CHAPTER 1

GENERAL INTRODUCTION
AND OUTLINE OF THE THESIS
The term diabetes mellitus, which roughly translates to excessive sweet urine, represents metabolic disorders of multiple aetiologies characterized by chronic elevated blood glucose levels. Diabetes is caused by defects in insulin secretion, insulin action, or both that result in disturbance of carbohydrate, fat and protein metabolism. Based on aetiology and pathology of diabetes, four different subgroups of diabetes can be distinguished. However, most cases fall into one of the two major subgroups, i.e. type 1 and type 2 diabetes. Type 1 diabetes, accounting for approximately 5%-10% of diabetic patients, is an autoimmune disease in which insulin production is lost due to the destruction of β-cells. In contrast, type 2 diabetes, accounting for approximately 90% of diabetic patients, is predominantly caused by peripheral insulin resistance, defined as a state of reduced responsiveness to circulating concentrations of insulin. Type 2 diabetes is most commonly caused by excess nutrition and lack of physical exercise, but also develops in elderly people of normal constitution. Diabetes mellitus is becoming a pandemic because of changes in lifestyle, population aging, growth and urbanization. According to the World Health Organization (WHO), at present more than 220 million people worldwide have diabetes mellitus. It is estimated that diabetes mellitus caused deaths will double until 2030, making diabetes a leading cause of global death by disease. Diabetes may present with characteristic symptoms such as thirst, polyuria, and blurring of vision. In type 2 diabetes however, symptoms are often not severe, or may be absent, and consequently hyperglycemia may be present for a long time before the diagnosis. Diabetes might also cause acute laps of blood glucose levels, i.e. ketoacidosis or a non-ketotic hyperosmolar state, leading to stupor, coma and, in absence of effective treatment, death. With the use of insulin and its analogues these acute complications of diabetes became preventable and instead, long-term complications of diabetes came to the fore. Chronic hyperglycemia leads to long-term damage of the entire vascular system thereby inducing dysfunction and failure of various organs. Generally, the injurious effects of diabetes are separated into those affecting macrovessels of the body and those damaging the microvasculature. Diabetic damage of macrovessels, represented by ateriosclerotic lesions, provokes stroke and heart attack and thereby causes death of nearly 50% of the diabetic patients. Among the microvascular complications of diabetes, diabetic retinopathy, neuropathy and nephropathy are the most common ones. Of importance, diabetic retinopathy is an important cause of blindness world wide and diabetic nephropathy (DN) is the leading causes of end stage renal disease (ERSD) and kidney failure. Since the number of patients with diabetes, especially with type 2 diabetes, has increased dramatically over the years, the
number of patients with long-term complications, such as DN, has increased in parallel. In fact, the prevalence of DN is expected to double within the next 10 years (1). It needs to be emphasized that the improved survival of diabetic patients, mainly due to substantial progress in cardiovascular therapy and better accessibility of renal replacement therapy, further contributes to the rapid increase in the prevalence of DN. The average progression rate to DN is approximately 2.5% per year, meaning that after 10 years 25% of the diabetic patients suffer from DN. Once DN is overt, 75% of the type 1 diabetics and 20% of the type 2 diabetics develop end stage renal disease (ESRD) with need for dialysis within 20 years. Therefore, DN is the most common cause for ESRD and is recognized as an urgent medical problem of world wide dimensions (2). The fact that, 50% of the patients on dialysis also have diabetes mellitus, further illustrates the severity of the problem. Moreover, mortality among dialysis patients with diabetes is 22% higher in the first year following the initiation of dialysis and 15% higher at 5 years than that among dialysis patients without diabetes (3). Given that health care costs caused by DN are already alarming and will continue to increase, there is an unmet demand for diabetes prevention. Currently, clinical practice lacks powerful diagnostic and predictive molecular approaches for accurate assessment of the risk for DN in individuals diagnosed with diabetes. Apart from that, the available therapies only slow-down the rate of progression, but do not arrest or reverse DN (4, 5).

