ROLE OF CIGARETTE SMOKE IN CHRONIC KIDNEY DISEASES

Ph.D. theses

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ABBREVIATIONS

ACE	angiotensin converting enzyme
Ach	acetylcholine
ACR	albumin-to-creatinine ratio
ADPKD	autosomal dominant polycystic kidney disease
AGEs	advanced glycation end products
amyl	amyloidosis
app	approximately
ARB	angiotensin receptor blocker
ATP	adenosine tri-phosphate
$BaCl_2$	barium chloride
BMI	body mass index
$CaCl_2{*}2H_2O$	calcium chloride
CAPD	continuous ambulantory peritoneal dialysis
$Ca_v1.2$ L-type calcium channel	L-type voltage-dependent calcium channel
CI	confidence interval
CO	carbon monoxide
CO_2	carbon dioxide
CS	cigarette smoking
CSB	cigarette smoke buffer
cyclic GMP	cyclic guanosine monophosphate
DM	diabetes mellitus
DNP	diabetic nephropathy
dsDNA	double-stranded deoxyribonucleic acid
eGFR	estimated glomerular filtration rate
ESRD	end-stage renal disease
ESRF	end-stage renal failure
FSGS	focal segmental glomerulosclerosis
GFR	glomerular filtration rate
GSH	glutathione
HbA_{1c}	glycosylated hemoglobin
HR	hazard ratio / heart rate
imt GP	immunotactoid glomerulopathy
ING	idiopathic nodular glomerulosclerosis
K_{ATP}	ATP-sensitive potassium channel
KCl	potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
KW	Kimmelstiel-Wilson
LiCl	lithium chloride
MAP	mean arterial pressure

MDRD	modification of diet in renal disease
mem GN	membranoproliferative glomerulonephritis
mg/c	milligram per cigarette
Mg_2SO_4	magnesium sulfate
MIDD	monoclonal immunoglobulin deposition disease
mmHg	millimeter of mercury
mM	millimol
NaCl	sodium chloride
NADPH-oxidase	nicotinamide adenine dinucleotide phosphate-oxidase
$NaHCO_3$	sodium bicarbonate
NCX	Na^+-Ca^{2+} exchanger
NGS	nodular glomerulosclerosis
NKF-KDOQI	National Kidney Foundation – Kidney Disease Outcomes Quality Initiative
nM	nanomol
NO	nitric oxide
non-diab NGS	non- diabetic nodular glomerulosclerosis
non-KW	non-Kimmelstiel-Wilson diabetic nephropathy
NS	not significant
µmol/l	micromol per liter
µm	micrometer
ODQ	oxadiazolo-quinoxalin-1
OR	odds ratio
O_2	oxygen
RAAS	renin-angiotensin-aldosterone system
RI	resistance index
RR	relative risk / Riva Rocci
SD	standard deviation
SH group	sulph-hydril group
SLE	systemic lupus erythematosus
SOD	superoxide dismutase
$TGF_{\beta 1}$,	transforming growth factor β1
TEA	tetraethylammonium
wCS	water-soluble components of cigarette smoke

INTRODUCTION

1. GENERAL ROLE OF SMOKING IN CHRONIC KIDNEY DISEASE AND DIABETIC NEPHROPATHY

The fight against cigarette smoking is a global challenge. Worldwide 1.2 billion people smoked in 2000 [1], a number that is projected to increase to 1.6 billion by 2030 [2]. Tobacco currently causes an estimated 5 million deaths annually and if the actual trends continue, the number of deaths will be doubled by 2030 [1]. Chronic cigarette consumption is harmful in both active and passive smokers and it has a role in the initiation and progression of certain chronic kidney diseases (CKD), type 2 diabetes mellitus and in the progression of diabetic nephropathy and cardiovascular complications of diabetes mellitus (DM). It is also evident that chronic kidney failure raises the risk of cardiovascular morbidity and mortality, thus tobacco use can be considered as a factor which induces or aggravates processes that diminish life quality or even shorten life expectancy. Noteworthy is the „memory for smoking" of the organism, namely the deleterious effects of tobacco consumption do not last only until the cessation of cigarette smoking but even years longer.

Tobacco consumption is involved in the initiation and progression of the most common causes of CKD [3] e.g. diabetic nephropathy, ischemic nephropathy, nephrosclerosis, IgA nephropathy - the most frequent primary glomerulonephritis - or autosomal dominant polycystic kidney disease - the most common cystic kidney disease [4, 5] and some less frequent diseases like lupus nephritis [6]. Smoking causes insulin resistance – thus increases the risk of metabolic syndrome [7-9] and type 2 diabetes [10, 11]. Both in type 1 and type 2 diabetes smoking increases the risk of the initiation and progression of nephropathy [8]. The pathomechanisms of these diseases are complex and not yet fully understood, therefore each step can

be crucial for the outcome of the disease for each individual. We would like to underline the facts and theories that support the fight against an addiction that can destroy one's life moreover the life of the smokers family, colleagues or other "innocent" bystanders.

1.1. SMOKING AND CHRONIC KIDNEY DISEASE

1.1.1. DEFINITION AND COMPLICATIONS OF CHRONIC KIDNEY DISEASE

The intended redefinition of chronic kidney disease (CKD) includes both kidney function and albuminuria [12]. On the basis of analyses of 45 cohorts including 1,555,332 subjects from general, high-risk and kidney disease populations, the attendees of a Controversies Conference organized by „Kidney Disease: Improving Global Outcomes" (KDIGO) in 2009 agreed that both glomerular filtration rate and stages of albuminuria should determine the classification of chronic kidney disease for a better estimation of the prognosis of the kidney patients [12].

The decline in glomerular filtration rate and/or presence of proteinuria increase the risk of cardiovascular diseases [13, 14]. According to the NKF-KDOQI Guidelines cardiovascular disease is the leading cause of death in non-diabetic patients with chronic kidney disease, moreover cardiovascular disease mortality is more likely than development of kidney failure in non-diabetic patients with chronic kidney disease [14], which means that these patients die due to fatal cardiovascular complications before they reach the end-stage renal failure. A prospective population-based cohort performed in Iceland with 16,958 people aged 33-81 years with a median follow-up of 24 years showed that even the earliest stage of chronic kidney disease is associated with higher risk of coronary heart disease [13].

It has been shown that albuminuria in a population with chronic stable coronary disease, including only partly diabetics, is an independent predictor of

cardiovascular and all-cause mortality [15]. Albuminuria is not only a cardiovascular risk factor but it is also associated with cancer incidence [16]. A 10.3 year follow-up of 5,425 subjects without diabetes or previous cancer in the Tromsø Study has showed that the albumin-to-creatinine ratio (ACR) at baseline significantly correlated with the incidence of cancer, even after adjustment for age, gender, body mass index, physical activity, and smoking ($P < 0.001$). Participants with ACR in the highest quintile were 8.3- and 2.4-fold more likely to receive bladder cancer and lung cancer, respectively, compared with those with ACR in the lowest quintile after similar adjustments [16].

1.1.2. ROLE OF TOBACCO IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

Tobacco consumption plays also a role in the progression of certain specific kidney diseases. The facts concerning the influence of smoking in autosomal dominant polycystic kidney disease (ADPKD) are somewhat controversial. ADPKD accounts for 5-10% of patients with end-stage renal disease [17]. A Turkish epidemiological study [17] included 1,139 patients with ADPKD, where 20.3% were current smokers and 15% were ex-smokers, which underlines the importance of the topic. In a study performed on 270 ADPKD patients, 32 subjects with established proteinuria had a significant larger pack-year smoking history, higher mean arterial pressure, larger renal volumes, lower creatinine clearances than did their non-proteinuric counterparts, moreover, smoking history was the only significant independent variable determing the level of proteinuria [18]. In a retrospective multicenter, matched case-control study including patients with primary renal disease (IgA nephropathy and ADPKD) a significant dose-dependent increase of the risk to progress to end-stage renal failure (ESRF) was found in male smokers compared with non-smokers or moderate smokers [19]. A small sample size and modest average tobacco consumption caused the subgroup of

women to be excluded from analysis [20]. Nevertheless, after adjustment to the ACE-inhibitor treatment, the risk of ESRF was not increased in heavy smokers, which suggests a pivotal role of the renin-angiotensin-aldosterone system in the pathomechanism of the smoking-induced kidney damage. A study of 554 patients with type 1 ADPKD did not find, however, that cigarette smoking influences the disease course [21].

1.1.3. SMOKING AND IGA GLOMERULONEPHRITIS

Cigarette smoking promoted in a dose-dependent manner the risk of progression to end-stage renal failure in male patients with IgA nephropathy in a retrospective case-control study [5, 19]. The pathomechanism – similar to that in ADPKD – probably involves the renin-angiotensin-aldosterone system because the history of ACE inhibitor treatment abolished the significance of the tobacco effect [5, 19]. Another clinical trial included 295 primer glomerulonephritis cases, 116 IgA nephropathy, 80 membranous nephropathy and 99 nephrotic syndrome with either minimal change nephropathy or focal segmental hyalinosis and 242 matched hospital controls [22]. In men the percentage of ever-smokers was significantly higher among cases with chronic renal failure than those without. Moreover, a dose-effect relationship was observed with both the daily and the cumulative dose of tobacco consumption, wich suggests a causative role of smoking [22]. Smoking was significantly related to chronic renal failure among cases who were older than 40 years and/or hypertensive and the results did not differ among the three histologic types mentioned above [22]. A single center retrospective study performed on 223 patients – both women and men – failed to confirm the influence of smoking on the progression of the disease [23], but the smoking habits were not well defined, women were included in a relevant number - of note the difference between the tobacco effects among the sexes is well defined [24] -, and past smokers seemed to have a poorer outcome of IgA nephropathy compared to

current smokers [25]. A recent study including patients suffering from IgA nephropathy, FSGS or membranous glomerulonephritis also failed to confirm the role of smoking in the progression to ESRD independent of the baseline estimated GFR [26]. The study design and the fact that minimum 75% of the patients received ACE inhibitor or ARB treatment may explain that finding. In a most recent retrospective cohort study including 971 IgA nephropathy patients in 3 major nephrology centers in Japan during a 5.8 years observational period, 117 participants progressed to a 50% increase in serum creatinine level and 47 advanced to ESRD [27]. Current smokers and number of cigarettes smoked in the period of kidney biopsy were significant predictors of outcomes and the association of current smoking with adverse outcomes was stronger in those with lower compared with higher estimated glomerular filtration rates which confirms that smoking is - in a dose-dependent manner - a key prognostic factor in IgA nephropathy [27]. Also, in a study performed in a Japanese population – 485 patients with stage 1 and stage 2 chronic kidney disease (IgA nephropathy, lupus nephritis, minimal change GN, FSGS etc.) - smoking habit was clearly related to accelerated disease progression [28].

1.1.4. CIGARETTE SMOKING – IMPORTANCE IN HYPERTENSIVE NEPHROPATHY

Smoking is a classical risk factor for the initiation of ischemic nephropathy and for the progression of hypertensive nephropathy. At the beginning of the 20th century it was known that cigarette smoking elicits an acute transient rise in blood pressure [29]. Although in the first analysis of a large epidemiological cross-sectional study (IRSA) lower systemic blood pressure was described among current smokers compared with non-smokers [30], a subsequent analysis in men revealed a higher risk of hypertension in smokers [31]. The examination of the albumin excretion in the morning urine of 631 hypertensive subjects showed by multivariate analysis that smoking was the most significant factor associated with

albuminuria [32]. In a prospective, 7-year-long study, performed on 225 hypertensive patients, GFR declined generally faster in smokers versus non-smokers, independent of urine albumin/creatinine ratio. However, the risk of GFR-decline increased robustly in subjects in whom the albumin/creatinine ratio was higher than 200 mg/g [33]. In patients with primary hypertension the prevalence of microalbuminuria is almost double in smoking than non-smoking lean patients and it has been shown that smoking is the strongest predictor for albuminuria [20]. The LIFE study has found that hypertensive and heavy smokers (> 20 cigarettes/day) with left ventricular hypertrophy had a 1.6-fold higher prevalence of microalbuminuria and a 3.7-fold higher prevalence of macroalbuminuria than never-smokers [20].

1.1.5. SMOKING: A COMMON ORIGIN OF ISCHEMIC NEPHROPATHY

Ischemic nephropathy or atherosclerotic renal artery stenosis is an important cause of end-stage renal failure in patients older than 65 years [24]. In a retrospective study of 218 subjects who underwent angiography to investigate peripheral vascular disease – which is common in smokers - the incidence of atherosclerotic renal artery disease was significantly higher in those patients with femoral artery atherosclerosis than in those without femoral lesion [34]. Smokers have also, not surprisingly, a higher risk of critical atherosclerotic renal artery stenosis [35]. In an arteriography study performed on 67 patients older than 50 years the percentage of smokers was 80.5% in the group with significant atheromatous stenoses of the renal artery and 44.4% in the group of smokers without significant stenoses [36]. The fact that the presence of atheromatous stenoses of renal arteries was connected to the number of cigarettes and the exposure time, and not to the current compartment of patients towards smoking suggested a cumulative effect of smoking [36]. In an observational multicenter Spanish study 69.8% of the elderly patients with bilateral renal artery stenosis and

chronic renal failure were smokers [37]. Both in unilateral and bilateral atherosclerotic renal artery stenosis the prevalence of smokers is higher compared to patients without stenosis [24]. Smoking is also a strong risk factor for cholesterol embolism, which could also contribute to the decline in renal function in patients with ischemic nephropathy [24]. In an Italian study of elderly patients with peripheral atherosclerosis multiple regression analysis showed that smoking and LDL cholesterol were associated with the decrease of renal plasma flow, the degree of the latter was parallel with the severity of the peripheral atherosclerotic lesion [38].

1.1.6. TOBACCO USE IN LUPUS NEPHRITIS

The effect of smoking on the course of lupus nephritis is an underexamined field. In a meta-analysis performed by Costenbader et al. [39] current smokers compared with non-smokers had a significantly elevated odds ratio (OR) for development of SLE (OR: 1.50, 95% CI, 1.09-2.08). Moreover, a study performed with black women supported an increased risk of SLE among smokers [40]. There is no clear evidence for the link between the initiation of lupus and smoking, but the latter has a great impact on the progression of lupus nephritis. In a retrospective cohort study of 160 adults with lupus nephritis the median time to ESRD among smokers was 145 months and among non-smokers it was greater than 273 months even in a multivariable analysis adjusting for differences in age, gender, socioeconomic status, renal histology, and immunosuppressive treatment [6]. The significant independent association between current smoking and dsDNA seropositivity (OR=3.5, 95% CI 1.2 to 10.5) in a multivariate cohort which included 410 white SLE patients [41] gives an indirect proof of the connection between lupus nephritis and smoking, given the well established association of dsDNA autoantibodies with lupus nephritis. At the same time it can serve as one potential plausible explanation for the pathomechanism of smoking-induced kidney damage

in lupus, e.g. the formation of DNA adducts with resultant autoantibodies to the damaged DNA. A study including 97 patients with lupus nephritis requiring renal transplantation and matched non-lupus controls revealed that subjects with lupus nephritis had inferior transplantation outcomes, with more than twice the risk of allograft loss compared with the control kidney transplant patients, moreover smoking status was associated with allograft loss in a multivariate model [42]. Because the life expectancy of lupus patients has improved from an approximately 4-year survival rate of 50% in the 1950s to a 15-year survival rate of 80% today, the bimodal pattern of mortality in lupus e.g. lupus or infection as main causes of death in the first period and myocardial infarction and stroke in the long term period [43] suggests that cigarette smoking due to direct macrovascular damage and indirectly via accelerating the progression of CKD in lupus nephritis can contribute to the mortality in this special autoimmune disease.

1.1.7. PATIENTS ON RENAL REPLACEMENT THERAPY (END-STAGE RENAL FAILURE; HEMODIALYSIS; CAPD; TRANSPLANTATION) – EFFECT OF SMOKING

In the Chronic Renal Impairment in Birmingham (CRIB) prospective cohort study of 382 stage 3-5 CKD patients with a mean follow-up of 4.1 years for ESRD and 6.0 years for death, smoking showed an association with ESRD independently of age and sex, moreover, current smoking was an independent predictor of death [44]. In hemodialysis patients active smoking causes a lower serum albumin level compared to non-smokers, of note, low albumin level is predictor of increased mortality in this CKD population [24]. Tobacco use increases cardiovascular mortality (myocardial infarction, carotid artery stenosis, peripheral vascular disease) and death both in non-diabetic and diabetic hemodialysis subjects [24]. In peritoneal dialysis patients smoking is a significant survival risk factor [24].

In a retrospective study of 1,334 transplant patients after adjustment for multiple predictors of graft failure, smoking more than 25 pack-years at transplantation was associated with a 30% higher risk of graft failure compared to those who smoked less intensively or did not smoke at all [45]. The increase in graft failure was due to an increase in deaths (adjusted RR 1.42; 95% CI: 1.08 - 1.87, P = 0.012) [45]. However, having quit smoking more than 5 years before transplantation reduced the relative risk of graft failure by 34% [45]. In a cohort study of 645 adult renal allograft recipients pretransplant smoking caused a reduced overall graft and death-censored graft survival [46]. Pretransplant smokers had kidney survival of 84%, 65%, and 48% at 1, 5, and 10 years, respectively; in contrast, non-smokers had an increased graft survival, namely 88%, 78%, and 62% (P = 0.007) [46].

1.1.8. HISTOLOGICAL ALTERATIONS OF THE KIDNEY DUE TO TOBACCO CONSUMPTION

Renal Vessels

Smoking-induced structural renal artery damage has been shown already in the 1980s. An autopsy study described an intima thickening in arterioles of smokers [47], whereas in another investigation the thickening of the renal arterioles was attributed to increase in collagen in smooth muscle [48]. Smoking promotes not only atherosclerosis of the vessels but it is also a risk factor for cholesterol microembolism [49].

Glomerular and Tubulointerstitial Alterations

It can probably also be learnt from histology results about the pathomechanism of cigarette-induced kidney damage. In case reports the histopathologic changes in the kidneys of non-diabetic smokers include focal segmental or focal global glomerulosclerosis, ischemic glomeruli, interstitial fibrosis, tubular atrophy, arterial sclerosis and arteriolar hyalinosis [50]. Electron microscopy showed glomerular

capillary wall thickening caused by subendothelial expansion by cellular elements and new basement formation resulting in segments of double contours [50].

