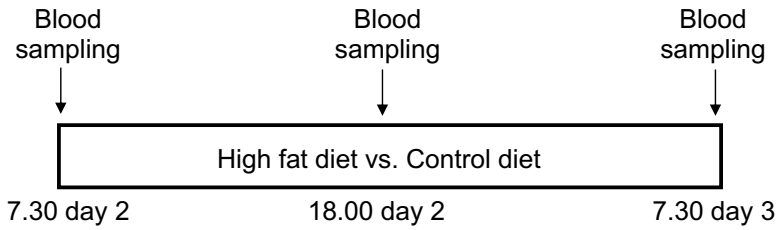

Figure 5. *Overview of Study B: High fat diet and Angiopoietin-2.*

2.2.3 Study C: Short late sleep and Angiopoietin-2

In study C, a total of 16 healthy men were included to assess the plasma Ang-2 levels under a short term of sleep deprivation. This analysis is part of a comprehensive study investigating the short-term effect of acute sleep loss on glucose homeostasis [32] and physical activity [137]. A detailed review of the inclusion and exclusion criteria based on medical history, physical examination, and venous blood sampling is shown below.

Inclusion criteria: Healthy young men, aged between 20 and 30 years, normal weight (BMI: 20.0 – 24.9 kg/m²) without diabetes history in their first-degree relatives were enrolled in this study.

Exclusion criteria: Chronic or acute illness, current medication of any kind, smoking, elevated alcohol (> 50 g per day) and caffeine (> 300 mg per day), psychiatric illness (such as depression or anxiety disorder), and physical or psychological stress, shift work, diet, during the past four weeks had traveled across time zones, and short habitual sleep duration (< 6 hours per day). Furthermore, abnormal findings on physical examination or routine laboratory testing were also exclusion criteria.

In the week preceding the experimental nights, subjects were asked to maintain a regular sleep schedule with a minimum of 7 hours of sleep per night. Physical activity, sleep behavior, nutrition, and subjective feelings during the day before experimental visits were assessed by a structured interview on each in-lab visit to ensure participants' adherence to the above-listed criteria.

Study timeline during the trial period: Participants underwent an adaptation night in the laboratory that included a standard overnight polysomnographic recording to exclude sleep disorders and for familiarization with the experimental setting.

On the test night (day 1), participants attended the research unit at 19.15 h. After a standardized light dinner (380 kcal) at 20.15 h, participants were prepared for polysomnographic recording. Thereafter, they were allowed to drink only water (max. 250 mL) until the next morning. Participants were examined for two sessions:

i) after a night with a regular sleep duration of eight hours ('regular sleep'; bedtime 22.15 – 06.45 h)

ii) after a night with partial sleep restriction of four hours in the first half of the night ('late short sleep'; bedtime 02.15 – 06.45 h).

The interval between the individual meetings was at least two weeks. Participants went to bed and lights turned off at 22.15 h and 22.30 h respectively, in the 'regular sleep' condition as control, while in the 'late short sleep' condition, they remained awake in a sitting position until 02.15 h. During the 'late short sleep' condition, participants were allowed to read and watch non-arousing movies while being monitored constantly by the experimenters. Light intensity in the laboratory was set at 300 lux.

An intravenous catheter was inserted into a vein of the participant's non-dominant distal forearm, to take blood during the test day. Ang-2 levels in the blood were analyzed in the evening before (day 1; 22.00 h) and in the morning (day 2; 07.00 h) after respective study conditions (**Figure 6**).

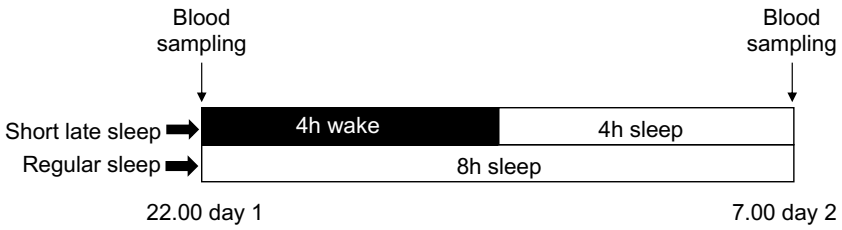

Figure 6. *Overview of Study C:* S*hort late sleep and Angiopoietin-2*.

The study was examined and approved by the ethics committee of the University of Lübeck (EC 10-109). All subjects were informed both orally and in writing about the course of the study and all associated risks and gave their written and oral consent prior to participation.

2.2.4 Study D: Obstructive sleep apnea and Angiopoietin-2

In study D, blood Ang-2 was assessed in a total of 17 patients with OSA in which the efficiency of UAS treatment after continuous positive airway pressure treatment failure was assessed [136].

Inclusion criteria: Between June 2014 and July 2015, adult patients with moderate-to-severe OSA (AHI 15–65 events per hr) who had continuous positive airway failure were implanted with an UAS system (Inspire Medical Systems, Maple Grove, MN, USA) were enrolled in this study.

Exclusion criteria: Patients were excluded if the BMI was above 35 kg/ m^2 and pronounced anatomical abnormalities preventing the effective use of assessment of the UAS were identified during a clinical examination (e.g., enlarged tonsils, complete concentric collapse at the retropalatal airway was observed on endoscopy performed during drug-induced sleep). Besides, patients were excluded with chronic obstructive pulmonary disease, severe cardiovascular diseases (e.g., New York Heart Association class III or IV heart failure, recent myocardial infarction, or severe cardiac arrhythmias), neuromuscular diseases, hypoglossal nerve palsy, persistent uncontrolled hypertension despite medication use, active psychiatric disease, and the foreseeable requirement of magnet resonance imaging [135].

Study timeline during the trial period: Eligible participants underwent surgery to implant the UAS system (Inspire Medical Systems, Maple Grove, MN, USA). One month after implantation, all participants had their devices activated and were told to use the controller to start and terminate treatment every night. After activation, participants arranged outpatient visits at month 1, 2, 3, 6, and 12 to obtain data on adverse events and conduct equipment inspections.

A home sleep test (HST; Somnocheck effort, Fa. Weinmann, Hamburg) was used to evaluate OSA severity before and 12 months after UAS device implantation. OSA-related parameters such as AHI, apnea index, hypopnea index, and oxygen desaturation index were estimated from HST. The outcome measurements and the classification of responders and non-responders were defined in dependence on the criteria postulated by Sher et al [138]. A response as measured by means of the AHI score was defined by a reduction of > 50 % and an absolute AHI score < 20 events per hour from baseline.

Metabolic parameters were assessed including an oral glucose tolerance test with measurements of glucose (Roche Diagnostics GmbH, Mannheim, Germany), insulin, and C-peptide (Immulite 2000, Siemens Healthcare Diagnostics, UK) from blood samples taken right before (fasting, 0 min), 60 min, and 120 min after the glucose load. Homeostatic model assessment of insulin resistance and C-peptide insulin resistance indices were calculated (Ohkura et al., 2013). Fasting leptin and acylated ghrelin were measured (RIA, Merk Millipore, Darmstadt, Germany) to assess markers of homeostatic regulation of hunger and appetite.

Plasma Ang-2 levels were assessed before (baseline) and after 12 months of treatment with a UAS system as described below (**Figure 7**).

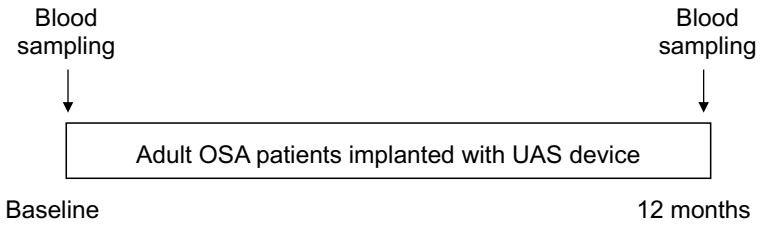

Figure 7. *Overview of Study D: Obstructive sleep apnea and Angiopoietin-2.*OSA, obstructive sleep apnea; UAS, upper airway stimulation.

The study was examined and approved by the ethics committee of the University of Lübeck (AZ 14-095). All subjects were informed both orally and in writing about the course of the study and all associated risks and gave their written and oral consent prior to participation.

2.3 Metabolic measurements

In study A-D, plasma samples were taken in pre-cooled blood EDTA tubes (S-Monovette® 2.7 ml; Servoprax, Wesel, Germany). Right after the blood taking, each plasma sample was immediately centrifuged at 4 ° C and 4000 revolutions (3112 g) (Laboratory Fugue 400R, Heraeus Instruments, Hanau), and the supernatant was then aliquoted. The aliquoted samples were initially stored at -20 ° C and transferred to a cooling device at -80 ° C after two weeks at the latest. Ang-2 values were determined from stored (-80°C) plasma samples by using commercial assays (Quantikine ELISA, R&D Systems, Inc, USA). The intra-assay precision coefficients of variability were 5.8 %, while the inter-assay precision was 9.0 %. The minimum detectable dose of human Ang-2 ranged from 1.20-21.3 pg/mL, and the mean minimum detectable dose was 8.29 pg/mL. The EDTA plasma sample in healthy volunteers provided by the manufacturer of this ELISA kit was 1964 ± 808 pg/mL.

Due to the research aim of the individual studies, anthropometrical and metabolic parameters, such as glucose homeostasis, lipid profile as well as inflammatory and hypoxic states of subjects have been assessed according to the respective study protocols.

2.4 Statistical analysis

The data was analyzed and displayed by SPSS Statistics 22.0 (SPSS Inc., Chicago IL), and GraphPad Prism Version 7.03 (GraphPad Software, La Jolla CA) for Windows®. Values are expressed as mean ± standard error of means (SEM) for distributed variables, and percentage for categorical variables. Statistical evaluation was carried out by means of the analysis of variance (ANOVA) for repeated measures with the time factor and, if necessary, condition. i.e. HFD vs. control diet as well as regular sleep vs. short late sleep, respectively. Paired t-tests were performed between two groups. Spearman's correlation analysis was used to assess the association between Ang-2 and AHI in patients with OSA. The changes of Ang-2 (d-Ang-2)-and AHI (d-AHI) over time were calculated (d-Ang-2 = Ang-2 at month 12 – Ang-2 at baseline; d-AHI = AHI at month 12 – AHI at baseline). A *P*-value < 0.05 was considered significant.

3 RESULTS

The first section describes the results for the narrative review of the association between Ang-2 and obesity and obese-related metabolic disorders. The second section presents the results of the experimental studies. In Study A, diurnal rhythm of Ang-2 was detected. Then, Ang-2 after short-term alterations in nutrition (Study B) and sleep (Study C) compared to control condition in healthy men are presented. Lastly, in Study D, the impact of OSA treatment on Ang-2 in subjects with OSA in long term was obtained.

3.1 Angiopoietin-2, obesity, and obesity-related metabolic disorders: A narrative review

In total, 86 publications were found using the above-mentioned research strategy. Twenty-three additional publications were identified through other sources and were manually included so that 109 publications were selected. After the first screening, 21 records were removed, while after reading the full text, 21 records were excluded. Thus, 67 publications were considered for the narrative review (see **Figure 8**). Data are summarized starting from cells and animal studies and ending up reporting human research concerning the expression of Ang-2, as well as the association between Ang-2 and the metabolic diseases (i.e., obesity, MetS, T2D, cardiovascular diseases, OSA, NAFLD, respectively) and its modulators such as HFD or hypoxia.

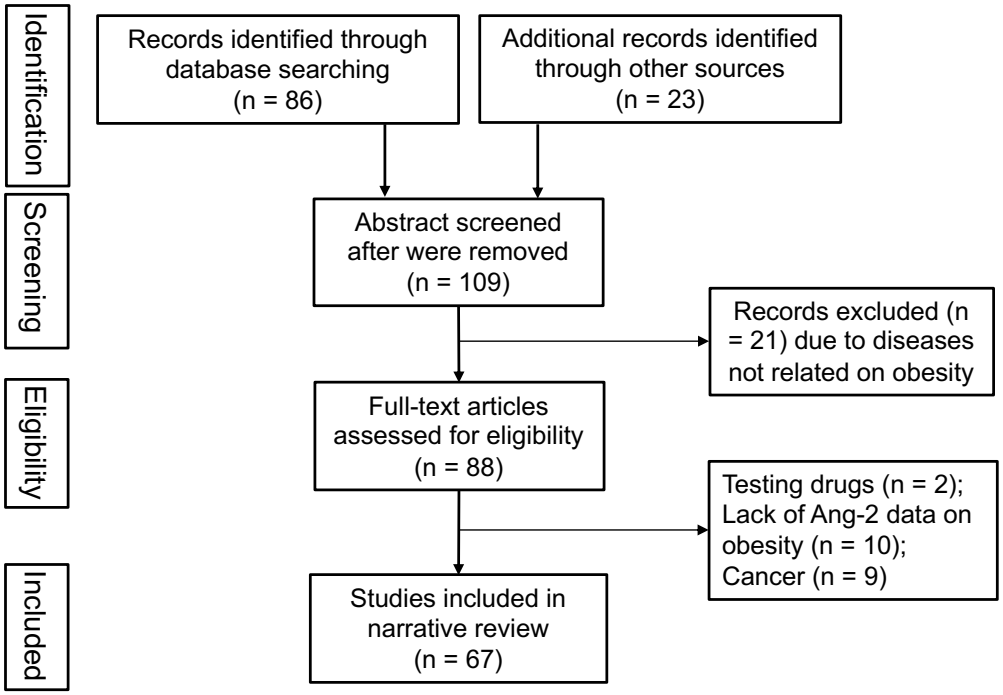

Figure 8. *Research strategy of the narrative review regarding the association between Angiopoietin-2 and obesity*.

