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Cardiovascular, inflammatory and circadian aspects of metabolic regulation in humans

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“A journey of a thousand miles begins with a single step”

Lao Tzu

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Abbreviations

ACOX3	Acyl-CoA oxidase 3
AMPK	5'-prime-AMP-activated protein kinase
ANP	Atrial natriuretic peptide
B2M	Beta-2-microglobulin
BMAL1 (ARNTL)	Aryl hydrocarbon receptor nuclear translocator-like
BMI	Body mass index
BNP	B-type natriuretic peptide
CCKAR	Cholecystokinin A receptor
CCN	Cyr61/CTGF/NOV protein family
CCR7	C-C chemokine receptor type 7
cGMP	Cyclic guanosine monophosphate
CID	Clinical investigation day
CLOCK	Clock circadian regulator
CNP	C-type natriuretic peptide
COX2	Cyclooxygenase 2
CPT1A	Carnitine palmitoyltransferase 1A
CREB1	cAMP responsive element binding protein 1
CRY	Cryptochrome circadian clock
EC	Hyperinsulinemic-euglycemic clamp
FASN	Fatty acid synthase
FXR	Farnesoid X receptor;
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GIPR	Glucose-dependent insulinotropic polypeptide receptor
GLP1R	Glucagon-like peptide-1 receptor
GPR41 (FFAR3)	G protein-coupled receptor 41
GPR43 (FFAR2)	G protein-coupled receptor 43
HC	Hyperinsulinemic-hyperglycemic clamp

HFD	High fat diet
HIC	Hepatic insulin clearance
HPRT1	Hypoxanthine phosphoribosyltransferase 1
IDE	Insulin degrading enzyme
IDH3A	Isocitrate dehydrogenase 3 alpha
IL1B	Interleukin 1, beta
ISI	Insulin sensitivity index
ISR	Insulin secretion rate
LPS	Lipopolysaccharide
MeSyBePo	Metabolic Syndrome Berlin Potsdam study
MR-proANP	Midregional proANP
NAMPT	Nicotinamide phosphoribosyltransferase
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
NOV	Nephroblastoma overexpressed protein
NP	Natriuretic peptide
NPR	Natriuretic peptide receptor
NR1D1 (RevErbα)	Nuclear receptor subfamily 1, group D, member 1
NTSR1	Neurotensin receptor 1
NUGAT	Nutrigenomic Analysis in Twins
OGTT	Oral glucose tolerance test
PBMC	Peripheral blood mononuclear cells
PER	Period circadian clock
PPAR	Peroxisome proliferator-activated receptor
PPIB	Peptidylprolyl isomerase B (cyclophilin B)
qRT-PCR	Quantitative real-time PCR
ROR	Retinoic acid-related orphan receptor
RPLP0	Ribosomal protein large protein 0

SAT	Subcutaneous adipose tissue
SCN	Suprachiasmatic nucleus
SIRT1	Sirtuin 1
SREBP-1	Sterol regulatory element binding transcription factor 1
SRF	Serum response factor
T2DM	Type 2 diabetes
TEF	Thyrotrophic embryonic factor
TGR5 (GPBAR1)	G protein-coupled bile acid receptor 1
VAT	Visceral adipose tissue
VIPR1	Vasoactive intestinal peptide receptor 1
VIPR2	Vasoactive intestinal peptide receptor 2
WISP1 (CCN4)	WNT-inducible signaling pathway protein-1
WNT	Wingless-type signaling pathway

1. Introduction

Obesity is an epidemic and still growing health problem of ”Western lifestyle” countries including Germany [1]. Obesity is associated with numerous metabolic disturbances including non-alcoholic fatty liver disease, metabolic syndrome, type 2 diabetes (T2DM), as well as cardiovascular disease and cancer and therefore results in substantially increased all-cause mortality [2]. Genetic predisposition, sedentary lifestyle and hypercaloric diet are main factors leading to the progression of obesity and accompanying metabolic diseases [3].

Despite epidemic proportions worldwide, enormous health care costs and thousands of scientific studies addressing aetiology of metabolic disturbances, many important pathophysiological mechanisms of metabolic regulation and response to nutritional changes remain unclear. A better understanding of molecular mechanisms will provide a key to developing targeted strategies of prevention and treatment of metabolic diseases. The present work describes several new cardiovascular, inflammatory and circadian mechanisms contributing to the human metabolic regulation in health and disease.

1.1. Metabolic syndrome and alterations of hepatic insulin clearance

“Insulin resistance” syndrome or “metabolic syndrome” represents a complex of metabolic and physiological disturbances such as dyslipidemia, insulin resistance, hyperinsulinemia, hyperglycemia, and high blood pressure and is associated with elevated risk of T2DM and cardiovascular diseases (**Table 1**) [4-6]. Moreover, some authors suggest to include microalbuminuria, hyperuricemia, inflammatory markers, abnormalities of hemostatic system [7], and elevated levels of liver enzymes [8] to the cluster of metabolic syndrome components. Reaven et al. [4] described the hyperinsulinemia as a predominant sign of metabolic syndrome developing as a compensative response to the insulin resistance. Two main processes, an increase of insulin secretion and a decrease of hepatic insulin clearance (HIC), contribute to the pathophysiology of hyperinsulinemia in the insulin-resistant state [9, 10].

Table 1. Criteria for clinical diagnosis of the metabolic syndrome [6]

Measure	Categorical cut points
Elevated waist circumference	Population- and country-specific definitions (≥94 cm in males; ≥80 cm in females for Europids)
Elevated triglycerides (drug treatment for elevated triglycerides is an alternate indicator)	≥150 mg/dL (1.7 mmol/L)
Reduced HDL-C (drug treatment for reduced HDL-C is an alternate indicator)	<40 mg/dL (1.0 mmol/L) in males; <50 mg/dL (1.3 mmol/L) in females
Elevated blood pressure (antihypertensive drug treatment in a patient with a history of hypertension is an alternate indicator)	Systolic ≥130 and/or diastolic ≥85 mm Hg
Elevated fasting glucose (drug treatment of elevated glucose is an alternate indicator)	≥100 mg/dL

Diminished HIC was described in insulin-resistant subjects [11], children with increased body weight [12], individuals with hypertension [10], elevated risk of T2DM [13] and non-alcoholic fatty liver disease [14]. We and others showed that genetic polymorphisms [15], hyperglycemia [16], and increased free fatty acids [17] may facilitate HIC dysregulation. Nevertheless, mechanisms contributing to this phenomenon are not entirely understood.

Surprisingly, relationships between HIC and components of the metabolic syndrome were investigated previously only in few studies [8, 11]. We examined systematically how HIC is associated with various components of metabolic syndrome and proved the hypothesis whether HIC predicts the risk of metabolic syndrome [18].

1.2. Natriuretic peptides in obesity

Numerous epidemiological studies clearly characterized the central obesity and metabolic syndrome as independent risk factors for cardiovascular disease [19] and heart failure [20]. Nevertheless, exact mechanisms underlying this phenomenon still need to be clarified.