1.1.9. CONCEPTIONS FOR THE PATHOMECHANISMS OF SMOKING-INDUCED KIDNEY DAMAGE IN CKD PATIENTS

The pathomechanism is probably multi-causative [51], but oxidative stress, alteration in cell membrane processes and signal transduction pathways likely play a pivotal role (***Table 1.1***)*.*

Nicotine

Nicotine has been „accused" as a link between cigarette smoking and the progression of renal injury by its mitogenetic effects and by inducing the production of extracellular matrix in human mesangial cells via reactive oxygen species [4, 52]. Nicotine also increases sympathetic activity via both stimulation of cathecolamine release from peripheral nerve endings and the adrenal medulla and via direct stimulation of postganglionic sympathetic nerve endings [8]. In a human study, the administration of nicotine gum to non-smokers was associated with increased mean arterial pressure and heart rate and renal vasoconstriction, the latter possibly through inhibition of a cyclic-GMP-dependent vasoactive mechanism [53]. However, in chronic smokers a tolerance to this renal effect of nicotine was observed despite the maintenance of the systemic response to nicotine [53]. The antidiuretic effect of the nicotine content of tobacco due to increased vasopressin secretion, and a possible increase in single-nephron GFR could also contribute to the deleterious consequences of tobacco use [8, 54]. Nicotinic acetylcholine receptors, which mediate cell proliferation, are expressed on human mesangial cells [52, 55]. Exposition of mesangial cells to cigarette smoke induced an increase in TGF-β_1, which is a major factor in the development of renal fibrosis [55]. In smokers, due to nicotine, the plasma concentration of another substance,

endothelin-1, a strong vasoconstrictor and at the same time a growth promoter of endothelial cells, vascular smooth muscle cells and mesangial cells [56] is increased [57, 58]. The consecutive glomerulomegaly, nephromegaly, and enlarged kidney size – the latter also observed in middle-aged smokers [59] - are established risk factors for kidney disease progression.

Oxidative Stress

Parallel to the deterioration of a chronic kidney disease the concentration of markers of oxidative stress (malondialdehyde, and hydrogen peroxide) rises, whereas protein SH groups (as important antioxidants) and activity of antioxidant enzymes (glutathione peroxidase, catalase and superoxide dismutase) decrease [60], hereby CKD patients are more susceptible to the oxidative stress either caused directly- or induced by cigarette smoke. Water-soluble constituents of cigarette smoke induce vascular reactive oxygen- and nitrogen species production, enhance inflammatory gene expression, and lead to endothelial dysfunction [61]. Nitric oxide (NO) bioavailability is reduced in smokers [62] and patients with chronic kidney disease have a reduced whole body NO production [63], which can play an important role in increasing renal vascular tone and perhaps also in mesangial cell proliferation [8].

Tubulointerstitial Injury

Both in diabetic and non-diabetic smokers a proximal tubular cell dysfunction and a tubular cell damage was observed [64], which is important in tubulointerstitial injury and thus in the progression of CKD. The excretion of N-acetyl-β-hexosaminidase, as a marker of proximal tubular cell damage, was dose-dependently elevated in smokers [64]. Cadmium – one of the app. 4,000 components of cigarette smoke – has also a toxic effect on proximal tubular cells in vitro [65], an observation confirmed by a population-based study, where diabetics were more susceptible to the toxic effects of cadmium [66]. Smoking 20 cigarettes

daily for a longer period leads to 45 to 70% higher accumulation dosages of cadmium in the renal cortex [55].

Gene Modification

Cadmium and strontium modify the expression of several genes in the endothelial cells, which can play a role in the pathogenesis of accelerated atherosclerosis induced by cigarette smoking [4]. In ADPKD patients a potential smoking-induced second PKD allele mutation in the unaffected parent could promote cyst formation, because loss of heterozygocity and intragenic mutations in the PKD-1 gene were already described, suggesting a two-hit model of cystogenesis due to inactivation of both copies of the gene [8].

Reversibility

The „point of no return" of smoking-induced kidney damage is not established yet, but there are some data about the reversibility of the process. A representative study performed in the general population revealed that cessation of cigarette smoking led to normalisation of urinary albumin excretion only in non-heavy smokers, namely in subjects who smoked less than 20 cigarettes per day [67]. Lifetable analyses were used to estimate gains in life expectancy in non-diabetics and diabetics of the Multiple Risk Factor Intervention Trial (MRFIT), and it was concluded that cessation of smoking would prolong life by a mean of around four years in a 45-year-old non-diabetic man and three years in a diabetic man, whereas aspirin and antihypertensive treatment would provide approximately one year of additional life expectancy in both categories [68]. This means that reversibility has its borders and probably both intensity, duration, form of tobacco consumption and associated diseases determine it.

Hyperfiltration

Parallel with the stimulation of the sympathetic nervous system, smoking causes a significant but transient (app. 30 minutes) increase of blood pressure, an effect which was observed among healthy subjects, hypertensive patients, type 1 and type 2 diabetics and also in patients with primary renal disease [20]. Also in chronic renal diseases hyperfiltration and glomerular hypertension accelerate the progression [69] according to Brenner's theory [70], which can be aggravated by cigarette smoking. In a study performed in an apparently "healthy" population subclinical inflammation (serum C-reactive protein level) was associated with cigarette smoking-induced hyperfiltration and proteinuria [71]. In IgA glomerulonephritis patients an increase in the urinary albumin/creatinine ratio was described, which could be also developed due to higher glomerular capillary pressure [8].

Table 1.1. **Patomechanisms of smoking-induced kidney damage in the general population and in patients suffering from chronic kidney disease**

- Hyperfiltration due to smoking
  - repetitive acute hyperperfusion + chronic endothelial dysfunction → hyperfiltration
- Nicotine
  - mitogenetic effects; reactive oxygen species → extracellular matrix overproduction mesangial cell proliferation → TGF-β_1* overproduction → renal fibrosis
  - sympathetic activity
    a/ rise in mean arterial pressure and heart rate
    b/ renal vasoconstriction → vasopressin secretion → antidiuresis
  - rise in single nephron GFR*
  - endothelin-1 →vasoconstriction and overgrowth of endothelial cells + vascular smooth muscle cells + mesangial cells → glomerulomegaly + nephromegaly → kidney disease progression
- Oxidative stress
  - parallel to CKD* development: rise in malondialdehyde + hydrogen peroxide
  - decrease in glutathione peroxidase + catalase + superoxide dismutase
  - water-soluble constituents of cigarette smoke
    → vascular reactive oxygen- and nitrogen species production
    → inflammatory gene expression → endothelial dysfunction
  - smokers: decrease in NO* bioavailability + CKD* patients: lower whole body NO production → elevated renal vascular tone + possible mesangial cell proliferation
- Tubulointerstitial injury
  - in smokers dose-dependent elevation in the excretion of N-acetyl-β-hexosaminidase = proximal tubular cell damage marker
  - cadmium – toxic effect on proximal tubular cells
- Gene modification
  - cadmium + strontium → modification of the expression of genes in endothelial cells → accelerated atherosclerosis
  - in ADPKD* potential smoking-induced second PKD* allele mutation → cyst formation
- Reversibility
  - unknown „point of no return" of smoking-induced kidney damage
  - in general population cessation of smoking → normalisation of urinary albumin excretion only in non-heavy smokers
  - cessation of smoking → prolongation of life by approximately 4 years in a 45- year-old non-diabetic man and 3 years in a diabetic man

* Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; CKD, chronic kidney disease; GFR, glomerular filtration rate; NO nitric oxide; PKD, polycystic kidney disease; TGF-β1, transforming growth factor β1.

1.2. SMOKING AND DIABETIC NEPHROPATHY

1.2.1. CIGARETTE SMOKING PROMOTES THE COMMENCEMENT OF DIABETIC NEPHROPATHY IN A MIXED POPULATION OF TYPE 1 AND TYPE 2 DIABETIC PATIENTS

Although there is a great body of evidence of the harmful effect of smoking on diabetic nephropathy, in the 2011 ADA recommendations for physicians this topic is perhaps not accentuated enough [72]. Both in type 1 and type 2 diabetes smoking increases the risk of development of nephropathy and almost doubles the rate of progression to end-stage renal failure [8]. Cigarette smoking causes not only a deterioration in renal function [73], but also elevates microalbuminuria and proteinuria in diabetes mellitus [8]. In a follow-up study of 185 – either type 1 or type 2 – diabetics (44 smokers and 141 non-smokers) without signs of overt renal disease, the GFR estimated with the MDRD formula remained constant during the minimum 3 years of follow-up in non-smokers (from 107±33ml/min baseline to 106±31ml/min), whereas GFR decreased significantly (from 95±26ml/min baseline to 83±22ml/min) in smokers [73]. This relationship persisted when adjusted for retinopathy, glycaemic control, age, body shape, ACE-inhibitor treatment, blood pressure control or severity of proteinuria [73].

1.2.2. CONTRIBUTION OF CIGARETTE SMOKING TO THE DEVELOPMENT OF DIABETIC NEPHROPATHY IN TYPE 1 DIABETES MELLITUS

A study including 668 type 1 diabetic patients confirmed that tobacco consumption is a risk factor for the development of nephropathy and revealed that the prevalence of nephropathy was higher among heavy smokers than among non-heavy smokers, 19.2% versus 12.1%, respectively. An increasing frequency of

nephropathy was found with increasing cigarette consumption [74]. A study group in Denver investigated 359 young patients with type 1 diabetes mellitus and they found that smoking was a significant risk factor of increased albumin excretion also in a logistic regression model controlled for duration of diabetes, glycohaemoglobin level, blood pressure, age and gender [75]. Also in a four year follow-up of a cohort of 137 insulin-dependent diabetics smoking was one of the significant determinants of persistent microalbuminuria [76]. In an ESRF retrospective study it was shown that tobacco consumption in a dose-dependent manner shortens the time period between the onset of diabetes and the commencement of albuminuria or proteinuria in type 1 diabetic patients [77].

1.2.3. CIGARETTE SMOKING – EFFECT ON THE PROGRESSION OF DIABETIC NEPHROPATHY OF TYPE 1 DIABETICS

Among type 1 diabetics with microvascular complications, albuminuria and retinopathy were found to progress more in smokers and the former improved significantly when subjects ceased smoking [75]. A case control study involved 192 cigarette-smoking patients with type 1 diabetes mellitus, who were compared with non-smoking controls pair-matched for sex, duration of diabetes and age [78]. Although the glycosylated haemoglobin values and the prevalence of hypertension were similar between the two groups, macroproteinuria was found significantly more often, in 19.3% of the smoking and in 8.3% of the non-smoking patients [78]. Moreover, proliferative retinopathy was present in 12.5% of the smoking and in 6.8% of the non-smoking patients [78]. Thus cigarette consumption seems to be a risk factor both for overt proteinuria and proliferative retinopathy in type 1 diabetics. In a retrospective study data of type 1 diabetic patients with end-stage diabetic nephropathy and a control group matched for sex, age and duration of diabetes were analysed [79]. Smokers – especially those with a large daily consumption – had an earlier onset of proteinuria than non-smokers; moreover, tobacco use was

proposed as a trigger for progression of incipient to overt nephropathy [79]. Tobacco consumption is also an independent variable associated with the rate of decrease of creatinine clearance in the predialysis phase both in type 1 and type 2 diabetic patients [80]. The rate of loss of glomerular filtration rate was 1.24±0.29ml/min/month in smokers versus 0.86±0.31ml/min/month in non-smoker type 1 diabetics; the respective values for subjects with type 2 diabetes mellitus were 1.21±0.34ml/min/month and 0.73±0.38ml/min/month [10, 80]. In a prospective, follow-up study over one year with treated hypertensive type 1 diabetics the progression of nephropathy – defined as an increase in proteinuria or serum creatinine or decrease in creatinine clearance – was more common in smokers (53%) and ex-smokers (33%) than in non-smokers (11%) [81]. In a prevalence survey of 3,250 men and women aged 15-60 years with type 1 diabetes mellitus from 31 diabetes centers in Europe 35% of the men and 29% of the women were smokers [82]. Current smokers had a higher prevalence of microalbuminuria and total retinopathy than did those who never smoked; moreover, ex-smokers had a higher prevalence of macroalbuminuria and proliferative retinopathy than did those who never smoked, but both had a similar prevalence of microalbuminuria [82], which can be perhaps interpreted that if someone quits smoking in time, the progression of diabetic nephropathy could be reduced to the level of that of a non-smoker. An observational extension of the randomized prospective Diabetes Control and Complications trial revealed that among 1,105 type 1 diabetics who had normal urine albumin excretion at baseline, a 4.3-fold greater rate of GFR decline could be observed in active versus non-active smokers (-0.77 versus -0.18 ml/min per 1.73m^2/year) [83].

1.2.4. INITIATION OF NEPHROPATHY IN TYPE 2 DIABETES MELLITUS DUE TO CHRONIC CIGARETTE SMOKING

In a cross-sectional study of 1,203 type 2 diabetic patients the prevalence of smokers was higher in patients with microalbuminuria [84], which was confirmed by another smaller study performed in Germany, where current smoking was significantly correlated with an increased risk of microalbuminuria [85]. A prospective study documented that smoking is also an independent predictor of the de novo development of microalbuminuria in type 2 diabetes [86]. In a population-based cohort of 1,574 type 2 diabetics cigarette smoking was an independent variable related to micro- and macroalbuminuria [87]; the latter was confirmed also by other authors [88].

1.2.5. TOBACCO AS A PROGRESSION PROMOTER OF DNP IN TYPE 2 DIABETES

A population-based prospective study in southern Wisconsin of individuals with type 2 diabetes showed that during a four-year follow-up the relative risk of developing gross proteinuria was 2- to 2.5-fold higher in heavy smokers compared to non-smokers [89]. In 933 type 2 diabetic patients using a multivariate logistic regression analysis controlling for diabetes duration, glycosylated hemoglobin, gender and race, one of the most significant predictors of microalbuminuria and macroalbuminuria was smoking pack-year [90]. In a prospective follow-up study of type 2 diabetic patients with normal renal function at the beginning, smokers had significantly faster decline of the creatinine-clearance (1.24±0.34ml/min/month) than non-smoking patients (0.99±0.35 ml/min/month), while systolic and diastolic blood pressure as well as serum cholesterol, triglycerides and HbA_{1c} were not significantly different in the two patient groups [91]. Another prospective study including type 2 diabetics with normal initial renal function, manifest nephropathy and with well-controlled blood pressure - partly due to ACE inhibitor treatment -,

the increase in serum creatinine was more pronounced in smokers as compared with non-smokers, i.e., from 93±7 µmol/L to 157±18µmol/L versus from 95±3 µmol/L to 117±4µmol/L [92]. Regression analysis (follow-up time, mean blood pressure and initial plasma creatinine) revealed that cigarette smoking was the only factor that significantly predicted the decline in GFR [92]. In a more recent prospective study involving 227 white patients with type 2 diabetes mellitus and nephropathy a faster rate of GFR decline was independently associated with heavy smoking during a follow-up of 6.5 years [93]. In a cross-sectional study involving 32,208 type 2 diabetic patients without known albuminuria smoking was an independent risk factor for increased urine albumin excretion [94]. A follow-up of 185 subjects with type 1 and type 2 diabetes with and without nephropathy showed that smoking was independently associated with a decrease in estimated GFR; moreover, the relation was independent of proteinuria [73].

1.2.6. DIABETIC SMOKERS WITH END-STAGE RENAL FAILURE AND RENAL REPLACEMENT THERAPY

In diabetic patients with end-stage renal failure, smoking decreases survival on commencement of dialysis [95]. The 1- and 5-year survival rates in diabetic patients with tobacco consumption were 68 and 9%, respectively, while in non-smoking patients these rates were 80 and 37%, respectively ($P<0.05$) [96]. As a potential explanation, hemodialyzed diabetic cigarette smokers showed higher fibrinogen and systemic blood pressure values and a higher incidence of myocardial infarctions when compared with non-smoker diabetic patients on hemodialysis [96].

1.2.7. SMOKING AS A RISK FACTOR FOR ALL-CAUSE MORTALITY IN TYPE 1 AND TYPE 2 DIABETIC PATIENTS WITH CKD

The relationship between CKD and all-cause mortality in type 1 diabetes was underlined in the Finnish Diabetic Nephropathy Study, which was a multicenter prospective study including 4,201 adult diabetics with a mean follow-up of 7 years [97]. The presence of microalbuminuria, macroalbuminuria, and end-stage kidney disease was associated with 2.8, 9.2, and 18.3 times higher standardized mortality ratio, respectively, compared to the general population [97]. In addition, the glomerular filtration rate was independently associated with mortality: both individuals with impaired kidney function and those demonstrating hyperfiltration had an increased risk of death [97].

The Casale Monferrato Study, a population-based cohort (n=1,538 type 2 diabetics; median age 68.9 years, 11 years follow-up) has found that chronic kidney disease (lower eGFR) conferred an increased risk of all-cause mortality of 23% and of cardiovascular mortality of 18% independently of both cardiovascular risk factors and albumin excretion rate. However, in an analysis stratified by albumin excretion rate categories, a significant increasing trend in risk with decreasing eGFR was evident only in people with macroalbuminuria [98]. Since cigarette smoking is a risk factor for the development of both diabetes and chronic kidney disease and the main complications of diabetes and CKD are the cardiovascular ones - the latter triggered also directly by tobacco use -, it is evident that chronic cigarette smoking is one of the major modifiable elements in the formation of potentially fatal illnesses.

1.2.8. POSSIBLE CONTRIBUTION OF SMOKING TO DIABETIC NEPHROPATHY

The potential pathomechanisms involved in the development of smoking-induced diabetic nephropathy are summerized in **Table 1.2.**

Podocyte Damage

Cigarette smoking also rises the urinary podocyte excretion [99]. First it may occur in patients with early diabetic nephropathy [100], and second, it can predict long-term urinary albumin excretion in type 2 diabetes and microalbuminuria [101]. Smoking increases urinary albumin level even at albumin concentrations below that of microalbuminuria [20]. In a prospective study including 80 type 2 diabetic patients and 30 healthy controls, urinary podocytes were detected by immunofluorescense microscopy in 35 diabetic subjects with microalbuminuria (27 smokers and 8 non-smokers, 1.4±0.7 cells/ml) but were not detected in the remaining 45 patients (23 smokers and 22 non-smokers) or the 30 healthy subjects [99]. More podocytes were excreted in the urine in smokers (27 of 50 patients) with microalbuminuria than in non-smokers (8 of 30 patients) with microalbuminuria ($P = 0.017$, χ^2 test). Interestingly, the urinary podocytes disappeared after 3 years in 77% of patients who had stopped smoking, whereas urinary podocytes increased in all patients who continued to smoke (from 1.1±0.8 to 1.7±0.4 cells/ml, $P<0.01$) [99]. These data suggest first that smoking may be associated with podocyte injuries in patients with early diabetic nephropathy [99], and second that podocyte excretion is probably an early and potentially reversible sign of diabetic nephropathy.

