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ORIGINAL ARTICLE

High Prolactin Excretion in Patients with Diabetes Mellitus and Impaired Renal Function

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SUMMARY

Background: **The metabolic clearance of prolactin (PRL) is partially executed by the kidney. Here, we investigate the urine excretion of PRL in patients with Diabetes Mellitus and renal impairment.**
Methods: **Serum and urine samples were collected from male, mestizo patients in central Mexico employing a cross-sectional study design. Ninety-eight individuals had either no diabetes and normal renal function (control), diabetes and normal renal function, or diabetes with impaired renal function. PRL was determined by a chemiluminescent immunometric assay; protein, albumin, and creatinine were evaluated using quantitative colorimetric assays. The results were analyzed using ANOVA-testing.**
Results: **Patients with Diabetes Mellitus and renal impairment had significantly higher urine PRL levels than patients with Diabetes Mellitus and normal renal function and control patients. Higher urine PRL levels were associated with lower glomerular filtration rates, higher serum creatinine, and higher urinary albumin-to-creatinine ratios (UACR). Urine PRL levels correlated positively with UACR. Serum PRL levels were similar among groups.**
Conclusions: **Patients with Diabetes Mellitus and impaired renal function demonstrate a high urinary PRL excretion. Urinary PRL excretion in the context of proteinuria could contribute to PRL dysregulation in renal impairment.**
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INTRODUCTION

Circulating levels of the pituitary hormone prolactin (PRL) usually do not exceed 20 ng/mL, except in pregnant or nursing women and, of course, in diseased states. The classical disease that leads to high PRL levels is prolactinoma, in which lactotropic tumor cells in the pituitary autonomously produce PRL. Another pathologic situation in which PRL levels rise is chronic kidney disease (CKD). In patients with CKD, there appears

to be an abnormal regulation of PRL secretion which features PRL hypersecretion, the failure of dopamine to suppress PRL levels, and a blunted PRL response to stimulatory agents including chlorpromazine, thyrotropin releasing hormone, vasoactive intestinal polypeptide, arginine, and insulin hypoglycaemia [1-6]. Also, in the presence of CKD, the physiological oscillations of serum PRL levels diminish, and the circadian rhythm of PRL secretion progressively disappears [7]. Besides the abnormal regulation of PRL secretion, PRL has a longer half-life and a lower metabolic clearance rate in the circulation of patients with CKD [5,8]. The latter may be due to impaired renal degradation, which is significant in the healthy kidney as the PRL concentration in the renal vein is approximately 16% lower than in the renal artery [9]. In CKD, the arteriovenous PRL concentration gradient may be lower due to a decline of functional kidney tissue, which could favor the rise in circulating PRL levels reported in these patients [9,10]. This possibility is also supported by the observation that after renal transplantation, or upon resolution of CKD for other reasons, PRL levels usually return to normal [6]. Here, we hypothesize that patients with Diabetes Mellitus and concomitant renal impairment demonstrate an altered renal metabolic clearance of PRL that leads to an increase of PRL levels in urine. In the light of the potential clinical relevance that PRL levels have in diabetes prevalence [11], diabetic retinopathy [12-14], and diabetic nephropathy [10], mechanisms of renal metabolic clearance of PRL deserve investigation.

MATERIALS AND METHODS

We designed an observational, cross-sectional, case-control study investigating male patients with either no diabetes and normal renal function, diabetes and normal renal function, or diabetes with impaired renal function. Female patients were not investigated because circulating PRL levels fluctuate throughout the reproductive cycle in women. The institutional review boards and ethics committees of each of the institutions involved approved the study protocol. Two urban, primary ambulatory care clinics in central Mexico served as recruitment centers, and all patients provided written informed consent before collection of samples.