**FUNCTIONAL CHANGES IN DIABETIC NEPHROPATHY**

The evolution of DN follows a typical time course and is similar in type 1 and type 2 diabetes. After the onset of diabetes, the kidneys start to become hypertrophic, as might be diagnosed on ultrasound evaluation, and the glomerular filtration rate initially starts to rise. At this stage the patient is still normoalbuminuric, although reversible albuminuria might appear under certain circumstances like fever, physical exercise or metabolic blunder. The next 5 to 15 years, a silent phase without novel clinical signs follows. Functional alteration of the kidney might only become apparent using specific tests, yet biopsy specimens already reveal structural changes at this stage of the disease. Next, microalbuminuria, defined as 30 to 300 mg albumin/g creatinine, develops. Although microalbuminuria virtually characterises an advanced stage of DN, it is the earliest diagnostic marker available. A frequent latency of several years between onset of diabetes and its diagnosis is responsible for the finding that as many as 7% of patients with type 2 diabetes already have microalbuminuria at the time they are diagnosed with diabetes (6-8). Without treatment, macroalbuminuria (>300 mg albumin/g creatinine) develops in 80% of the patients after 10 to 15 years. Approximately half of the
macroalbuminuric patients will develop a gradual decline in GFR, become proteinuric and end up with endstage renal disease within another 5 years. Interestingly, cerebro- and cardiovascular complications develop more frequently in diabetics with DN compared to those without, accounting for the excessively elevated morbidity and mortality in these patients. Thus, microalbuminuria can be considered as an early marker not only for kidney damage but also for damage in the cardiovascular system (9-11).

HISTOPATHOLOGY OF DIABETIC NEPHROPATHY

The glomerular lesions characteristic for diabetic nephropathy (DN) were first described in 1936 by the British physician Clifford Wilson and the German-born American physician Paul Kimmelstiel (12). Histologically, three major changes occur in the glomeruli, which are strongly related with the clinical manifestation of DN: diffuse mesangial expansion, GBM thickening and progressive glomerular scarring (Fig. 1).

Fig. 1. Histopathology of diabetic nephropathy. This picture shows a PAS stained hypertrophic glomerulus with mesangial expansion and GBM thickening forming typical PAS positive nodula as first described by Kimmelstiel and Wilson. Magnification 200x. From: The Internet Pathology Laboratory for Medical Education, Florida State University College of Medicine. http://library.med.utah.edu/WebPath/RENAHTML/RENAL028.html
In addition, the glomeruli appear hypertrophic with typical hyaline arteriosclerosis of afferent and efferent arterioles. As revealed by electron microscopy, podocytes are key targets for glomerular injury at the onset of DN (13-16). The so called diabetic podocytopathy is characterized by podocyte foot process effacement and a gradual decline of podocytes number per glomerulus (14). Podocytes loss from the glomerulus occurs either by apoptosis or by cell detachment from the glomerular basement membrane (17). As a result, the denuded GBM adheres to Bowman’s capsule, initiating the development of glomerulosclerosis. Loss of Podocytes is highly predictive for both progressive glomerular injury and long-term albumin excretion in diabetic patients (18). Thus, podocyte pathology seems to precede the development of DN and determines at least in part kidney function in diabetic patients. Apart from the glomerular lesions, the diabetic kidney is characterised by tubulo-interstitial sclerosis, usually represented by interstitial fibrosis and tubular atrophy (IFTA).

**BIOCHEMICAL CHANGES IN THE DIABETIC KIDNEY**

The general mechanism by which chronic hyperglycemia causes tissue injury is still elusive. Because glucose uptake is not restricted in cells that have non-insulin sensitive glucose transporters on their surface, e.g. endothelial cells, mesangial cells and Schwann cells, these cells are in particular vulnerable to damage by chronic hyperglycemia (19). The increased supply of glucose will consequently lead to an increased glucose metabolism and hence, increases in electron donors (NADH and FADH2) are generated. As more electrons are donated into the intra mitochondrial electron transport chain, the inner membrane voltage gradient increases until electrons start to leak and react with molecular oxygen, resulting in the production of so called reactive oxygen species (ROS). Superoxide, one of the important ROS species, inhibits a key enzyme of glycolysis, i.e. glyceraldehyde-3 phosphate dehydrogenase (GAPDH). Consequently, accumulation of the upstream metabolites occurs, disturbing the normal cell metabolism. One of these metabolites, glyceraldehyde-3-phosphate, is degraded into the advanced glycation end product (AGE) precursor methylglyoxal. Subsequently, intracellular AGEs emerge by a non-enzymatic and ROS promoted reaction between methylglyoxal and certain proteins. AGEs can directly damage the cell by modification of intracellular proteins, such as transcription factors, or indirectly by modifications of matrix molecules. Because the extra-cellular matrix is an important signalling interface for cells, the latter may severely alter normal cell behaviour. AGEs can activate specific cell surface receptors that are linked to inflammation. Through the production of cytokines or growth factors this can further perpetuate cell damage in an auto-
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and paracrine manner. Glyceraldehyde-3-phosphate can also be metabolized to diacylglycerol, an intracellular signalling molecule that activates protein kinase-C (β, δ, and α), thereby possibly affecting gene expression, e.g. transforming growth factor-β (TGF-β) and plasminogen activator inhibitor-1 (PAI-1).