Hyperfiltration and Limited or Abolished Glomerular Autoregulation

In insulin-treated diabetics a higher prevalence of hyperfiltration was found in smokers than in non-smokers, moreover, the glomerular filtration rate was directly dependent on the intensity of smoking [102]. The fact that in the same study no correlation could be shown in users of oral snuff suggested that another

component of tobacco apart from nicotine was responsible for the hyperfiltration [102]. It supports our theory [103] that not only nicotine but other components of cigarette smoke are also crucial in the hyperfiltration process, namely hydrogen peroxyde reduces the vasomotor tone of renal arteries, which could lead to hyperperfusion of kidneys also in diabetics. The elevated mean arterial pressure due to cigarette smoke can harm the glomeruli of patients with diabetic nephropathy in a greater extent than non diabetics, because autoregulation of GFR is impaired or abolished both in type 1 [104] and type 2 [105] diabetic patients with diabetic nephropathy.

Nicotine

Animal experiments and human studies indicated that nicotine exposure could induce a reduction of insulin release, and negatively affect insulin action, suggesting that this substance of cigarette could be a cause of insulin resistance [11]. Animal and human studies suggest that either acute or chronic nicotine exposures could negatively affect insulin action both in smokers preceding type 2 diabetes mellitus and in type 2 diabetic patients, which means that nicotine can contribute to type 2 diabetes development and aggravation of the disease through enhancing insulin resistance [11]. It has been already shown that functional nicotinic receptors are present in pancreatic islets and beta cells, so nicotine could, at least in part, negatively affect beta-cell function [11]. Moreover, nicotine increases apoptosis of islet β-cells [11]. Mitochondrial dysfunction, oxidative stress, and inflammation are involved as underlying mechanisms of nicotine-induced pancreatic β-cell loss [11].

Oxidative stress, angiotensin-II, advanced glycation end products, $TGF_{\beta 1}$

Both smoking-induced insulin resistance and a direct decrease of NO bioavailability due to cigarette smoking increase oxidative stress and contribute to the progression of diabetic nephropathy [106]. Smoking also increases serum

concentration of angiotensin-II in diabetics, and this could lead via NADPH-oxidase and enhanced oxidative stress to the observed higher level of $TGF_{\beta 1}$ in diabetic patients [107]. Tobacco consumption enhances the accumulation of advanced glycation end products similar to hyperglycemia [106] and thereby increases also indirectly the expression of $TGF_{\beta 1}$, which is one of the key factors of the deterioration of diabetic nephropathy and the development of renal fibrosis.

Genetic Predisposition

A cross-sectional analysis in 1,209 normo-albuminuric type 2 diabetics has shown a genetic predisposition to develop albuminuria in smokers, who carried the DD-genotype of the ACE gene [108].

Heavy Metals

In diabetic patients low-level cadmium exposure has been also associated with early onset of diabetic nephropathy [55].

Impaired Vasodilation

Smoking also impairs the responsiveness of intrarenal arteries to vasodilators, which is one of the potential mechanisms behind the progression of diabetic nephropathy [109].

Elevated Resting Energy Expenditure

Smoking is an independent risk factor for elevated resting energy expenditure in patients with diabetic nephropathy and since resting energy expenditure is not attributable to heightened oxidative stress and inflammation, it provides an additional mechanism by which smoke may lead to poor outcomes in subjects with diabetic nephropathy [110].

Reversibility

In type 1 diabetics with nephropathy and with adequate control of blood pressure and glycemia, the progression of nephropathy slowed down among diabetic subjects, who had stopped smoking [75]. Cigarette smoking-induced increased TGF-β_1 excretion (the promoter of renal fibrosis) was reduced after smoking cessation, which can underline the beneficial consequences of quitting smoking [55].

Table 1.2. **Possible contribution of smoking to diabetic nephropathy**

- Oxidative stress, angiotensin-II, AGEs, $TGF_{\beta 1}$
  - tobacco consumption → higher insulin resistance + decreased NO bioavailability → oxidative stress → progression of DNP
  - smoking → increased serum concentration of $TGF_{\beta 1}$ and angiotensin-II → NADPH-oxidase stimulation → oxidative stress
  - smoking → accumulation of AGEs → oxidative stress → $TGF_{\beta 1}$ → progression of DNP, renal fibrosis
- Podocyte damage
  - cigarette smoking → rise in urinary podocyte excretion (early sign of diabetic nephropathy; prediction of long-term urinary albumin excretion in type 2 DM* and microalbuminuria;
  - possible reversible stage
- Hyperfiltration and limited or abolished glomerular autoregulation
  - among insulin-treated diabetics higher prevalence of hyperfiltration in smokers than in non-smokers + GFR* directly dependent on the intensity of smoking
  - cause: non-nicotinic component of smoke (no correlation in users of oral snuff)
  - impaired or abolished autoregulation of GFR* both in type 1 and type 2 diabetics with diabetic nephropathy → harm of glomeruli due to elevated mean arterial pressure caused by smoking
- Nicotine
  - higher insulin resistance → development and aggravation of type 2 diabetes
  - presence of functional nicotinic receptors in pancreatic islets and beta cells → negative impact on beta-cell function (mitochondrial dysfunction, oxidative stress, and inflammation) + apoptosis of islet β-cells
- Genetic predisposition
  - carriers of the DD-genotype of the ACE* gene in normo-albuminuric type 2 diabetic smokers → predisposition to develop albuminuria
- Heavy metals
  - low-level cadmium exposure - association with early onset of diabetic nephropathy
- Impaired vasodilation
  - smoking → impaired responsiveness of intrarenal arteries to vasodilators
- Elevated resting energy expenditure
  - smoking: independent risk factor for elevated resting energy expenditure in patients with diabetic nephropathy → poorer outcome of diabetic nephropathy
- Reversibility
  - in type 1 diabetics with nephropathy and with adequate control of blood pressure and glycemia after stopping smoking → slow down of the progression of nephropathy
  - smoking cessation → reduction in cigarette smoking-induced increased TGF-β_1* excretion → possible reduced renal fibrosis

* Abbreviations: ACE, angiotensin converting enzyme; DM, diabetes mellitus; GFR, glomerular filtration rate; TGF-β1, transforming growth factor β1; DNP, diabetic nephropathy; NADPH oxidase, nicotinamide adenine dinucleotide phosphate-oxidase; AGEs, advanced glycation end products.

1.2.9. HISTOLOGICAL SIGNS OF CIGARETTE SMOKING IN DIABETIC NEPHROPATHY AND METABOLIC SYNDROME

Histology may also add some information to the understanding of the pathomechanism in diabetic nephropathy. Chronic cigarette smoking could contribute to the development of „idiopathic" nodular glomerulosclerosis [111], and noteworthy, diabetic nodular glomerulosclerosis is a separate entity in the histopathologic classification of diabetic nephropathy [112]. In a small sample study with metabolic syndrome patients 2 out of 3 individuals with histology-proven nodular glomerulosclerosis were smokers [113]. Histopathologic features in smoking-induced renal damage in 18 type 1 diabetic patients involved a more pronounced matrix volume and greater ratio in mesangial - to - glomerular volume and greater basement membrane thickness in smokers than in non-smokers [69]. The increase in basement membrane thickness in 96 type 2 diabetics was found to be a dose-dependent alteration (non-smokers: 398.0±92.5 nm, moderate smokers: 438.6±80.9 nm, heavy smokers: 471.0±113.3 nm) [114]. In a Scandinavian study, where changes of kidney function, microalbuminuria and kidney biopsy-proven structural glomerular parameters, namely glomerular volume, matrix/glomerular volume fraction, mesangial/glomerular volume fraction and the basement membrane thickness were analysed in type 1 diabetics in an 8-year period, the smoking group had a definitely higher rise in albumin ecretion rate and a tendency to larger decline in GFR than the non-smokers. Moreover, smoking was an independent risk factor for decline in GFR in a multivariate analysis [115]. We have also data about the reversal of established lesions of diabetic nephropathy after pancreas transplantation [116], which can give hope for the patients who decide to quit smoking, so that the smoking-induced alterations could be also reversible until a certain degree of damage.

AIMS

1) **First, we aimed to test the hypothesis that cigarette smoking elicits acute changes in hemodynamic parameters and vasomotor tone of renal arteries in humans.**

    a. **Investigation of the acute effect of nicotinic cigarette smoke**
        i. **on the changes in the resistance index (RI) of segmental renal arteries.**
        ii. **on the mean arterial pressure.**
        iii. **on the heart rate.**

    b. **Evaluation of the acute effect of nicotine free cigarette smoke in the same conditions for testing the role of nicotine.**

    c. **Checking the consequences of sham smoking under the same circumstances for the investigation of potential influence of the parasympatho-mimetic activation.**

2) **Second, we investigated the acute effects of water-soluble components of cigarette smoke (wCS) on the isometric tension of isolated rat segmental renal arteries in order to elucidate the underlying mechanisms.**

    a. **Testing the effect of different concentrations of nicotinic cigarette smoke on isolated rat renal vessels.**

    b. **Investigation of the role of nicotine by adding nicotine free cigarette smoke buffer to rat isolated renal arteries.**

c. **Clear the role of endothelium in the nicotinic cigarette smoke-induced relaxations of rat isolated renal arteries.**

d. **Evaluation of the potential contribution of carbon monoxide (CO) and/or nitric oxide (NO) in the smoking- induced relaxation of renal arteries.**

e. **Elucidation of the role of free radicals, as known modulators of the vascular tone, in the relaxation caused by cigarette smoke.**

f. **Investigation of the potential role of certain ion channels, $Ca_V1.2$ L-type calcium channels and the Na^+-Ca^{2+} exchanger, in the smoke-induced response of rat renal arteries.**

3) **Our third aim was to test the role of chronic cigarette smoking in male patients with diabetic nodular glomerulosclerosis.**

a. **Retrospective systematic revision of the native kidney biopsy databank (n=644) between 2001 and 2011, and comparison of the smoking habits and other clinical settings in three groups:**

i. **men with type 2 diabetes mellitus and Kimmelstiel-Wilson lesion (KW)**
ii. **men with non-KW diabetic nephropathy (non-KW; negative control)**
iii. **male patients with non-diabetic nodular glomerulosclerosis (non-diab NGS; positive control).**

METHODS AND RESULTS

3.1. In vivo investigation of the acute effects of cigarette smoke on human renal arteries.

In the present study we aimed to test the hypothesis that cigarette smoking elicits acute changes in hemodynamic parameters and vasomotor tone of renal arteries. In order to test this hypothesis, first we investigated by ultrasound the acute effect of cigarette smoke on the changes in the resistance index (RI) of segmental renal arteries. At the same time we tested the acute consequences of cigarette smoking on the systemic mean arterial pressure and heart rate in the same subjects.

3.1.1. Materials and methods

Eight healthy male smoker volunteers with ages ranging 21 to 41 years were examined. The subjects smoked daily 17.7±6.9 cigarettes and the duration of smoking was 13.3±5.0 years. Renal disease was excluded based on history, physical examination, laboratory tests (serum creatinine (91±9 μmol/l), estimated GFR (Mayo quadratic equation: 120±12 ml/min), microalbuminuria (9±7 mg/ml) urine analysis and renal ultrasound. All individuals were instructed to stop smoking 8 hours before the study. Before the examinations they rested in the supine position in a quiet room for 15 minutes. The subjects smoked one commercial available cigarette (brand: Camel filters, tar: 10 mg/c, CO: 10 mg/c, nicotine: 0.8 mg/c) for 5 minutes. One segmental renal artery was chosen by a trained physician and the Doppler spectrum was detected. The resistance index [RI= (peak systolic velocity – end diastolic velocity)/peak systolic velocity] was measured in the same

artery 3 minutes before, in every minute during, and 3 minutes after smoking the cigarette. The results 3 minutes before lighting and 3 minutes after finishing the

cigarette and the lowest value during smoking were recorded. The average coefficient of variation of the eight patients for the three consecutive resistance index measurements on segmental renal arteries before lighting a cigarette was 1.6±1.0%. Also, systemic blood pressure and heart rate were measured in the same period. In another series of experiments on separate days the same protocol was repeated with the same subjects, but instead of the nicotinic cigarette first sham smoking, i.e., suction of a dummy cigarette, and second a nicotine-free – according to the description of the manufacturer, brand: HoneyRose De Luxe – cigarette was used. The changes in the RI, mean arterial pressure (MAP) or heart rate (HR) during and after smoking were expressed as the percentage of the values before smoking. The study was approved by the local ethics committee (N°. 3344). All subjects gave written informed consent.

Statistics

Statistical significance was calculated using Student's *t*-test in the human study and the animal experiments. All distributions were normal, data are means ± SD. The tests were performed with the SPSS program package, Version 15.0 (SPSS, Chicago, IL, USA), considering P values of 0.05 or less to be significant.

3.1.2. Results: human study

An original record shows that in healthy young men, smoking a nicotinic cigarette caused a transient reduction in the resistance index of a segmental renal artery (***Figure 3.1.1***).

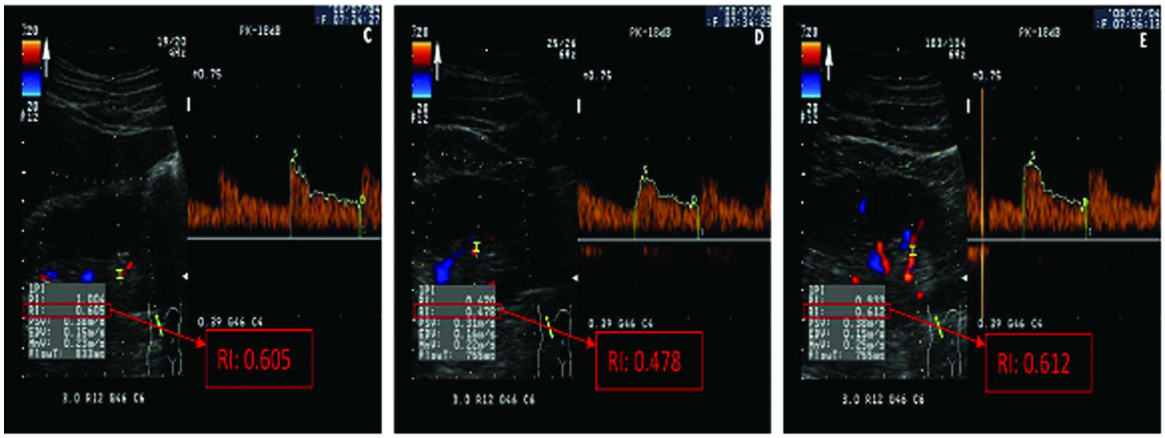

Figure 3.1.1.
Original Doppler spectrum and acute changes in resistance index of a human segmental renal artery in response to smoking.

Abbreviations are: RI, resistance index.

Summary data indicate that this reduction was significant (P<0.05, ***Figure 3.1.2***.).

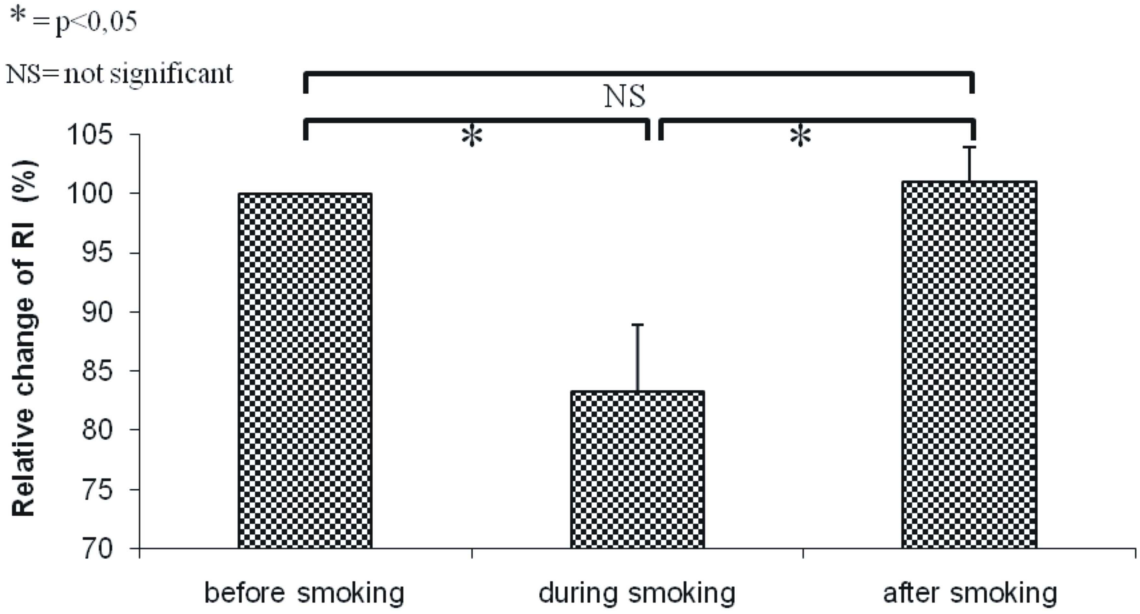

Figure 3.1.2. **Reduction in the resistance index (RI) of the segmental renal arteries due to nicotinic cigarette smoking (n=8).**

Nicotine free cigarette smoking elicited a similar reduction in RI (P<0.05, ***Figure 3.1.3.***) as the nicotinic cigarette did.