Patients and samples (Figure 1)

Male, mestizo subjects visiting the clinics who were 18 - 90 years old were approached in a randomized fashion, and a detailed medical history was taken and recorded on a recruitment form. Based on the reported concordance rate between impaired renal function and proliferative diabetic retinopathy (PDR) [15,16], we expected the retinal status to be a sensitive tool for the non-invasive and reliable stratification of patients with normal or impaired renal function. Hence, to non-invasively identify patients with a high probability of impaired renal function [lower estimated glomerular filtration rate (eGFR), proteinuria, and elevated serum creatinine], all patients underwent comprehensive ophthalmologic assessment. Dilated indirect fundoscopy was performed, and the absence or presence of diabetic retinopathy was staged according to the International Clinical Diabetic Retinopathy and Diabetic Macular Edema Disease Severity scale [17]. According to the presence or absence of Diabetes Mellitus and retinal status, e.g., presence or absence of PDR, patients were assigned to the control-group, or to the groups with diabetes and anticipated normal or impaired renal function. Exclusion criteria were: medical history of liver cirrhosis, prolactinoma, other pituitary adenoma, thyroid dysfunction, thoracic lesions during the last three months, sarcoidosis, tuberculosis, histiocytosis, lymphocytic hypophysitis, acute infections, or medication with antipsychotics (phenothiazine, haloperidol, risperidone), antidopaminergics (metoclopramide, domperidone), antihypertensives with a known effect on prolactin secretion (methyldopa, verapamil), oestrogens, opiates, or cimetidine. The absence of diabetes was defined as having no diabetes symptoms, no medical history of hyperglycemia (defined as fasting plasma glucose levels > 7 mmol/L, random venous plasma glucose level > 11.1 mmol/L), lack of antihyperglycemic medications, and HbA1C levels < 6.5% (48 mmol/mol). Conversely, the existence of diabetes was defined as the presence of diabetes symptoms, a positive medical history of hyperglycemia, the use of antihyperglycemic medications, or a glycosylated hemoglobin level > 6.5% (48 mmol/mol). Urine and serum samples were taken between 08:00 a.m. and 12:00 noon, aliquoted, and stored at -80°C.

Determination of PRL in urine and serum

Seven and a half mL of urine was concentrated to a volume of 300 µL using centrifuge filters with a molecular weight cut-off of 5 kDa (Vivaspin 20, Vivaproducts, Inc., Littleton, MA, USA). One hundred and twenty µl of urine concentrate, or non-processed serum was assayed for PRL by the Immulite 1000 Immunoassay System (Siemens, Munich, Germany).

Clinical parameters

Protein, albumin, and creatinine were determined in serum and urine using quantitative colorimetric assays (Spinreact S.A./S.A.U. Ctra. Santa Coloma, Spain). Serum HbA1C was evaluated by the automated boronate affinity test (Afinion, AS100 Analyzer, Axis-Shield, Dundee, Scotland).

Statistical analyses

Descriptive statistics were used to report the demographic and clinical characteristics of the study population. Numerical parameters with parametric distributions were analyzed by an ordinary one-way ANOVA followed by Tukey's multiple comparisons test. Non-parametric data were analyzed by the Kruskal-Wallis test, followed by Dunn's multiple comparison test. Multiplicity-adjusted p-values are reported, and p-values