3.1.1 Expression of Angiopoietin-2 in adipose cells, blood, and adipose tissues

In vitro studies: Ang-2 has been detected in human adipose-derived stromal cells [139]. Ang-2 mRNA was dramatically elevated concomitant with elevated Ang-1, and VEGF mRNA strongly enhanced the endothelial cells numbers in the process of adipogenic differentiation [139], and during liver regeneration [140]. Under inflammatory conditions, the expression of Ang-2 was increased along with decreased expression of Ang-1 in premature adipocytes [141]. Co-cultured with rat AT-derived stem cells, human umbilical vein endothelial cells showed increased Ang-2 expression with significantly improved endothelial tube formation [142]. Moreover, specific challenges such as intermittent fluid flow, hypoxia (1%), and oxidative stress lead to the increased secretion of Ang-2 in human AT-derived stem cells [133].

In animal studies: Expression of Ang-2 was found in AT in wild-type mice [14–16]. Among different AT depots, Ang-2 is more pronounced in SAT compared with VAT and BAT [16]. In Ang-2 adipocyte-specific knockout mice, weight of SAT was significantly decreased [16]. Besides, the expression of Ang-2 was also found in pig SAT, which was more pronounced in the middle SAT compared to outer SAT, and Ang-2 expression in SAT was unchanged between 90 to 210 d of age [143].

Leptin induces increased murine AT Ang-2 expression without an increase in VEGF both *in vivo* via subcutaneous infusion [14,55], and *in vitro* via 24h leptin treatment in mouse adipocytes [14], with a loss of fat mass [14,55], and apoptosis of endothelial cells in AT [14]. In SAT of female $^{ob/ob}$mice (the transcriptionally leptin-deficient mice), Ang-2 mRNA expression was lower compared with age-matched wild-type mice [14]. In contrast, a study in male $^{ob/ob}$mice demonstrated a 2-fold increase of Ang-2 mRNA expression in inguinal SAT, with a decreased SAT blood vessel density compared with wild-type mice [144]. However, a further male $^{ob/ob}$mice study reported no association between Ang-2 mRNA expression and the epididymal AT growth and regression [145].

In addition to leptin, forkhead box protein C2 (FOXC2) as an important transcription factor [146] can partly modulate the adipocytic Ang-2 [147]. In adipocytes-specific FOXC2 expression mice, Ang-2 increased nearly 6-fold in epididymal, and inguinal WAT compared with wild-type mice. A nearly 50% reduction of Ang-2 expression was detected in preadipocytes In knockout FOXC2 mice [147].

Clinical and experimental research in humans: In circulations, plasma from healthy humans contains levels of Ang-2 between 1-3 ng/mL and seldom more than 4 ng/mL [39]. Higher blood Ang-2 levels were seen in adults [18–21], children and adolescents with obesity compared to lean subjects [148–150]. Accordingly, a positive relationship was found between serum Ang-2 and BMI after excluded gender and age [19]. Besides, waist circumference, VAT, pericardial fat, and periaortic fat except for SAT were positively associated with circulating Ang-2 [151]. In addition, the odds of

suffering from several metabolic disorders such as T2D, systolic blood pressure, and MetS were positively associated with serum Ang-2 [152]. Gender was found to contribute to the disparity of Ang-2 levels as well, with higher serum Ang-2 in women than men [19,152]. The more marked differences between normal weight and obesity were in female subjects [19].

Regarding human AT, Ang-2 expression is increased in SAT of patients with obesity compared to those of normal weight [17,153], and SAT Ang-2 mRNA is positively related to serum leptin and fasting insulin [17]. In children aged between 0 and 9 years, Ang-2 was also found expressed in SAT, but the expression of Ang-2 is not correlated with BMI [154].

3.1.2 Angiopoietin-2 and obesity-related metabolic diseases

3.1.2.1 Metabolic syndrome

A large community-based study including 3,205 participants (42% having MetS) shows a positive correlation between Ang-2 and the prevalence of MetS. In addition, MetS components such as triglycerides and high non-fasting glucose increased as Ang-2 levels risen [27]. However, in patients with MetS and erectile dysfunction, serum Ang-1 but not Ang-2 increased. The increased Ang-1 contributed to vessel stabilization, as a compensatory attempt before cardiovascular events take place [155]. In postmenopausal women, serum Ang-2 showed no difference between those with and without MetS [156].

3.1.2.2 Diabetes and diabetic angiopathy

In *vitro* and animal studies: In *vitro,* a 3.2-fold increase of Ang-2 was seen in human pancreatic β-cells under glucolipotoxicity conditions [111]. In *vivo*, myocardial Ang-2 concentrations have been found elevated in diabetic mice [157]. Besides, the renal cortex Ang-2 expression has been evaluated in diabetic $^{db/db}$mice as compared to non-diabetic control, revealing that the impaired renal function reversed by applying an Ang-2 inhibitor [158].

Clinical and experimental research in humans: In pre-diabetic status, a study showed higher blood Ang-2 levels in men with hyperinsulinemia than in those with normal insulin levels [31]. Patients with impaired glucose tolerance (IGT) showed increased blood Ang-2 levels as compared to subjects with normal glucose tolerance [81]. Additionally, Ang-2 levels correlated with the severity of glucose intolerance [81]. In line with this, a multiple regression analysis revealed that fasting blood glucose and HbA1c were independently related to serum Ang-2 in humans [159].

Focusing on diabetes state, higher levels of blood Ang-2 were seen in patients with diabetes compared with non-diabetes [84]. This finding was paralleled to several other human studies [28,29], controls [81–85]. In addition, a positive association between blood levels of Ang-2 and the

prevalence of T2D was found [152]. Greulich and his colleagues have shown that myocardial Ang-2 concentrations were elevated in patients with T2D [160].

In the context of diabetic angiopathy, T2D with either macroangiopathy or microangiopathy showed higher levels of serum Ang-2 compared with those without angiopathy [30]. In multivariable logistic regression, positively independent associations were seen between blood Ang-2 levels and patients with diabetic vascular complications [30]. Furthermore, multivariate analysis shows that increased blood levels of Ang-2 are a significant predictor of new-onset microalbuminuria in T2D [161]. In addition, serum Ang-2 levels were found independently associated with carotid and aortic intima-media thickness [159]. In patients with T2D, higher serum Ang-2 levels were seen in diabetic retinopathy as compared to non-retinopathy [162]. Furthermore, urinary Ang-2 was also found associated with the levels of albuminuria in patients with T2D [163]. Regarding comorbidities, patients with T2D and additionally suffering from comorbidities including hypertension [152,164] and heart failure [165] showed higher circulating Ang-2 levels in respect to T2D without such comorbidities. However, another study showed no difference between diabetes with and without cardiovascular diseases [82]. Study in 80 patients with diabetes and after controlling age and BMI, Ang-2 levels were found to be correlated with sTie-2, diastolic blood pressure, plasma insulin, homeostasis model assessment of insulin resistance, creatinine, glomerular filtration rate, and gamma-glutamyl transferase [166]. Besides, the presence of diabetic macrovascular complications, polyneuropathy, and insulin therapy was positively associated with Ang-2 levels [166].

A clear association had been shown between circulating Ang-2 and different states of the process of diabetes, including hyperinsulinemia [31], IGT [81], diabetes [28,29,81–85,152], and diabetes with diabetic angiopathy [30,159,161–163,166] or with comorbidities [82,152,164,165]. These studies proposed that Ang-2 had been involved in the impairment of glucose homeostasis and starting from the very early phase. Related blood levels of Ang-2 are shown in **Table 1**.

Table 1. *Circulating Angiopoietin-2 expression in diabetes-related human studies (pg/mL)*

Specimen	Sample Size	Ang-2 levels						ELISA assay
		Non-diabetes	Hyper-insulinemia	IGT	Diabetes	Diabetes with Complications/ Comorbidities		
Serum [31]	260	1299.0 ± 54.3	1792.0 ± 197.1 *	— —	— —	— —	— —	R&D systems
Blood [84]	121	2700 ± 900	— —	— —	4100 ± 1300 *	— —	— —	BioSource International Inc.
Blood [81]	130	1462 ± 856	— —	1907 ± 855 *	3741 ± 1429 *#	— —	— —	R&D Systems
Plasma [82]	131	4000 (2500-5000)	— —	— —	6000 (4000-9200) *	5500 (3400-7400) *	Cardiovascular Diseases	R&D Systems
Serum [85]	250	597 (274–1005)	— —	— —	838 (473–1241) *	— —	— —	R&D Systems
Serum [28–30]	120	800 ± 200	— —	— —	1600 ± 400 *	2900 ± 700 *+ (macro) 2200 ± 600 *+ (micro)	Angiopathy	R&D Systems
Serum [159]	90	80 (20-100)	— —	— —	700 (600-800) *	1500 (1300-1600) *+	Not shown	Elabscience Biotechnology Co., Ltd,
Plasma [161]	260	— —	— —	— —	1850 ± 910	2300 ± 1300 +	Micro-albuminuria	R&D systems
Serum [162]	100	— —	— —	— —	2700 ± 1400 *	4400 ± 2510 * (non-proliferative) 6600 ± 4200 (proliferative)	Diabetic retinopathy	Not shown
Serum [164]	200	467 (180.0–877.7)	— —	— —	710.4 (419.0–1084.1) *	875.6 (515.2–1482.4) *+	Hypertension	R&D systems
Plasma [166]	80	— —	— —	— —	2955 (1349-9000)	— —	— —	R&D systems

Ang-2, Angiopoietin-2; IGT, impaired glucose tolerance; ELISA, enzyme-linked immunosorbent assay. *, $P < 0.05$ in compared to non-diabetes; #, $P < 0.05$ in compared to IGT; +, $P < 0.05$ in compared to diabetes

3.1.2.3 Cardiovascular diseases

In *vitro* studies: The accumulation of Ang-2 caused a dose-dependent reduction in cardiomyocyte contractile function [160]. In the acute phase of cardiac hypertrophy, enhanced angiogenesis was associated with myocardial VEGF and Ang-2 expression in mice [75]. In atherosclerotic model mice, injection of Ang-2 antibody induces a decreased artery plaque formation [167]. However, Ang-2 blockage had no effects on the size or composition of pre-existing plaques in atherosclerosis [167].

In human studies: several lines of evidence suggest that blood Ang-2 could be a predictor and a biomarker of cardiovascular diseases [22–26]. In patients with coronary artery disease, Ang-2 was overexpressed in perivascular AT depots with more extensive lymphangiogenesis exacerbated inflammation states, and fibrosis [168]. In the acute phase of angina pectoris and acute myocardial infarction, plasma Ang-2 levels in these patients were increased compared to healthy control [24]. Besides, patients with acute congestive heart failure showed more pronounced plasma Ang-2 levels compared with patients with chronic congestive heart failure [22]. Blood Ang-2 has been proposed as an independent predictor of myocardial infarction in hypertensive participants [25]. Furthermore, it has been shown that Ang-2 acts as an independent predictor of poor outcomes in patients with acutely decompensated heart failure [23], and in patients with cardiogenic shock complicated with acute myocardial infarction [26].

3.1.2.4 Obstructive sleep apnea

It has been shown that 1% of hypoxia intervention on human adipose-derived stromal cells elicited an increase in Ang-2 [133]. Plasma Ang-2 and Tie-2 levels are higher in children with OSA compared with non-OSA peers [134]. Besides, the induced Ang-2 was more prominent in children with obesity than children with normal weight, particularly when endothelial dysfunction and insulin resistance were present [134]. Moreover, a reduction of Ang-2 and Tie-2 levels was observed after adenotonsillectomy treatment [134].

3.1.2.5 Non-alcoholic fatty liver disease

In patients with severe obesity and with NAFLD, VAT expression of Ang-2 was positively correlated with lobular inflammation [80]. Serum Ang-2 levels were higher in patients with histological nonalcoholic steatohepatitis compared with those of simple steatosis [74]. In the nonalcoholic steatohepatitis mice model as a severe form of NAFLD, both serum and hepatic Ang-2 expressions increased, and Ang-2 inhibition reversed nonalcoholic steatohepatitis and attenuate hepatocellular carcinoma progression [74].

3.1.3 Angiopoietin-2 and high fat diet as a risk factor for obesity

In *vitro* studies: After being treated with saturated fatty acid (i. e. palmitic acid) for 24 hours, Ang-2 expression increased by 2.0- and 2.7-fold in mice and humans SAT adipocytes, respectively [16]. *Vice versa*, fat adipose uptake increased in human umbilical vein endothelial cell compared with vehicle after 30 min of Ang-2 (2.5ug/ml) stimulation [16].

In animal studies: Animals fed with HFD tend to consume more calories [108]. In wild-type mice, increased SAT Ang-2 expression was shown after 3-7 days of HFD (60% kcal fat) [16]. In accordance, perigonadal WAT Ang-2 and VEGF mRNA increased in wild-type mice after 16 weeks of HFD (58% kcal fat) in comparison with control mice (11% kcal fat) [113]. Interestingly, compared with male mice, this pattern was more pronounced in female mice, and with higher adipose vascular density and insulin sensitivity [113]. Furthermore, an elevated Ang-2 was observed before marked changes in blood lipids occurred after HFD with high calories (45.7% kcal fat, 19.5 kj/g) intervention in the corpus cavernosum of rats compared to control (3.8% kcal fat, 12.1 kj/g) [112]. However, blood levels of Ang-2 were unchanged under 3-7 days [16] or 2-8 weeks [169] of HFD (60% kcal fat).