Natriuretic peptides (NPs), i.e. atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), are important regulators of the cardiovascular homeostasis and also demonstrate growth-regulating properties [21]. ANP and BNP are produced by the cardiomyocytes of cardiac atria and ventricles, respectively,

whereas CNP is secreted predominantly in the brain and additionally in chondrocytes, vascular endothelial cells and in other tissues [21, 22]. ANP and BNP directly regulate blood pressure and body fluid homeostasis [21, 22]. Three NP receptors are known – natriuretic peptide receptor A (NPRA), natriuretic peptide receptor B (NPRB), and natriuretic peptide receptor C (NPRC). NPRA and NPRB represent membrane-bound guanylyl cyclase receptors which activation leads to the intracellular increase of cyclic guanosine monophosphate (cGMP) and alteration of the activity of cGMP-dependent protein kinases and ion channels **(Figure 1)** [21, 22]. NPRA shows an affinity to ANP and to a lesser degree to BNP, while CNP is a ligand for NPRB. NPRC binds all three natriuretic peptides (ligand affinity ANP>BNP>CNP) [22, 23]. This receptor demonstrates no guanylyl cyclase activity but provides the receptor-mediated internalization and degradation and in this way regulates tissue concentration of NPs **(Figure 1)** [22, 23].

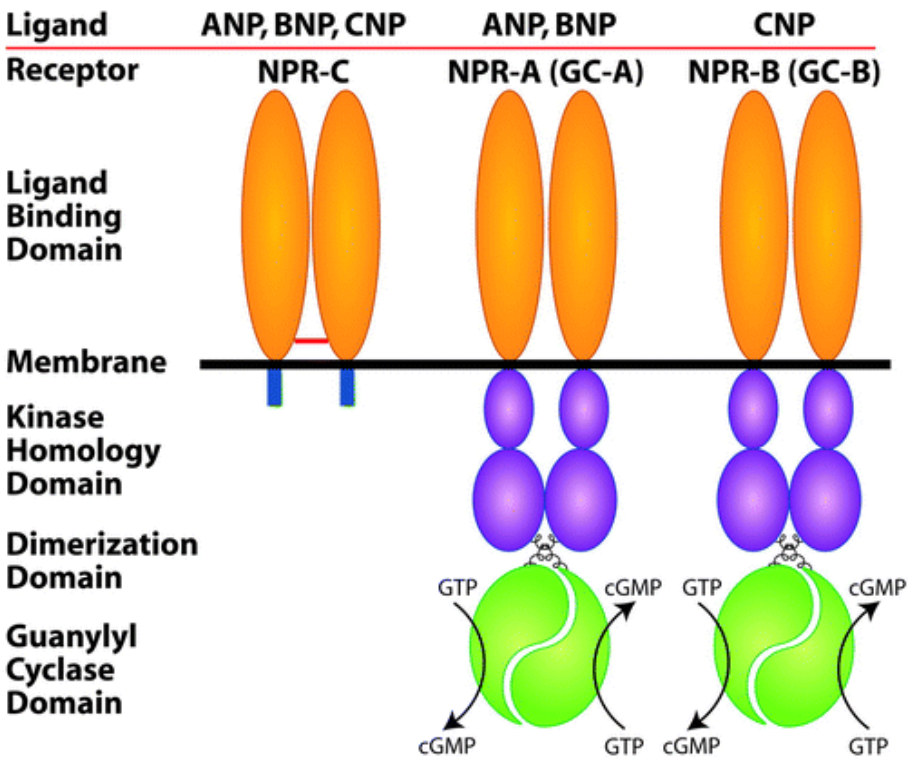

Figure 1. Natriuretic peptide receptors and their ligands.
Natriuretic peptides are ligands for three receptors, NPRA, NPRB, and NPRC. NPRA and NPRB are characterized by the membrane-bound guanylyl cyclase activity and consist of an extracellular ligand binding domain, hydrophobic transmembrane region, and intracellular kinase homology, dimerization, and carboxyl-terminal guanylyl cyclase domains. NPRC has approximately 30% identity to NPRA and NPRB in the extracellular ligand-binding domain but contains only 37 intracellular amino acids [22].

Beside of their renal and cardiovascular actions, NPs have a range of metabolic functions including activation of lipolysis, lipid oxidation, and mitochondrial respiration [24]. These effects induce browning of white adipose tissue, increase muscular oxidative capacity, and are protective against diet-induced obesity and insulin resistance [24].

High blood levels of NPs are known to be cardiovascular risk markers [25]. In subjects with hypertension and particularly heart failure or myocardial ischemia, ANP and BNP rise dramatically because the organism tries to compensate corresponding dysfunction [21, 22]. In opposite, the NP decrease referred to as “natriuretic handicap” was found in obesity [26-28]. This is counterintuitive because in obesity characterized by salt retention and higher cardiac output, increased NP levels would be expected. The unexpected NP suppression in obesity may be explained by an increased expression of NPRC in adipose tissue which leads to the elevated NP clearance in obese subjects [28]. A decrease of cardiovascular protective hormones may increase a long-term risk of cardiovascular disease such as occurs for ANP in the metabolic syndrome [29].

Central obesity is usually characterized by the increased insulin levels resulted from insulin resistance [4]. In recently published observation in rodents, insulin is supposed to be a major regulator of the NPRs expression in the organism [30]. We elucidated this pathophysiological mechanism in humans investigating the NPR expression in the adipose tissue and their regulation by insulin [29].

1.3. Adipose tissue inflammation

Immune system plays an important role in the pathogenesis of obesity-associated diseases [31]. Indeed, the chronic low-grade systemic inflammation was described in obesity and T2DM which is characterized by the macrophage infiltration of adipose tissue and contributes to the development of insulin resistance [32, 33]. Interactions between adipocytes, macrophages and endothelial cells lead to the deterioration of the inflammation and to the elevated secretion of proinflammatory cytokines, chemokines, adipokines and angiogenic factors [32].

Macrophage infiltration results from activation of circulating monocytes, their transmigration into adipose tissue and differentiation into macrophages [31]. Two polarized subtypes of adipose tissue macrophages are described - classically activated "proinflammatory" M1 and alternatively activated "antiinflammatory" M2 [33] **(Figure 2)** which demonstrate differences in their phenotype, metabolism and morphology, and play various roles in the immune response [33, 34]. M1 cells are characterized by the IL-12^{high}, IL-23^{high}, IL-10^{low} phenotype, produce proinflammatory cytokines, reactive oxygen and nitrogen intermediates, participate in polarized T helper type 1 responses, and contribute to the parasite and tumor resistance. M2 cells have various subtypes demonstrating the IL-12^{low}, IL-23^{low}, IL-10^{high} phenotype, produce antiinflammatory cytokines, participate in polarized T helper type 2 reactions, promote tissue repair and remodelling, and have immunoregulatory