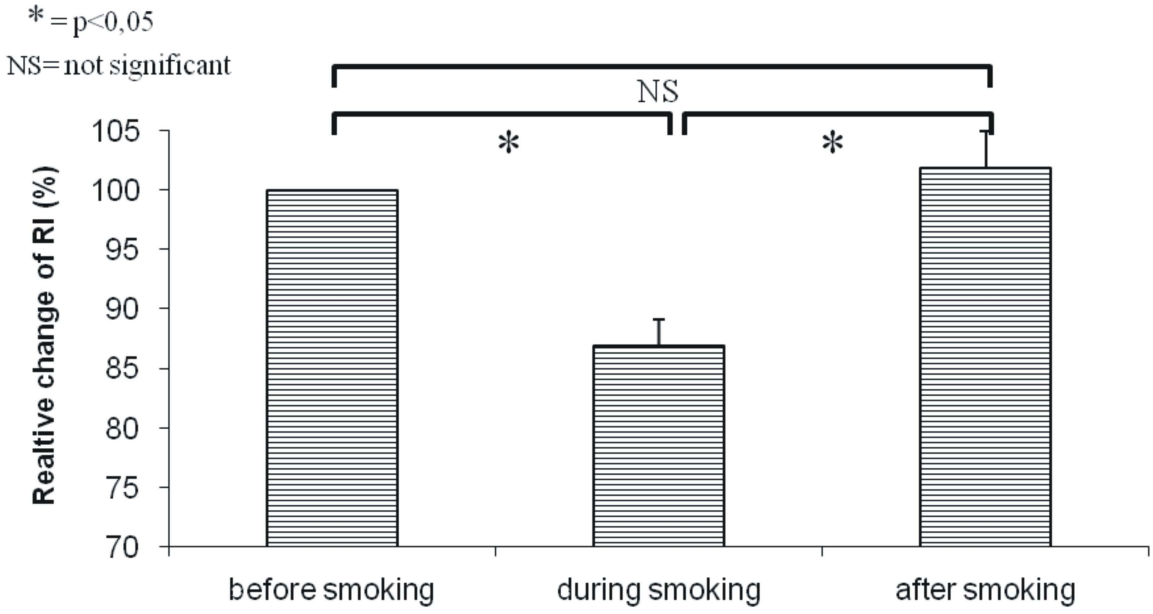

Figure 3.1.3. **Reduction in the resistance index (RI) of the segmental renal arteries due to nicotine-free cigarette smoking (n=8).**

Sham smoking did not elicit significant changes in these parameters (***Figure 3.1.4.***).

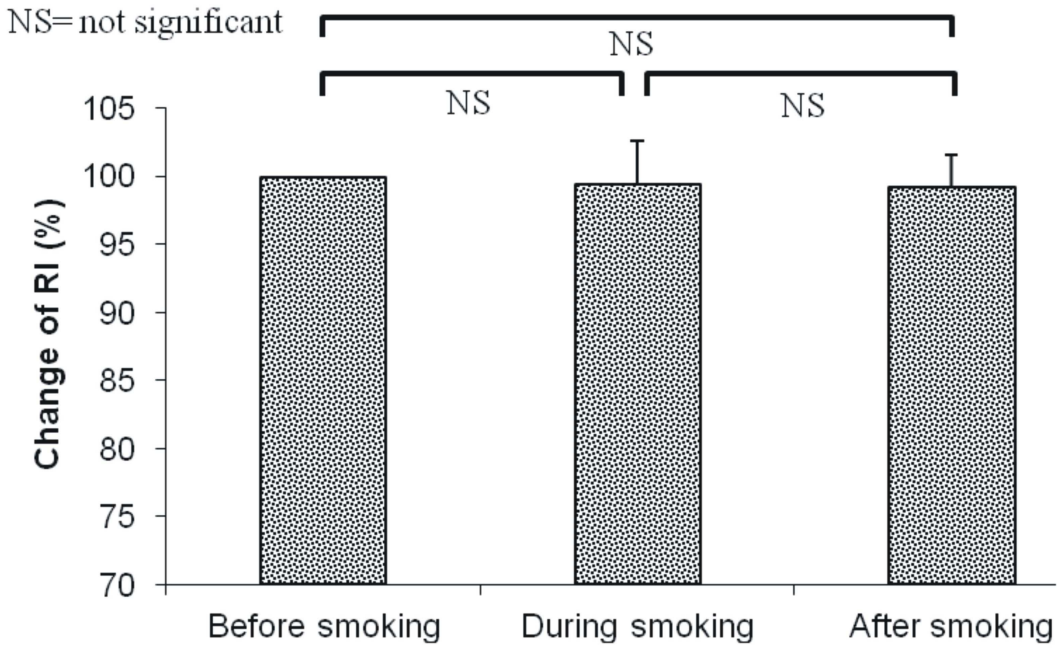

Figure 3.1.4. **Changes in resistance index of human segmental renal arteries in vivo due to sham cigarette smoking (n=8).**

Due to nicotinic cigarette smoke a transient elevation in MAP (P<0.05, ***Figure 3.1.5.***) and an elevation in HR (P<0.05, ***Figure 3.1.6.***) were also observed. However neither nicotine free cigarette smoking nor sham smoking changed MAP and HR significantly (***Figures 3.1.5. and 3.1.6.***).

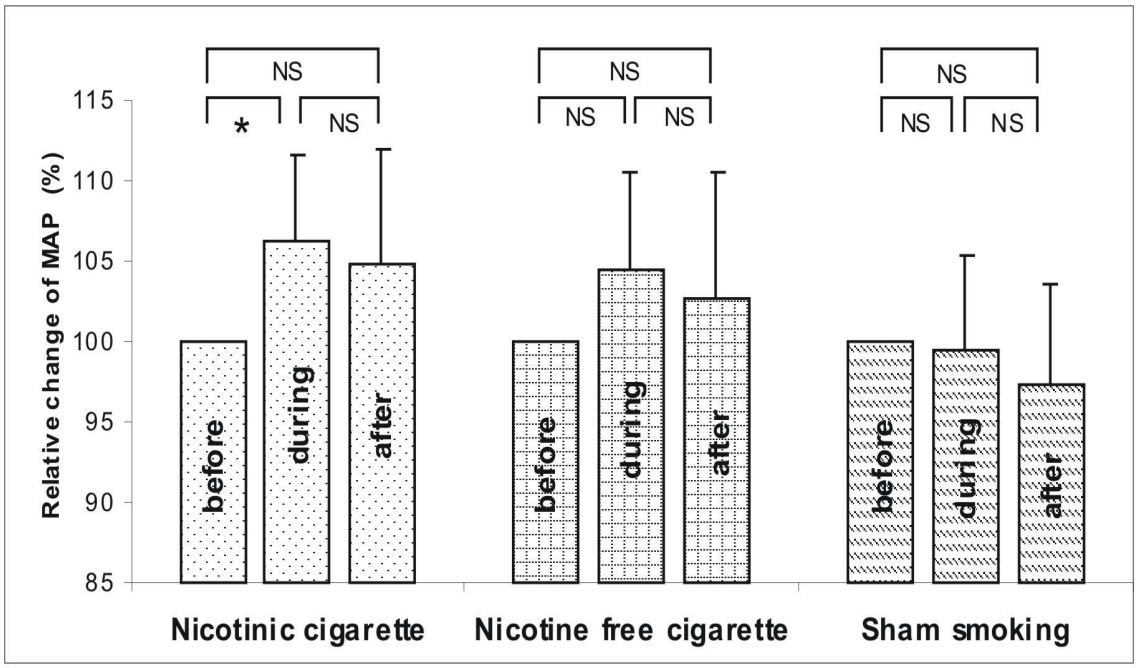

Figure 3.1.5

Acute changes in the systemic mean arterial pressure due to smoking (n=8) of either nicotinic or nicotine free cigarette or sham smoking. Each measurement before smoking was set to be 100 percent.

Abbreviations are: MAP, mean arterial blood pressure; NS, not significant; *denotes a significant difference, P<0.05.

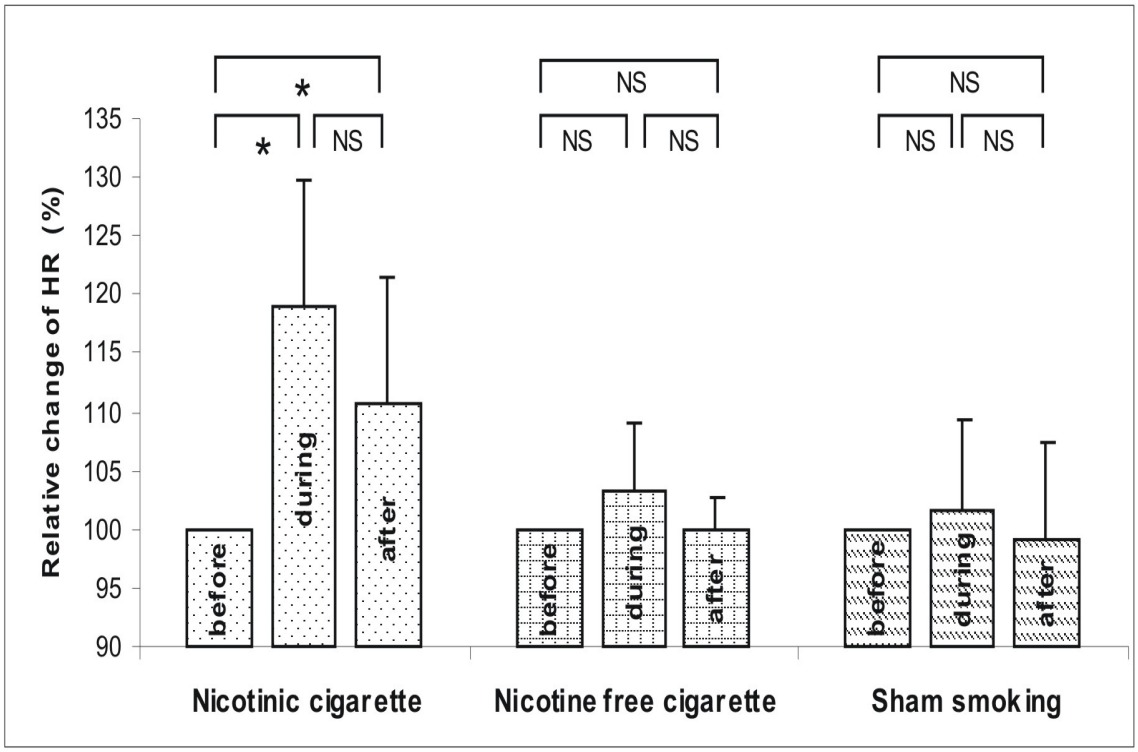

Figure 3.1.6.
Acute changes in heart rate due to smoking (n=8) of either nicotinic or nicotine free cigarette or sham smoking. Each measurement before smoking was set to be 100 percent.

Abbreviations are: HR, heart rate; NS, not significant; *denotes a significant difference, P<0.05.

3.2. Ex vivo investigation of the acute effects of cigarette smoke on rat renal arteries.

In the previous study we have shown that cigarette smoking elicits acute and transient decrease in the resistance index of human renal arteries, which is indicative for a dilation of these vessels. In the present ex vivo experiments we investigated the acute effects of water-soluble components of cigarette smoke (wCS) on isolated rat segmental renal arteries in order to elucidate the underlying mechanisms.

3.2.1. Materials and methods

The modified method of Fésüs et al. [117] was used. Cigarette smoke buffer (CSB) stock solution was prepared by passing the smoke of one commercially available cigarette through 5ml Krebs buffer (containing 119 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 1.2 mM Mg_2SO_4, 11.1 mM glucose, 1.6 mM $CaCl_2{*}2H_2O$, pH 7.4), for 5 min in order to trap its water-soluble components [118] (***Figure 3.2.1.***).

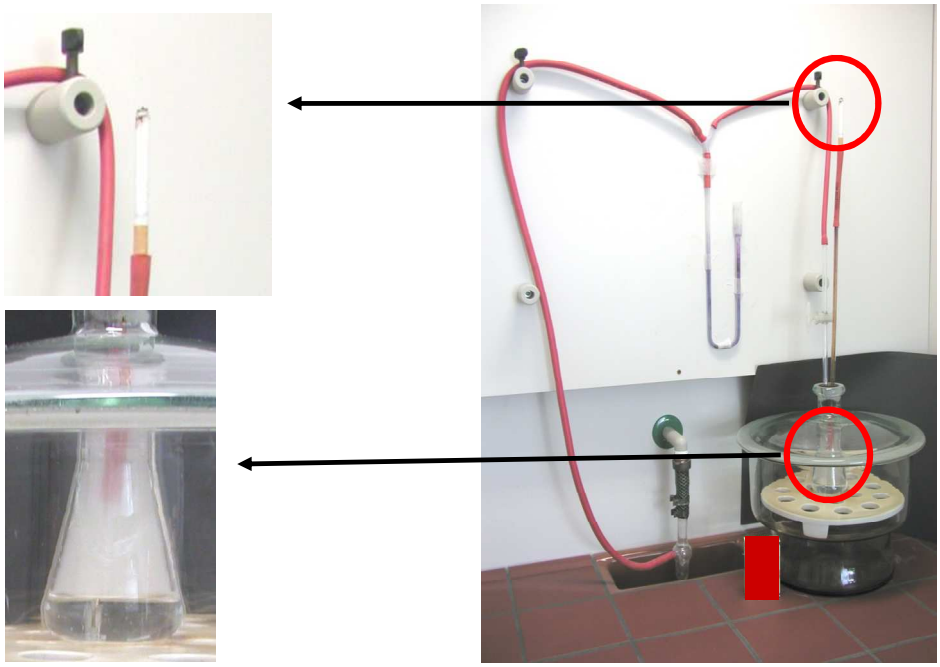

Figure 3.2.1.

Preparation of cigarette smoke buffer (CSB) stock solution by passing the smoke of one commercially available cigarette through 5ml Krebs buffer, for 5 min in order to trap its water-soluble components.

Adult (10-12 week-old, 300-350 g, altogether 86 specimens) male CFY rats – a strain of Sprague-Dawley rat – were kept on regular diet. On the day of experiments they were deeply anaesthetized with ether and then decapitated with a guillotine. After preparation of 2 mm long first order renal arteries (~150-200 µm in diameter) in ice-cold Krebs buffer, rings were mounted on two stainless steel wires (40 µm in diameter) in a Danish Multimyograph (Model 610M; ***Figure 3.2.2.***).

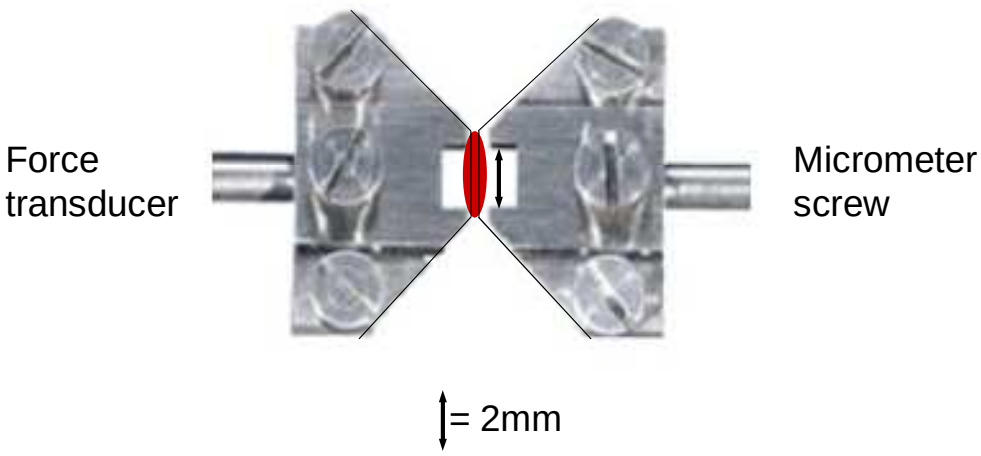

Figure 3.2.2.

Danish Multimyograph system (Model 610M). Between the force transducer (for measuring isometric tension) and the micrometer screw a first order renal artery (marked with red; 2mm long) is mounted on two stainless steel wires.

Vessels were bathed in Krebs buffer and gassed with 5% CO_2 and 95% O_2 at 37˚C, pH 7.4. The resting tension/internal circumference relationship for each vessel was determined, then the internal circumference was set to 0.9 x L_{100}, where L_{100} is the internal circumference that the vessel would have had *in vivo* when relaxed under a transmural pressure of 100 mmHg. After this normalization procedure vessels were allowed to stabilize for 30 min, and then isometric tension was continuously recorded. The contractile capacity was assessed by exposing the arterial segments to an isotonic 60 mM KCl solution prepared by substituting NaCl in the normal Krebs buffer with an equimolar amount of KCl. Vessels producing less then 10 mN force were discarded. The presence of functional endothelium was assessed in all preparations by the ability of acetylcholine (ACh $3x10^{-6}$ M) to induce more than 50% relaxation of 60mM KCl precontracted vessels. The preparations were washed three times with Krebs buffer and rested for 20 minutes. When necessary, endothelium-denuded vessels were prepared by gently rubbing a hair through the lumen and verified by loss of response to acetylcholine. Then washing procedure was repeated. To give the vessels the possibility both to relax or contract, rings were preconstricted with 100nM epinephrine (series #1) which, in previously performed concentration response curves experiments (data not shown) showed 60% contraction force of the 60mM KCl contraction. After reaching a stable contraction plateau, increasing doses of cigarette smoke buffer (1%, 5%, or 10% final concentrations) were administered into the vessels chamber. Because the isometric force of vessels treated by epinephrine diminishes with time, the relaxation rate of the precontracted vessels was normalized to contractile force of a parallel “control” artery. The rate of relaxation caused by the water-soluble components of cigarette smoke (wCS) was expressed as the percentage of the control (100%) vessel. Soluble guanylate cyclase inhibitor oxadiazolo-quinoxalin-1 (ODQ, 5 µM), inhibitor of the ATP-sensitive potassium (K_{ATP}) channel glibenclamide (10 µM), or large-conductance calcium-activated potassium channel blocker tetraethylammonium (TEA, 2 mM), were added to the vessel chamber 30

minutes before getting the epinephrine induced contraction plateau. Free radical scavengers, such as glutathione (GSH, 4 mM), catalase (1000 U/ml) or superoxide dismutase (SOD, 200 U/ml) were added both to the wCS solutions and to the vessel chamber 30 minutes before establishing the epinephrine induced contraction plateau.

In another series of experiments contractions evoked by modified depolarizing Krebs buffers were measured (series #2). After washing the vessels 3 times with Krebs buffer and waiting for 20 minutes $CaCl_2$ was substituted with $BaCl_2$ (3,2 mM) to obtain a control contraction [119]. Then the preparations were washed 3 times with Krebs buffer and after another 20 minutes resting period the $BaCl_2$ contractions were repeated alone or in the presence of either 5% cigarette smoke buffer or the specific L-type calcium channel blocker nifedipine (10 nM). The peak responses to $BaCl_2$ in control conditions and in the presence of cigarette smoke buffer or nifedipine were measured and the ratio of the CSB/control and nifedipine/control contractions was calculated. The results were normalized by correcting the ratio with the percentage of 2 following “non-treated” $BaCl_2$-induced contractions.