A study reported that wild-type mice after HFD (60% kcal fat) challenge showed reduced vascularization in subcutaneous WAT, associated with glucose tolerance impairment and lipid clearance [116]. Whereas in AT-specific Ang-2 overexpressed mice model, HFD treatment evoked an increased SAT vascularization, displayed a healthier expansion pattern with increased anti-inflammatory macrophage infiltration, improved glucose, and lipid metabolic homeostasis compared with wild-type mice. The Ang-2 neutralizing antibodies caused reducing of AT blood vessel formation [116]. In addition, a study in Ang-2 adipocyte-specific knockout mice revealed that, HFD (60% kcal fat) intervention derived a proper fat distribution towards glucose metabolizing organs (e.g., liver and skeletal muscle) instead of towards SAT [16]. Whereas, the overexpressed Ang-2 in β-cells showed deteriorated endothelial inflammatory states which may directly lead to β-cells apoptosis in pancreatic β-cells-specific Ang-2 overexpressed model mice, after HFD (58% kcal fat) challenge [111].

A study in wild-type mice showed that the average adipocyte areas and total blood content (measured by radiolabeled tracer) of inguinal SAT and gonadal VAT were dramatically increased, but the blood mass/AT mass ratios (blood vessel density) was decreased compared to control mice (13% kcal fat and 10.9 kJ/g) after 15 weeks HFD and high calory treatment (42% kcal fat with 20.1 kj/g) [144]. However, the mRNA of Ang-2 was unchanged during the HFD intervention process. By contrast, Ang-1 mRNA was descended in both inguinal SAT and gonadal VAT [144].

Clinical and experimental studies in humans: Human studies of Ang-2 expression under HFD intervention are scarce. Plasma Ang-2 levels decreased 3 hours post-prandial after a single-meal HFD (63% kcal fat, 12 kcal/kg), but Ang-2 levels returned back to baseline after 6 hours post-prandial in healthy subjects [117].

In contrast to studies using HFD as an intervention, several studies are focusing on calorie restriction. One *in vitro* study showed that decreased human umbilical vein endothelial cells proliferation caused by energy deficit was not related to serum concentrations of VEGF and Ang-2 in patients with obese [170]. Whereas, in *vivo* study in patients with obesity, after 3-6- [17] and 12-months [21] of weight loss surgery (sleeve gastrectomy [17,21], adjustable gastric banding surgery [21]), reduced body weight was followed by a decrease in serum Ang-2 levels [17,21], and the expression of Ang-1 and Ang-2 was absent in SAT [17]. Serum Ang-1 and the Ang-1/Ang-2 ratio was decreased along with reduced bodyweight in subjects with obesity but metabolically healthy following 8 weeks of a very low energy diet, while Ang-2 levels were not reported [171].

3.2 Experimental results

3.2.1 Study A: Diurnal rhythm of Angiopoietin-2

In study A, 19 healthy young men were included in the analysis. Subjects were aged between 18 and 35 and were of normal weight and fat mass. The characteristics of these subjects are shown in **Table 2.**

Table 2. *Subjects' characteristics at baseline in study A: Diurnal rhythm of Angiopoietin-2*.

Variable	**Subjects (n = 19)**
Age (years)	25.1 ± 0.8
Body height (m)	1.80 ± 0.02
Body weight (kg)	76.0 ± 2.1
BMI (kg/m^2)	22.8 ± 0.4
Fat mass (%)	18.4 ± 1.1

Data are shown as mean ± SEM; BMI, body mass index

The plasma Ang-2 levels over 24 hours ranged between 1315 pg/mL and 1425 pg/mL, but revealed no statistical difference (P = 0.372 by ANOVA time effect). **(Figure 9**).

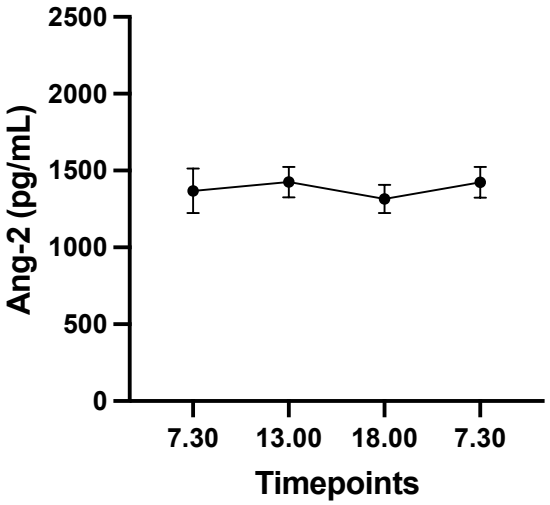

Figure 9. *Plasma Angiopoietin-2 over 24 hours.*
Data are shown as mean ± SEM. Ang-2, Angiopoietin-2.

3.2.2 Study B: High fat diet and Angiopoietin-2

For study B, analysis was performed using the same study sample as in study A (see characteristics in **Table 2**). The concentration of Ang-2 after HFD did not change as compared to the control diet over 24 hours (P = 0.369 for ANOVA treat x time interaction; **Figure 10**).

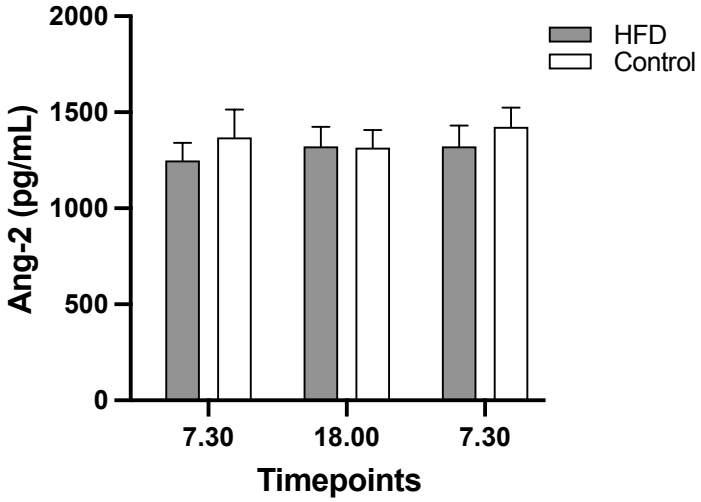

Figure 10. *Plasma Angiopoietin-2 under high fat diet.*
Data are shown as mean ± SEM. HFD, high fat diet; Ang-2, Angiopoietin-2.

3.2.3 Study C: Short late sleep and Angiopoietin-2

A total of 16 healthy young and with normal weight were analyzed in this study (**Table 3**).

Table 3. *Subjects' characteristics at baseline in study C: Short Late Sleep and Angiopoietin-2*.

Variable	Subjects (n = 16)
Age (years)	24.6 ± 0.7
BMI (kg/m^2)	23.5 ± 0.4

Data are shown as mean ± SEM; BMI, body mass index

Plasma Ang-2 levels did not change overnight in both short late sleep and regular sleep condition (P = 0.442 for ANOVA treat x time interaction; **Figure 11**).

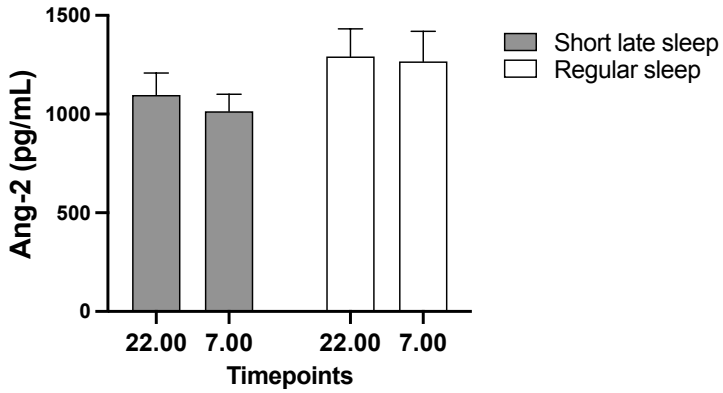

Figure 11. *Plasma Angiopoietin-2 under short late sleep intervention.*
Data are shown as mean ± SEM. Ang-2, Angiopoietin-2.

3.2.4 Study D: Obstructive sleep apnea and Angiopoietin-2

A total of 17 (15 male, 2 female) subjects with OSA were included in study D. Four of them were diagnosed as obese, 9 suffer from hypertension, and 4 suffer from dyslipidemia. Twelve subjects had a history of smoking (**Table 4**).

Table 4. *Subject's characteristics at baseline in study D: Obstructive sleep apnea and Angiopoietin-2.*

Variable	**Subjects (n = 17)**
Age (years)	52.2 ± 2.3
Gender, male (%)	88
Body height (m)	1.78 ± 0.03
Bodyweight (kg)	89.5 ± 3.5
BMI (kg/m^2)	28.1 ± 0.9
Body waist circumference (cm)	104 ± 3
Obesity, n (%)	24
Hypertension, n (%)	53
Dyslipidemia, n (%)	24
Smoking, n (%)	70

Data are shown as mean ± SEM or percentage for categorical variables; BMI, body mass index.

After 12 months of UAS treatment, the mean of Ang-2 levels was no different compared to baseline (P = 0.130 by paired t-test). Individual changes in Ang-2 are depicted in **Figure 12**, ranging from - 56.2 % to + 37.9 %.

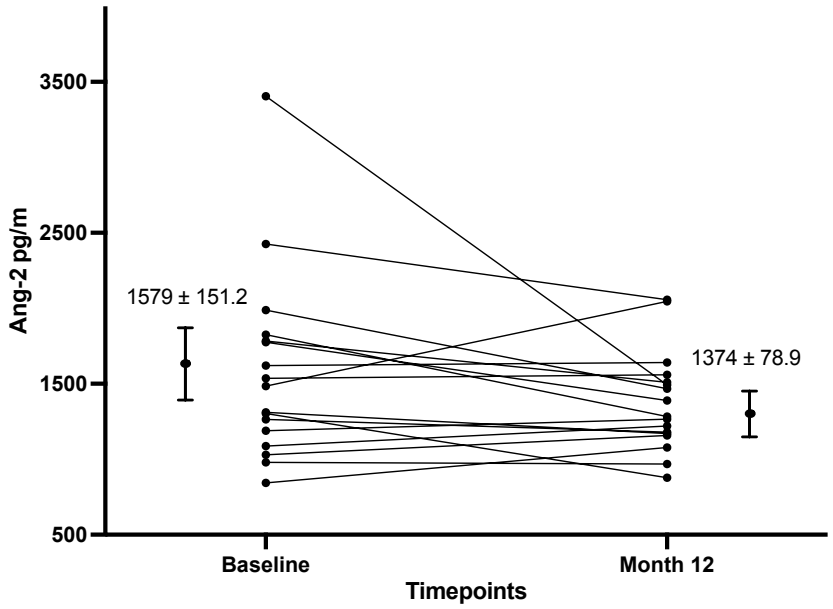

Figure 12. *Plasma Angiopoietin-2 before and 12 months after upper airway stimulation intervention in patients with obstructive sleep apnea.*

Data are shown as individual levels of Ang-2 or mean ± SEM. Ang-2, Angiopoietin-2.

After UAS treatment, the OSA-related respiratory parameter AHI was improved as reported previously [136], whereas there was no correlation between Ang-2 levels and AHI at baseline, month 12, as difference between timepoints (all $P \geq 0.349$; **Figure 13 a-c)**.

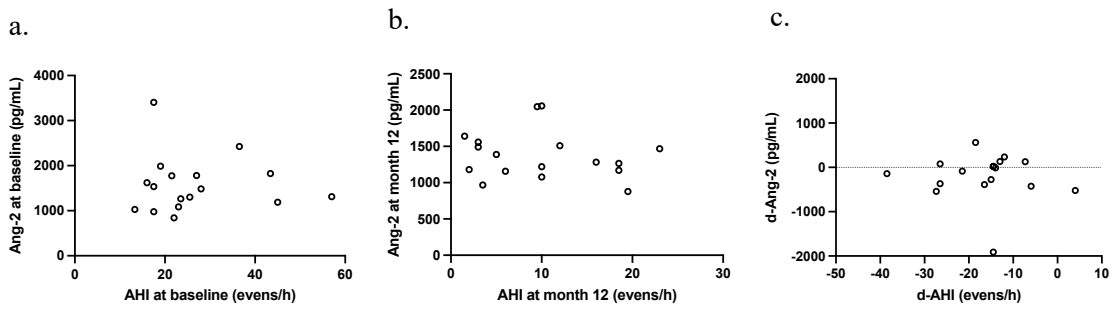

Figure 13. *The correlation between plasma levels of Angiopoietin-2 and apnea hypopnea index in patients with obstructive sleep apnea.*

a. Correlation between Ang-2 and AHI at baseline; b. Correlation between Ang-2 and AHI at month 12; c. Correlation between d-Ang-2 and d-AHI; Ang-2, Angiopoietin-2; AHI, apnea hypopnea index; d-Ang-2 = (Ang-2 at month 12 – Ang-2 at baseline); d-AHI = (AHI at month 12 – AHI at baseline).

4 DISCUSSION

This doctoral thesis focused on the association between Ang-2 and obesity and related metabolic diseases. Not only the first narrative review typically focuses on Ang-2 in obese and obesity-related metabolic disorders will be demonstrated, but also further the link between Ang-2 and several common obesity related risk factors explored.