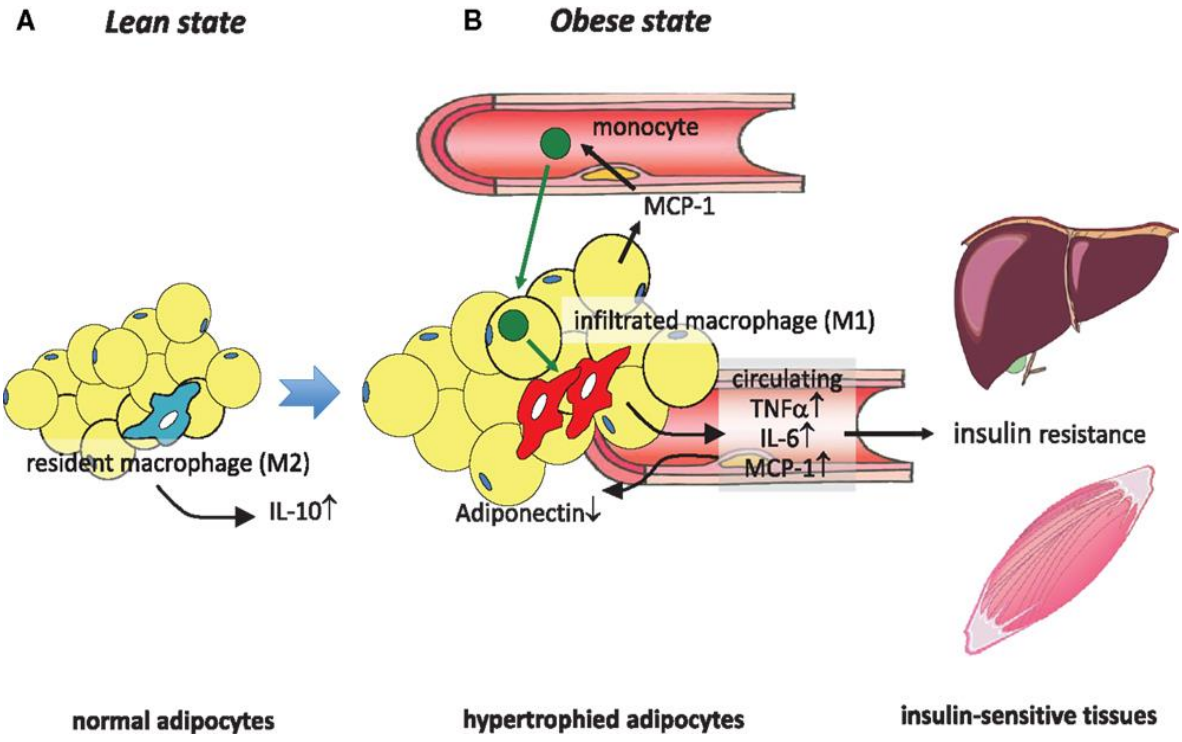

Figure 2. Macrophage infiltration into adipose tissue in obesity.
(**A**) In a lean state, most resident macrophages in adipose tissue represent M2 macrophages which promote insulin sensitivity particularly by IL-10 secretion. (**B**) In the obese state, large hypertrophic adipocytes secret MCP-1 to the circulation that recruits circulating monocytes to adipose tissue. Infiltrated monocytes differentiate into classically activated M1 macrophages secreting proinflammatory cytokines such as TNFα, IL-6, and MCP-1, which facilitate the low-grade inflammation in adipose tissue and a decrease of adiponectin. Secreted cytokines act as insulin resistance-inducing adipokines and contribute to the development of insulin resistance in skeletal muscle and liver. Figure adapted from [35].

functions [34]. M1 and M2 cells express different patterns of chemokine and cytokine receptors [36]. Interestingly, obesity shifts M1/M2 ratio in adipose tissue to M1 subtype [37] **(Figure 2)**. However, mechanisms of the low-grade inflammation are not fully understood.

In particular, numerous nutrition-associated factors such as food components, metabolites, and gastrointestinal neuropeptides and hormones may influence immune cell functions [38-41] and in this way contribute to the pathogenesis of low-grade inflammation [42]. In our recent study, we characterized in detail the expression of twelve receptors of such nutrition-associated factors in human blood monocytes and monocyte-derived macrophages [42].

A novel interesting player in the pathogenesis of the adipose tissue inflammation is a Wingless-type (WNT) signaling pathway contributing to the regulation of adipogenesis and macrophage infiltration in obese mice [43, 44]. WNT proteins are secreted glycoproteins with autocrine and paracrine action contributing to the regulation of cell proliferation and death, cell migration and embryonic development [45, 46]. One of the targets of the canonical WNT pathway, WNT-inducible signaling pathway protein-1 (WISP1, also known as CCN4), belongs to the Cyr61/CTGF/NOV (CCN) family of extracellular matrix proteins [47]. Several CCN family members, e.g. WISP2 and nephroblastoma overexpressed protein (NOV), contribute to the pathogenesis of obesity [43, 48-50], but nothing is known about the role of WISP1 in obesity. WISP1 expression was found in heart, pancreas, lung, kidney, small intestine, ovaries, spleen and brain. WISP1 regulates skeletal growth, bone repair [51], mesenchymal proliferation, osteoblastic and chondrogenic differentiation [52]. Anti-apoptotic effects of WISP1 through PI3K and Akt pathways were described in some tissues [53]. WISP1 is overexpressed in different types of cancer including invasive of cholangiocarcinoma [54]. Thus, WISP1 is an important regulator of apoptosis and autophagy in healthy state and in acute or chronic degenerative diseases [53]. Our research characterized WISP1 as a novel adipokine linking obesity to inflammation and insulin resistance in humans [55].

1.4. Day-time-dependent regulation of human metabolism

Circadian clock described in most of living organisms synchronizes energy intake and expenditure with the day/night cycle and controls major components of energy homeostasis [56]. The master clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus adjusts subordinate clocks in peripheral tissues and organs via nervous and humoral mechanisms [56, 57] **(Figure 3)**.

Two interlocked feedback loops are major parts of the molecular clock – (1) the main loop enclosing genes PER and CRY which are up-regulated by transcription factors CLOCK and BMAL1 and down-regulated by PER and CRY proteins and (2) an additional loop including BMAL1 and CLOCK activators RORα/RORγ and repressors REV-ERBα/REV-ERBβ [56]. This machinery orchestrates functions of numerous tissue-specific genes including clock-controlled transcription factors [56, 57]. A lot of components of carbohydrate and lipid homeostasis, detoxification pathways, and immune reactions (in total, 5-25% of the transcriptome and metabolome) are under the circadian control [58-60].

Both human studies in shift workers and animal models with genetic knockout of clock components elucidated the importance of circadian clocks in the regulation of metabolism [61-63]. Conversely, clock system is itself regulated by metabolic signals modulating circadian gene expression and behavior. Rodent studies showed that beside of light, food quantity and feeding time are dominant external stimuli entraining peripheral clocks [64, 65] **(Figure 3)**. In particular, a calorically dense high-fat diet (HFD) induces alterations of behavioral and molecular circadian rhythms in mice [60, 64, 66]. Many metabolic disturbances like obesity, T2DM, metabolic syndrome and cardiovascular disease are associated with alteration of clock oscillations [67-69]. In human adipose tissue, diurnal rhythms of gene expression are acutely changed by food intake or fasting [59]. As shown in mice, insulin represents one of major humoral signals contributing to the feeding-induced circadian entrainment in liver and adipose tissue [70]. However, little is known about the effect of food composition on circadian mechanisms in humans and its role in metabolic regulation.

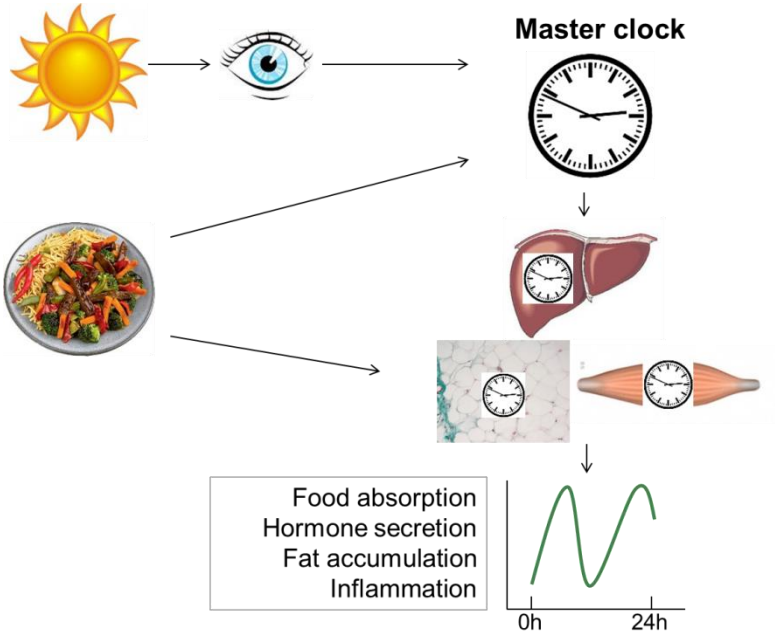

Figure 3. Mammalian central and peripheral clocks.
Circadian clock in mammals includes master clock in SCN of hypothalamus and peripheral clock. SCN rhythms are entrained by light which is absorbed through the retina and is transmitted to the SCN. The master clock then adjusts rhythms of peripheral oscillators via humoral factors or autonomic innervation. As a result, many physiological processes including food absorption, hormone secretion, fat accumulation and inflammation exhibit circadian oscillations. Caloric restriction and feeding time can also entrain circadian rhythms affecting peripheral clocks or the central clock in the SCN. Figure adapted from [57].