Other metabolites of glycolysis, like fructose-6 phosphate, can also accumulate in the diabetic cells. Fructose-6 phosphate increases the flux through the nutrition sensitizing hexosamine pathway, in which it is converted to glucosamine-6 phosphate by glutamine fructose-6 phosphate amidotransferase (GFAT). Glucosamine-6 phosphate is further processed to uridine diphosphate N-acetyl glucosamine (UDP-GlcNAc), the major substrate for N- and O-glycosylation of proteins and for the production of glycosaminoglycans. An increased flux through the hexosamine pathway therefore might result in changes in gene expression via changes in intra- and extra cellular signalling events (20-23) but also might affect secretion of proteins (24).

Apart from accumulation of these intermediates of glycolysis, glucose itself accumulates intracellularly. The polyol pathway reduces excess glucose to the sugar alcohol sorbitol in a NADPH consuming reaction. Since NADPH is generally needed to regenerate the intracellular antioxidant glutathione, activation of the polyol pathway increases the cell’s susceptibility to oxidative stress. In summary, chronic hyperglycemia has a deleterious and toxic effect on susceptible cells by five major mechanisms, i.e. ROS production, AGE production, stimulation of protein kinases, activation of the hexosamine pathway, and the NAPDH consumption by the polyol pathway.

GROWTH FACTOR ALTERATIONS IN DIABETIC NEPHROPATHY

The aforementioned pathways of hyperglycaemia induced biochemical dysfunction are causally linked to the development of DN (25-27). In addition, mechanical stress due to osmosis driven hyperfiltration associated with hypertension contribute to the development of glomerulosclerosis. These metabolic and mechanical changes either directly damage kidney cells or stimulate resident and non-resident cells to produce growth factors and cytokines, such as angiotensin II (ANG II), vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β). TGF-β, belonging to the superfamily of multifunctional cytokines (28), plays a key role in a cascade of events that ultimately lead to kidney sclerosis and hypertrophy (29). All cell types of the glomerulus and the proximal tubular produce TGF-β and are a target of TGF-β action (30). TGF-β signal transduction involves three subclasses of
Smad proteins, i.e., receptor-activated (R-Smads), common-partner (Co-Smads) and inhibitory Smads (I-Smads). In addition, TGF-ß might signal in a Smad independent manner involving the TGF-ß-activated kinase 1/MAPK signaling pathway. The common final effect of TGF-ß signaling is the regulation of genes involved in matrix deposition and cell growth. Activation of the TGF-ß pathway in diabetic kidneys might be causally related to the pathological findings of fibrosis, although definite proof for this assumption is lacking. Nonetheless its effect to stimulate matrix accumulation and to modulate the expression of matrix receptors clearly can contribute to the structural abnormalities found in diabetic kidneys.

**GENETIC ASSOCIATIONS OF DIABETIC NEPHROPATHY**

Apart from hyperglycemia-induced biochemical damage and deregulation of growth factors, genetic susceptibility has been proposed as an important factor for the development and progression of diabetic nephropathy. While the prevalence of diabetic retinopathy continues to rise with the duration of hyperglycemia, diabetic nephropathy develops in only about one-third of the patients, irrespective of glycemic control (31). A significant proportion of diabetic patients do not develop DN despite long-standing severe hyperglycaemia whereas others develop DN even under intensive insulin therapy (4). This indicates that other factors apart from chronic hyperglycemia per se may equally determine susceptibility for development of DN. Factors like hypertension, poor glycemic and lipid control, and smoking (32) clearly increase the risk to develop DN. Therapies aimed to improve blood pressure and lipid control, or discontinuation of nicotine abuse delay the onset and slow down the progression of DN. It is clear however that control of these risk factors alone is not sufficient to prevent DN. Based on a number of observations made in diabetic patients, a genetic predisposition to develop DN in type 1 and type 2 diabetic patients has been suggested. Siblings of type 1 diabetic patients with diabetic nephropathy have a fourfold increased risk for developing DN (33). Relatives of type 2 diabetic patients on dialysis who develop end stage renal disease (ESRD) have a five times higher risk to develop DN than relatives of type 2 diabetic patients without nephropathy (7%) (34). Aggregation of type 2 diabetes was also observed in families of Pima Indians. If both parents presented with DN, it was also observed in 46% of the offspring. In contrast, only 23% of Pima Indian offspring progressed to diabetic nephropathy if only one parent was proteinuric and 14% if neither parent had proteinuria (35). Moreover, in type 2 diabetes the frequency of renal complications depends very much on race. Pima, Navajo, Winnebago and Omaha Indians show the highest prevalence (35), whereas
Caucasians of European origin demonstrate the lowest prevalence (36). In African Americans the prevalence of diabetic nephropathy is four times higher than in a population of non-hispanic whites (37). The high ethnic variance not only points to geographic and dietary factors, but likely also reflects genetic heterogeneity.