From the narrative review, low levels of Ang-2 were found in blood but expressed the most in SAT of both mice and humans than other metabolic organs. According to literature research, Ang-2 is correlated with BMI, age, and several cytokines such as FOXC2, leptin, adiponectin, VEGF, TNF-α. Pro-longed high Ang-2 concentration deteriorated endothelial inflammation and impaired vessel forming capacity and functions in multiple organs. Besides, elevated Ang-2 levels were found in obesity and several obese-related diseases in humans such as diabetes, MetS, OSA, cardiovascular disease, and NAFLD. Increased Ang-2 was found associated with poor prognosis in most of these diseases. The narrative review sheds light on the association between Ang-2 and obesity and proposed Ang-2 as a potential biomarker of obese-related metabolic disorders. Notably, several obesity related risk factors have been found associated with the expression of Ang-2 in animal and *in vitro* studies such as high fat intake, short sleep, and hypoxia status. However, relevant human studies are scarce. In addition, there was no study aimed at exploring the diurnal rhythm of blood Ang-2 levels.

Thus, to further explore the association between Ang-2 and obese related risk factors, human experimental studies were designed as second part of this thesis. For this, impact of fat intake and short late sleep in short term interventions, as well as hypoxia status as long-term intervention on circulating Ang-2 in humans were assessed. The major findings are as follows:

i) there is no plasma diurnal rhythm of Ang-2 in healthy young men.

ii) one day of HFD did not change plasma Ang-2 as compared to control diet in healthy young men.

iii) one night of late short sleep in healthy young men did not change plasma Ang-2 levels as compared to regular sleep.

iv) 12 months of UAS treatment and thus improved hypoxia status did not change plasma Ang-2 levels in patients with OSA. Ang-2 levels were not associated with AHI.

4.1 Narrative review of Angiopoietin-2 in obesity

The narrative review gave the first hint of a close association between Ang-2 and obesity and proposed Ang-2 as a potential biomarker of obese-related metabolic disorders. However, several divergent views are presented, which will be discussed in the following section.

4.1.1 Expression of Angiopoietin-2 in animal studies

Ang-2 was found to play a role in adipogenesis, and leptin as one of the most essential adipokines modulates the expression of Ang-2 in adipocytes [14,55]. However, divergence findings obtained in $^{ob/ob}$mice were found [14,144,145]. A study on female $^{ob/ob}$mice showed that Ang-2 mRNA SAT was decreased with adipose endothelial cells apoptosis compared to wild type mice [14]. In this case, the Ang-2 is reduced by the lack of leptin, and this process seems one of the events leading to SAT regression. However, in studies in male $^{ob/ob}$mice, Ang-2 mRNA expression increased in inguinal SAT [144], whereas Ang-2 expression in epididymal AT [145].

Distinct regions of AT were analyzed between these studies might contribute to the inconsistency of results. Unlike WAT concentrates the largest deposit of AT in mature humans [172], BAT constitutes a small portion of AT and is majorly located in the supraclavicular area [173]. Of note, BAT was not seen in the large subcutaneous depots in humans [173]. However, in small mammals such as mice and rats, a wider distribution of BAT was seen than in humans, such as in the upper (interscapular, subscapular, axillary, and cervical) and lower (inguinal) subcutaneous depots, and the perirenal and mediastinal depots, especially in young animals [56]. It was known that the Ang-2 expression is significantly higher in WAT than in BAT [16]. The Ang-2 related animal studies mentioned above utilized different areas of AT such as inguinal [144], and epididymal AT [145]. In the distinct regions of AT depots, the BAT proportion might be disparity, which likely contributes to the inconsistent patterns of Ang-2.

4.1.2 Expression of Angiopoietin-2 upon of high fat diet intervention in animals

In *vitro* study, expression of Ang-2 showed a clear increase after being treated with saturated fatty acid (palmitic acid) in both mice and humans SAT adipocytes [16]. However, divergence views have been observed in animal studies. After HFD, the expression of Ang-2 has been increased in SAT [16], and perigonadal WAT [113] in mice. But, mRNA of Ang-2 was unchanged in both inguinal SAT and gonadal VAT during the 16-week HFD intervention [144].

Analogous to the distribution of WAT and BAT we mentioned before, different areas of AT in mice may cause these distinct results. In addition, these divergence findings may be partly due to gender differences. Animal study showed that female mice display greater expression of Ang-2 and VEGF and vascularity in WAT than males in response to HFD [113]. The greater endothelial cells content

of WAT in female mice was found compared to weight-matched males reinforcing the concept of sex-dependent differences expression of Ang-2 [113]. Thus, both gender [16,113], or only male mice [144] were included might have the chance to influence the results of Ang-2.

Furthermore, between these HFD studies, the proportions of fat in the diet were inconsistent. The calories of fat are between 42%-60%. Besides, the total calory intake was not reported in most of these animal studies [16,111,113,116,169]. Divergent calories between studies are highly possible. Thus, whether HFD or high calories intake impacted the results cannot be distinguished and distinct energy intakes might largely contribute to the conflict views.

Regarding the functions of Ang-2 under HFD, overexpressed Ang-2 seems playing distinct roles in AT depots compared with other metabolic organs. It has been known that in addition to energy-storing, another essential function of WAT is to effectively isolate lipids to prevent lipotoxicity in other tissues such as liver, heart, and muscle, avoiding ectopic lipid accumulation [174]. In patients with obesity, the redistribution of the lipids seems to occur besides the expansion of WAT [174], which contributes to metabolic disorders such as systemic inflammation or insulin resistance [174]. In AT-specific Ang-2 overexpressed model mice, HFD treatment displayed a healthier expansion pattern compared with wild-type mice [116]. Whereas, under HFD, the overexpressed Ang-2 in β-cells showed deteriorated endothelial inflammation [111]. Furthermore, in the corpus cavernosum of rats, an elevated Ang-2 was observed before marked changes in blood lipids occur after HFD intervention [112], which indicates that the increased local Ang-2 expression increased before the circulation lipids alter. Uniformly, in *vitro* study has demonstrated that Ang-2 can uptake the fatty acids in AT depots [16]. This suggests that Ang-2 derived from adipocytes can regulate the redistribution of free fatty acid, and the circulating elevated Ang-2 might be a sign of ectopic lipid accumulation that will contribute to metabolic disorders.

Collectively, the above evidence suggests that changes in eating behavior (towards a higher fat diet) may regulate Ang-2 expression thereby inducing metabolic disorders. However, human-related research is warranted to explore the effect of an unbalanced diet on Ang-2 regulations, which will be discussed later in 4.2.2.

4.1.3 Circulation of Angiopoietin-2 in humans with obesity

Compared to the expression in AT, human studies were mainly focused on changes of Ang-2 in circulation. From the narrative review, a positive association between Ang-2 levels and obesity have indicated in several studies, which presented elevated Ang-2 levels with increased bodyweight [18–21,148–150], BMI [19], waist circumference, VAT, pericardial fat, and periaortic fat size [151]. At first glance, data seem to be not conflicting. Nevertheless, inconsistent Ang-2 levels were shown between these studies. Several confounders might contribute to the different results.

It has been shown that circulating Ang-2 can be impacted by age with higher levels in older age [152]. In line with this, there are three studies with children and adolescent subjects [148–150], which demonstrate remarkably lower Ang-2 levels from the adult-related studies [18–21]. Besides, gender was found may also contribute to the disparity of Ang-2 levels with higher serum Ang-2 in women than men [152]. This is parallel to the animal study that higher WAT expression of Ang-2 in females compared to males. In the narrative review, exclusively all considered studies in humans were performed in both genders [18–21,148–152], which may contribute to the fluctuation of Ang-2 levels between studies.

In addition, blood levels of Ang-2 showed remarkable positive associations with major vascular risk factors such as MetS [27], diabetes [28–30], systolic blood pressure [152], as well as cardiovascular disease [22–26]. Each of these diseases is precipitated by the dysfunction of the vascular endothelial cells [67]. It has been known that the impairment of endothelial cells has presented in a very early phase of obesity [3]. As an endothelial cells' impairment marker, Ang-2 levels are probably significantly higher in patients with obese and vascular diseases than patients with simple obesity. In our review, however, there is no study that was designed to focus only on obesity and thus exclude patients with obesity and other vascular risk factors. This might to a great extent impact Ang-2 levels.

Last, the measurement kits from different companies used studies could also dramatically impact the Ang-2 values. However, it can be assumed that within one study only one type of kit was used which lower in turn the expected variance. In consequence, demographical differences in combination with variations in methodology in studies may account for inconsistencies in the study results that focus on Ang-2 in humans with obesity.

4.2 Experimental studies of circulating Angiopoietin-2 in humans

In our experimental study, we found that Ang-2 shows no diurnal rhythm in circulations, the high fat diet and sleep intervention in short term and the treatment of OSA in long term did not change Ang-2 levels in adults. These data are to some extent contradicted to our hypothesis and disparity to some existing evidence as discussed below.

4.2.1 The diurnal rhythm of Angiopoietin-2 in humans

It has been shown that asynchrony of the circadian clock is associated with increased incidence of obesity [121]. As an obese-related factor, I hypothesis that Ang-2 may also display a diurnal rhythm. However, plasma Ang-2 levels had no change between 4 different time points for 24 hours in our study. We have aforementioned that the circulating leptin exhibits a clear diurnal rhythm [98]. And Ang-2 mRNA in SAT of morbidly obese patients (BMI >35 kg/m^2) was positively related to serum leptin [17]. It seems that leptin levels could modulate the local expression of Ang-2 in AT, but could not further influence the Ang-2 levels in circulations. Besides, until now, there is no direct association between circulating Ang-2 and other hormones with diurnal rhythm such as corticosteroids, melatonin, growth hormone reported. Thus, stable levels of Ang-2 in circulation were indicated which provided insight for the following studies that there are no time effects on Ang-2 levels in healthy control.

4.2.2 Angiopoietin-2 under high fat diet in humans

There is evidence that Ang-2 increases upon HFD in *vitro* [16] and in animal studies [16,113]. Thus, we hypothesized that plasma Ang-2 levels would increase after one day of HFD including three times of meals. However, no difference in Ang-2 has been observed in comparison to the control diet. This result is in parallel to a study reporting that a single HFD meal induced no differences in Ang-2 6h post-meal [117]. Due to the deficient of HFD related human studies on Ang-2, a few calories restriction studies in humans provided some evidence regarding the impact of calory intake on Ang-2. In obese patients after 3-6-[17] and 12-months [21] of weight loss surgery, serum Ang-2 levels dramatically decreased [17,21] with reduced bodyweight [17]. These calories restriction human studies implicating that energy restriction and bodyweight changes could regulate circulating Ang-2 levels. In the above cited single meal study, the amount of the high-fat meal was calculated by 12 kcal per kg of body mass with a mean of 927 kcal of the participants, which was generally characteristic of the standard meal [117]. In this case, the high calories were excluded which avoided the cross-influence in calorie changes and fat proportion changes. However, in the single meal study, standard meal as a control group is lacking. Thus, to make the results more convincing, in our study, an HFD group and a control diet group were included, and with identical calories between groups. In addition, we calculated the meals based on subjects resting energy expenditure and corrected them

with the physical activity level of 1.3 [175]. Furthermore, to avoid the influence by age, gender, and extra energy expenditure, we included only healthy young men, with no excessive exercise in the week before and with low physical activity during the study. Thus, unchanged Ang-2 levels between HFD and control diet in our study suggested that Ang-2 levels might not be modified by high fat intake without increasing calorie intake, at least in healthy young men. However, whether high calories could modulate the levels of Ang-2 in humans remains still unclear. To further verify this question, two more conditions are required in further studies: a) high calories with high-fat proportion (74% fat, 11% carbohydrate, 15% protein); b) high calories with standard macronutrients (30% fat, 55% carbohydrate, 15% protein). Therefore, that would be helpful to clarify whether high fat or/and high calories are associated with Ang-2 levels.

In addition, the length of intervention might also contribute to the unchanged Ang-2 levels. An animal study has shown that the circulating Ang-2 is more stable than Ang-2 expression in AT [16]. Under 3 to 7 days of HFD challenge, the expression of Ang-2 in adipocytes increased, but Ang-2 was unchanged in the systemic circulation [16]. It seems that Ang-2 levels in circulation are less activated than in AT depots. One day of HFD intervention may not be long enough to induce an increase in plasma Ang-2 levels. Thus, further studies are required to clarify the modification of Ang-2 levels under a longer intervention time in humans. To eliminate the bodyweight effects, the proper length of HFD time would be just before the bodyweights get changed or statistically adjusted the impacts of bodyweight changes.

4.2.3 Angiopoietin-2 under short late sleep in humans

Study C is the first study, to our knowledge that analyzed the effects of Ang-2 under sleep disruption condition. After one night of late short sleep, plasma Ang-2 levels were unchanged, which suggests that one night of sleep disruption might not be strong enough to influence the Ang-2 levels.

First step to get deeper knowledge on Ang-2 upon short sleep conditions would analyze within a cross sectional study, if Ang-2 is higher in short sleeper than normal sleeper and if Ang-2 is higher in subjects working on rotating shift as compared to subjects working only during daytime.

Ang-2 has aforementioned more stable in circulation than in local AT [16]. The study in our lab has shown that WAT under a day of late sleep is not long enough to induce inflammatory and metabolic disorders, but the gene expression has been modified [126]. The retinol-binding protein 4 is linked to inflammation and MetS [176]. The study from our lab found that retinol-binding protein 4 expression did not increase after one day of late sleep loss, but SAT gene expression was elevated [126]. Ang-2 has been found to express the most in SAT [16] and is closely related to inflammation [9]. Whether Ang-2 mRNA in WAT had been elevated under a short sleep and involved in the modulation of inflammatory factors would be interesting to explore and need further study to verify.