1.5. Objectives

Main objectives of selected research work were:

1) to examine the relationship between hepatic insulin clearance (HIC) and different components of metabolic syndrome and to test the hypothesis that HIC may predict the risk of metabolic syndrome;

2) to investigate whether insulin may acutely regulate the expression of natriuretic peptide (NP) receptors in the adipose tissue and in this way affect NP levels;

3) to characterize the expression of receptors to nutrition-associated factors (nutrient components, neuropeptides involved in the control of gastrointestinal functions, and gastrointestinal hormones) in human peripheral blood monocytes and monocyte-derived macrophages and to elucidate their functional significance in the regulation of the adipose tissue inflammation;

4) to elucidate a role of CCN family member WISP1 as a novel adipokine related to insulin resistance and adipose tissue inflammation and to clarify its functions in human adipocytes and macrophages;

5) to study whether isocaloric changes of food composition may entrain central and peripheral clock in humans and in this way contribute to the metabolic and inflammatory regulation.

2. Results of selected original papers

2.1. Hepatic insulin clearance is closely related to metabolic syndrome components

Original paper 1

Pivovarova O*, Bernigau W*, Bobbert T, Isken F, Möhlig M, Spranger J, Weickert MO, Osterhoff M, Pfeiffer AF, Rudovich N. Hepatic insulin clearance is closely related to metabolic syndrome components. *Diabetes Care*. 2013 Nov; 36(11):3779-85.

* shared first authorship

http://dx.doi.org/10.2337/dc12-1203

Insulin clearance is decreased in T2DM but mechanisms of this effect are unknown [10, 12-14]. Individuals with metabolic syndrome demonstrate hyperinsulinemia and an increased T2DM risk. In our study, we examined how HIC is associated with various components of metabolic syndrome and proved the hypothesis whether HIC predict the risk of metabolic syndrome [18]. For this, we studied nondiabetic individuals from the Metabolic Syndrome Berlin Brandenburg (MeSyBePo) study (800 subjects at the baseline and 189 subjects from the MeSyBePo recall study). An oral glucose tolerance test (OGTT) was performed in all study subjects and insulin secretion (insulin secretion rate [ISR]) was assessed using the two-compartment model of C-peptide kinetics [71]. The Harmonizing Criteria of the Metabolic Syndrome were used for the discrimination of metabolic syndrome [6]. Two indices of HIC were calculated - $HIC_{C\text{-}peptide}$ ($AUC_{C\text{-}peptide\ 0–120\ min}/AUC_{insulin\ 0–120\ min}$) and HIC_{ISR} ($AUC_{ISR\ 0–120\ min}/AUC_{insulin\ 0–120\ min}$) [13] where AUC is an incremental area under the curve.

At baseline, both HIC indices were lower in subjects with metabolic syndrome ($P < 0.001$) [18]. We found inverse relationships of HIC indices with waist circumference, triglycerides, diastolic blood pressure, fasting glucose, and insulin secretion index assessed in OGTT [72], and positive HIC correlation with OGTT-derived insulin sensitivity index (Gutt $ISI_{0,120}$) [73]. At the follow-up after 5.1 ± 0.9 years, 47 new cases of metabolic syndrome and 33 new cases of impaired glucose metabolism were detected. We also observed a trend towards an association of both HIC indices with elevated risk of metabolic syndrome (odds ratio 1.13 [95% CI 0.97–1.31], $P = 0.12$ and 1.38 [0.88–2.17], $P = 0.16$ for $HIC_{C\text{-}peptide}$ and

HIC_{ISR}, respectively) and impaired glucose metabolism (odds ratio 1.12 [0.92–1.36], *P* = 0.26 and 1.31 [0.74–2.33], *P* = 0.36 for $HIC_{C\text{-}peptide}$ and HIC_{ISR}, respectively) [18].

We concluded that HIC is associated with various components of metabolic syndrome, markers of insulin sensitivity and insulin secretion, and risk of metabolic syndrome [18]. Thus, HIC decrease may be a novel pathophysiological mechanism of the metabolic syndrome.

2.2. Insulin up-regulates natriuretic peptide clearance receptor expression in the subcutaneous fat depot in obese subjects

Original paper 2

Pivovarova O, Gögebakan Ö, Klöting N, Ernst A, Weickert MO, Haddad I, Nikiforova VJ, Bergmann A, Kruse M, Seltmann A, Blüher M, Pfeiffer AFH, Rudovich. N. Insulin up-regulates natriuretic peptide clearance receptor expression in the subcutaneous fat depot in obese subjects: a missing link between CVD risk and obesity? *J Clin Endocrinol Metab.* 2012; 97(5):E731-9.

http://dx.doi.org/10.1210/jc.2011-2839

Natriuretic peptide levels are decreased in obesity [26-28]. Central obesity is typically accompanied by a hyperinsulinemia induced by insulin resistance and resulting in a range of metabolic disturbances [4]. Here, we hypothesized that insulin may acutely regulate expression of NPRs in human adipose tissue [29]. To investigate this, we firstly measured mRNA levels of *NPRA, NPRB* and *NPRC* genes in paired samples of visceral (VAT) and subcutaneous (SAT) adipose tissue from 157 subjects (108 with T2DM) using quantitative real-time PCR (qRT-PCR). Expression of *NPRA* and *NPRC* was higher in VAT than in SAT ($p<0.01$). *NPRC* mRNA expression in VAT and SAT showed a strong correlation with fasting insulin levels ($r=0.65$, $p=0.04X10^{-3}$ and $r=0.54$, $p=0.002$, respectively). *NPRB* expression was lower in VAT than in SAT in subjects with T2DM and was lower compared with nondiabetic subjects [29].

Furthermore, the acute effect of insulin on *NPR* expression in SAT was studied in euglycemic-hyperinsulinemic and hyperglycemic-hyperinsulinemic clamp experiments in non-diabetic male subjects with moderate obesity (n=14) [29]. In the hyperinsulinemic-euglycemic clamp experiments, effects of high insulin concentrations in the presence of normal glucose concentration were studied, and in hyperinsulinemic-hyperglycemic clamp experiments, simultaneous effects of high insulin and high glucose concentrations were analyzed. *NPRC* mRNA expression was increased in both euglycemic- and hyperglycemic-hyperinsulinemic clamps (74.7%, $P = 0.038$; and 26.2%, $P = 0.048$, respectively). We additionally analyzed

plasma levels of MR-proANP, a stable N-terminal fragment of proANP [74]. In both clamp experiments, a significant reduction of MR-proANP by about 20% from basal values was observed. Similar results were received in the other cohort of the normotensive subjects with normal glucose tolerance underwent the hyperinsulinemic-euglycemic clamp (n=25) [29]. In this cohort, we observed correlations of fasting MR-proANP levels with age, diastolic blood pressure, and insulin sensitivity index ($r = 0.539$, $P = 0.005$; $r = -0.396$, $P = 0.049$; and $r = 0.410$, $P = 0.042$, respectively).