In a third set of experiments (series #3), in the Krebs buffer, NaCl was substituted by equimolar LiCl to activate Na^+-Ca^{2+} exchanger. The protocol used was similar to series #2. Contractions were studied using either 1% cigarette smoke buffer or a specific blocking agent of the Na^+-Ca^{2+} exchanger SEA0400 (2 µM). These data were normalized as described in series #2.

All drugs were purchased from Sigma Chemicals (St Louis, MO, USA) except for SEA0400, which was produced in the Institute of Pharmaceutical Chemistry, University of Szeged, Hungary.

The experiments were performed with the permission of the Hungarian Local Animal Experiment Committee (Nº. BA02/2000-1/2008).

3.2.2. Results: animal experiments

In isolated first order branches of rat renal arteries, water-soluble components of nicotinic cigarette smoke elicited a dose-dependent relaxation (***Figure 3.2.3*** right panel and ***Figure 3.2.4.***) compared to control (***Figure 3.2.3.*** left panel).

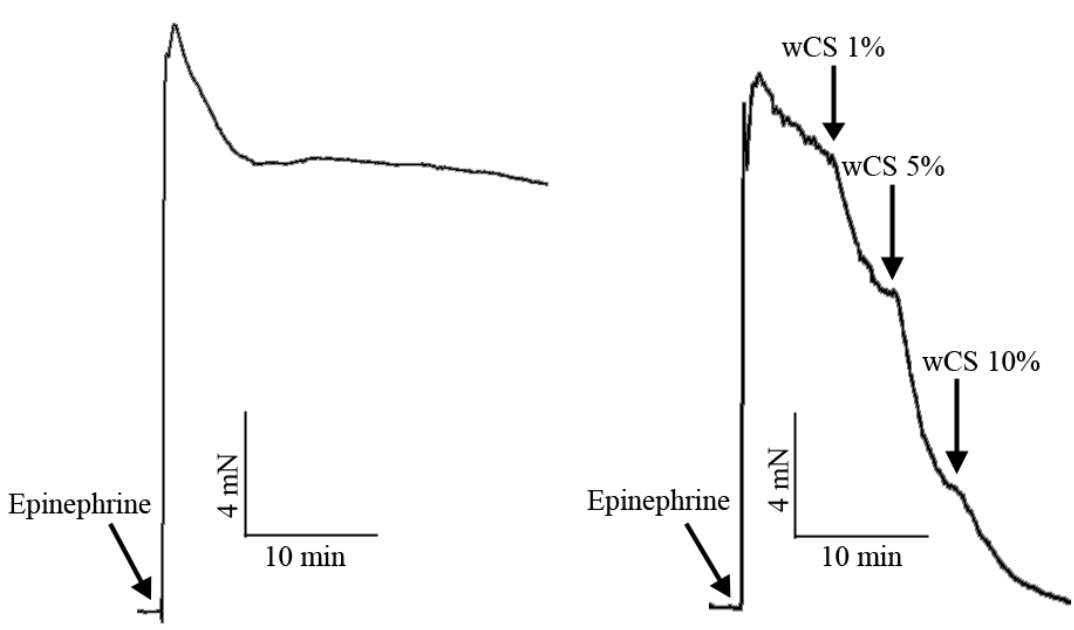

Figure 3.2.3.

Original record of the response of an isolated rat renal artery to water-soluble components of nicotinic cigarette smoke (right panel) compared to a control vessel (left panel).

Abbreviations are: wCS, water-soluble components of cigarette smoke.

Water soluble components of nicotine free cigarette had a similar effect (***Figure 3.2.4.***).

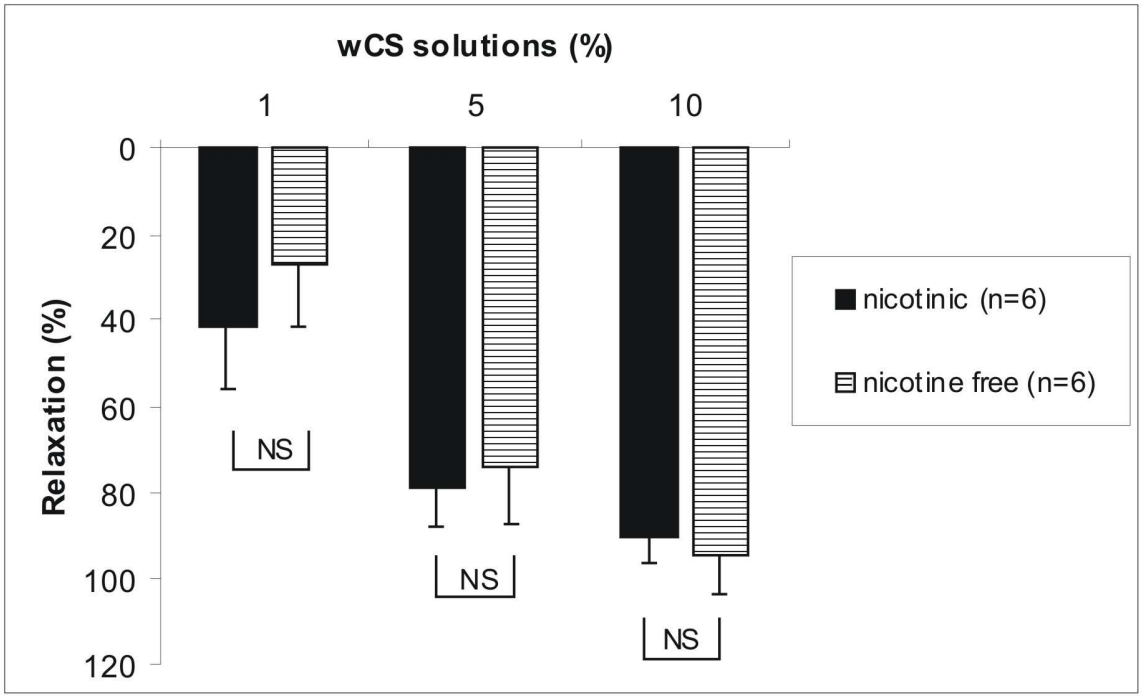

Figure 3.2.4.

Relaxation of rat renal arteries to different dilutions of the water-soluble components (wCS) of either nicotinic or nicotine free cigarette smoke (n=6).

Abbreviation: NS, not significant.

The water-soluble components of the nicotinic tobacco smoke without cigarette paper elicited a similar relaxation as the water-soluble components of an intact nicotinic cigarette did (***Figure 3.2.5.***), whereas the water soluble components of the smoke of the cigarette paper without tobacco caused a significantly smaller relaxation compared to the nicotinic wCS (***Figure 3.2.5.***).

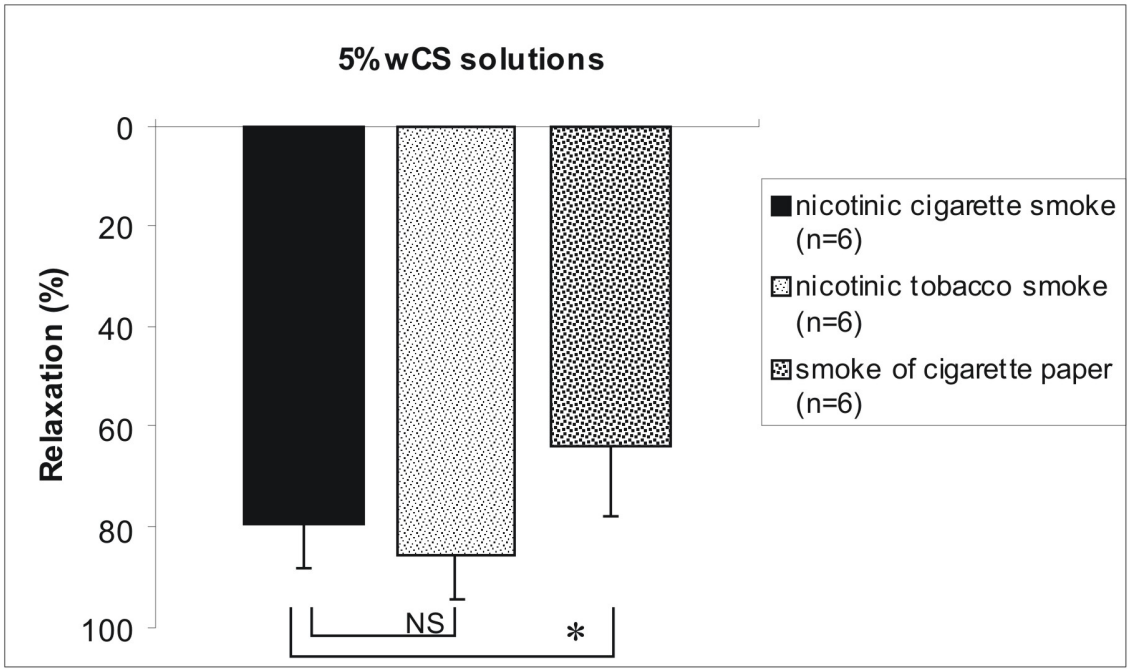

Figure 3.2.5.

Relaxation of rat renal arteries to wCS of nicotinic *tobacco* smoke or cigarette *paper* compared to nicotinic cigarette smoke (n=6).

NS, not significant; *denotes a significant difference, P<0.05.

Relaxation of isolated renal arteries by adding wCS into the chambers was still present after removal of the endothelium (***Figure 3.2.6.***). We also found that relaxation of vessels to nicotinic wCS was not significantly affected by prior incubation of the vessels with agents interfering with known mechanisms of smooth muscle relaxation, such as oxadiazolo-quinoxalin-1, tetraethylammonium or glibenclamide (***Figure 3.2.6.***).

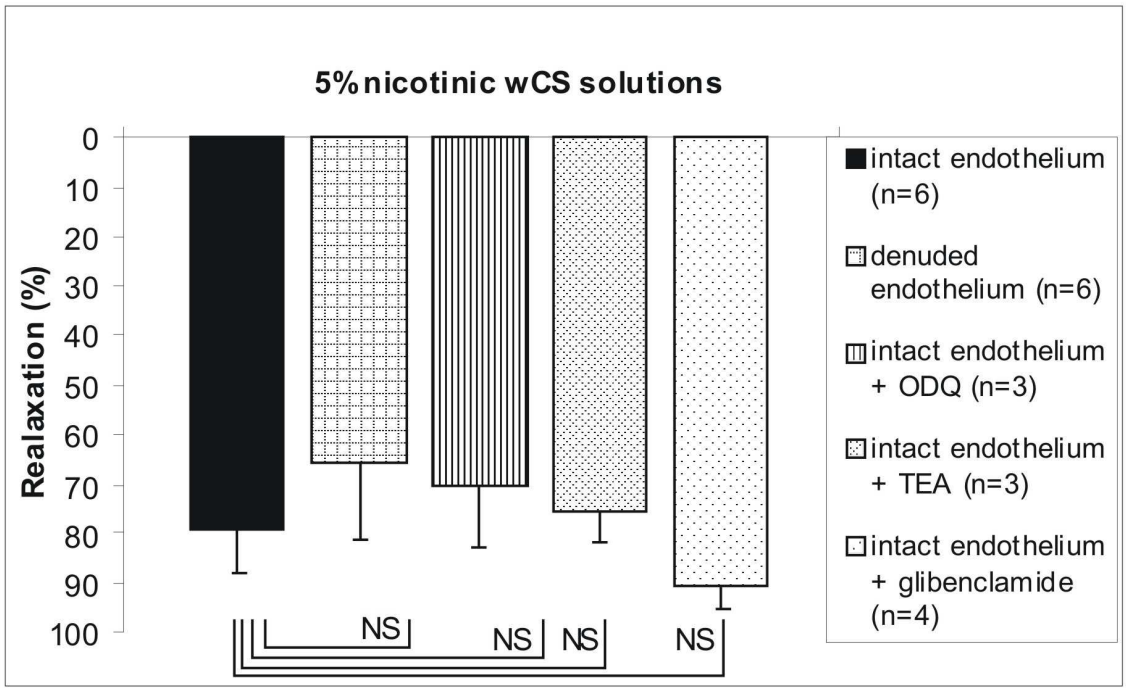

Figure 3.2.6.

Relaxation of renal arteries with intact (n=6) or removed (n=6) endothelium to water-soluble components of nicotinic cigarette smoke (nicotinic wCS), and the effect of oxadiazolo-quinoxalin-1 (n=3), tetraethylammonium (n=3) or glibenclamide (n=4) on the relaxation of intact renal arteries caused by nicotinic wCS.

Abbreviations are: ODQ, oxadiazolo-quinoxalin-1; TEA, tetraethylammonium; NS, not significant.

In contrast, the hydrogen peroxide-scavenger catalase significantly diminished the relaxation of renal arteries caused by nicotinic wCS (***Figure 3.2.7.***), whereas superoxide dismutase (SOD) significantly enhanced relaxation (***Figure 3.2.7.***). Also, presence of reduced glutathione (GSH) significantly diminished the relaxation of vessels to nicotinic wCS (***Figure 3.2.7.***).

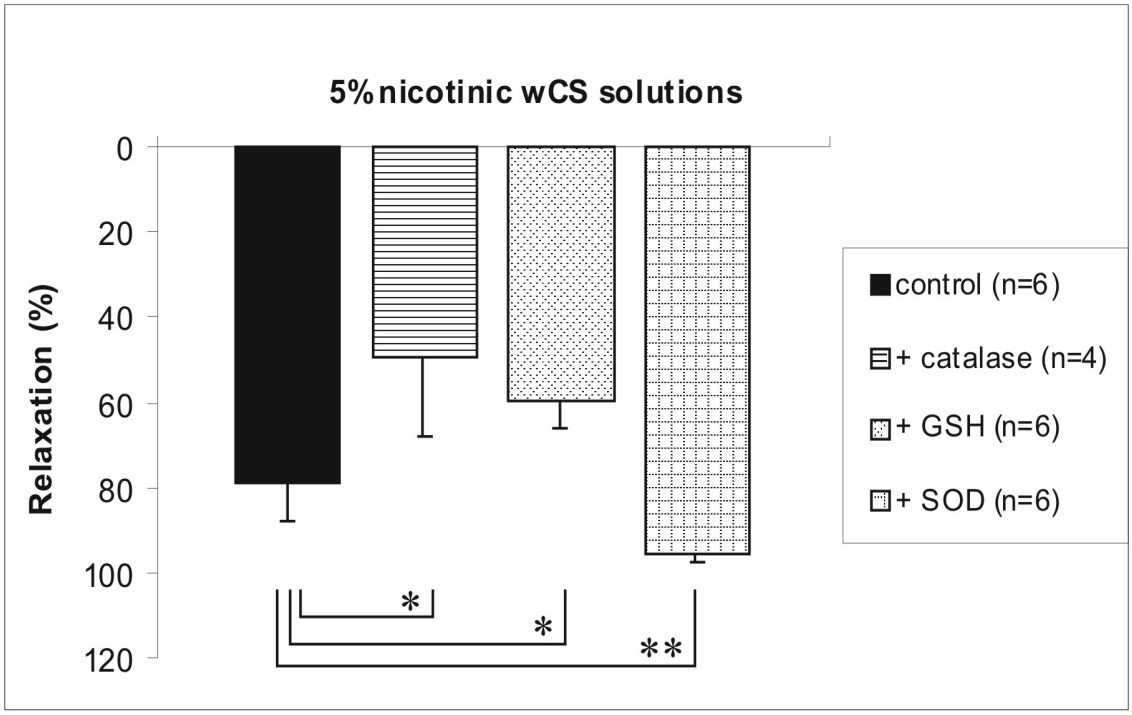

Figure 3.2.7.

Effect of the free radical scavengers catalase (n=4), reduced glutathione (n=6) or superoxide dismutase (n=6) on the relaxation of rat renal arteries caused by nicotinic wCS.

Abbreviations are: GSH, reduced glutathione; SOD, superoxide dismutase; *denotes a significant difference, $P<0.05$.

Depolarizing Krebs solution caused a transient contraction of rat renal arteries, which was reduced by nicotinic wCS (5%). It is noteworthy, that the reduction was not different compared to the effects of the L-type calcium channel blocker nifedipine (***Figure 3.2.8.***).

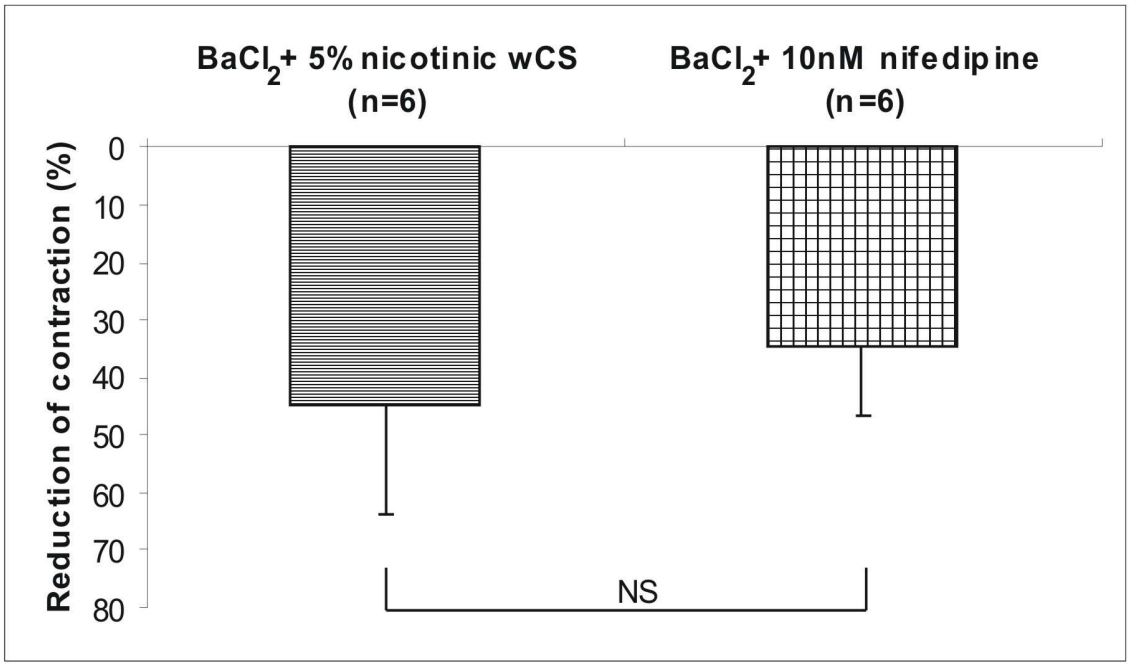

Figure 3.2.8.