4.2.4 Angiopoietin-2 levels in patients with obstructive sleep apnea

It is known that hypoxia status, low-grade inflammation, and oxidative stress are the basic pathologic status in OSA [90]. Besides, OSA is closely associated with metabolic and cardiovascular risks [177]. It has been shown that Ang-2 was observed positively related to inflammation, hypoxia status [42,132], and oxidative stress *in vitro* [133]. Therefore, we hypothesized that the effects of OSA treatment might impact plasma Ang-2 levels toward lower levels thereby predicting an improvement of vascular impairments. However, plasma Ang-2 levels did not reach a significant difference over a year of UAS treatment. Further, there was no association between Ang-2 and AHI.

In contrast to the present study, a close association between blood Ang-2 and AHI in OSA has been demonstrated in 126 children (age: 7.4±2.0 years) [134]. In this study, plasma Ang-2 and Tie-2 levels were higher in children with OSA as compared to those without OSA. After 12-16 weeks of adenotonsillectomy, blood Ang-2 decreased commitment with an improvement in respiratory consequences of OSA [134]. In the present study part D, 12 months of UAS treatment was utilized, and thus, the intervention timeline is much longer than in the pediatric study. However, the decrease of AHI was approximately 50% in our study, while as much as 80% of AHI decreased in the pediatric study [134]. Therefore, the distinct results in Ang-2 are presumably due to the different degrees of the AHI improvement. The 50% of AHI improvement may not be strong enough to impact the changes of Ang-2 in circulation in adult OSA patients. Besides, the subjects we included were limited to moderate-to-severe OSA patients (AHI 15–65 /h) rather than the randomized patients including all different severity. Thus, the limited group of subjects might also influence the results. A cohort including all stages of OSA patients is required to reduce the bias.

Moreover, as aforementioned, blood Ang-2 levels are associated with gender [152], obesity [19], and metabolic disorders [152]. In our study, a total of 17 patients with OSA included both males and females. In addition, several subjects who had been diagnosed with obesity, hypertension, dyslipidemia were included. Thus, characteristics of subjects might influence the consequence to some extent. Thus, a larger number of subjects with well-controlled confounders is in need.

4.3 Strengths and limitations

However, the limitations of this study should be noted. First, owing to the relatively small number of cases, results must be confirmed in larger studies. In our experimental study parts A, B, C, the unchanged Ang-2 levels might partly be due to the characteristics of subjects that were enrolled, i.e. healthy young men. The normal range of Ang-2 levels in metabolic healthy young men might be lower than in older, female, and metabolic unhealthy subjects as discussed on review results before. Thus, the short diet and sleep intervention were not strong enough to influence the Ang-2 levels towards significantly higher levels. Longer well-controlled studies under free living conditions are needed to better understand association with Ang-2 in the longer term. In addition, female subjects showed more marked differences between normal weight and obesity compared to male subjects [19]. Therefore, the male subjects might not be as sensitive as female subjects after HFD and short late sleep interventions.

Lastly, a control group is lacking in study D to compare Ang-2 between patients with OAS and controls without OSA. In addition, a longer intervention time with a more dramatic decrease of AHI is in need to further explore the association between Ang-2 and OSA. Finally, larger numbers of patients with OSA and with different severity, i.e. mild sleep apnea ($5 \leqslant AHI < 15$), moderate sleep apnea ($15 \leqslant AHI < 30$), and severe sleep apnea ($AHI \geqslant 30$), respectively, [178], and controls the other confounders are warranted to explain the association between Ang-2 levels and OSA in adults.

4.4 Conclusion

As an endothelial-specific expressed cytokine, Ang-2 has been found closely associated with inflammation and pathologic angiogenesis in obesity and obesity-related metabolic disorders. *In vitro* and animal studies had shed light on the link between Ang-2 and obesity related risk factors such as high fat intake, sleep loss, and hypoxia states. From our experimental study, circulating Ang-2 shows no diurnal rhythm. Further, one day of HFD, and sleep intervention did not change Ang-2 levels in healthy youth men. Lastly, a 50% improvement of AHI cannot impact Ang-2 levels in adult patients with OSA, and no correlation was found between Ang-2 and AHI. Our study has raised a link between Ang-2 and obese and obese related risk factors, but Ang-2 levels seem stable in circulation under some of the obese related risk factors.

5 SUMMARY

Background: The prevalence of overweight and obesity is increasing worldwide. Impaired endothelial function initiates in a very early phase of obesity and contributed to major mortalities in obesity. However, the circulating biomarkers of endothelial impairment have not yet been fully understood. The core cytokine Angiopoietin-2 in the Angiopoietin/Tie pathway has been shown to play a role in endothelial cells survival and it was found that Ang-2 could induce endothelial cells impairment. It is known that Angiopoietin is positively correlated with body weight. Therefore, it can be hypothesized that monitoring circulating Angiopoietin-2 could be relevant to understand obese-related vascular impairment. However, studies in circulating Angiopoietin-2 in obesity and its risk conditions in humans are scarce.

Methods: This thesis consisted of two parts. Firstly, a narrative review mainly focused on Angiopoietin-2 in obesity has been carried out. Then, prior studies of our lab had been used to investigate Angiopoietin-2 blood levels under four approaches: 1) whether circulating Angiopoietin-2 has circadian rhythmicity in healthy men; 2) whether blood Angiopoietin-2 can be impacted by a short high fat diet; 3) whether blood Angiopoietin-2 is altered by short sleep deprivation; 4) whether blood Angiopoietin-2 decreases after obstructive sleep apnea treatment.

Results: In the narrative review, we demonstrated that Angiopoietin-2 was found weakly expressed in blood and expressed the most in subcutaneous adipose tissues than other metabolic organs of both mice and humans. Angiopoietin-2 was found to be influenced by bodyweight, age, and several cytokines. Pro-longed high Angiopoietin-2 concentration deteriorates endothelial inflammation and impairs vessel forming capacity and functions in multiple organs. However, Angiopoietin-2 is necessary for the lipid accumulation in adipose tissues. Furthermore, elevated Angiopoietin-2 levels were found in obesity and several obese-related diseases in humans and the increased Angiopoietin-2 were found associated with poor prognosis in most of these diseases. In studies on humans, we found: i) There is no plasma diurnal rhythm of Angiopoietin-2 in healthy young men. ii) One day of high fat diet did not affect plasma Angiopoietin-2 compared to control diet in healthy young men. iii) One night of late short sleep in healthy young men could not change the plasma Angiopoietin-2 levels as compared to regular sleep. iv) After 12 months of upper airway stimulation treatment, plasma Angiopoietin-2 levels did not decrease in patients with obstructive sleep apnea.

Discussion: The evidence provided in this thesis will help to a better understanding of the role of Angiopoietin-2 in obesity and the pattern of blood Angiopoietin-2 in the conditions of several obese related risk factors. However, the divergence of findings was expressed. In our experimental study, we found that the levels of Angiopoietin-2 were stable in circulations. But human studies with longer term intervention with more participants are needed to further explore role of Angiopoietin in obese related disease.

6 REFERENCES

1. GBD 2017 Risk Factor Collaborators. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Lond Engl. 2018;392:1923–1994.
2. Prospective Studies Collaboration, Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, Qizilbash N, Collins R, Peto R. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet Lond Engl. 2009;373:1083–1096.
3. Karpoff L, Vinet A, Schuster I, Oudot C, Goret L, Dauzat M, Obert P, Perez-Martin A. Abnormal vascular reactivity at rest and exercise in obese boys. Eur J Clin Invest. 2009;39:94–102.
4. Vlachopoulos C, Xaplanteris P, Aboyans V, Brodmann M, Cífková R, Cosentino F, Carlo MD, Gallino A, Landmesser U, Laurent S, Lekakis J, Mikhailidis DP, Naka KK, Protogerou AD, Rizzoni D, Schmidt-Trucksäss A, Bortel LV, Weber T, Yamashina A, Zimlichman R, Boutouyrie P, Cockcroft J, O'Rourke M, Park JB, Schillaci G, Sillesen H, Townsend RR. The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology Working Group on peripheral circulation: Endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) Society. Atherosclerosis. Elsevier; 2015;241:507–532.
5. Fagiani E, Christofori G. Angiopoietins in angiogenesis. Cancer Lett. 2013;328:18–26.
6. Thurston G, Daly C. The Complex Role of Angiopoietin-2 in the Angiopoietin-Tie Signaling Pathway. Cold Spring Harb Perspect Med. 2012;2:a006650–a006650.
7. Papadopoulos KP, Kelley RK, Tolcher AW, Razak ARA, Van Loon K, Patnaik A, Bedard PL, Alfaro AA, Beeram M, Adriaens L, Brownstein CM, Lowy I, Kostic A, Trail PA, Gao B, DiCioccio AT, Siu LL. A Phase I First-in-Human Study of Nesvacumab (REGN910), a Fully Human Anti-Angiopoietin-2 (Ang2) Monoclonal Antibody, in Patients with Advanced Solid Tumors. Clin Cancer Res Off J Am Assoc Cancer Res. 2016;22:1348–1355.
8. Koga K, Todaka T, Morioka M, Hamada J, Kai Y, Yano S, Okamura A, Takakura N, Suda T, Ushio Y. Expression of angiopoietin-2 in human glioma cells and its role for angiogenesis. Cancer Res. 2001;61:6248–6254.
9. Fiedler U, Reiss Y, Scharpfenecker M, Grunow V, Koidl S, Thurston G, Gale NW, Witzenrath M, Rosseau S, Suttorp N, Sobke A, Herrmann M, Preissner KT, Vajkoczy P, Augustin HG. Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. Nat Med. 2006;12:235–239.
10. Schuldt EA, Lieb W, Dörr M, Lerch MM, Völzke H, Nauck M, Friedrich N. Circulating angiopoietin-2 and its soluble receptor Tie-2 concentrations are related to inflammatory markers in the general population. Cytokine. 2018;105:1–7.
11. Rutkowski JM, Davis KE, Scherer PE. Mechanisms of obesity and related pathologies: the macro- and microcirculation of adipose tissue. FEBS J. 2009;276:5738–5746.
12. Christiaens V, Lijnen HR. Angiogenesis and development of adipose tissue. Mol Cell Endocrinol. 2010;318:2–9.
13. Mancuso P. The role of adipokines in chronic inflammation. ImmunoTargets Ther. 2016;5:47–56.
14. Cohen B, Barkan D, Levy Y, Goldberg I, Fridman E, Kopolovic J, Rubinstein M. Leptin

induces angiopoietin-2 expression in adipose tissues. J Biol Chem. 2001;276:7697–7700.

15. Lee HJ. Exercise training regulates angiogenic gene expression in white adipose tissue. J Exerc Rehabil. 2018;14:16–23.

16. Bae H, Hong KY, Lee C-K, Jang C, Lee S-J, Choe K, Offermanns S, He Y, Lee HJ, Koh GY. Angiopoietin-2-integrin α5β1 signaling enhances vascular fatty acid transport and prevents ectopic lipid-induced insulin resistance. Nat Commun. 2020;11:2980.

17. Figueroa-Vega N, Jordán B, Pérez-Luque EL, Parra-Laporte L, Garnelo S, Malacara JM. Effects of sleeve gastrectomy and rs9930506 FTO variants on angiopoietin/Tie-2 system in fat expansion and M1 macrophages recruitment in morbidly obese subjects. Endocrine. 2016;54:700–713.

18. Piecuch J, Mertas A, Nowowiejska-Wiewiora A, Zurawel R, Gregorczyn S, Czuba Z, Wiewiora M. The relationship between the rheological behavior of RBCs and angiogenesis in the morbidly obese. Clin Hemorheol Microcirc. 2019;71:95–102.

19. Silha JV, Krsek M, Sucharda P, Murphy LJ. Angiogenic factors are elevated in overweight and obese individuals. Int J Obes. 2005;29:1308–1314.

20. Wiewiora M, Mertas A, Nowowiejska-Wiewiora A, Kozlowski A, Czuba Z, Piecuch J. The effects of venous hemodynamics on angiogenesis in morbid obese. Clin Hemorheol Microcirc. 2019;71:347–356.

21. Wiewiora M, Mertas A, Gluck M, Nowowiejska-Wiewiora A, Czuba Z, Piecuch J. Effect of Weight Loss Surgery on Biomarkers of Angiogenesis in Obese Patients. Obes Surg. 2020;30:3417–3425.

22. Chong AY, Caine GJ, Freestone B, Blann AD, Lip GYH. Plasma angiopoietin-1, angiopoietin-2, and angiopoietin receptor tie-2 levels in congestive heart failure. J Am Coll Cardiol. 2004;43:423–428.

23. Pöss J, Ukena C, Kindermann I, Ehrlich P, Fuernau G, Ewen S, Mahfoud F, Kriechbaum S, Böhm M, Link A. Angiopoietin-2 and outcome in patients with acute decompensated heart failure. Clin Res Cardiol Off J Ger Card Soc. 2015;104:380–387.

24. Lee KW, Lip GYH, Blann AD. Plasma angiopoietin-1, angiopoietin-2, angiopoietin receptor tie-2, and vascular endothelial growth factor levels in acute coronary syndromes. Circulation. 2004;110:2355–2360.

25. Patel JV, Lim HS, Varughese GI, Hughes EA, Lip GYH. Angiopoietin-2 levels as a biomarker of cardiovascular risk in patients with hypertension. Ann Med. 2008;40:215–222.