To address interactions between adipocytes and infiltrating macrophages in adipose tissue [75], we additionally conducted experiments in the culture of primary human macrophages and blood monocytes as macrophage precursor cells and examined the influence of insulin and glucose on the NPR expression. All three types of *NPR* were expressed in macrophages but only *NPRB* and *NPRC* expression was found in monocytes. Simultaneous insulin and glucose treatment increased *NPRC* expression in monocytes (70.2%, p=0.01), but not in mature macrophages [29].

We concluded that insulin can acutely increase NPRC expression in SAT independently of circulating glucose concentrations. Thus, an insulin-induced suppression of circulating NP via up-regulation of NPRC expression may be a novel link between hyperinsulinemia and obesity [29].

2.3. Novel regulators of adipose tissue inflammation

Obesity, T2DM, and associated metabolic diseases are characterized by the low-grade systemic inflammation which involves interplay of nutrition and monocyte/macrophage functions [31, 35]. Monocytes play a pivotal role in immune functions and metabolic regulation [75, 76]. They are able to respond to nutrient-related hormonal stimuli such as free fatty acids [77] and present macrophage precursor cells. Therefore, human primary blood monocytes and monocyte-derived macrophages were used in following two studies as an exciting model for the investigation of molecular mechanisms of the low-grade inflammation in adipose tissue.

Original paper 3

Pivovarova O, Hornemann S, Weimer S, Lu Y, Murahovschi V, Zhuk S, Seltmann A, Malashicheva A, Kostareva A, Kruse M, Busjahn A, Rudovich N, Pfeiffer AFH. Regulation of nutrition-associated receptors in blood monocytes of normal weight and obese humans. *Peptides.* 2015 Mar;65:12-19.

http://dx.doi.org/10.1016/j.peptides.2014.11.009

In this study [42], we suggested that some nutrition-associated factors may influence immune cell functions and in this way contribute to the pathogenesis of metabolic diseases. We therefore measured the mRNA expression of twelve nutrition-associated receptors in peripheral blood mononuclear cells (PBMC), isolated monocytes and monocyte-derived macrophages using qRT-PCR. The mRNA expression of receptors for short chain fatty acids (*GPR41, GPR43*), bile acids (*TGR5*), incretins (*GIPR, GLP1R*), cholecystokinin (*CCKAR*), neuropeptides VIP and PACAP (*VIPR1, VIPR2*), and neurotensin (*NTSR1*) was detected in PBMC and monocytes, while *GPR41, GPR43, GIPR, TGR5*, and *VIPR1* were found in macrophages [42].

We also compared receptor expression patterns in two polarized subtypes of monocyte-derived macrophages, GM and M macrophages, which demonstrate characteristics similar to M1 and M2 adipose tissue macrophages, respectively [78-80]. We

found higher *GPR43* and lower *TGR5* and *VIPR1* expression in M macrophages compared with GM macrophages. During the *in vitro* differentiation, expression levels of all studied receptors decreased dramatically compared to the monocyte expression already at day 1 of differentiation in both macrophage subtypes [42]. After the lipopolysaccharide (LPS) treatment, we found an up-regulation of *GIPR, GPR41, GPR43,* and *VIPR1* in monocytes and/or macrophages [42]. In most experiments, these effects were observed already by the lowest LPS concentration employed (1 ng/ml).

Furthermore, we analysed receptor expression in monocytes of thirty non obese individuals with normal glucose tolerance. Receptor expression in monocytes correlated with a range of metabolic and inflammatory markers such as body fat content, fasting glucose, total cholesterol, leucocyte count, and serum IL6 levels ($p<0.05$) [42]. The dietary switch from the high-carbohydrate low-fat diet to the low-carbohydrate high-fat isocaloric diet induced the increase of *GPR43* and *VIPR1* expression in monocytes by 28% ($p=0.030$) and 12% ($p=0.041$), respectively. However, no significant differences of receptor expression between normal weight and moderately obese subjects were found (n=16) [42].

In conclusion, our study characterized for the first time expression patterns of nutrition-associated receptors in primary human monocytes and macrophages and elucidated possible links between metabolic responses and immune functions in pathogenesis of the adipose tissue inflammation.

Original paper 4

Murahovschi V*, **Pivovarova O***, Ilkavets I, Dmitrieva RM, Döcke S, Keyhani-Nejad F, Gögebakan O, Osterhoff M, Kemper M, Hornemann S, Markova M, Klöting N, Stockmann M, Weickert MO, Lamounier-Zepter V, Neuhaus P, Konradi A, Dooley S, von Loeffelholz C, Blüher M, Pfeiffer AF, Rudovich N. WISP1 is a novel adipokine linked to inflammation in obesity. *Diabetes.* 2015 Mar;64(3):856-66.

* shared first authorship

http://dx.doi.org/10.2337/db14-0444

WISP1, a target gene of the WNT signaling pathway, is a member of the CCN family of secreted extracellular matrix-associated proteins [47]. Other CCN family members are shown to be closely linked to adipogenesis [43, 48, 50], but no data existed about the role of WISP1/CCN4 in the pathogenesis of obesity and associated diseases. In our study, we validated WISP1 as a novel adipokine [55].

We found that *WISP1* mRNA expression and WISP1 protein secretion in the culture medium were up-regulated during the *ex vivo* differentiation of human adipocytes from mesenchymal stem cells [55]. Further, we investigated the *WISP1* mRNA expression in human paired SAT and VAT samples from lean and overweight subjects with normal glucose tolerance (n=75). *WISP1* was highly expressed in VAT and moderately expressed in SAT. We observed negative correlations of *WISP1* mRNA levels with insulin sensitivity and blood adiponectin levels and positive correlations with fasting insulin, macrophage infiltration in SAT and VAT and visceral fat content ($p<0.05$) [55].

In the macrophage culture, but not in adipocytes, we found that WISP1 induces the dose-dependent increase of IL6, TNFA, IL1B, and IL10 mRNA expression and secretion in the culture medium [55]. Furthermore, the WISP1 treatment (500 ng/ml for 24h) increased the expression of M1 markers *CCR7* and *COX2* and suppressed the expression of antiinflammatory M2 markers which suggests the modulation of the macrophage polarization towards proinflammatory M1 phenotype [55].

After the weight loss (at least 8% after 8 weeks of the hypocaloric diet), *WISP1* mRNA expression in SAT (n=49) decreased in whole cohort, and circulating WISP1 levels

were reduced only in female participants, but not in males [55]. In mice, *WISP1* expression was up-regulated in the epididymal fat tissue, liver and muscle after 6 weeks of HFD (n=7). In patients with nonalcoholic fatty liver disease, no associations between disease activity score, liver fat content, and hepatic *WISP1* mRNA expression were found (n=47) [55].

Insulin (100nM for 4 h) increased *WISP1* expression in human adipocytes *in vitro*. However, no acute insulin effect on *WISP1* expression in SAT were found in overweight subjects in hyperinsulinemic clamp experiments (n=14) [55].

Our data suggest that WISP1 may contribute to the link between obesity, inflammation and insulin resistance and could be a perspective molecular target for obesity treatment.