Effect of water-soluble components of nicotinic cigarette smoke (nicotinic wCS, n=6) or nifedipine (n=6) on $BaCl_2$ induced contraction in renal arteries.

Abbreviations are: $BaCl_2$, barium-chloride; NS, not significant.

Use of a Na^{+}-Ca^{2+} exchanger activator Krebs solution also elicited a transient contraction in rat renal vessels. The Na^{+}-Ca^{2+} channel blocker SEA0400 reduced this contraction, similar to nicotinic wCS (1%) (***Figure 3.2.9.***).

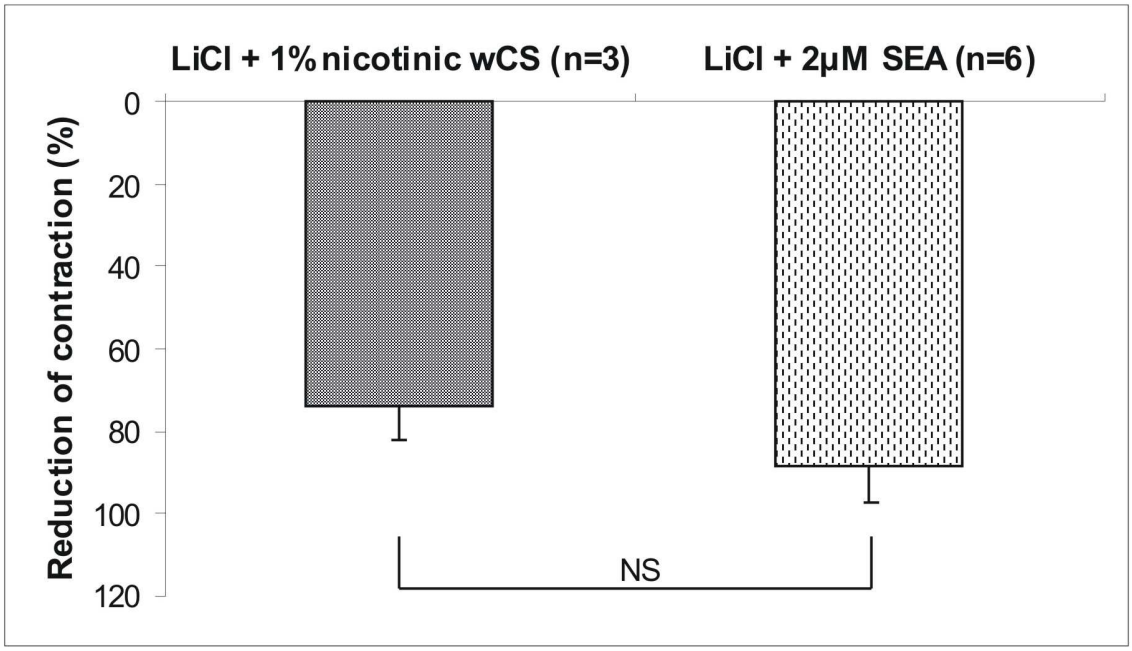

Figure 3.2.9.

Effect of nicotinic wCS (n=3) or SEA0400 (n=6) on LiCl induced contraction in renal arteries.

Abbreviations are: SEA0400, a specific inhibitor of the Na^{+}-Ca^{2+} exchanger; LiCl, lithium-chloride; NS, not significant.

3.3. Retrospective clinical study to investigate the role of chronic cigarette smoking in the development of diabetic nodular glomerulosclerosis (Kimmelstiel-Wilson lesion).

In this study we reviewed our clinic's native renal biopsy databank between 2001 and 2011 and we formed three groups of male patients: (i) all men with type 2 diabetes mellitus and Kimmelstiel-Wilson (KW) lesion; (ii) all diabetic men with non-KW diabetic nephropathy (non-KW); and (iii) all male patients without any carbohydrate metabolic disorder but with non-diabetic nodular glomerulosclerosis (non-diab NGS). Afterwards we compared among these groups the smoking habits and the retrospective available clinical data that have an impact on the development of diabetic nephropathy and nodular glomerulosclerosis.

3.3.1. Subjects and methods

All native renal biopsy specimens of male patients (n=644) available in the Renal Pathology Laboratory at the 2nd Department of Internal Medicine and Nephrological Center of the University of Pécs from 2001 to 2011 were reviewed retrospectively. The pathologist who established the diagnosis was blinded to the patients' smoking history. Type 2 diabetic patients with either KW lesion (***Figure 3.3.1.b***) or other classes of diabetic nephropathy and patients with non-diab NGS were further analyzed. All renal biopsy specimens were examined at once using a dissecting microscope. The overall 15±8 glomeruli obtained in accordance with existing research evidence [120] ascertained a sample adequate for examination by light microscopy, immunofluorescence, and electron microscopy. Type 2 diabetes mellitus and KW were defined following the literature by: (i) the evidence of diabetes mellitus [72], (ii) the histological finding of nodular mesangial sclerosis in at least one glomerulus (without global glomerulosclerosis in more than 50% of the glomeruli) [112, 121], and (iii) the exclusion of chronic membranoproliferative

glomerulonephritis, chronic thrombotic microangiopathy, amyloidosis, monoclonal immunoglobulin deposition disease, fibrillary glomerulonephritis, and immunotactoid glomerulopathy [122]. In the non-KW group, there were type 2 diabetics who met the criteria both of diabetes mellitus and diabetic nephropathy [112], not including KW lesion. The non-diab NGS group represented men with NGS but without any carbohydrate metabolic disorder proven by an oral glucose tolerance test, HBA_{1c} or fasting plasma glucose. Moreover, during a follow-up of between two and nine years after renal biopsy, none of these patients developed diabetes mellitus. Apart from a thorough review of the histological descriptions, factors potentially responsible for either the worsening of diabetic nephropathy or the development of NGS were also analyzed. These recorded clinical settings (age, body mass index (BMI), duration of diabetes mellitus, prevalence of hypertension (RR>140/90Hgmm), duration of hypertension, percentage of renin-angiotensin-aldosterone system (RAAS) blocker treatment (angiotensin-converting-enzyme inhibitor, angiotensin receptor blocker or spironolactone), serum cholesterol, serum triglyceride, glycemic control (HbA_{1c}), and estimated glomerular filtration rate (eGFR) (MDRD-175 formula) at the time of kidney biopsy were compared between the groups. The smoking habits of the different groups were analyzed by thorough revision of the patients' medical documents and, in order to acquire a deeper evaluation, a standardized telephone questionnaire was also performed. Both diabetic and non-diabetic patients were divided into two groups: ever-smokers and non-smokers. Cigarette consumption was expressed in pack-years (1 pack-year = consumption of 20 cigarettes / day for 1 year).

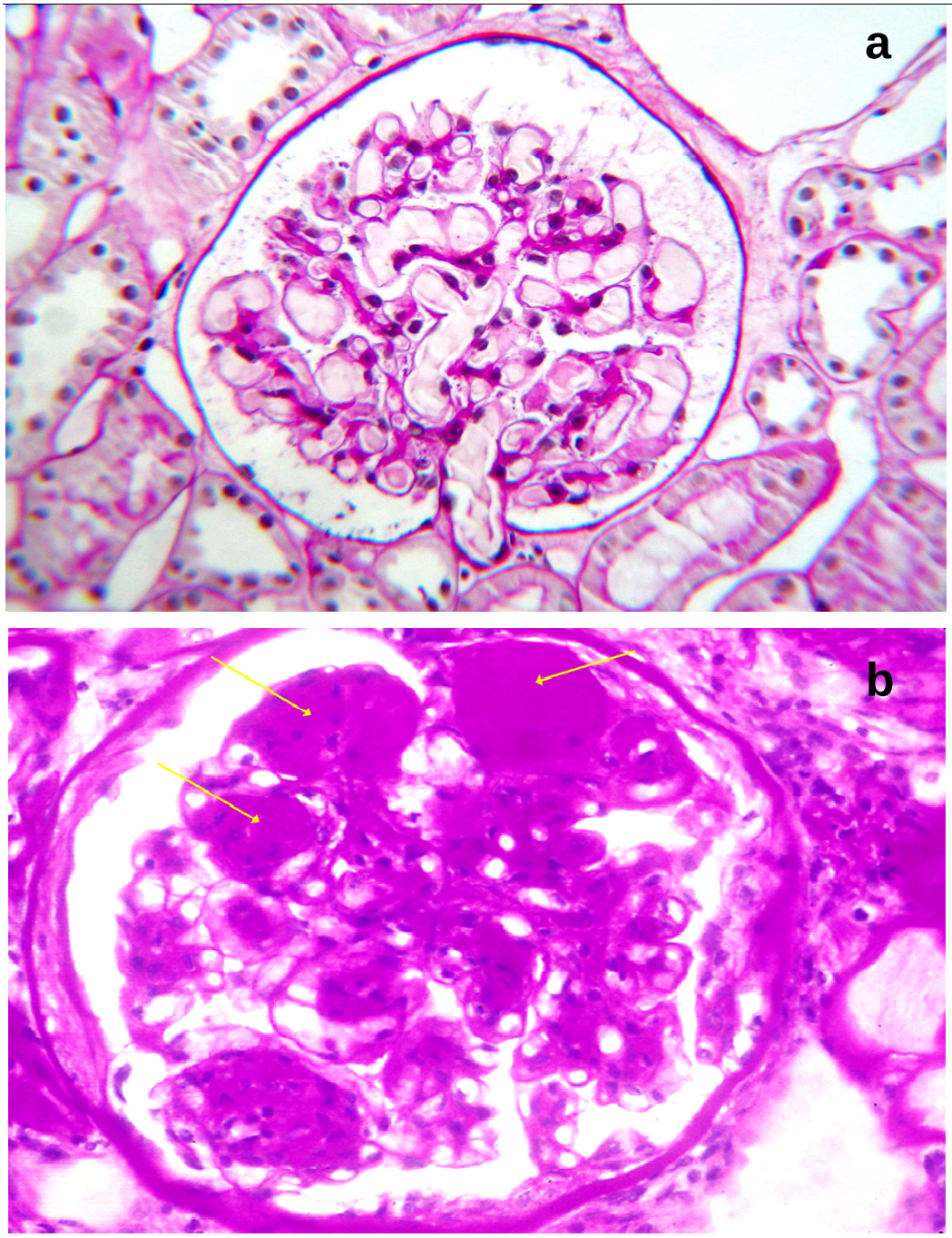

Figure 3.3.1. **Histology pattern of Kimmelstiel Wilson lesion**

(a) normal glomerulus; **(b)** glomerulus with KW lesion; arrows indicate nodular sclerotic lesions; periodic acid Schiff reaction; 400x magnification; with permission from the histology database of Peter Degrell MD. PhD.

Statistics

Statistical significance was calculated by using analysis of variance (ANOVA), chi square or Kruskal-Wallis tests as appropriate. Multiple group comparisons were carried out using ANOVA and Bonferroni's post hoc tests. All distributions were normal, with the exception of serum cholesterol, triglyceride, eGFR and pack-years. Data are expressed as mean ± SD. The tests were performed using the SPSS program package, Version 17.0 (SPSS, Chicago, IL, USA), considering P values of 0.05 or less to be significant.

3.3.2. Results: retrospective clinical case – control study

Among male patients who underwent a native kidney biopsy in the period between 2001 and 2011 in our nephrological center (n=644), the proportion of type 2 diabetic men with diabetic nephropathy was 9.5% (n=61), 2.3% (n=15) with KW and 7.2% (n=46) with non-KW. The percentage of non-diab NGS was 1.1% (n=7), including two patients (0.3%) with ING and five (0.8%) with non-diab NGS of different known origins (***Figure 3.3.2.***).

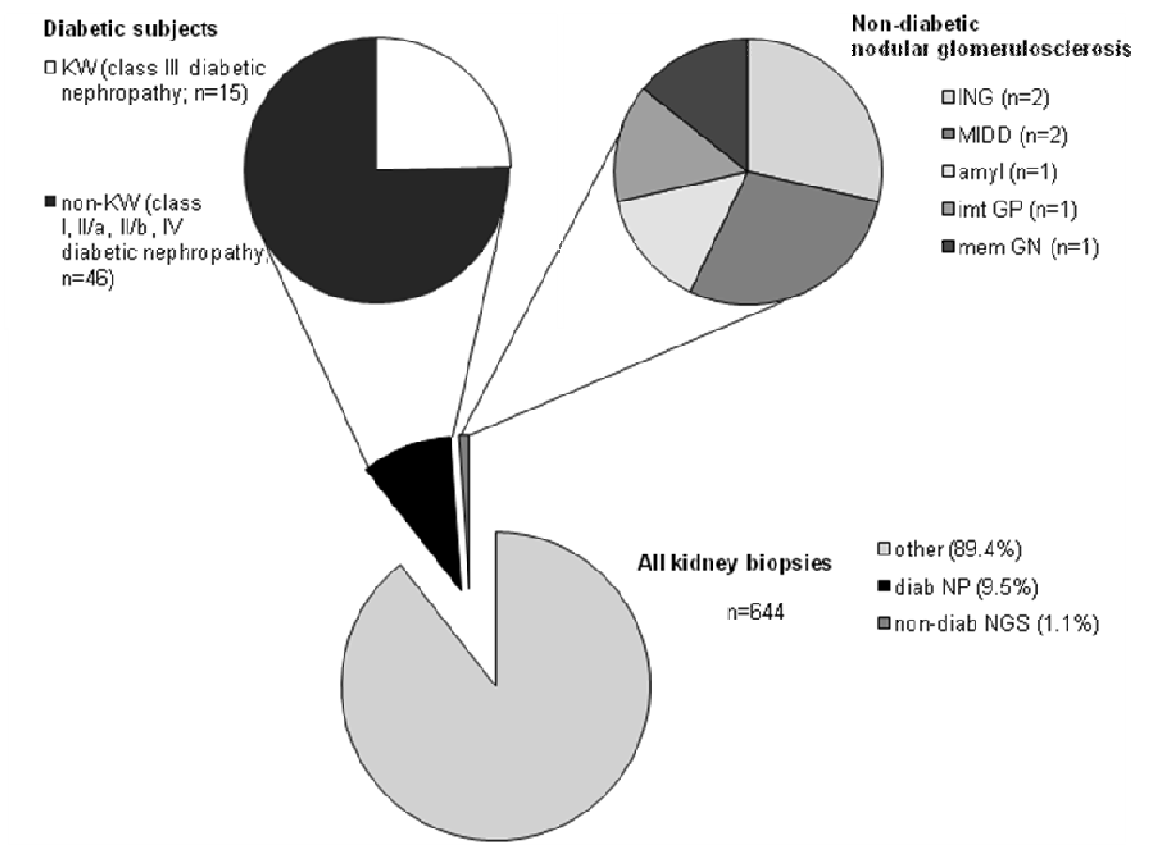

Figure 3.3.2.

Distribution of diagnoses among 644 male patients' kidney biopsy samples in a single center between 2001 and 2011.

Abbreviations are: diab NP, diabetic nephropathy in type 2 diabetic men; non-diab NGS, non-diabetic nodular glomerulosclerosis in males; KW, Kimmelstiel-Wilson lesion (class III diabetic nephropathy); non-KW, non-KW diabetic nephropathy (class I, II/a, II/b, IV diabetic nephropathy); ING, idiopathic nodular glomerulosclerosis; MIDD, monoclonal immunoglobulin deposition disease; amyl, amyloidosis; imt GP, immunotactoid glomerulopathy; mem GN, membranoproliferative glomerulonephritis.

The indications for renal biopsy in the three patient groups were various and all of the following indications were present in each group: nephrotic syndrome, nephritic syndrome, isolated proteinuria, hematuria glomerular type, azotemia, or their combinations, and acute renal failure. Members of all three groups (KW, non-KW, non-diab NGS) did not differ from each other in several parameters: they were middle-aged (mean age: 56±1, 56±9, 55±1 years, respectively; p=0.935), obese (BMI: 30±5, 31±5, 28±6 kg/m^2; p=0.538) patients with hypercholesterolaemia (5.2 {4.0-6.3}, 5.5 {4.3-7.2}, 5.7 {4.7-9.9} mmol/l; p=0.500), hypertriglyceridaemia (2.0 {1.4-2.7}, 2.4 {1.6-3.1}, 3.4 {1.1-3.6} mmol/l; p=0.784), impaired renal function (eGFR, 25 {13-48}, 42 {20-70}, 42 {23-77} ml/min; p=0.483), high prevalence of hypertension (93, 87, 86%; p=0.782), and a long duration of hypertension (11±8, 13±10, 11±6 years; p=0.948; ***Table 3.3.1.***).

Table 3.3.1.

Clinical and laboratory findings in patients with Kimmelstiel-Wilson lesion, non-KW diabetic nephropathy and non-diabetic nodular glomerulosclerosis

	KW (n=15)	non-KW (n= 46)	non-diab NGS (n=7)	P
Age (years)	56 ± 1	56 ± 9	55 ± 1	0.935
BMI (kg/m^2)	30 ± 5	31 ± 5	28 ± 6	0.538
HTNprev (%)	93	87	86	0.782
HTNdur (years)	11 ± 8	13 ± 10	11 ± 6	0.948
chol (mmol/l)	5.2 (4.0-6.3)	5.5 (4.3-7.2)	5.7 (4.7-9.9)	0.500
tri (mmol/l)	2.0 (1.4-2.7)	2.4 (1.6-3.1)	3.4 (1.1-3.6)	0.784
eGFR (ml/min)	25 (13-48)	42 (20-70)	42 (23-77)	0.483
proteinuria (g/day)	3.7 ± 1.7	2.3 ± 1.6	5.8 ± 4.2	*
RAAS-blocker (%)	100	87	100	0.222

Abbreviations are: KW, Kimmelstiel-Wilson lesion; non-KW, non-KW diabetic nephropathy; non-diab NGS, non-diabetic nodular glomerulosclerosis; HTNprev, prevalence of hypertension; HTNdur, duration of hypertension; chol, serum cholesterol; tri, serum triglyceride; eGFR, estimated glomerular filtration rate (MDRD-175 formula); RAAS-blocker, renin-angiotensin-aldosterone system-blocker treatment. * for the logarithmic values, Bonferroni: KW vs. non-KW, p = 0.153; non-KW vs. non-diab NGS, p = 0.042; KW vs. non-diab NGS p = 1.0.