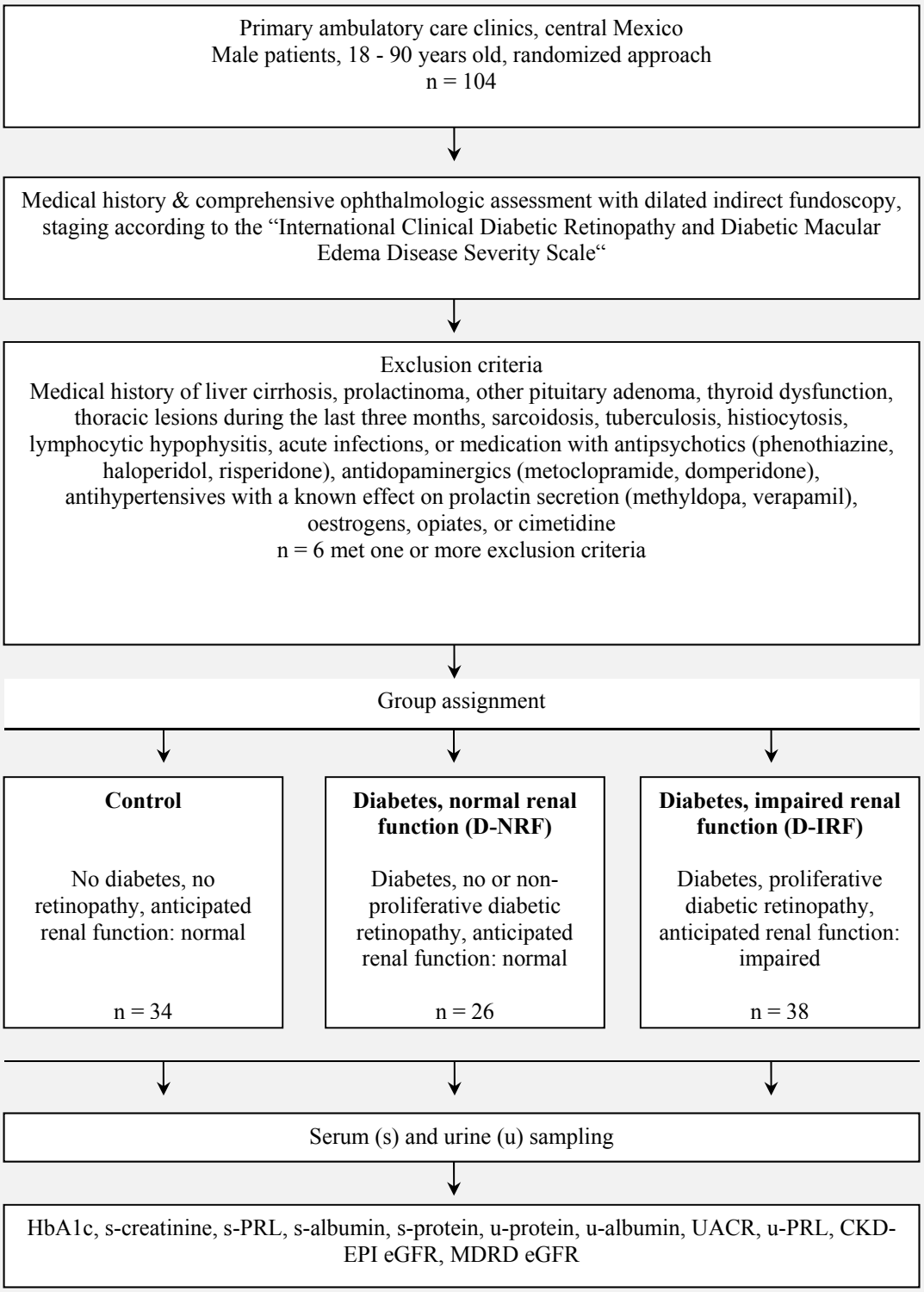

Figure 1. Research design and recruitment scheme.

< 0.05 were considered statistically significant. Spearman's nonparametric correlation test was performed to test correlations. All statistical analyses were performed using Prism 6 software (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS AND DISCUSSION

Description of the study population

Of 104 recruited patients, six were excluded: one due to a pre-existing diagnosis of tuberculosis, two because of acute systemic infection, and three failed to keep the appointment (Figure 1). Table 1 summarizes demographic and clinical characteristics of the groups studied. Age, body mass index (BMI), and time after diabetes diagnosis were similar among all groups and diabetic groups. HbA1C levels were significantly higher in the diabetic groups compared to the control group. Retinopathy was absent in the control group and diagnosed as proliferative (PDR) in all diabetic patients with anticipated impaired renal function (D-IRF). Retinopathy was absent in 80% of the patients in the diabetic and anticipated normal renal function group (D-NRF), whereas the remaining 20% displayed mild non-proliferative diabetic retinopathy (NPDR).

Patients with proliferative diabetic retinopathy have impaired renal function with proteinuria and lower eGFR (Table 2)