2.4. Changes of dietary fat and carbohydrate content alter central and peripheral clock in humans

Original paper 5

Pivovarova O*, Jürchott K*, Rudovich N*, Hornemann S, Lu Y, Möckel S, Murahovschi V, Kessler K, Seltmann A-C, Maser-Gluth C, Mazuch J, Kruse M, Busjahn A, Kramer A, Pfeiffer AFH. Changes of dietary fat and carbohydrate content alter central and peripheral clock in humans. *J Clin Endocrinol Metab*. 2015 Jun;100(6):2291-302.

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The circadian clock coordinates numerous metabolic processes to adapt physiological responses to light-dark and feeding regimens and is itself regulated by metabolic cues. However, it is unknown whether alterations of food composition influence circadian rhythms in humans.

We examined whether an isocaloric change of the food composition affects central and peripheral circadian clock in humans [81]. For this, we performed detailed analysis of diurnal oscillations of salivary cortisol and of the gene expression in human blood monocytes as a minimally invasive method of monitoring human clock. Because monocyte samples were available only at three time points during the investigation day (at 8.00 am, 11.15 am, and 3.45 pm), we established the mathematical method of the prediction of the 24h diurnal rhythms based only on the three-point-sampling. We fitted data of all donors together to the sinus models, allowing the estimation of averaged values for mesor, amplitude and peak time [81]. Moreover, we additionally evaluated our method using bootstrapping and simulation approaches and the comparison with frequently sampled microarray data set [82]. Using the procedure of diurnal rhythm prediction by three-time-point data, we described diurnal oscillations of core clock genes and metabolic and inflammatory genes in monocytes of twenty-nine non obese healthy individuals before and after the switching from high-carbohydrate low-fat to low-carbohydrate high-fat isocaloric diet [81].

Salivary cortisol rhythm used for the assessment of the central clock function showed a phase delay and an amplitude increase one and six weeks after the dietary switch. The change of the food composition also affected diurnal oscillations of peripheral clock genes (*PER1, PER2, PER3* and *TEF*) and inflammatory genes (*CD14, CD180*, *NFKBIA*, and *IL1B*) in blood monocytes [81]. Particularly, we found that the mesor and amplitude of *PER1, PER2,* and *PER3* oscillations increase after the dietary switch without any shifts of acrophase. The dietary switch also affected the expression of non-oscillating fat oxidation genes *ACOX3* (acyl-CoA oxidase 3) and *IDH3A* (isocitrate dehydrogenase 3 alpha) and energy metabolism gene *SIRT1* [81]. Clock gene expression in monocytes, but not salivary cortisol levels, tightly correlated with blood lipid levels (total and LDL cholesterol, and triglycerides) and with expression of metabolic and inflammatory genes [81].

We concluded that isocaloric changes of the dietary fat and carbohydrate content modulate the function of the central and peripheral circadian clocks in humans [81].

3. Discussion

3.1. Role of decreased insulin clearance in the pathophysiology of metabolic syndrome

Decreased HIC is found in T2DM, metabolic syndrome and associated diseases and can be considered as an early phenotypical marker of insulin metabolism disturbances [10, 12-14]. In our study [18], we found an association of two HIC indices derived in OGTT with different components of metabolic syndrome, insulin secretion and insulin sensitivity markers, and a trend towards possible association of HIC with an increased risk of metabolic syndrome. In obesity, a first step in the development of insulin resistance is obviously the disturbance of hepatic insulin metabolism [83]. In opposite, HIC increases upon the weight loss in both humans [83] and animals [84]. In our study, we found lower HIC in glucose tolerant subjects with metabolic syndrome compared with subjects without metabolic syndrome [18]. This points out that the alteration of insulin clearance may precede the manifestation of glucose metabolism disturbances. In our hyperinsulinemic-euglycemic clamp experiments published previously [15], we showed a strong correlation of the OGTT-derived HIC with metabolic insulin clearance. Therefore, HIC assessment may be useful for the early recognition of subjects with high risk of metabolic syndrome showing no other signs of impaired glucose metabolism.

Decreased HIC may enhance insulin resistance due to the long-term increase of blood insulin concentrations in fasting and postprandial state [17, 85, 86]. Our finding that insulin secretion negatively correlates with HIC is in accordance with previously published data [87, 88] and may be a physiological mechanism of the HIC regulation by insulin secretion. In metabolic syndrome, decreased HIC rather represents an additional element of insulin metabolism disturbance dependent on alterations of insulin secretion, but not the compensatory response to the decreased insulin sensitivity. The HIC decrease may also be involved in the effects diets stimulating insulin secretion (e.g. diets with a high glycemic index or high consumption of soft drinks) [89]. Nevertheless, we cannot exclude that HIC is not one

of determinant components of metabolic syndrome and only cluster with metabolic syndrome [18].

Changes of insulin degradation in humans obviously have complex underlying mechanisms which are not elucidated in detail [85]. ~75% insulin is removed from blood during the first passage through the portal vein, therefore, liver thought to be the main organ contributing to the insulin clearance [85, 88]. The major enzyme responsible for insulin degradation is the insulin-degrading enzyme (IDE) [85]. Insulin clearance is a highly heritable trait [11], and genetic variations within IDE locus are associated with elevated T2DM risk and diminished HIC in subjects without diabetes [15]. We showed previously that hyperglycemia inhibits the insulin-induced activation of the IDE in HepG2 hepatoma cells [16] and this may by a mechanism of the decrease of IDE activity in T2DM [85].

The tight correlation of HIC with HDL cholesterol, a marker of liver fat metabolism, was found [18]. Kotronen et al. observed the negative HIC correlation with fat content and glucose production in liver of subjects with and without diabetes [14]. To summarize, decreased HIC is probably the earliest marker of hepatic insulin resistance and is directly linked to insulin effects on glucose and lipid metabolism in the liver. Thus, HIC decrease may be a novel mechanism of the metabolic syndrome pathophysiology and could be additionally applied for early identification of subjects with high risk of metabolic syndrome [18].

3.2. Natriuretic peptides as a link between cardiovascular and metabolic functions

NPs play an important role in cardiovascular homeostasis and contribute to the regulation of metabolic functions [24]. Central obesity associated with insulin resistance is characterized by decreased circulating levels of NP or "natriuretic handicap" [26-28]. Our study demonstrated that insulin acutely increases expression of NPRC in human SAT independently of blood glucose levels [29]. Expression of NPR in fat depots was studied previously in animals, but not in humans [30]. In primates but not in rodents, the *NPRA* to *NPRC* expression ratio in adipose tissue affect the ANP stimulated lipolysis [22]. High-fat diet associated with hyperinsulinemia induces the increase of the expression of all three NPR

types in the white and brown adipose tissue of mice and rats [90, 91]. Obese subjects with hypertension exhibit elevated levels of *NPRC* expression in SAT as shown in the first human observation [28]. Oppositely, moderate weight loss in humans induces the decrease of the *NPRA* expression in SAT [92]. Furthermore, insulin-deficient mice showed the strong reduction of the *NPRC* expression [30]. In human VAT, *NPRA* and *NPRC* expression levels are higher than in SAT suggesting that VAT is the target organ for NP effects. Interestingly, inhibitory effect of ANP on the proliferation of visceral adipocytes *in vitro* was found [93]. Mice models with increased ANP half-life [23] or elevated levels of the other NPRA ligand, BNP [90], demonstrate a lean phenotype. Notably, ANP induces lipolysis in SAT via the pathway which is not directly affected by insulin coapplication [94]. Obviously, ANP acts as a physiological antagonist of insulin action in the adipose tissue [29].