The initial proteinuria was high in all the three groups (***Table 3.3.1.***). There was a similarity in the KW and non-diab NGS groups (KW: 3.7±1.7 g/day; non-diab NGS: 5.8±4.2g/day; p=1.0 {for the logarithmic values, Bonferroni}) and it was somewhat lower in the non-KW group (non-KW: 2.3±1.6g/day). There was no statistical difference in the initial proteinuria between the KW and non-KW groups (p=0.153 {for the logarithmic values; Bonferroni}). The difference only just reached significance between the non-KW and non-diab NGS groups (p=0.042 {for the logarithmic values, Bonferroni}). The percentage of RAAS-blocker treatment, as a confounding factor both in the course of diabetic nephropathy [123] and non-diab NGS [50], did not differ significantly among the three investigated groups (KW: 100, non-KW: 87, non-diab NGS: 100%; p=0.222; ***Table 3.3.1.***). Between the two diabetic groups, the duration of diabetes (KW: 11±6, non-KW: 10±7 years; p=0.617) and glycemic control (HbA_{1c}: 6.5±1, 6.0 ±1%; p=0.88) was similar (***Table 3.3.2.***).

Table 3.3.2.

Clinical and laboratory findings in patients with Kimmelstiel-Wilson lesion and non-KW diabetic nephropathy

	KW (n=15)	non-KW (n=46)	P
DM duration (years)	11 ± 6	10 ± 7	0.617
HbA_{1c} (%)	6.5 ± 1	6.0 ± 1	0.88

Abbreviations are: DM duration, duration of diabetes mellitus; HbA_{1c}, hemoglobin A_{1c}.

However, among type 2 diabetic men with KW, the majority (13/15= 87%) were smokers, unlike the non-KW group (16/46= 35%; p=0.001 vs. KW) but similar to the non-diab NGS group (7/7= 100%; p=1.0 vs. KW; ***Figure 3.3.3.***).

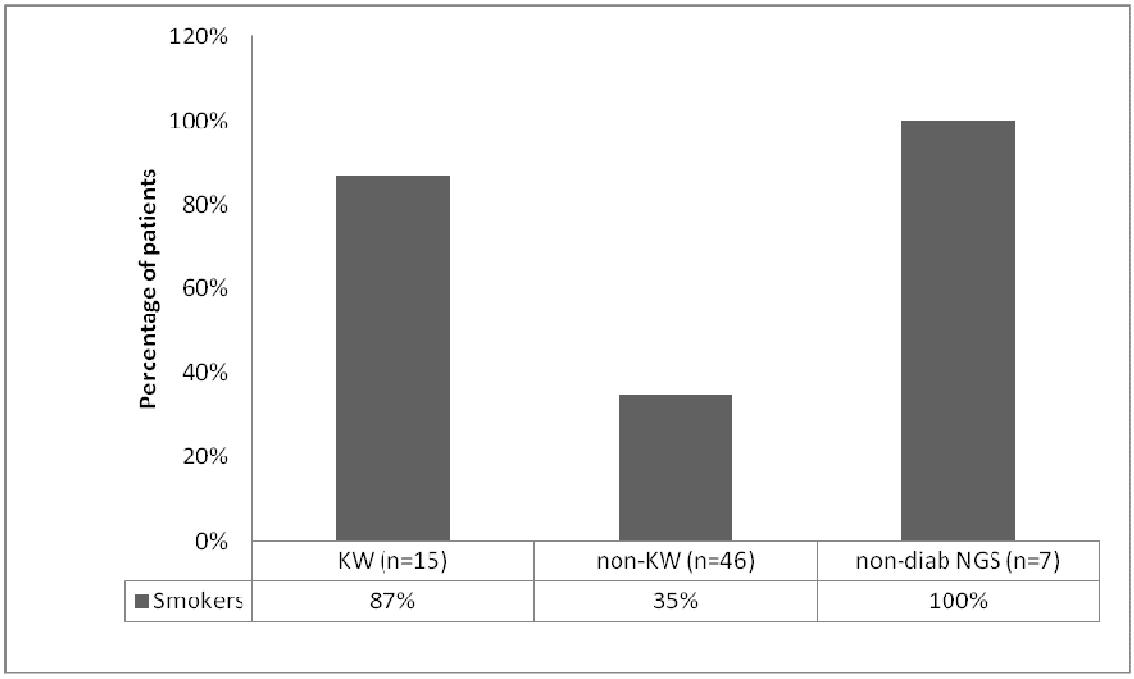

Figure 3.3.3.

Prevalence of smokers among patients with KW, non-KW and non-diab NGS

Abbreviations are: KW, Kimmelstiel-Wilson lesion; non-KW, non-KW diabetic nephropathy; non-diab NGS, non-diabetic nodular glomerulosclerosis. Chi square test (KW vs. non-KW), p=0.001; (KW vs. non-diab NGS), p=1.0.

Cigarette consumption also differed among the groups and showed the same pattern as smoking prevalence (KW: 15 {6-30} pack-years; non-KW: 0 {0-21} pack-years; non-diab NGS: 30 {16-33} pack-years; p=0.010 non-KW vs. KW; p=0.008 non-KW vs. non-diab NGS). Moreover, there was no difference in cigarette consumption between the two groups with NGS (p=0.185).

DISCUSSION

Large epidemiological cross-sectional studies (IRSA, PREVEND) [30, 124, 125] have shown that in the general population there is a higher glomerular filtration rate (GFR) and an elevated risk for albuminuria or proteinuria in smokers compared to the non-smokers. Although in the first analysis of the “IRSA” study lower systemic blood pressure was described among current smokers compared to non-smokers [30], a subsequent analysis in men revealed a higher risk of hypertension in smokers [31]. However, in otherwise healthy individuals smoking a single cigarette causes a transient elevation in blood pressure [29] and a reduction in GFR [126], while the change in the normal range of albuminuria is not known [126]. It was also suggested that glomerular pressure during smoking may be increased due to acute elevation of systemic blood pressure and changes of the renal autoregulation [127].

The salient findings of the present *in vivo* investigation of the acute effects of cigarette smoke on *human renal arteries* and *ex vivo* investigation of the acute effects of cigarette smoke on *rat renal arteries* are two-fold. 1) In healthy individuals smoking of a nicotinic or a nicotine free cigarette causes a transient reduction in the resistance index of the segmental renal arteries. 2) Water-soluble components of nicotinic or nicotine free cigarette smoke elicit dose-dependent relaxations of rat renal arteries. These effects were not affected by removal of the endothelium, inhibition of the soluble guanylate cyclase, blocking of large-conductance calcium-activated potassium channels or ATP-sensitive potassium channels. However, the relaxations were reduced by catalase and enhanced by superoxide dismutase.

Previous studies have shown that human airway blood flow is transiently increased after smoking a cigarette [128]. Also, Doppler ultrasound revealed an increased diameter of the portal vein during smoking [129]. Based on these

findings, we hypothesized that wCS cause relaxation of renal vessels, which together with the elevated mean arterial pressure during smoking and both endothelial cell injury and endothelial dysfunction [118,130,131] could elicit hyperfiltration and increased albuminuria in smokers [30,124,125]. Accordingly, in humans we studied resistance indices (RIs) of segmental renal arteries during smoking, known to reflect alterations in vessel diameter [132]. The potential influence of the parasympatho-mimetic activation due to deeper inhalations was excluded by sham smoking. It is known that tachycardia can lower the RI; however, our data shows that the non-nicotinic cigarette caused similar reductions in RI as the nicotinic cigarette did, without elevation in heart rate. In the present study, we provided clear evidence for a transient decrease in RI during cigarette smoking, which is indicative of dilation of the examined segmental renal arteries and/or more distal renal resistance vessels. The results of the present study are different from the findings of Ritz et al., where smoking caused an acute increase in renal vascular resistance and a fall in GFR [126]. This could be due to the difference in the study design, e.g. in the former study [126] there were both female and male volunteers, with a limited chronic cigarette consumption (10cig./day), whereas we studied only male volunteers with a history of more severe cigarette consumption (17.7±6.9cig./day). In addition, because of the different measurement techniques, the time intervals where the effects of smoking were studied were also different. Despite the fact, that former studies found an age- and gender-related difference in the functional and pathological detrimental renal effects of smoking in the general population [30, 133], a recent collaborative analysis of 57 prospective studies revealed that cigarette smoking doubles all-cause mortality included renal mortality in both genders [134]. Beyond the clear different hormonal status before menopause, the milder and shorter cigarette consumption of females in the past [135] can partly explain these different findings.

Investigation of isolated rat renal arteries showed dose-dependent relaxations in response to water-soluble components of cigarette smoke. Nicotine, the most

investigated component of cigarette smoke [52] is unlikely to be responsible for the observed response, since wCS of nicotine free cigarettes induced similar relaxations of vessels compared to wCS of nicotinic cigarettes. We have also found that nicotine free cigarettes did not elicit significant alterations in systemic BP and HR, findings which are consistent with the literature [136]. We could also exclude the potential contribution of carbon monoxide (CO) because inhibition of soluble guanylate cyclase or large-conductance calcium-activated potassium channels – the main targets of CO [137,138] – did not affect relaxation of vessels to wCS. In addition, previous human studies have shown that CO levels remain high even 30 minutes after smoking a single cigarette [136], whereas dilation of renal vessels occurred early and transiently (~5 min). Also, because removal of the endothelium or inhibition of the soluble guanylate cyclase by ODQ (5 μM) did not influence significantly the relaxation of vessels to cigarette smoke we could exclude the contribution of nitric oxide produced by endothelial nitric oxide synthase, as well.

It is known that free radicals found either in cigarette smoke or generated in the vascular tissue can modulate vascular tone [139,140]. Hydrogen peroxide can induce dilation via endothelial and smooth muscle-dependent mechanisms [141]. Incubation of rat carotid arteries with wCS for 6 hours increased vascular production of hydrogen peroxide [139]. Others found that wCS acutely elicited a biphasic vasomotor response on isolated precontracted porcine coronary arteries [142]. In these vessels an initial weak contraction was followed by a marked dose-dependent relaxation. The latter was augmented by the pre-incubation of vessels with SOD [142]. In endothelium–denuded arterioles of rat skeletal muscle, exogenous hydrogen peroxide at $6x10^{-5}$ - $3x10^{-4}$M also elicited a biphasic response [143]. In the present study, renal arteries were incubated with 1-10% solutions of wCS, which is similar to the concentrations used by others [139,142]. We found that the hydrogen peroxide scavenger catalase significantly diminished smoke-induced renal artery relaxation, whereas SOD, which dismutates superoxide to hydrogen peroxide, enhanced the relaxation. Thus, relaxation of renal arteries by

wCS can be – at least in part – mediated by hydrogen peroxide present in the wCS-containing bath solution and/or produced by SOD in the vessel wall. Moreover, our findings that relaxations were present after removal of the endothelium suggest that primarily smooth muscle dependent mechanisms are involved. Reactive oxygen species are known to regulate ion channels and transporters [144]. For example, thiol oxidation has been reported to activate a redox-regulated vasodilator mechanism involving inhibition of Ca^{2+} influx through L-type calcium channels in coronary arteries [144]. In the present study, GSH (an inhibitor of thiol oxidation) diminished the relaxation caused by wCS.

It is known that barium ions block potassium channels [145] and induce smooth muscle depolarization with increased cation influx through voltage-dependent $Ca_v1.2$ L-type calcium channels [145]. Accordingly, we observed that renal artery contractions induced by $BaCl_2$ were reduced by the $Ca_v1.2$ L-type calcium channel blocker nifedipine. Noteworthy, we observed similar inhibitory effects by 5% solution of wCS. The striking similarity of inhibition of barium-induced contractions by wCS and nifedipine suggest that voltage-dependent $Ca_v1.2$ calcium channels might be involved in the action of wCS. These data support the idea that inhibition of $Ca_v1.2$ L-type calcium channels occurs *via* a redox-regulated mechanism.

A modified isotonic Krebs buffer by replacing NaCl with equimolar LiCl causes a transient contraction in smooth muscle *via* Ca^{2+} influx through the Na^+-Ca^{2+} exchanger [146]. In the present experiments, SEA0400 - a specific blocker of the Na^+-Ca^{2+} exchanger [147] - elicited a reduction of Li^+-induced contraction to a similar extent as 1% solution of wCS, suggesting that Na^+-Ca^{2+} exchange mechanism may also play a role in the vasomotor action of cigarette smoke. It is of note that H_2O_2 induces oxidative modification of thiols on the Na^+-Ca^{2+} exchanger in cardiomyocytes [148].

Taken together, we suggest that hydrogen peroxide present in water-soluble components of cigarette smoke and/or formed from superoxide anion in the vessel wall could, in part, explain the relaxation of rat renal arteries to wCS, a mechanism,

which may also operate in renal vessels of humans. It is also likely that $Ca_v1.2$ L-type calcium channels and the Na^+-Ca^{2+} exchanger are also involved in smoke-induced vasomotor response, but further investigations are needed to substantiate their role.

The relevance of the results and facts of our human study and animal experiments are supported by several epidemiological studies. These were performed on healthy individuals and confirm the relationship between chronic cigarette smoking and both the initiation and the progression of chronic kidney disease in the general population. The acute detrimental effects of cigarette smoke i.e. on renal hemodynamics mean a 5/ 10/ 40 times repetitive harm for the kidneys in a smoker who smokes 5/ 10/ 40 cigarettes a day.

There are more and more evidences accumulating in the field of the connection between cigarette smoking and CKD. Cigarette smoking is a risk factor for chronic kidney disease [149] and a predictor for the risk of end-stage kidney failure in the general population both in women and men [150]. A community-based, prospective observational study of 20 years duration in 23,534 men and women in Washington County, Maryland revealed a significant association between current cigarette smoking and the risk of CKD in both women and men (in women hazard ratio [HR] 2.9; 95% confidence interval [CI]: 1.7 - 5.0; and in men HR 2.4; 95% CI: 1.5 - 4.0) [149]. A prospective open cohort study using general practitioners databases with the goal of developing risk algorithms for estimating the individual 5-year risk of moderate-severe CKD and end-stage kidney failure in a primary care population (775,091 women and 799,658 men aged 35-74 years, contribution of 4,068,643 and 4,121,926 person-years of observation respectively) involved cigarette smoking as one of the main factors in the models [150]. A comprehensive review showed an overall evidence for current smoking as a risk factor for incident chronic kidney disease [151]. An increased risk of developing chronic kidney disease among smokers was significantly associated with male gender (relative risk [RR] 2.4, 95% CI: 1.2-4.5), >20 cigarettes smoked per day (odds ratio [OR] 1.51, 95%

CI: 1.06-2.15, and relative risk 2.3, 95% CI 1.2-4.3), and smoking >40 years (OR 1.45, 95% CI: 1.00-2.09) [151]. In the Chronic Renal Insufficiency Cohort (CRIC) Study (3,612 participants, 46% women, 47% diabetics) among others, previous cigarette exposure was associated with lower eGFR [152]. Results from the Italian Longitudinal study of Aging (ILSA) revealed that during a 3.6 years follow-up of 2,981 subjects, aged 65-84 years, heavy current smoking (> 20 cigarettes/day) showed to be a risk factor for pathological loss of renal function (OR 2.3, 95% CI: 1.0-5.3) in a multiple logistic regression analysis model [153]. The third National Health and Nutration Examination Survey – a cross-sectional analysis of 15,719 adults - in the US revealed an association between cigarette smoking and albuminuria [154]. Current smoking was more common in persons with albuminuria (26%) compared to normal albumin-to-creatinine ratio (21%), and after adjusting for other risk factors, among hypertensives, current smokers were 1.85 (95% CI: 1.29 - 2.64) times more likely to have albuminuria than never smokers [154], moreover current smokers with more than 40 pack-years were at highest risk for albuminuria. The role of passive smoking was also highlighted, among non-smoking hypertensives, those exposed to passive smoke (highest vs. lowest quartile of serum cotinine) were 1.41 (95% CI: 1.04 - 1.90) times more likely to have albuminuria, and surprisingly the association between tobacco use and albuminuria disappeared in former smokers among hypertensives if they stopped smoking for at least 1 year [154]. An age-associated decline in renal function is more marked in patients with co-existent cardiovascular risk factors, among these smoking seems to have an important role in the detrimental effect on renal function also in individuals without co-presence of other cardiovascular risk factors or renal diseases [155]. In the cross sectional PREVEND (Prevention of REnal and Vascular ENd stage Disease) study with 7,476 participants, compared to non-smokers, current smokers had higher median albumin excretion, and were more likely to have microalbuminuria and high-normal albuminuria with either elevated or decreased GFR, all differences showed a dose-dependent manner (above or

below than 20cigarettes/day) [156]. In an analysis of 12,866 randomly assigned men of the The Multiple Risk Factor Intervention Trial (MRFIT) current smoking had an adjusted hazard ratio of 1.84 (95%CI: 1.35 - 2.51) for end-stage renal disease after 25 years [157]. A 10-year follow-up study with 123,764 (male: 41,012, female: 82,752) adults aged 40 years and over showed that smoking was a predictor of CKD in both genders (RR for CKD stages 3 and 4 1.13 and 1.16, respectively) [158]. In a Norwegian population-based cross-sectional study involving 30,485 men and 34,708 women a significant, dose-dependent elevation in risk for CKD (GFR<45ml/min per 1.73 m^2) was found above a cumulative lifetime exposure of 25 pack-years (adjusted RR 1.42 for 25 to 49 pack-years and 2.05 for >50 pack-years, respectively) [159]. The results of these studies - summerized in ***Table D.1.*** – also suggest a great benefit when a healthy subject or a kidney patient quits smoking.