As demonstrated before and consistent with the high concordance rate between PDR and renal impairment [15,16], patients with PDR had impaired renal function revealed by elevated s-creatinine and reduced eGFR. Reduced eGFR, elevated s-creatinine level, and the high degree of proteinuria in patients of the D-IRF group demonstrates that assessment of the retinal status was an adequate tool to non-invasively identify patients having a high likelihood for impaired renal function. Patients in the D-IRF group demonstrated a high degree of proteinuria with elevated protein and albumin levels. Correspondingly, their UACR was significantly elevated. The prevalence of microalbuminuria in control and patients with diabetes and normal renal function was 71% (n = 22) and 67% (n = 16), respectively, which is far above the average of approximately 6% for non-diabetic patients and 25% for patients with diabetes 10 years after diagnosis [18-20]. Similarly, the prevalence of macroalbuminuria in the control and D-NRF group was 6% (n = 2) and 25% (n = 6), respectively, and it exceeded the expected prevalence of 0% in the non-diabetic population and approximately 5% in patients with diabetes 10 years after diagnosis [18-20]. The extremely high proportion of patients with macroalbuminuria in the D-IRF group (76%, n = 28) was remarkable, as was the massive proteinuria with a mean UACR of 1885. However, an extremely high prevalence of macroalbuminuria in patients with PDR is not uncommon and has been reported previously [21]. Also, Mexican American diabetic subjects have a higher prevalence of proteinuria than non-Hispanic White diabetic subjects [22]. The extraordinarily high degree of proteinuria of the participants in this study is consistent with the perception that proteinuria in subjects of Mexican origin may be more prevalent and more severe than in subjects of other ethnic origins. In view of the surprisingly high prevalence of proteinuria in this small case-control study, another, large epidemiological study investigating the prevalence of proteinuria in the Mexican population seems necessary.

Patients with diabetes, impaired renal function and proteinuria excrete more PRL than patients with diabetes and normal renal function or without diabetes (Table 2)

PRL urinary excretion is physiological [23] and has also been demonstrated in patients with preeclampsia [24] and glomerulonephritis [25]. Consistent with a previous study [23], our data demonstrate that PRL levels in the urine-concentrates (uc-PRL) of healthy male subjects (control group) is relatively low (0.70 ± 0.16 ng/mL). Similar levels of uc-PRL (0.79 ± 0.31 ng/mL) were detected in D-NRF patients, which are comparable in terms of renal function and proteinuria levels. However, the uc-PRL concentration was 18 to 20-fold higher in the D-IRF group (14.10 ± 35.72 ng/mL).

PRL excretion directly correlates with proteinuria and impaired renal function (Table 3)

Uc-PRL levels positively correlated with UACR, u-protein, and u-albumin levels, demonstrating that an increase in proteinuria is accompanied by an increase in urinary excretion of PRL. Further, uc-PRL correlated positively with s-creatinine and inversely with eGFR, illustrating a strong association, and possibly a functional relationship, between urinary PRL and renal function. In contrast, s-PRL levels correlated neither with s-creatinine nor with eGFR. These results show that PRL levels can remain within the normal range in spite of an impaired eGFR, as demonstrated in the D-IRF group, where patients had an eGFR of 65.18 ± 29.66. This is in contrast with previous studies implying that PRL accumulates in the circulation as a consequence of reduced intrarenal degradation in renal impairment and lower eGFR [10]. In none of the groups nor in the total study population did uc-PRL levels correlate with s-PRL levels, indicating that renal elimination of PRL represents a stable level, rather than a fraction dependent on s-PRL concentration (Table 2). A logical explanation for an increase in uc-PRL levels in patients with diabetes and renal impairment, however, is the assumption that in the course of marked proteinuria, the amount of PRL filtrated exceeds renal metabolization, utilization, and tubular reabsorption and consecutively leads to higher amounts of PRL in urine.

Table 1. Demographic and clinical characteristics of the study population.

Parameter	Control	D-NRF	D-IRF
	n = 34	n = 26	n = 38
Age, years	46.53 ± 17.76	56.04 ± 13.92	55.82 ± 10.94
BMI	26.15 ± 4.84	26.70 ± 5.23	25.10 ± 3.05
Years since DM diagnosis	-	10.69 ± 11.02	16.31 ± 8.74
HbA1C, %	5.02 ± 1.00	7.94 ± 2.31 *	7.46 ± 2.19 *
HbA1C, mmol/mol n	31 ± 10.9 20	63 ± 25.3 * 18	57 ± 23.9 * 31
DM medication, %, (n)			
no medication	100 (34)	8 (2)	-
oral medication	-	92 (24)	28 (11)
insulin	-	-	72 (27)
DR stage, %, (n)			
no apparent retinopathy	100 (34)	80 (21)	-
NPDR	-	20 (5)	-
PDR	-	-	100 (38)

Data are mean ± SD. Patients were male mestizos without diabetes and retinopathy (control), with Diabetes Mellitus and anticipated normal renal function (D-NRF), or with Diabetes Mellitus and anticipated impaired renal function (D-IRF). Body mass index (BMI), glycosylated hemoglobin (HbA1C), Diabetes Mellitus (DM), diabetic retinopathy (DR), nonproliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR). * p < 0.001 vs. control.