The phenomenon of “natriuretic handicap” in obesity was hypothesized by Dessì-Fulgheri *et al.* [28] who observed the inverse relationship between ANP levels in circulation and elevated *NPRC* expression in adipose tissue of hypertensive obese patients. In subjects without manifest cardiovascular disease, the decline of cardioprotective hormones ANP and BNP in circulation may elevate a long-term risk of cardiovascular disease similarly to the metabolic syndrome and central obesity [19-21]. In our study, we found the strong correlation of the *NPRC* expression in VAT and SAT with fasting insulin levels, which was independent of other anthropometric parameters or glycemic control, pointing out that insulin may directly regulate *NPRC* expression in adipose tissue [29]. In our clamp experiments performed in obese subjects, an acute insulin infusion increased *NPRC* expression in SAT independent of glucose concentration which supports this hypothesis [29]. Nakatsuji et al. showed *in vitro* that insulin can increase *NPRC* expression due to the activation of the phosphatidylinositol phosphate-3 kinase pathway [30], and the same pathway is involved in the inhibition of adipocyte lipolysis. The *NPRC* up-regulation by insulin could accelerate the ANP removal from circulation which contributes to the opposed effects of ANP and insulin in the regulation of lipolysis [95]. In a like manner, the sodium-retaining actions of insulin counteracts with natriuretc effect of ANP in hyperinsulinemic state [94].

Our additional experiments in the culture of primary human monocytes and macrophages revealed that simultaneous insulin and glucose treatment increases the *NPRC* expression in monocytes, but not in mature macrophages [29]. Hence, the *NPRC* increase in SAT observed in our *in vivo* experiments arises most likely from adipocytes or adipocyte-macrophage interactions but not from adipose tissue macrophages alone [29].

To conclude, acute insulin stimulation increases the *NPRC* expression in SAT and down-regulates MR-proANP levels in circulation independent of blood glucose concentrations. Therefore, circulating levels of NPs in obesity may be suppressed by insulin via increase of *NPRC* expression. Thus, our study revealed a novel direct link between hyperinsulinemia and cardiovascular components of the metabolic syndrome [29].

3.3. Nutrition-dependent mechanisms of adipose tissue inflammation

Mechanisms of the low-grade inflammation in adipose tissue associated with several metabolic diseases are not fully understood. In our study [42], we hypothesized that some nutrition-associated factors may influence immune cell functions in humans.

We demonstrated the mRNA expression of receptors for short chain fatty acids, bile acids, incretins, cholecystokinin, neuropeptides VIP and PACAP, and neurotensin in PBMC and monocytes [42]. Moreover, we provided the first evidence that *GIPR, GPR41,* and *GPR43* mRNA are expressed in primary human macrophages. Thus, we extended the expression profiling of these receptors previously described in other types of human immune cells or rodent macrophages [96-98]. In humans, short chain fatty acid receptors *GPR41* and *GPR43* were shown to be expressed in PBMC, dendritic cells, lymphocytes, monocytes, and neutrophiles [99-101]. *GIPR* mRNA was described previously only in the human U937 monocyte cell line [102]. In opposite to literature data, we detected no *FXR* mRNA in any of studied cell types [103, 104].

Interestingly, during the macrophage differentiation, the mRNA expression of all studied receptors was dramatically down-regulated and was very low in mature

macrophages [42]. This may point to a more important role of these receptors in circulating monocytes rather than in mature cells.

To mimic the systemic inflammation state, we also studied receptor expression in monocytes and macrophages after the 24h LPS treatment [42]. Bacterial endotoxin LPS is an effective activator of an immune response, macrophage migration and maturation *in vitro* and *in vivo* [105, 106]. In human serum, bacterial endotoxin activity is associated with the components of the metabolic syndrome - dyslipidemia, insulin resistance, obesity, and chronic inflammation [107]. An increase of plasma endotoxin concentration was recently showed in the postprandial state in humans [108].

In our study, the LPS treatment in monocytes and macrophages induced an increase of *GIPR, GPR41, GPR43,* and *VIPR1* expression observed mainly already by the lowest LPS concentration employed [42]. Therefore, a potential physiological role of these receptors in monocytes and in tissue resident macrophages during systemic inflammation can be assumed. Particularly, the up-regulation of *GPR41* and *GPR43* by LPS may enhance the antiinflammatory effects of short chain fatty acids [99] in the postprandial state, representing an unknown compensatory self-regulating mechanism.

Furthermore, we observed a range of relationships of receptor expression in monocytes with metabolic and inflammatory markers. In particular, the *GPR43* expression was associated with body fat content and serum IL6 level [42]. GPR43 is activated by short-chain fatty acids which are produced by microbial fermentation of dietary fiber in the gut. GPR43 is involved in the regulation of host energy homeostasis in the gastrointestinal tract and adipose tissues [109] and in the regulation of inflammatory reactions by short chain fatty acids [99, 110]. In our study, we provided the evidence that the isocaloric change of food composition can change the *GPR43* expression in blood monocytes and in this way may affect the inflammatory reactions [42].

We also found the correlation of the *VIPR1* expression with fasting glucose, total and LDL cholesterol and an increase of *VIPR1* mRNA levels after the switch to the isocaloric HFD [42]. VIPR1 agonists showed antihyperglycemic, antioxidant and antiinflammatory

effects in streptozotocin-induced diabetic mice [111] and prevented elevations in body weight, plasma glucose, cholesterol and triglycerides in the model of HFD-induced obesity [112]. Interestingly, *VIPR1* expression in monocytes was increased 6h and 24 h after the LPS injection in humans [113]. Therefore, the dietary-induced *VIPR1* increase found in our study could be associated with low-grade endotoxemia induced by the consumption of high-fat meals [108].

In conclusion, our data suggest that nutrition-associated receptors and corresponding signaling pathways may play an important role in the regulation of low-grade systemic inflammation [42]. Further experiments are needed to elucidate the functional significance of these receptors in monocytes and macrophages in humans and their contribution to the pathophysiology of metabolic diseases.

In the other study [55], we characterized for the first time WISP1, a CCN family member, as a novel adipokine which may be involved in the regulation of adipose tissue inflammation via the stimulation of cytokine response in macrophages.

We found that *WISP1* mRNA expression and WISP1 protein secretion are up-regulated during the human adipocyte differentiation [55]. In opposite to WISP2, another CCN family member [49], *WISP1* expression in VAT is higher than in SAT. Based on the found *WISP1* correlations with insulin sensitivity, adiponectin levels and visceral fat content, WISP1 could be a used as a marker of insulin resistance and visceral fat accumulation [55]. Additionally, a positive correlation of *WISP1* expression with macrophage number in both SAT and VAT was observed [55].

Moreover, WISP1 treatment induced a dose-dependent increase of proinflammatory cytokine production in human differentiated macrophages, but not in in adipocytes [55]. Interestingly, macrophage polarization was shifted by WISP1 stimulation towards proinflammatory M1 phenotype [55]. In murine RAW 264.7 macrophages, WISP1 induced a dose-dependent increase of the mRNA expression of extracellular matrix degrading enzyme [114]. We hypothesize that WISP1 secreted by adipocytes may hereby regulate function and

migration of macrophages in adipose tissue. In consideration of the association with insulin sensitivity, WISP1 may be characterized as an adipokine involved in the control of macrophage function [55].