26. Pöss J, Fuernau G, Denks D, Desch S, Eitel I, de Waha S, Link A, Schuler G, Adams V, Böhm M, Thiele H. Angiopoietin-2 in acute myocardial infarction complicated by cardiogenic shock-a biomarker substudy of the IABP-SHOCK II-Trial: Angiopoietin-2 in acute myocardial infarction complicated by cardiogenic shock. Eur J Heart Fail. 2015;17:1152–1160.

27. Lorbeer R, Baumeister SE, Doerr M, Nauck M, Grotevendt A, Schlesinger S, Teumer A, Voelzke H, Grabe H-J, Wallaschofski H, Vasan RS, Lieb W. Angiopoietin-2, its Soluble Receptor Tie-2, and Metabolic Syndrome Components in a Population-Based Sample. Obesity. 2016;24:2038–2041.

28. Li L, Yu ZH-Q, Qian L, Xu J-Y, Liu F, Zhao G-C, Zhang L, Gu H-M, Zhang S-J, Meng J. Interleukin-19 and angiopoietin-2 can enhance angiogenesis of diabetic complications. J Diabetes Complications. 2016;30:386–387.

29. Li L, ZHeng-Qing Y, Juan-Yu H, Jian-Yong X, Fan L, Guang-Chun Z, Lei Z, Hui-Ming G, Si-Jing Z, Meng J. Association between interleukin-19 and angiopoietin-2 with vascular complications in type 2 diabetes. J Diabetes Investig. 2016;7:895–900.

30. Li L, Qian L, Yu Z-Q. Serum angiopoietin-2 is associated with angiopathy in type 2 diabetes

mellitus. J Diabetes Complications. 2015;29:568–571.

31. Chandel S, Sathis A, Dhar M, Giri H, Nathan AA, Samawar SKR, Gupta A, Gopal J, Harish R, Mohan V, Dixit M. Hyperinsulinemia promotes endothelial inflammation via increased expression and release of Angiopoietin-2. Atherosclerosis. 2020;307:1–10.

32. Wilms B, Chamorro R, Hallschmid M, Trost D, Forck N, Schultes B, Mölle M, Sayk F, Lehnert H, Schmid SM. Timing modulates the effect of sleep loss on glucose homeostasis. J Clin Endocrinol Metab. 2019;104:2801–2808.

33. Yuan HT, Suri C, Landon DN, Yancopoulos GD, Woolf AS. Angiopoietin-2 is a site-specific factor in differentiation of mouse renal vasculature. J Am Soc Nephrol JASN. 2000;11:1055–1066.

34. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, Pober JS, Wick TM, Konkle BA, Schwartz BS, Barnathan ES, McCrae KR, Hug BA, Schmidt AM, Stern DM. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood. 1998;91:3527–3561.

35. Eelen G, de Zeeuw P, Treps L, Harjes U, Wong BW, Carmeliet P. Endothelial Cell Metabolism. Physiol Rev. 2018;98:3–58.

36. Carmeliet P. Angiogenesis in health and disease. Nat Med. 2003;9:653–660.

37. Carmeliet P. Angiogenesis in life, disease and medicine. Nature. 2005;438:932–936.

38. Hanahan D. Signaling vascular morphogenesis and maintenance. Science. 1997;277:48–50.

39. Lukasz A, Kümpers P, David S. Role of angiopoietin/tie2 in critical illness: promising biomarker, disease mediator, and therapeutic target? Scientifica. 2012;2012:160174.

40. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN, Yancopoulos GD. Angiopoietin-2, a Natural Antagonist for Tie2 That Disrupts in vivo Angiogenesis. Science. 1997;277:55–60.

41. Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, Ryan TE, Bruno J, Radziejewski C, Maisonpierre PC, Yancopoulos GD. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell. 1996;87:1161–1169.

42. Mandriota SJ, Pepper MS. Regulation of angiopoietin-2 mRNA levels in bovine microvascular endothelial cells by cytokines and hypoxia. Circ Res. 1998;83:852–859.

43. Fiedler U, Scharpfenecker M, Koidl S, Hegen A, Grunow V, Schmidt JM, Kriz W, Thurston G, Augustin HG. The Tie-2 ligand Angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. Blood. 2004;103:4150–4156.

44. David S, Kumpers P, van Slyke P, Parikh SM. Mending Leaky Blood Vessels: The Angiopoietin-Tie2 Pathway in Sepsis. J Pharmacol Exp Ther. 2013;345:2–6.

45. Davis S, Papadopoulos N, Aldrich TH, Maisonpierre PC, Huang T, Kovac L, Xu A, Leidich R, Radziejewska E, Rafique A, Goldberg J, Jain V, Bailey K, Karow M, Fandl J, Samuelsson SJ, Ioffe E, Rudge JS, Daly TJ, Radziejewski C, Yancopoulos GD. Angiopoietins have distinct modular domains essential for receptor binding, dimerization and superclustering. Nat Struct Biol. 2003;10:38–44.

46. Fiedler U, Augustin HG. Angiopoietins: a link between angiogenesis and inflammation. Trends Immunol. 2006;27:552–558.

47. Imhof BA, Aurrand-Lions M. Angiogenesis and inflammation face off. Nat Med. 2006;12:171–172.

48. Sun C, Li J, Wang B, Shangguan J, Figini M, Shang N, Pan L, Zhang Z. Tumor angiogenesis and bone metastasis - Correlation in invasive breast carcinoma. J Immunol Methods. 2018;452:46–52.

49. Reiss Yvonne, Droste Jasmin, Heil Matthias, Tribulova Silvia, Schmidt Mirko H.H., Schaper Wolfgang, Dumont Daniel J., Plate Karl H. Angiopoietin-2 Impairs Revascularization After Limb Ischemia. Circ Res. American Heart Association; 2007;101:88–96.

50. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multi-step paradigm. Cell. 1994;76:301–314.

51. Xue L, Shuyan T, Xiaoli L, Zilong L, Qiuling F, Lining W, Yanqiu L, Li Y. Glomerular Proteomic Profiles in the NZB/W F1 Hybrid Mouse Model of Lupus Nephritis. Med Sci Monit Int Med J Exp Clin Res. 2019;25:2122–2131.

52. Kurosaka D, Hirai K, Nishioka M, Miyamoto Y, Yoshida K, Noda K, Ukichi T, Yanagimachi M, Furuya K, Takahashi E, Kingetsu I, Fukuda K, Yamada A. Clinical significance of serum levels of vascular endothelial growth factor, angiopoietin-1, and angiopoietin-2 in patients with rheumatoid arthritis. J Rheumatol. 2010;37:1121–1128.

53. Westra J, de Groot L, Plaxton SL, Brouwer E, Posthumus MD, Kallenberg CGM, Bijl M. Angiopoietin-2 is highly correlated with inflammation and disease activity in recent-onset rheumatoid arthritis and could be predictive for cardiovascular disease. Rheumatol Oxf Engl. 2011;50:665–673.

54. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer. 2008;8:592–603.

55. Zhang W, Della-Fera MA, Hartzell DL, Hausman D, Baile CA. Adipose tissue gene expression profiles in ob/ob mice treated with leptin. Life Sci. 2008;83:35–42.

56. Cinti S. The adipose organ: morphological perspectives of adipose tissues. Proc Nutr Soc. 2001;60:319–328.

57. Liakouli V, Cipriani P, Marrelli A, Alvaro S, Ruscitti P, Giacomelli R. Angiogenic cytokines and growth factors in systemic sclerosis. Autoimmun Rev. 2011;10:590–594.

58. Mensink GBM, Schienkiewitz A, Haftenberger M, Lampert T, Ziese T, Scheidt-Nave C. Übergewicht und Adipositas in Deutschland. Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz. 2013;56:786–794.

59. Blüher M. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol. 2019;15:288–298.

60. Bosy-Westphal A, Müller MJ. Diet and Nutrition in the Prevention of Non-Communicable Diseases (NCD). Dtsch Med Wochenschr 1946. 2021;146:389–397.

61. Kolotkin RL, Meter K, Williams GR. Quality of life and obesity. Obes Rev. 2001;2:219–229.

62. Aird WC. Endothelium as an organ system. Crit Care Med. 2004;32:S271-279.

63. Cao Y. Angiogenesis and vascular functions in modulation of obesity, adipose metabolism, and insulin sensitivity. Cell Metab. 2013;18:478–489.

64. Crewe C, An YA, Scherer PE. The ominous triad of adipose tissue dysfunction: inflammation, fibrosis, and impaired angiogenesis. J Clin Invest. 2017;127:74–82.

65. Bhupathiraju SN, Hu FB. Epidemiology of Obesity and Diabetes and Their Cardiovascular Complications. Circ Res. 2016;118:1723–1735.

66. Franklin KA, Lindberg E. Obstructive sleep apnea is a common disorder in the population—a review on the epidemiology of sleep apnea. J Thorac Dis. 2015;7:1311–1322.

67. Endemann DH, Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol JASN. 2004;15:1983–1992.

68. Chia PY, Teo A, Yeo TW. Overview of the Assessment of Endothelial Function in Humans. Front Med. 2020;7:542567.

69. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and

clinical relevance. Circulation. 2007;115:1285–1295.

70. Daquinag AC, Zhang Y, Kolonin MG. Vascular targeting of adipose tissue as an anti-obesity approach. Trends Pharmacol Sci. 2011;32:300–307.

71. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S. Effects of Recombinant Leptin Therapy in a Child with Congenital Leptin Deficiency. N Engl J Med. Massachusetts Medical Society; 1999;341:879–884.

72. Considine RV, Caro JF. Leptin and the regulation of body weight. Int J Biochem Cell Biol. 1997;29:1255–1272.

73. Söderberg S, Ahrén B, Jansson JH, Johnson O, Hallmans G, Asplund K, Olsson T. Leptin is associated with increased risk of myocardial infarction. J Intern Med. 1999;246:409–418.

74. Lefere S, Van de Velde F, Hoorens A, Raevens S, Van Campenhout S, Vandierendonck A, Neyt S, Vandeghinste B, Vanhove C, Debbaut C, Verhelst X, Van Dorpe J, Van Steenkiste C, Casteleyn C, Lapauw B, Van Vlierberghe H, Geerts A, Devisscher L. Angiopoietin-2 Promotes Pathological Angiogenesis and Is a Therapeutic Target in Murine Nonalcoholic Fatty Liver Disease. Hepatology. 2019;69:1087–1104.

75. Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao RL, Colucci WS, Walsh K. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. J Clin Invest. 2005;115:2108–2118.

76. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106:3143–3421.

77. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology. 2001;121:91–100.

78. Clain DJ, Lefkowitch JH. Fatty liver disease in morbid obesity. Gastroenterol Clin North Am. 1987;16:239–252.

79. Luyckx FH, Desaive C, Thiry A, Dewé W, Scheen AJ, Gielen JE, Lefèbvre PJ. Liver abnormalities in severely obese subjects: effect of drastic weight loss after gastroplasty. Int J Obes Relat Metab Disord J Int Assoc Study Obes. 1998;22:222–226.

80. Wolfs MGM, Gruben N, Rensen SS, Verdam FJ, Greve JW, Driessen A, Wijmenga C, Buurman WA, Franke L, Scheja L, Koonen DPY, Shiri-Sverdlov R, van Haeften TW, Hofker MH, Fu J. Determining the association between adipokine expression in multiple tissues and phenotypic features of non-alcoholic fatty liver disease in obesity. Nutr Diabetes. 2015;5:e146.

81. Anuradha S, Mohan V, Gokulakrishnan K, Dixit M. Angiopoietin-2 levels in glucose intolerance, hypertension, and metabolic syndrome in Asian Indians (Chennai Urban Rural Epidemiology Study-74). Metabolism. 2010;59:774–779.

82. Lim HS, Lip GYH, Blann AD. Angiopoietin-1 and angiopoietin-2 in diabetes mellitus: relationship to VEGF, glycaemic control, endothelial damage/dysfunction and atherosclerosis. Atherosclerosis. 2005;180:113–118.

83. Tsai Y-C, Kuo P-L, Hung W-W, Wu L-Y, Wu P-H, Chang W-A, Kuo M-C, Hsu Y-L. Angpt2 Induces Mesangial Cell Apoptosis through the MicroRNA-33-5p-SOCS5 Loop in Diabetic Nephropathy. Mol Ther - Nucleic Acids. 2018;13:543–555.

84. Nomura S, Shouzu A, Omoto S, Inami N, Ueba T, Urase F, Maeda Y. Effects of eicosapentaenoic acid on endothelial cell-derived microparticles, angiopoietins and adiponectin in patients with type 2 diabetes. J Atheroscler Thromb. 2009;16:83–90.

85. Yeboah K, Agyekum JA, Baafour EK, Antwi DA, Adjei AB, Boima V, Gyan B. Circulating Angiogenic Growth Factors in Diabetes Patients with Peripheral Arterial Disease and Exertional Leg Pain in Ghana. Int J Vasc Med. 2017;2017:2390174.

86. Puddu A, Sanguineti R, Maggi D, Nicolò M, Traverso CE, Cordera R, Viviani GL. Advanced Glycation End-Products and Hyperglycemia Increase Angiopoietin-2 Production by Impairing Angiopoietin-1-Tie-2 System. J Diabetes Res. 2019;2019:6198495.

87. Advani A, Gilbert RE. The endothelium in diabetic nephropathy. Semin Nephrol. 2012;32:199–207.

88. Rangasamy S, Srinivasan R, Maestas J, McGuire PG, Das A. A potential role for angiopoietin 2 in the regulation of the blood-retinal barrier in diabetic retinopathy. Invest Ophthalmol Vis Sci. 2011;52:3784–3791.