Major advances in molecular genetics have enabled the exploration of loci involved in diabetic nephropathy in genome-wide association studies. In Pima Indians, four loci on chromosomes 3, 7, 9 and 20 were identified (38). Additional DN susceptibility loci were found on chromosomes 7q21.3, 10p15.3 and 14q23.1 (39). Vardarli et al. performed a genetic linkage analysis in 18 large Turkish families (368 subjects examined) with recurrence of type 2 diabetes and diabetic nephropathy, and found a highly significant linkage of diabetic nephropathy with a locus on chromosome 18 (18q22.3–23, LOD score of 6.1) (40). This locus was subsequently tested and confirmed in an analysis of 101 affected sibling Pima Indian pairs. Others also found a susceptibility locus on 18q22.3–23 in Afro-Americans (41, 42), suggesting that this locus harbours important genes that confers predisposition to DN. An association between locus 18q22.3–23 and pathological fasting glucose, independent of body weight and most likely related to insulin resistance has also been reported (43). Of note, all of the mentioned studies showed that the locus on chromosome 18q22.3–23 contains an autosomal-dominant inherited mutation.

In 2005 Janssen et al. (44) performed a case control study on a cohort of European whites from Germany, the Netherlands, Prague and Arabic individuals from Qatar. 135 cases (diabetics with DN) and 105 controls (diabetics without DN) were included. Inclusion criteria for cases were the presence of macroalbuminuria (>300 mg/24h) and diabetic retinopathy to exclude patients with proteinuria of other causes. Moreover, patients with biopsy proven DN were included in the study. The inclusion criteria for the controls were duration of diabetes for more than 15 years, normoalbuminuria and no intake of ACE-inhibitors, AT1-antagonists or NSAID. In his study the susceptibility locus on chromosome 18q22.3–23 could be narrowed down to a single gene, i.e. the CNDP1 gene, encoding the serum carnosinase protein (CN-1). The association between the CNDP1 gene polymorphism and susceptibility to develop DN could also be demonstrated in European Americans (38). Based on the number of CTG repeats, 5 different genotypes of CNDP1 can be found that encode for a hydrophobic stretch of 4, 5, 6, 7 or 8 leucines (4L–8L alleles) in the NH2-terminal signal peptide sequence of the CN-1 precursor. The signal peptide enables the nascent CN-1 protein to be targeted to the endoplasmatic reticulum and thereby into the secretory pathway. Thus, signal peptide is of utmost importance for CN-1, since CN-1 has to be secreted to become a serum enzyme.
Individuals homozygous for \textit{CNDP1} containing 5 CTG copies (5L-5L) were at a 2.56-fold reduced risk for DN when compared with all other genotypes. Since the first patient found to be homozygous for 5L came from Mannheim, this allele was designated as protective "Mannheim allele". As the association to DN was only present when 5L-5L was compared to all other genotypes, the 5L allele is assumed to function in a recessive manner.