After the weight loss, *WISP1* mRNA expression in SAT and circulating WISP1 levels were reduced in female subjects [55]. Hepatic *WISP1* expression was not up-regulated in the subjects with nonalcoholic fatty liver disease [55]. Furthermore, no correlation links between hepatic *WISP1* expression and biochemical and anthropometrical obesity markers were found pointing out that WISP1 apparently does not contribute to the fat accumulation in liver [55]. Obviously, alterations of circulating WISP1 levels induced by the weight loss originate from adipose tissue rather than from the liver or other organs. Interestingly, an association of NOV, another CCN family member, with obesity was described, and its plasma levels were reduced by the weight loss after the bariatric surgery [48]. The weight loss induced decrease of CCN proteins in circulation may be explained by down-regulation of the WNT signalling pathway in muscle and adipose tissue. Further, WISP1 up-regulation is associated with different cancer types [115]. Central obesity and metabolic syndrome are independent risk factors for cancer [20], and therefore WISP1 could be also used as a marker of cancer risk in obesity, but this speculation needs further investigation.

Insulin controls numerous aspects of adipocyte differentiation and function [116]. Experiments in murine preadipocytes showed that insulin and WNT signalling pathways can interact at multiple levels [117]. The PI3K/Akt pathway mediates both antiapoptotic and proliferative WISP1 effects [53], therefore we hypothesized that WISP1 affects insulin pathway. However, we did not found WISP1 effects on the insulin signaling *in vitro*; conversely, activation of insulin signalling augmented *WISP1* expression in human adipocytes [55]. Nevertheless, *in vivo*, *WISP1* expression in SAT was not affected by short hyperinsulinemia during clamp experiments [55]. Possibly, chronic insulin exposure is needed to induce significant changes of the WISP1 expression *in vivo*.

To conclude, our study characterized WISP1 as a novel adipokine with high expression in VAT from obese humans which is associated with insulin resistance and

adipose tissue inflammation. WISP1 expression in adipose tissue and circulating WISP1 levels are regulated by weight changes. We therefore suggest that WISP1 represents a novel link between inflammation and obesity and could be a perspective molecular target for obesity treatment [55].

3.4. Circadian mechanisms in human metabolic responses to food intake

Effects of food and feeding regimens on circadian clock are intensively studied in rodents, but not in humans. In our study [81], we performed analysis of diurnal oscillations of salivary cortisol and of the gene expression in blood monocytes and their response to the change of food composition in humans.

For this, we used the procedure of diurnal rhythm prediction by three-time-point data [81] similarly to approaches described in recently published human chronobiological studies [63, 118]. Bootstrapping and simulation approaches used for additional evaluation of our method confirmed that the procedure allows analysing rhythmic parameters of clock oscillations with a reasonable accuracy and a good amplitude-to-noise ratio. Our method can be used for studying human circadian clock in outpatient departments and other conditions when the 24h frequent blood sampling is technically not feasible.

Using this method we showed for the first time that the alteration of the dietary fat and carbohydrate content affects central and peripheral circadian clock in humans [81]. In animals, circadian rhythms of clock genes and metabolic genes and eating behaviour are showed to be changed upon the feeding with a hypercaloric HFD [60, 64, 66, 119]. Notably, important clock components, genes *Period* and *TEF*, were affected by dietary switch in our study which confirms their implication in the metabolic regulation described previously in mice [120, 121]. Per2-/- mice developed significant obesity on a hypercaloric HFD [62]. In human adipose tissue, *Period* expression levels correlate with waist circumference, blood cholesterol levels [122], expression of metabolic genes and cytokines [59], and this is in agreement with our findings in human monocytes.

In our study, we also demonstrated diurnal oscillations of key fat metabolism genes in human monocytes. In the afternoon, the expression of fatty acid synthase (*FASN*), the key enzyme of fatty acid biosynthesis, was up-regulated and the expression of carnitine palmitoyl transferase 1 (*CPT1A*), the rate limiting enzyme of mitochondrial fat oxidation, was decreased [81]. Obviously, these changes of gene expression reflect diurnal variations of the fat oxidation and fat synthesis previously characterized in rodents and probably also existing in humans [123]. Other components of fat metabolism, e.g. intestinal lipid transport, *de novo* lipid synthesis and adipokine secretion, are also regulated by circadian clock [124-126]. Moreover, expression levels of non-oscillating fat metabolism genes were also altered by the dietary switch. We found down-regulation of the *ACOX3* expression and up-regulation of *IDH3A* expression involved in peroxisomal and mitochondrial fatty acid oxidation, respectively. Numerous connections of clock genes with fat oxidation genes *IDH3A* and *CPT1A* were additionally confirmed in our network analysis.

Further, in human monocytes, we observed diurnal variations of genes involved in the LPS-triggered response (*CD14, CD180, NFKBIA*, and *IL1B*) which were showed previously in murine macrophages [58]. Both immune cell number and function including cytokine expression, phagocytosis and lytic activity, are under the clock control [58, 127] and are affected by circadian disruption [128]. Indeed, we observed remarkable relationships of clock genes with cytokines in the correlation analysis. Moreover, we showed that the change of the food composition alters diurnal oscillations of inflammatory genes in monocytes [81]. Even in healthy subjects, fatty meal consumption is known to induce the postprandial low-grade inflammatory response [108]. This can lead to the dysregulation of peripheral circadian rhythms, because systemic inflammation can reset the peripheral circadian clock [129, 130]. However, whether dietary switch to HFD firstly affect clock functions and in this way indirectly change metabolic and inflammatory pathways in monocytes, or vice versa, is not clarified.

Murine metabolome and transcriptome studies demonstrated that the HFD consumption lead to the profound reorganisation of metabolic pathways including the disruption of normal circadian cycles and genesis of *de novo* oscillating transcripts [60]. This reprogramming is

mediated by the impairment of CLOCK:BMAL1 chromatin recruitment and cyclic activation of surrogate pathways via transcription factors PPARɣ, SREBP-1, CREB1, and SRF [60]. Changes of the activity of NAMPT/SIRT1 pathway, PPARα/PPARγ [131, 132], AMPK, and redox state [133-135] are also involved in disruption circadian rhythmicity by HFD.

In conclusion, we demonstrated that the dietary fat and carbohydrate content alters diurnal rhythms of central and peripheral clock and inflammatory genes in humans. Our data confirm the tight relationship between molecular clock and metabolic and inflammatory pathways involved in adaptation of energy metabolism to changes of food composition [81]. Further studies are needed to investigate exact mechanisms of the effects of food composition and the role of clock genes in metabolic and immune responses to nutritional signals in humans.

4. Summary

Many important pathophysiological aspects of metabolic disturbances remain poorly understood. The present work elucidated following mechanisms of metabolic regulation in humans:

1) Hepatic insulin clearance is associated with several components of metabolic syndrome and markers of insulin secretion and insulin sensitivity. Decreased insulin clearance may be a novel mechanism of the metabolic syndrome pathophysiology and could be additionally applied for early identification of subjects with high risk of metabolic syndrome.

2) Insulin increases expression of natriuretic peptide clearance receptor (NPRC) in subcutaneous adipose tissue and decreases circulating levels of MR-proANP independently of circulating glucose concentrations. Thus, insulin may reduce natriuretic peptide levels in circulation via up-regulation of NPRC expression providing a new link between hyperinsulinemia and obesity.

3) Novel mechanisms contributing to the progression of the adipose tissue inflammation were characterized. Firstly, human monocytes and macrophages were found to express a panel of nutrition-associated receptors suggesting that an activation of corresponding signaling may regulate the low-grade inflammation in adipose tissue. Secondly, WISP1 was validated as a novel human adipokine which is released by adipocytes and stimulates cytokine responses in macrophages.

4) Dietary fat and carbohydrate content alters diurnal rhythms of central and peripheral clock in humans. The tight crosstalk between clock genes and metabolic and inflammatory pathways demonstrated for the first time the important role of molecular clock in metabolic adaptations to food composition changes in humans.

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7. Declaration

Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,

- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,

- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

…… ……..

Datum Unterschrift