Table D.1. **Smoking as a risk factor for initiation and progression of chronic kidney disease in the general population**

Author	number of patients	study design	follow-up (years)	primary end-point	hazard ratio/ relative risk/odds ratio (95%CI)	conclusion
Haroun et al.	23,534 women and men	po*	20	kidney disease/ end-stage renal disease	HR 2.9 in women (CI:1.7-5)HR 2.4 in men (CI:1.5-4)	association between current smoking and risk of CKD
Hippisley-Cox et al.	777,091 women 799,658 men	poc*	5	moderate/ severe CKD, ESRF	-	smoking is a main risk factor for CKD
Jones-Burton et al.	74-157,377	cr*	2-18.5	incident CKD	OR 1.51[a] (CI:1.06-2.15)	>20 cigarettes smoked/day[a]
					RR 2.3[a] (CI: 1.2-4.3) OR 1.45[b] (CI:1.0-2.09)	&>40 years duration[b] are main risks for incident CKD
Lash et al.	3,612	pc*	8	progression of CKD	-	lower eGFR associated with previous smoking
Baggio et al.	2,981	l*	3.6	pathological increase of renal function >26.5 µmol/l of SCr	OR 2.3 (CI:1.0-5.3)	current smokers >20 cigarettes smoked per day have pathological rise in GFR
Hogan et al.	15,719rp*	cs*	-	risk of albuminuria in hypertensives &	OR 1.85 (CI:1.29-2.64)	current smokers have increased risk for albuminuria
				non-hyper-tensives	OR 1.41 (CI:1.04-1.90)	passive smoking increased risk for albuminuria

Table D.1. **(Continued)**

Author	number of patients	study design	follow-up (years)	primary end-point	hazard ratio/ relative risk/odds ratio (95%CI)	conclusion
Pinto-Sietsma et al.	7,476	cs*	-	association between smoking and albuminuria and abnormal renal function		smokers *vs* non-smokers assoc. between smoking albuminuria and altered GFR
				-high normal albuminuria	RR 1.33 *vs* (CI:1.1-1.61)	<20cigarettes/day
					RR 1.98 (CI:1.49-2.64)	>20cigarettes/day
				microalbuminuria		
					RR 1.92 *vs* (CI:1.54-2.39)	<20cigarettes/day
					RR 2.15 (CI:1.52-3.03)	>20cigarettes/day
				-elevated GFR		
					RR 1.82 *vs* (CI:1.31-2.53)	<20cigarettes/day
					RR 1.84 (CI:1.12-3.02)	>20cigarettes/day
				-decreased GFR		
					RR 1.53 *vs* (CI:1.04-2.24)	<20cigarettes/day
					RR 1.83 (CI:1.05-3.20)	>20cigarettes/day
Ishani et al.	12,866 men	it*	25	ESRD	HR 1.84 (CI:1.35-2.51)	elevated risk for ESRD in current smokers *vs* non-smokers
Yamagata et al.	123,764 gp*	fu*	10	development of CKD	HR 1.4[c] (CI:1.16-1.69) HR 1.26[d] (CI:1.14-1.41) HR 1.13[e] (CI:1.05-1.22) HR 1.16[f] (CI:1.06-1.26)	current smoking rised risk for CKD stage I/II *in women*[c] and *in men*[d] & elevated risk for CKD st. III/IV *in women*[e] and *in men*[f]
Hallan et al	30,485 men 34,708 women	pbcs*	-	risk for CKD	RR 1.52 (CI:1.13-2.06)	elevation in risk of CKD lifetime exposure of >25 pack-years

* Abbreviations: assoc, association; cr, comprehensive review; cs, cross-sectional; f, follow-up; gp, general population; it, intervention trial; l, longitudinal; pc, prospective cohort; po, prospective observational; poc, prospective open cohort; pbcs, population-based cross-sectional; rp, representative population.

The above summarized studies present a clear evidence for the association between cigarette smoking and CKD, because: (a) results for dose- and time-dependent detrimental effects of smoking on CKD are available; (b) both active and passive smoking are associated with CKD; (c) both gender are involved; (d) cigarette smoking is in connection with both altered GFR and elevated albuminuria; (e) tobacco consum rises the risk of all stages of CKD.

A very important “representative” cause of CKD is diabetic nephropathy, which leads similar to chronic cigarette smoking to an elevated risk for cardiovascular diseases and cardiovascular mortality. It is known that smoking causes insulin resistance - thus increases the risk of developing type 2 diabetes [10, 11] - and elevates the risk of the initiation of diabetes mellitus and metabolic syndrome [7-9]. However, it is not established yet if chronic cigarette smoking is only one risk factor for diabetic nephropathy or if it has a pivotal role in the development of certain stages of diabetic nephropathy.

The prevalence of diabetes, predominantly type 2, is increasing worldwide, and diabetic nephropathy is the leading cause of end-stage renal failure [160]. The basics of the clinical development and histological course of diabetic nephropathy are known [123], yet the exact pathogenesis remains uncertain. Kidney biopsies are relatively rarely performed in diabetic nephropathy, despite the fact that bleeding complications occur less frequently in diabetic nephropathy compared to other common kidney disorders [161]. In general practice, the biopsy procedure in diabetic nephropathy is only indicated if a non-diabetic renal disease is suspected [162, 163], and this situation probably occurs more often in type 2 than in type 1 diabetic patients [164]. A recent consensus of pathologic classification of diabetic nephropathy deals with Kimmelstiel-Wilson (KW) lesion [165] as a separate class (class III) [112]. The same histological pattern as that of KW lesion was also described in patients without the clinical evidence of diabetes mellitus [50, 166], and named “idiopathic nodular glomerulosclerosis” (ING). ING is an extremely rare and distinct clinicopathologic entity, which is linked to hypertension and chronic

cigarette smoking. The name “smoking-associated nodular glomerulosclerosis” also arose as a possibility for the entity [167] and is much more common in men than in women [168]. There are several factors, such as smoking, hypertension, obesity, hyperlipidaemia, and renal insufficiency, that can contribute both to the development of ING [166, 168] and to the progression of diabetic nephropathy [112, 169, 170]. However, among patients with KW, the exact prevalence of these conditions and altered laboratory parameters have yet to be described.

Bearing in mind the fact that the majority of patients suffering from ING are male, that there is a well documented difference in smoking habits between the two genders in Hungary (a prevalence of chronic smokers in males 38.3% and 23% in females) [171], and in order to ensure homogeneity, only men were investigated in the subsequent analysis. The aim was to systemically review our native kidney biopsy databank (n=644) and compare the smoking habits and other clinical settings within three groups: (i) men with type 2 diabetes mellitus and KW, (ii) men with non-KW diabetic nephropathy (non-KW), and (iii) male patients with non-diabetic nodular glomerulosclerosis (non-diab NGS). The last group included patients with ING, as an extreme rare entity [168], and patients with known, non-diabetic diseases associated with NGS.

In this single centre reptrospective study, we have described, for the first time, a very high prevalence of smokers (87%) among type 2 diabetic men with KW lesion in contrast to diabetic males with non-KW diabetic nephropathy of whom the prevalence of smoking was 35%. The latter is similar to the occurance of smoking in the general population [171]. Other risk factors responsible for the progression of diabetic nephropathy were common both in patients with KW and in type 2 diabetics with other classes of diabetic nephropathy; however, only the smoking habit differed clearly between the KW and non-KW groups. The link between chronic cigarette smoking and NGS was strengthened by the fact that, in our databank, we found 100% prevalence of smokers among patients with non-diab NGS; this group served as a positive control. Cigarette consumption was also

significantly more frequent in the two separate patient groups with NGS (KW and non-diab NGS) compared to the group without (non-KW). Interestingly, we have found chronic cigarette consumption not only in patients with ING, similar to the literature [50, 166, 168], but in all male patients with non-diab NGS of known origins, like monoclonal immunoglobulin deposition disease, amyloidosis, immunotactoid glomerulopathy and membranoproliferative glomerulonephritis. Several potential mechanisms arose for the explanation of smoking-induced kidney damage in diabetic nephropathy [169], and our study group strengthened the role of hyperfiltration previously reported [103]. It is known that cigarette smoking acutely elevates blood pressure [29], and recently we described, for the first time, that cigarette smoking also causes an acute relaxation of the renal arteries [103]. The chronic alteration of the filtration barrier, because of endothelial cell injury [118], endothelial dysfunction [130, 131] and podocyte damage due to smoking [172], and the repetitive elevation of intraglomerular pressure and hyperfiltration might all play an important role in the patomechanism of NGS. These processes together could lead to the hyperfiltration and increased albuminuria observed in smokers without diabetes [30, 124, 125] and with type 2 diabetes mellitus [114, 116]. We have found a parallelism between the severity of chronic cigarette consumption and the grade of proteinuria between the different groups. This suggests a role of tobacco consumption in the development of proteinuria and is in agreement with the literature [89, 90, 124]. Our previously published hypothesis of the continuity of the mesangial channel network with the glomerular basement membrane and intraglomerular subendothelial space [173] may add a new aspect to the pathophysiology of KW. Elevated intraglomerular pressure due to smoking could, in this way, directly affect the mesangial space and partly explain mesangiolysis, an important initial process in the development of nodular sclerotic lesions [112]. Our data suggest that, among the many progression promoters of diabetic nephropathy, chronic cigarette smoking could have a pivotal role in the development of KW lesion. This study has its limitations, as it is an observational

retrospective case-control study and cannot provide direct evidence for the causative role of chronic cigarette smoking in the development of KW lesion. Prospective cohort studies dealing with larger sample sizes should be carried out in order to confirm the present suggestions.

FUTURE PERSPECTIVES

In the future having tools to help the understanding of the pathomechanism and the detection of the stage of the kidney damage caused by cigarette smoking would be of great help. Specifically, for the detection of the probably initial step, e.g. hyperfiltration, not only measuring the glomerular filtration rate via creatinine clearance would be important, but perhaps pointing out of potential specific signs of the damage by ultrasound or specific biomarkers would be of great importance. Among members of the medical society a broader knowledge of the importance of tobacco consumption in the initiation and progression of renal diseases will hopefully come to a more succesfull motivation of patients both in the prevention and the cessation of cigarette smoking. It is worth to quit smoking in time, because in some cases, like idiopathic nodular glomerulosclerosis or in the renal transplant it is proven that quitting smoking reduces the rate of progression of renal failure [109], moreover, cessation of smoking alone may reduce the risk of progression in the decline of GFR by 30% also in patients with type-2 diabetes [9]. However, the role of smoking both in the development and progression of membranous nephropathy, FSGS or minimal change glomerulonephritis and the „point of no return” in other renal diseases are not established yet. Chronic kidney diseases and diabetes mellitus should be prevented, therefore intervention in the smoking habit - as a modifiable risk factor - should begin very early!

THESES

1) **In otherwise healthy male chronic smokers, cigarette smoking acutely reduces the resistance index (RI) of segmental renal arteries.**

2) **The reduction of the RI of segmental renal arteries due to cigarette smoke in healthy volunteers is transient and the process is not dependent on nicotine.**

3) **Water-soluble components of cigarette smoke – either nicotinic or nicotine free – elicit dose-dependent relaxations of rat isolated renal arteries.**

4) **The nicotinic cigarette smoke-induced relaxations of rat isolated renal arteries happen primarily due to smooth muscle dependent mechanisms and it is unlikely that either nitric oxide or carbon monoxide play a major role in this phenomenon.**

5) **The relaxations of rat renal arteries due to cigarette smoke are reduced by catalase and enhanced by superoxide dismutase, which suggest that cigarette smoke reduces the vasomotor tone of renal arteries in part via hydrogen peroxide, present in water-soluble components of cigarette smoke and/or formed from superoxide anion in the vessel wall.**

6) **It is likely that $Ca_v1.2$ L-type calcium channels and the Na^+-Ca^{2+} exchanger are also involved in smoke-induced vasomotor response of rat renal arteries.**

7) **Chronic cigarette smoking could play a pivotal role in the development of Kimmelstiel-Wilson lesions in type 2 diabetic men.**

PUBLICATIONS (in English) RELATED TO THE Ph.D.THESES:

Halmai R, Szijártó IA, Fehér E *et al*. Cigarette smoke elicits relaxation of renal arteries. *European Journal of Clinical Investigation.* 2011;41 (2):195-202. (impact factor (IF): 3.018)

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Cumulative impact factor of all publications:

full papers: 8.666; abstracts: 42.63

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ÖSSZEFOGLALÁS

Bevezetés és célkitűzés:

Epidemiológiai vizsgálatok alapján a dohányzás – valószínűleg hiperperfúzió előidézésével – a glomeruláris filtrációs ráta emelkedéséhez vezet. Ez alapján azt feltételeztük, hogy a cigarettafüst megváltoztathatja a veseerek tónusát. Első, *in* vivo human vizsgálatunk célja az volt, hogy nyomon kövessük a cigarettázás hemodinamikai paraméterekre és a veseerek tónusára kifejtett akut hatását. Második, *ex vivo* kísérletsorozatunk során a cigarettafüst vízoldékony komponenseinek patkány veseerekre kifejtett akut hatását elemeztük a lehetséges patomechanizmus feltárása céljából.

A noduláris glomeruloszklerózis (NGS) szövettani képe nemcsak diabéteszes betegeknél (Kimmelstiel-Wilson lézió; KW) hanem ritkán ugyan, de nem diabéteszes pácienseknél (non-diab NGS) is előfordulhat. Míg a krónikus dohányzás a nem diabéteszes noduláris glomeruloszklerózis egyik lehetséges oki tényezője, a Kimmelstiel-Wilson lézióval bíró cukorbetegek között a dohányzás gyakorisága nem ismert. Harmadik, *retrospektív klinikai vizsgálatunkban* a krónikus dohányzás Kimmelstiel-Wilson lézióban betöltött szerepét vizsgáltuk.

Betegek és módszerek:

Első vizsgálatunk során egészséges önkéntesekben ultrahang segítségével vizsgáltuk egy-egy kiválasztott szegmentális renális artéria rezisztencia indexét nikotin tartalmú, ill. nikotinmentes cigaretta elszívása előtt, alatt és után, valamint un. áldohányzás során. Ezzel párhuzamosan a szisztémás artériás középnyomás és a szívfrekvencia változását is nyomon követtük.

Második vizsgálatunkban a dohányfüst vízoldékony komponenseinek (Dvíz) izolált patkányveseartériák izometrikus feszülésére kifejtett hatását az alábbi körülmények között vizsgáltuk: (a) különböző koncentrációjú nikotinos Dvíz

alkalmazása; (b) nikotinmentes cigarettafüst hatása; (c) endotélmentes erek tesztelése; (d) különböző ioncsatornagátlók alkalmazása; (e) szabadgyökfogók alkalmazása.

Harmadik vizsgálatunk során adatbázisunkból a 2001 és 2011 között natív vesebiopszián átesett férfibetegeket (n=644) válogattuk ki, majd három csoportba soroltuk őket: diabéteszes nefropátiás betegek Kimmelstiel-Wilson lézióval (n=15), diabéteszes nefropátiás betegek Kimmelstiel-Wilson lézió nélkül (n=46), nem diabéteszes betegek noduláris glomeruloszklerózissal (n=7). Retrospektív elemzésünk során a három csoportban számos klinikai paramétert és a betegek dohányzási szokásait hasonlítottuk össze.

Eredmények

Első vizsgálatunkban egészséges önkéntesekben mind a nikotin tartalmú, mind a nikotinmentes cigaretta a szegmentális renális artériák rezisztencia indexének átmeneti csökkenését eredményezte (a kiindulási érték 83.25±5.67%-ára, $P<0.05$). A nikotin tartalmú cigaretta elszívása a szisztémás artériás középnyomásban és a szívfrekvenciában egyaránt átmeneti emelkedéshez vezetett.

Második vizsgálatunk során az állatkísérletes modellben mind a nikotin tartalmú-, mind a nikotinmentes cigarettafüst vízoldékony komponensei a patkányveseartériákon dózisfüggő relaxációt okoztak (1% -os nikotin tartalmú dohányfüstpuffer: 41,18 ± 14,86%-os relaxáció; 5%-os puffer: 79,28 ± 8,91%-os relaxáció; 10%-os puffer: 90,3 ± 6,1%-os relaxáció, $P<0,05$), melyet sem az endotél eltávolítása, sem a szolubilis guanilát cikláz inhibitor oxadiazoloquinoxalin-1 , sem a nem-specifikus kálium csatorna blokkoló tetraetilammónium, sem a kálium-dependens ATP csatorna blokkoló glibenclamid nem befolyásolt szignifikánsan. Ugyanakkor a relaxációt a hidrogén-peroxidot elbontó kataláz csökkentette (1000 U/mL kataláz + 5% nikotin tartalmú dohányfüstpuffer: 49,71 ± 18,4%, $P<0,05$), a hidrogén-peroxid képződéséhez vezető szuperoxid diszmutáz pedig növelte (200 U/mL SOD + 5% nikotinos dohányfüstpuffer: 95,7 ± 2,3%, P <

0,05). A redukált glutation (a tiol oxidáció egyik gátlója) a Dvíz-okozta érrelaxációt szintén szignifikánsan csökkentette. A nikotin tartalmú cigarettafüst az L-típusú kálciumcsatornablokkoló nifedipin- és a Na^{+}-Ca^{2+} cseretranszportert gátló SEA400-hoz hasonló módon csökkentette a veseartériákon előidézett specifikus kontrakciót.

Harmadik vizsgálatunkban mind a KW mind a non-diab NGS csoport többsége krónikus dohányosnak bizonyult (KW: 13/15= 87%, non-diab NGS: 7/7=100%, P=1.0) a non-KW csoporttal ellentétben (16/46= 35%; p=0.001 vs. KW). A cigaretta csomagévek száma a három csoportban a dohányzási szokásokhoz hasonló arányokat mutatott (KW: 15 {6-30}, non-KW: 0 {0-21}, non-diab NGS: 30 {16-33}; P=0.010 non-KW vs. KW, P=0.008 non-KW vs. non-diab NGS). A diabéteszes nefropátia progressziójához, ill. a nem diabéteszes noduláris glomeruloszklerózis kialakulásához vezető egyéb tényezőkben (kor; testtömeg index; diabetes tartam; HbA_{1c}; hipertónia előfordulása és időtartama; szérum összkoleszterin és triglicerid szint; becsült glomeruláris filtrációs ráta; renin-angiotenzin-aldoszteron-rendszert gátló gyógyszeres kezelés) nem találtunk szignifikáns különbséget a három csoport között.

Következtetések

Feltételezzük, hogy a dohányzás hozzájárulhat az „egészséges dohányzó populációban” megfigyelt emelkedett glomeruláris filtrációs rátához.

A dohányfüst vízoldékony komponensei-okozta patkányveseartéria relaxációt részben a füstben fellelhető, ill. az érfalban szuperoxid anionból képződő hidrogén-peroxid okozhatja. A relaxáció létrjöttében valószínűleg az L-típusú kálciumcsatornának és a Na^{+}-Ca^{2+} cseretranszporternek is szerepe lehet.

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