\section*{SERUM CARNOSINASE}

Carnosinases are enzymes belonging to the large family of metallocarboxypeptidases. Two isoforms of carnosinase have been characterized, i.e. a cellular and a secreted carnosinase, that result from different gene product and that differ not only in their distribution but also in their enzymatic properties. The cytosolic form of carnosinase (CN-2, EC 3.4.13.18) was first isolated from porcine kidney by Hanson and Smith (45) in 1949 and subsequently found widely distributed in tissues of rodents and higher mammals (46-50). Lenney et al. (51) were the first to isolate human CN-2 from kidney tissue. Human CN-2 is an intracellular nonspecific dipeptidase with broad substrate specificity (52). In 1968 Perry et al. (53) described for the first time that human serum hydrolyses carnosine, suggesting the presence of an extracellular carnosinase. Lenney et al (54) isolated the enzyme 14 years later, and it was designated as serum carnosinase (CN1, EC 3.4.13.20). CN-1 is an enzyme of a molecular mass of 57 kDa, carries a typical signal peptide sequence and three N-glycosylation sites. CN-1 is expressed in the adult central nervous system where it is secreted into CSF and in liver, which is responsible for its secretion in serum (55). Remarkably, CN-1 activity in CSF is only about 10\% of that found in serum, although CN-1 expression is 10 times higher in brain than elsewhere (56). In contrast to CN-2 which probably has little or no activity for carnosine in vivo because of its high pH optimum (9.5) and its high \(K_m\) value (20 mmol/l carnosine) (56), CN-1 is generally characterized by its narrow substrate specificity for carnosine, anserine and homocarnosine. CN-1 is absent in nonprimate mammals except in the Syrian golden hamster (56). However, rats and mice have a CN-1 orthologue in kidneys lacking a signal peptide and thus being a cellular enzyme (55). CN-1 is activated by Cd\(^{2+}\) and citrate ions and seems to be present as a homodimer in serum (54). Interestingly, CN-1 activity is not detectable in newborns and is higher for females compared to males (46).

The involvement of CN-1 in some important pathological conditions in the central nervous system and in certain muscle disorders has already been demonstrated (53, 57-59). Apart from this, CN-1 seems to be involved in the susceptibility to develop DN (44). CN-1 enzyme activity in 5L homozygous individuals is significantly lower compared to all other genotypes.
(Fig 2). Diabetic patients homozygous for 5L show a reduced risk to develop DN, suggesting that CN-1 activity in diabetic patients is implicated in the susceptibility to develop DN.

FIGURE 2

![Bar graph showing CN-1 activities for different CNDP1 genotypes detected in serum of 45 healthy individuals. The lowest CN-1 activity was found in (CTG)$_5$ allele homozygous individuals.]

*Fig. 2. Mean CN-1 activities and SD shown for the different CNDP1 genotypes detected in serum of 45 healthy individuals. Of note, the lowest CN-1 activity was found in (CTG)$_5$ allele homozygous individuals.*


CARNOSINE

Carnosine was isolated for the first time from Liebig’s meat extract and was subsequently identified as β-alanyl-L-histidine in 1900 (60). Throughout the years, the function of carnosine has been studied intensively, but the complete physiologic role of this dipeptide is still unknown.

Carnosine, along with related compounds (e.g. anserine and homocarnosine), is an endogenously synthesized peptide that can be found in kidney, stomach, olfactory bulbs, cardiac muscle, and in surprising high concentrations up to 20 mM in brain and in skeletal muscles (61-63). Carnosine is synthesized from β-alanine and L-histidine by carnosine-
synthetase (EC 6.3.2.11; (64)), which is also present in muscle (61). Tissue carnosine concentrations are influenced by diet, primarily by meat intake (65). Part of the ingested carnosine is however already degraded in the intestine mucosa cells before it is absorbed in the jejunum (66-69). In addition, the presence of carnosinase in serum makes it difficult to detect carnosine in human serum after oral supplementation (70). Nonetheless, supplementation with high concentrations of dietary carnosine increases skeletal carnosine concentrations (71), illustrating that tissue distribution of carnosine can be influenced by food intake.

The skeletal muscle has long been in the focus of carnosine researchers. 1953, Severin et al. (72) described that the frog muscle working capacity was restored after addition of carnosine in the medium surrounding a fatigued skeletal muscle. This phenomenon, was not mechanistically understood for a long time, but pointed out that carnosine specifically participates in muscle metabolism. Muscle work is associated with the activation of glycolysis and the generation of lactate and H⁺. It is believed that carnosine acts as a natural physicochemical buffer during muscle exercise (72, 73), preventing accumulation of H⁺ and lactate. Moreover, carnosine appears to regulate a variety of enzymes (74, 75), activates myosin ATPase (76) and might be a regulator of channel activity for the release of calcium from the sarcoplasmic reticulum (77). It has also been suggested that carnosine has an activating effect on the aforementioned key enzyme involved in glycolysis, i.e. glyceraldehyde-3-phosphate dehydrogenase (78).