Table 2. Renal function, proteinuria, and serum and urinary PRL levels.

Parameter	Control	D-NRF	D-IRF	Comparison	
	n = 34	n = 26	n = 38		
CKD-EPI eGFR n	95.21 ± 20.88 34	92.79 ± 18.73 24	65.18 ± 29.66 * 38	D-IRF vs. D-NRF D-IRF vs. Control	p = 0.0001 p = < 0.0001
MDRD eGFR n	97.68 ± 27.45 34	97.88 ± 23.48 24	65.95 ± 30.74 * 38	D-IRF vs. D-NRF D-IRF vs. Control	p = < 0.0001 p = < 0.0001
s-creatinine mg/dL n	0.95 ± 0.24 34	0.91 ± 0.20 24	1.70 ± 1.28 * 38	D-IRF vs. D-NRF D-IRF vs. Control	p = < 0.0001 p = 0.0008
u-protein mg/dL n	17.97 ± 12.95 31	37.58 ± 61.52 24	174.2 ± 133.9 * 37	D-IRF vs. D-NRF D-IRF vs. Control	p = < 0.0001 p = < 0.0001
u-albumin mg/dL n	10.26 ± 7.78 31	30.91 ± 52.32 24	114.4 ± 96.42 * 37	D-IRF vs. D-NRF D-IRF vs. Control	p = 0.0003 p = < 0.0001
UACR n	113.9 ± 163.8 31	440.4 ± 828.4 24	1885 ± 2445 * 37	D-IRF vs. D-NRF D-IRF vs. Control	p = 0.0007 p = < 0.0001
normo-/micro-/macro-albuminuria %	23/71/6 7/22/2	8/67/25 2/16/6	0/24/76 0/9/28		
Urinary PRL levels					
uc-PRL, ng/mL n	0.70 ± 0.16 30	0.79 ± 0.31 24	14.10 ± 35.72 * 35	D-IRF vs. D-NRF D-IRF vs. Control	p = 0.040 p = 0.007
Circulating PRL, albumin and protein levels					
s-PRL, ng/mL n	9.39 ± 5.08 34	15.82 ± 17.78 25	10.52 ± 6.69 38	D-IRF vs. D-NRF D-IRF vs. Control	NS NS
s-albumin, g/dL n	6.02 ± 0.76 33	6.12 ± 0.80 23	5.38 ± 1.22 * 32	D-IRF vs. D-NRF D-IRF vs. Control	p = 0.026 p = 0.041
s-protein, µg/µL n	54.34 ± 17.98 29	75.02 ± 20.42 * 19	51.70 ± 16.64 27	D-NRF vs. Control D-NRF vs. D-IRF	p = 0.0007 p = 0.0002

Data are mean ± SD. Patients without diabetes (control), with diabetes and anticipated normal renal function (D-NRF), or with diabetes and anticipated impaired renal function (D-IRF). Estimated glomerular filtration rate according to formula by the chronic kidney disease epidemiology collaboration [26] (CKD-EPI eGFR) and modification of diet in renal disease [27] (MDRD eGFR). Urinary albumin-to-creatinine ratio (UACR). PRL in serum (sPRL), PRL in urine-concentrates (uc-PRL). * Significantly different vs. control and D-NRF. Non-significant (NS).

Table 3. Within- and cross-compartment correlations.

Parameter	Control	D-NRF	D-IRF	All patients	Interpretation
	correlation coefficient & p-values				
UACR/uc-PRL	-0.362 * 0.049 *	0.417 * 0.043 *	0.547 * 0.0007 *	0.393 * 0.0001 *	uc-PRL increases with UACR
u-protein/uc-PRL	-0.187 0.32	0.391 0.059	0.588 * 0.0002 *	0.439 * < 0.0001 *	uc-PRL increases with uc-protein
u-albumin/uc-PRL	-0.330 0.075	0.343 0.101	0.538 * 0.0009 *	0.399 * 0.0001 *	uc-PRL increases with uc-albumin
eGFR/uc-PRL	0.086 0.65	-0.287 0.17	-0.250 0.147	-0.270 * 0.010 *	uc-PRL increases with decreasing eGFR
s-creatinine/uc-PRL	0.051 0.78	0.338 0.10	0.585 * 0.0002 *	0.406 * < 0.0001 *	uc-PRL increases with s-creatinine
uc-PRL/s-PRL	0.050 0.79	-0.012 0.95	0.068 0.69	0.053 0.621	uc-PRL levels are not related with s-PRL
s-PRL/s-creatinine	0.164 0.35	-0.265 0.21	0.159 0.34	0.032 0.76	s-PRL levels are not related with s-creatinine
eGFR/s-PRL	0.053 0.76	0.192 0.36	-0.228 0.169	-0.013 0.89	s-PRL levels are not related with eGFR