89. Lim HS, Blann AD, Chong AY, Freestone B, Lip GYH. Plasma vascular endothelial growth factor, angiopoietin-1, and angiopoietin-2 in diabetes: implications for cardiovascular risk and effects of multifactorial intervention. Diabetes Care. 2004;27:2918–2924.

90. Farooqi IS, O'Rahilly S. Genetic factors in human obesity. Obes Rev Off J Int Assoc Study Obes. 2007;8 Suppl 1:37–40.

91. Cordero P, Li J, Oben JA. Epigenetics of obesity: beyond the genome sequence. Curr Opin Clin Nutr Metab Care. 2015;18:361–366.

92. Speakman JR. Thrifty genes for obesity and the metabolic syndrome — time to call off the search? Diab Vasc Dis Res. SAGE Publications; 2006;3:7–11.

93. Tong X, Yin L. Circadian rhythms in liver physiology and liver diseases. Compr Physiol. 2013;3:917–940.

94. Bass J, Takahashi JS. Circadian integration of metabolism and energetics. Science. 2010;330:1349–1354.

95. Li J, Lv S, Qiu X, Yu J, Jiang J, Jin Y, Guo W, Zhao R, Zhang Z-N, Zhang C, Luan B. BMAL1 functions as a cAMP-responsive coactivator of HDAC5 to regulate hepatic gluconeogenesis. Protein Cell. 2018;9:976–980.

96. Guillaumond F, Gréchez-Cassiau A, Subramaniam M, Brangolo S, Peteri-Brünback B, Staels B, Fiévet C, Spelsberg TC, Delaunay F, Teboul M. Kruppel-like factor KLF10 is a link between the circadian clock and metabolism in liver. Mol Cell Biol. 2010;30:3059–3070.

97. Shi D, Chen J, Wang J, Yao J, Huang Y, Zhang G, Bao Z. Circadian Clock Genes in the Metabolism of Non-alcoholic Fatty Liver Disease. Front Physiol. 2019;10:423.

98. Licinio J, Mantzoros C, Negrão AB, Cizza G, Wong ML, Bongiorno PB, Chrousos GP, Karp B, Allen C, Flier JS, Gold PW. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat Med. 1997;3:575–579.

99. Jebb SA, Prentice AM. Is obesity an eating disorder? Proc Nutr Soc. 1995;54:721–728.

100. Prentice AM, Jebb SA. Fast foods, energy density and obesity: a possible mechanistic link. Obes Rev Off J Int Assoc Study Obes. 2003;4:187–194.

101. Astrup A. Healthy lifestyles in Europe: prevention of obesity and type II diabetes by diet and physical activity. Public Health Nutr. 2001;4:499–515.

102. Aoqui C, Chmielewski S, Scherer E, Eissler R, Sollinger D, Heid I, Braren R, Schmaderer C, Megens RT, Weber C, Heemann U, Tschöp M, Baumann M. Microvascular dysfunction in the course of metabolic syndrome induced by high-fat diet. Cardiovasc Diabetol. 2014;13:31.

103. Sternson SM, Eiselt A-K. Three Pillars for the Neural Control of Appetite. Annu Rev Physiol. 2017;79:401–423.

104. Brobeck JR. Mechanism of the development of obesity in animals with hypothalamic lesions.

Physiol Rev. 1946;26:541–559.

105. Scholtz S, Miras AD, Chhina N, Prechtl CG, Sleeth ML, Daud NM, Ismail NA, Durighel G, Ahmed AR, Olbers T, Vincent RP, Alaghband-Zadeh J, Ghatei MA, Waldman AD, Frost GS, Bell JD, le Roux CW, Goldstone AP. Obese patients after gastric bypass surgery have lower brain-hedonic responses to food than after gastric banding. Gut. 2014;63:891–902.

106. Carnell S, Gibson C, Benson L, Ochner CN, Geliebter A. Neuroimaging and obesity: current knowledge and future directions. Obes Rev Off J Int Assoc Study Obes. 2012;13:43–56.

107. Sharma AM, Padwal R. Obesity is a sign – over-eating is a symptom: an aetiological framework for the assessment and management of obesity. Obes Rev. 2010;11:362–370.

108. Woods SC, Seeley RJ, Rushing PA, D'Alessio D, Tso P. A controlled high-fat diet induces an obese syndrome in rats. J Nutr. 2003;133:1081–1087.

109. Woods SC, D'Alessio DA, Tso P, Rushing PA, Clegg DJ, Benoit SC, Gotoh K, Liu M, Seeley RJ. Consumption of a high-fat diet alters the homeostatic regulation of energy balance. Physiol Behav. 2004;83:573–578.

110. Ahrén B, Gudbjartsson T, Al-Amin AN, Mårtensson H, Myrsén-Axcrona U, Karlsson S, Mulder H, Sundler F. Islet perturbations in rats fed a high-fat diet. Pancreas. 1999;18:75–83.

111. Shah P, Lueschen N, Ardestani A, Oberholzer J, Olerud J, Carlsson P-O, Maedler K. Angiopoetin-2 Signals Do Not Mediate the Hypervascularization of Islets in Type 2 Diabetes. Claret M, editor. PLOS ONE. 2016;11:e0161834.

112. Tomada I, Tomada N, Almeida H, Neves D. Energy restriction and exercise modulate angiopoietins and vascular endothelial growth factor expression in the cavernous tissue of high-fat diet-fed rats. Asian J Androl. 2012;14:635–642.

113. Rudnicki M, Abdifarkosh G, Rezvan O, Nwadozi E, Roudier E, Haas TL. Female Mice Have Higher Angiogenesis in Perigonadal Adipose Tissue Than Males in Response to High-Fat Diet. Front Physiol. 2018;9:1452.

114. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. J Biol Chem. 2003;278:45021–45026.

115. Bruce CR, Mertz VA, Heigenhauser GJF, Dyck DJ. The stimulatory effect of globular adiponectin on insulin-stimulated glucose uptake and fatty acid oxidation is impaired in skeletal muscle from obese subjects. Diabetes. 2005;54:3154–3160.

116. An YA, Sun K, Joffin N, Zhang F, Deng Y, Donzé O, Kusminski CM, Scherer PE. Angiopoietin-2 in white adipose tissue improves metabolic homeostasis through enhanced angiogenesis. eLife. 2017;6:1–22.

117. Emerson SR, Sciarrillo CM, Kurti SP, Emerson EM, Rosenkranz SK. High-Fat Meal-Induced Changes in Markers of Inflammation and Angiogenesis in Healthy Adults Who Differ by Age and Physical Activity Level. Curr Dev Nutr. 2019;3:098.

118. Schultes B, Schmid S, Peters A, Born J, Fehm HL. Sleep loss and the development of diabetes: a review of current evidence. Exp Clin Endocrinol Diabetes Off J Ger Soc Endocrinol Ger Diabetes Assoc. 2005;113:563–567.

119. Czeisler CA. Impact of sleepiness and sleep deficiency on public health--utility of biomarkers. J Clin Sleep Med JCSM Off Publ Am Acad Sleep Med. 2011;7:S6-8.

120. Cappuccio FP, Taggart FM, Kandala N-B, Currie A, Peile E, Stranges S, Miller MA. Meta-analysis of short sleep duration and obesity in children and adults. Sleep. 2008;31:619–626.

121. Schmid SM, Hallschmid M, Schultes B. The metabolic burden of sleep loss. Lancet Diabetes Endocrinol. 2015;3:52–62.

122. Archer SN, Oster H. How sleep and wakefulness influence circadian rhythmicity: effects of insufficient and mistimed sleep on the animal and human transcriptome. J Sleep Res.

2015;24:476–493.

123. Archer SN, Laing EE, Möller-Levet CS, van der Veen DR, Bucca G, Lazar AS, Santhi N, Slak A, Kabiljo R, von Schantz M, Smith CP, Dijk D-J. Mistimed sleep disrupts circadian regulation of the human transcriptome. Proc Natl Acad Sci U S A. 2014;111:E682-691.

124. Schulz H. Phasic or transient? Comment on the terminology of the AASM manual for the scoring of sleep and associated events. J Clin Sleep Med JCSM Off Publ Am Acad Sleep Med. 2007;3:752.

125. Kenneth P. Wright J. Influence of Sleep Deprivation and Circadian Misalignment on Cortisol, Inflammatory Markers, and Cytokine Balance. Brain Behav Immun. 2015;47:24–34.

126. Wilms B, Leineweber EM, Mölle M, Chamorro R, Pommerenke C, Salinas-Riester G, Sina C, Lehnert H, Oster H, Schmid SM. Sleep Loss Disrupts Morning-to-Evening Differences in Human White Adipose Tissue Transcriptome. J Clin Endocrinol Metab. 2019;104:1687–1696.

127. Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of sleep apnea. Physiol Rev. 2010;90:47–112.

128. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The Report of an American Academy of Sleep Medicine Task Force. Sleep. 1999;22:667–689.

129. Young T, Finn L, Peppard PE, Szklo-Coxe M, Austin D, Nieto FJ, Stubbs R, Hla KM. Sleep disordered breathing and mortality: eighteen-year follow-up of the Wisconsin sleep cohort. Sleep. 2008;31:1071–1078.

130. Coughlin SR, Mawdsley L, Mugarza JA, Calverley PMA, Wilding JPH. Obstructive sleep apnoea is independently associated with an increased prevalence of metabolic syndrome. Eur Heart J. 2004;25:735–741.

131. Somers VK, White DP, Amin R, Abraham WT, Costa F, Culebras A, Daniels S, Floras JS, Hunt CE, Olson LJ, Pickering TG, Russell R, Woo M, Young T. Sleep apnea and cardiovascular disease: an American Heart Association/American College of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council on Cardiovascular Nursing. J Am Coll Cardiol. 2008;52:686–717.

132. Yuan HT, Yang SP, Woolf AS. Hypoxia up-regulates angiopoietin-2, a Tie-2 ligand, in mouse mesangial cells. Kidney Int. 2000;58:1912–1919.

133. Bravo B, Garcia de Durango C, Gonzalez A, Gortazar AR, Santos X, Forteza-Vila J, Vidal-Vanaclocha F. Opposite Effects of Mechanical Action of Fluid Flow on Proangiogenic Factor Secretion From Human Adipose-Derived Stem Cells With and Without Oxidative Stress. J Cell Physiol. 2017;232:2158–2167.

134. Gozal D, Khalyfa A, Qiao Z, Smith DL, Philby MF, Koren D, Kheirandish-Gozal L. Angiopoietin-2 and soluble Tie-2 receptor plasma levels in children with obstructive sleep apnea and obesity. Obesity. 2017;25:1083–1090.

135. Strollo PJ, Soose RJ, Maurer JT, de Vries N, Cornelius J, Froymovich O, Hanson RD, Padhya TA, Steward DL, Gillespie MB, Woodson BT, Van de Heyning PH, Goetting MG, Vanderveken OM, Feldman N, Knaack L, Strohl KP, STAR Trial Group. Upper-airway stimulation for obstructive sleep apnea. N Engl J Med. 2014;370:139–149.

136. Chamorro R, Steffen A, Buyny L, Windjäger A, Wilms B, Hasselbacher K, Wollenberg B, Lehnert H, Schmid SM. Upper airway stimulation in obstructive sleep apnea improves glucose metabolism and reduces hedonic drive for food. J Sleep Res. 2018;e12794.

137. Wilms B, Kuhr M, Chamorro R, Klinsmann N, Spyra D, Mölle M, Kalscheuer H, Schultes B, Lehnert H, Schmid SM. Chronobiological aspects of sleep restriction modulate subsequent

spontaneous physical activity. Physiol Behav. 2020;215:112795.

138. Sher AE, Schechtman KB, Piccirillo JF. The efficacy of surgical modifications of the upper airway in adults with obstructive sleep apnea syndrome. Sleep. 1996;19:156–177.

139. Verseijden F, Jahr H, Posthumus-van Sluijs SJ, Ten Hagen TL, Hovius SER, Seynhaeve ALB, van Neck JW, van Osch GJVM, Hofer SOP. Angiogenic Capacity of Human Adipose-Derived Stromal Cells During Adipogenic Differentiation: An In Vitro Study. Tissue Eng Part A. 2009;15:445–452.

140. Jiao Z, Ma Y, Zhang Q, Wang Y, Liu T, Liu X, Piao C, Liu B, Wang H. The adipose-derived mesenchymal stem cell secretome promotes hepatic regeneration in miniature pigs after liver ischaemia-reperfusion combined with partial resection. Stem Cell Res Ther. 2021;12:218.

141. Shibata C, Nakai K, Ozaki M, Koshi R, Tanaka H, Morita T, Maeno M, Kawato T. Effects of interleukin-6 and tumor necrosis factor-α on the expression of angiogenic and collagenolytic factors in premature and mature adipocytes. Biochem Biophys Res Commun. 2020;531:297–304.

142. Mohammadi E, Nassiri SM, Rahbarghazi R, Araghi A. Endothelial juxtaposition of distinct adult stem cells activates angiogenesis signaling molecules in endothelial cells. Cell Tissue Res. 2015;362:597–609.

143. Hausman GJ, Barb CR, Dean RG. Patterns of gene expression in pig adipose tissue: Insulin-like growth factor system proteins, neuropeptide Y (NPY), NPY receptors, neurotrophic factors and other secreted factors. Domest Anim Endocrinol. 2008;35:24–34.

144. Voros G, Maquoi E, Demeulemeester D, Clerx N, Collen D, Lijnen HR. Modulation of Angiogenesis during Adipose Tissue Development in Murine Models of Obesity. Endocrinology. 2005;146:4545–4554.