For long time carnosine has been envisaged as anti-aging/rejuvenating drug (79-81). This was based on the findings of McFarland et al. (82, 83) who reported that cell division cycles were extended and lifespan was prolonged by 20-30% of human fibroblasts when cell culture medium was conditioned with carnosine. Similarly, it could be shown that carnosine delays aging in senescence-accelerated mice (84). Mice that were supplemented with carnosine in the drinking water had an extended mean lifespan by 20%. This was also found in male Drosophila flies (85, 86). In humans there is some evidence suggesting that tissue levels of carnosine may decline with age (87-89) and that the formation of altered proteins related to ageing is suppressed by carnosine (90).

The protective properties of carnosine seem to involve its biochemical characteristics. Carnosine is an antioxidant (91-93) and antiglycating agent that inhibits sugar-mediated protein crosslinking (94-96). It also has the propensity to chelate a number of metal ions (97) thereby reducing metal ion induced oxidative stress. There is evidence showing that carnosine can protect against ischemia induced tissue damage when added either prior to or even after
an ischemic insult (98-102). Carnosine reacts with methylglyoxal (103, 104) and it has been described as a glyoxalase mimetic (105). The dipeptide can react with a number of deleterious aldehyde products of lipid peroxidation and thereby suppressing their toxicity (106-109). Carnosine also reacts with glycated proteins and inhibits advanced glycation end product formation (110). Moreover, there is evidence from animal studies that carnosine can inhibit some of the deleterious effects of a high fructose diet (111). Given these properties of carnosine, it is conceivable that a low carnosinase activity would be beneficial for diabetic patients to limit diabetic associated complications such as DN. Indeed, a number of studies have suggested that carnosine suppresses the progression of the secondary complications of diabetes (44, 91, 112, 113). It is also worth mentioning the findings that carnosine can act as natural ACE inhibitor (114-116). Since pharmacologic inhibition of the renin-angiotensin-system is known to decline progression of DN (117), this gives an alternative explanation for the beneficial effect of high tissue carnosine concentrations or low carnosinase activity on the development of DN.

OUTLINE OF THIS THESIS

This thesis is based on the recent finding of Janssen et al (44) that a polymorphic CTG repeat within CNDP1 gene is associated with susceptibility to develop DN, for which a sound biological interpretation still missing. Therefore, we sought to assess the functionality of the CNDP1 gene polymorphism and investigated how CN-1 secretion and activity is regulated. In addition, we studied the influence of carnosine on hyperglycaemia induced extracellular matrix (ECM) accumulation in more detail and tested carnosine in a rat model of diabetes.

Since the CN-1 polymorphism is located in the signal peptide of CN-1, low CN-1 enzyme activity in 5L homozygous individuals might result from a poor CN-1 secretion in these individuals. In chapter 2 we transfected Cos-7 cells with the different CNDP-1 cDNA variants and tested the influence hereof on CN-1 secretion. CN-1 is a heavily glycosylated protein with 3 putative N-glycosylation sites at asparagine N322, N382 and N402. In chapter 3, we tested the influence of the N-glycosylation sites on CN-1 secretion and activity. Moreover, we hypothesized that under hyperglycemic conditions N-glycosylation of CN-1 is affected as a consequence of an increased glucose flux through the hexosamine pathway resulting in an increased GlcNAc production. This in turn leads to a more efficient N-glycosylation of CN-1 and thereby affects CN-1 secretion and enzyme activity.
CN-1 activity in human serum might also be influenced by factors other than secretion. Hence, measurement of CN-1 activity is not necessarily in concordance to CN-1 protein concentrations. In chapter 4, we therefore aimed to develop a method to quantitatively determine CN-1 in human serum. In chapter 5 we subsequently studied the relevance of allosteric CN-1 conformations and homocarnosine concentration on CN-1 activity. CN-1 activity and CN-1 protein concentrations in serum of diabetic patients in relation to metabolic changes in these patients was investigated in chapter 6.

The importance of carnosine for prevention of typical lesions observed in DN is subject of the chapters 7 and 8. In chapter 7, we studied the influence of carnosine on ECM accumulation and by what mechanism this was mediated. In chapter 8, we tested the effect of oral carnosine administration on early diabetic changes in STZ-induced diabetic rat kidneys, i.e. biochemical imbalance, glomerular cell apoptosis and podocyte loss.