Upper value in each row shows the correlation coefficient and lower value the respective p-value. * Statistically different, indicating positive or negative correlations. Patients without diabetes (control), with diabetes and anticipated normal renal function (D-NRF), or with diabetes and anticipated impaired renal function (D-IRF). Estimated glomerular filtration rate according to formula by the chronic kidney disease epidemiology collaboration [26] (eGFR), urinary albumin-to-creatinine ratio (UACR), serum (s), urine (u), urine concentrated prolactin (uc-PRL).

High PRL excretion is compensated in the circulation

The normal s-PRL levels in patients with D-IRF and the lack of correlation between s-PRL and uc-PRL levels, suggest that PRL from some other source compensates for the higher urinary PRL excretion. This source is likely to be the anterior pituitary gland, since the major fraction of circulating PRL is of anterior pituitary origin, and an increase in anterior pituitary PRL secretion is a common phenomenon in CKD [5]. In CKD patients, PRL hypersecretion may be due to the failure of dopamine to suppress s-PRL levels [1]. In addition, it is reported that PRL has a longer half-life and a lower metabolic clearance rate in the circulation of patients with CKD [5,8]. However, our observation that s-PRL levels did not correlate with s-creatinine or with the eGFR seems to contradict the long-held view that s-PRL levels rise in CKD as a result of reduced renal elimination [10]. Instead of s-PRL levels, uc-PRL levels increased with a greater degree of proteinuria, higher s-creatinine, and lower eGFR. Thus, the accumulation of PRL in the circulation expected from reduced eGFR and higher s-creatinine [10] did not occur, possibly due to the increased PRL excreted in the urine. Of note, previous studies that addressed circulating PRL levels in CKD did not assess urinary PRL levels and proteinuria. Thus, besides pituitary PRL hypersecretion and intrarenal degradation, urinary PRL excretion in the context of proteinuria may contribute to PRL dysregulation in renal impairment.

Limitations

The study design did not allow confirmation of CKD and its underlying cause in D-IRF patients, since no longitudinal observation of s-creatinine and eGFR was performed, and no renal biopsies were taken. However, the patients did not suffer from any acute illnesses or injuries at the time of recruitment. Thus, the occurrence of elevated s-creatinine, reduced eGFR, and severe proteinuria in PDR patients indicated a high likelihood for the presence of CKD, irrespective of the specific cause.

CONCLUSION

In summary, this study demonstrates that proteinuria in patients with diabetes and impaired renal function associates with higher urinary excretion of PRL, and that PRL urinary levels correlate with total proteinuria and increasing UACR. It appears then that not only intrarenal degradation but also urinary excretion are involved in the renal elimination of PRL. Accordingly, total renal PRL elimination should be regarded as a product of intrarenal PRL degradation by kidney tissue and elimination by glomerular filtration and subsequent excretion in the urine. This is consistent with the view that total renal organ clearance is the net result of glomerular filtration, tubular secretion, and tubular reabsorption. It is likely that damage of glomerular filtration increases PRL filtration to an extent that exceeds tubular reabsorption, consecutively resulting in higher urinary PRL levels. Understanding the elimination of PRL in the pre-

sence of renal impairment could help elucidate the interconnection between PRL levels and renal impairment and warrants further investigation.

Author Contributions:
CC and JT conceived and directed the study and wrote the manuscript. JT performed experiments and data analysis. AIMV contributed to experimental design and to performing experiments. MVM, RGF, ELS, OBH, and DO conducted and supervised patient recruitment and served as clinical advisors. TB contributed to data analysis, provided scientific advice and edited the manuscript. YM and GME served as scientific advisors and contributed to data analysis and experimental design.

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Declaration of Interest:
The authors declare no conflict of interest.

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