145. Dallabrida SM, Zurakowski D, Shih SC, Smith LE, Folkman J, Moulton KS, Rupnick MA. Adipose tissue growth and regression are regulated by angiopoietin-1. Biochem Biophys Res Commun. 2003;311:563–571.

146. Seo S, Fujita H, Nakano A, Kang M, Duarte A, Kume T. The forkhead transcription factors, Foxc1 and Foxc2, are required for arterial specification and lymphatic sprouting during vascular development. Dev Biol. 2006;294:458–470.

147. Xue Y, Cao R, Nilsson D, Chen S, Westergren R, Hedlund E-M, Martijn C, Rondahl L, Krauli P, Walum E, Enerback S, Cao Y. FOXC2 controls Ang-2 expression and modulates angiogenesis, vascular patterning, remodeling, and functions in adipose tissue. Proc Natl Acad Sci. 2008;105:10167–10172.

148. Liu W, Ji Y, Chu H, Wang M, Yang B, Yin C. SFRP5 mediates downregulation of the wnt5a/caveolin-1/JNK signaling pathway. J Endocrinol. 2020;247:181–190.

149. Yin C, Hu W, Wang M, Xiao Y. The role of the adipocytokines vaspin and visfatin in vascular endothelial function and insulin resistance in obese children. BMC Endocr Disord. 2019;19:127.

150. Yin C, Hu W, Wang M, Lv W, Jia T, Xiao Y. Irisin as a mediator between obesity and vascular inflammation in Chinese children and adolescents. Nutr Metab Cardiovasc Dis NMCD. 2020;30:320–329.

151. Kaess BM, Pedley A, Massaro JM, Larson MG, Corsini E, Hoffmann U, Smith HM, Sawyer DB, Vasan RS, Fox CS. Relation of Vascular Growth Factors with CT-Derived Measures of Body Fat Distribution: The Framingham Heart Study. J Clin Endocrinol Metab. 2012;97:987–994.

152. Lieb W, Zachariah JP, Xanthakis V, Safa R, Chen M-H, Sullivan LM, Larson MG, Smith HM, Yang Q, Mitchell GF, Vita JA, Sawyer DB, Vasan RS. Clinical and Genetic Correlates of Circulating Angiopoietin-2 and Soluble Tie-2 in the Community. Circ Cardiovasc Genet.

2010;3:300–306.

153. Skopkova M, Penesova A, Sell H, Radikova Z, Vlcek M, Imrich R, Koska J, Ukropec J, Eckel J, Klimes I, Gasperikova D. Protein array reveals differentially expressed proteins in subcutaneous adipose tissue in obesity. Obesity. 2007;15:2396–2406.

154. Gaebler N, Haggenmüller B, Kapapa M, Serra A, Tews D, Funcke J-B, Brandt S, Ioannidis V, Schön M, Möller P, Debatin K-M, Wabitsch M, Fischer-Posovszky P. Age- and BMI-Associated Expression of Angiogenic Factors in White Adipose Tissue of Children. Int J Mol Sci. 2019;20:5204.

155. Tomada N, Tomada I, Botelho F, Pacheco-Figueiredo L, Lopes T, Negrão R, Pestana M, Cruz F. Endothelial function in patients with metabolic syndrome and erectile dysfunction: a question of angiopoietin imbalance? Andrology. 2013;1:541–548.

156. Chedraui P, Escobar GS, Pérez-López FR, Palla G, Montt-Guevara M, Cecchi E, Genazzani AR, Simoncini T, Research Group for the Omega Women's Health Project. Angiogenesis, inflammation and endothelial function in postmenopausal women screened for the metabolic syndrome. Maturitas. 2014;77:370–374.

157. Gonzalez-Quesada C, Cavalera M, Biernacka A, Kong P, Lee D-W, Saxena A, Frunza O, Dobaczewski M, Shinde A, Frangogiannis NG. Thrombospondin-1 induction in the diabetic myocardium stabilizes the cardiac matrix in addition to promoting vascular rarefaction through angiopoietin-2 upregulation. Circ Res. 2013;113:1331–1344.

158. Ichinose K, Maeshima Y, Yamamoto Y, Kinomura M, Hirokoshi K, Kitayama H, Takazawa Y, Sugiyama H, Yamasaki Y, Agata N, Makino H. 2-(8-hydroxy-6-methoxy-1-oxo-1h-2-benzopyran-3-yl) propionic acid, an inhibitor of angiogenesis, ameliorates renal alterations in obese type 2 diabetic mice. Diabetes. 2006;55:1232–1242.

159. El-Asrar MA, Elbarbary NS, Ismail EAR, Bakr AA. Circulating angiopoietin-2 levels in children and adolescents with type 1 diabetes mellitus: relation to carotid and aortic intima-media thickness. Angiogenesis. 2016;19:421–431.

160. Greulich S, Maxhera B, Vandenplas G, de Wiza DH, Smiris K, Mueller H, Heinrichs J, Blumensatt M, Cuvelier C, Akhyari P, Ruige JB, Ouwens DM, Eckel J. Secretory Products From Epicardial Adipose Tissue of Patients With Type 2 Diabetes Mellitus Induce Cardiomyocyte Dysfunction. Circulation. 2012;126:2324–2334.

161. Scurt FG, Menne J, Brandt S, Bernhardt A, Mertens PR, Haller H, Chatzikyrkou C, ROADMAP Steering Committee. Systemic Inflammation Precedes Microalbuminuria in Diabetes. Kidney Int Rep. 2019;4:1373–1386.

162. Khalaf N, Helmy H, labib H, Fahmy I, Abd El Hamid M, Moemen L. Role of Angiopoietins and Tie-2 in Diabetic Retinopathy. Electron Physician. 2017;9:5031–5035.

163. Chen S, Li H, Zhang C, Li Z, Wang Q, Guo J, Luo C, Wang Y. Urinary angiopoietin-2 is associated with albuminuria in patients with type 2 diabetes mellitus. Int J Endocrinol. 2015;2015:163120.

164. Yeboah K, Kyei-Baafour E, Antwi DA, Asare-Anane H, Gyan B, Amoah AGB. Circulating angiogenic factors in diabetes patients in a tertiary hospital in Ghana. J Diabetes Metab Disord. 2016;15:44.

165. Florijn BW, Valstar GB, Duijs JMGJ, Menken R, Cramer MJ, Teske AJ, Ghossein-Doha C, Rutten FH, Spaanderman MEA, den Ruijter HM, Bijkerk R, van Zonneveld AJ. Sex-specific microRNAs in women with diabetes and left ventricular diastolic dysfunction or HFpEF associate with microvascular injury. Sci Rep. 2020;10:13945.

166. Rasul S, Reiter MH, Ilhan A, Lampichler K, Wagner L, Kautzky-Willer A. Circulating angiopoietin-2 and soluble Tie-2 in type 2 diabetes mellitus: a cross-sectional study. Cardiovasc Diabetol. 2011;10:55.

167. Theelen TL, Lappalainen JP, Sluimer JC, Gurzeler E, Cleutjens JP, Gijbels MJ, Biessen EAL, Daemen MJAP, Alitalo K, Yla-Herttuala S. Angiopoietin-2 blocking antibodies reduce early atherosclerotic plaque development in mice. Atherosclerosis. 2015;241:297–304.

168. Drosos I, Pavlaki M, Ortega Carrillo MDP, Kourkouli A, Buschmann K, Konstantinou F, Gogiraju R, Bochenek ML, Chalikias G, Tortopidis C, Vahl CF, Mikroulis D, Tziakas D, Münzel T, Konstantinides S, Schäfer K. Increased Lymphangiogenesis and Lymphangiogenic Growth Factor Expression in Perivascular Adipose Tissue of Patients with Coronary Artery Disease. J Clin Med. 2019;8:E1000.

169. Bar A, Kieronska-Rudek A, Proniewski B, Suraj-Prażmowska J, Czamara K, Marczyk B, Matyjaszczyk-Gwarda K, Jasztal A, Kuś E, Majka Z, Kaczor A, Kurpińska A, Walczak M, Pieterman EJ, Princen HMG, Chlopicki S. In Vivo Magnetic Resonance Imaging-Based Detection of Heterogeneous Endothelial Response in Thoracic and Abdominal Aorta to Short-Term High-Fat Diet Ascribed to Differences in Perivascular Adipose Tissue in Mice. J Am Heart Assoc. 2020;9:e016929.

170. Korybalska K, Swora-Cwynar E, Łuczak J, Kanikowska A, Czepulis N, Rutkowski R, Bręborowicz A, Grzymisławski M, Witowski J. Association of endothelial proliferation with the magnitude of weight loss during calorie restriction. Angiogenesis. 2016;19:407–419.

171. Cullberg KB, Christiansen T, Paulsen SK, Bruun JM, Pedersen SB, Richelsen B. Effect of weight loss and exercise on angiogenic factors in the circulation and in adipose tissue in obese subjects. Obes Silver Spring Md. 2013;21:454–460.

172. Vishvanath L, Gupta RK. Contribution of adipogenesis to healthy adipose tissue expansion in obesity. J Clin Invest. 2019;129:4022–4031.

173. Leitner BP, Huang S, Brychta RJ, Duckworth CJ, Baskin AS, McGehee S, Tal I, Dieckmann W, Gupta G, Kolodny GM, Pacak K, Herscovitch P, Cypess AM, Chen KY. Mapping of human brown adipose tissue in lean and obese young men. Proc Natl Acad Sci U S A. 2017;114:8649–8654.

174. Hafidi ME, Buelna-Chontal M, Sánchez-Muñoz F, Carbó R. Adipogenesis: A Necessary but Harmful Strategy. Int J Mol Sci. 2019;20:3657.

175. Westerterp KR. Physical activity and physical activity induced energy expenditure in humans: measurement, determinants, and effects. Front Physiol. 2013;4:90.

176. Shechter A, Rising R, Albu JB, St-Onge M-P. Experimental sleep curtailment causes wake-dependent increases in 24-h energy expenditure as measured by whole-room indirect calorimetry. Am J Clin Nutr. 2013;98:1433–1439.

177. Barbé F, Durán-Cantolla J, Sánchez-de-la-Torre M, Martínez-Alonso M, Carmona C, Barceló A, Chiner E, Masa JF, Gonzalez M, Marín JM, Garcia-Rio F, Diaz de Atauri J, Terán J, Mayos M, de la Peña M, Monasterio C, del Campo F, Montserrat JM, Spanish Sleep And Breathing Network. Effect of continuous positive airway pressure on the incidence of hypertension and cardiovascular events in nonsleepy patients with obstructive sleep apnea: a randomized controlled trial. JAMA. 2012;307:2161–2168.

178. Ruehland WR, Rochford PD, O'Donoghue FJ, Pierce RJ, Singh P, Thornton AT. The New AASM Criteria for Scoring Hypopneas: Impact on the Apnea Hypopnea Index. Sleep. 2009;32:150–157.

7 ETHICS APPROVAL

The present human-experimental study part A, B are part of the study titled "Chronophysiologische Genexpressionsmuster des weißen Fettgewebes in Abhängigkeit vom Kohlenhydrat- und Fettgehalt der Nahrung – funktionelle Implikationen für Glukosestoffwechsel und hedonische Aspekte des Essverhaltens" was presented to the head of the study, Prof. Dr. Sebastian M. Meyhöfer was approved by the ethics committee of the University of Lübeck on April 6th, 2017, under the file number EC 16-343.

The present human-experimental study part C is part of the study titled "Einfluss von Schlafdauer-Restriktion in der frühen vs. späten Nachthälfte auf die Verarbeitung von Essensreizen und den Glukosestoffwechsel" was presented to the head of the study, Prof. Dr. Sebastian M. Meyhöfer was approved by the ethics committee of the University of Lübeck on July 5th, 2010, under the file number EC 10-109.

The present human-experimental study part D is part of the study titled "Upper airway stimulation (UAS) bei obstruktiver Schlaf Apnea – Metabolische Effekte" was presented to the head of the study, Prof. Dr. Armin Steffen was approved by the ethics committee of the University of Lübeck on July 9th, 2014, under the file number AZ 14-095.

The medical and legal responsibility of the head of the clinical study and the doctors participating in the study remain unaffected by our opinion in accordance with the advisory function of the ethics committee.

8 ACKNOWLEDGMENT

First, I would like to thank my supervisor, Prof. Dr. med. Sebastian Meyhöfer, for offering me the opportunity to undertake my thesis in his group, and for his constant support, valuable advice, knowledge and understanding throughout my postgraduate research.

I would also like to extend my sincere thanks to my co-supervisor, PD Dr. Britta Wilms for her valuable advice, and kind help on proposal writing, data analysis, dissertation revising, as well as the endless care in my daily life in Germany.

In addition, thank Dr. Rodrigo Chamorro for his consistent support during my whole experiment process even after he went back to Chile. Further, I must thank Susanne Behling for the technical support with blood measurement and the kindly help in my daily life. I also give my special and warmest thanks to everyone in the Metabolic Core Unit of CBBM for their kind help in accomplishing each of the steps on my doctoral study, and everybody who has helped, supported me throughout the years of this work. I will never forget the life I spent in Lübeck with all of you. Many thanks to all the friends I've made in Lübeck in the past years. All of you made my life here so colorful and delightful.

Thank Dr. Med. Siyi Xin, my best friend. Thanks for always being with me in this foreign country of Germany, and thanks for supporting in my work and life. Without you, I would not have had the courage to make this decision to study abroad and become a better of me.

Most importantly, I must thank my parents for their support, encouragement, and endless love. No matter what happened, you were always behind me. I love you all!