

# Treatment with insulin analogs, especially Glargine and Lispro, associates with better renal function and higher hemoglobin levels in Type 1 diabetic patients with impaired kidney function

*Ther Adv Endocrinol Metab*

2016, Vol. 7(4) 166–177

DOI: 10.1177/

2042018816644393

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## Abstract

**Objectives:** The influence of type of insulin treatment - insulin analogs versus human insulin - on the development of diabetes related vascular complications has been sparsely investigated. We examine here possible differences regarding kidney function and hemoglobin levels.

**Methods:** Multiple linear regression was used to investigate the relationship between the following characteristics measured in 509 type 1 diabetic patients who were recruited in an outpatient practice: current clinical status and treatment modalities, type of injected insulin and the routine laboratory parameters hemoglobin, HbA1c, serum creatinine, eGFR, hs CRP and urinary albumin/creatinine ratio.

**Results:** Compared with human insulin, multiple regression analysis taking into account possible confounders revealed that treatment with insulin analogs was associated with increased eGFR (+7.1 ml/min; P=0.0002), lower urinary albumin/creatinine ratio (ratio logarithm -0.4; P=0.003) and higher hemoglobin concentration (+0.31 g/dl; P=0.04).

Stratification by type of insulin showed the best renal status for treatment with insulins Glargine and Lispro. Differences were consistent both for patients with normal (eGFR → 90 ml/min) and with an impaired (eGFR ← 90 ml/min) kidney function.

**Conclusions:** Present results suggest that treatment of type 1 diabetic patients with normal and impaired renal function with insulin analogs, especially Glargine and Lispro, is associated with better kidney function, lower urinary albumin/creatinine ratio and lower hemoglobin concentration compared to therapy with human insulin. If confirmed by other studies, treatment with insulin analogs may be a further possibility in delaying progression of nephropathy and in preventing early hemoglobin decline.

**Keywords:** albumin excretion, aspart, detemir, diabetic nephropathy, glargine, hemoglobin, insulin analog, kidney function, lispro, type 1 diabetes

## Background

Insulin analogs (IAs) are widely used in the treatment of insulin-dependent diabetic patients. Compared with human insulin (HI), short-acting IAs decrease postprandial blood glucose peaks by their faster onset of action. Long-acting IAs

decrease the risk of hypoglycemia by their balanced pharmacokinetics. Owing to these effects, IAs do not just improve glycemic control but may also lower the risk of vascular complications. Despite its clinical relevance, the relationship between the type of insulin therapy and the onset

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of diabetic complications has been poorly investigated. In a previous study, we reported IA to be associated with a better kidney function and smaller decrease in hemoglobin concentrations with declining kidney function than HI [Hasslacher *et al.* 2010]. Due to the small number of patients, differentiation considering types of IAs was not possible in this early publication. Present analyses rely on data from approximately 500 patients, thus allowing stratification by the type of IA and/or insulin combinations. The primary investigated endpoints are kidney function and Hb level with declining kidney function in type 1 diabetic patients.

### Methods

A total of 509 patients with type 1 diabetes who attended the outpatient clinic of St. Josefs hospital Heidelberg between 2006 and 2012 and agreed to participate in the study were included if they fulfilled the following inclusion criteria: age 20–85 years, duration of diabetes at least 3 years, and no change in the insulin therapy during the previous 12 months. Patients affected by severe illnesses including malignoma, liver cirrhosis, congestive heart failure, renal insufficiency [estimated glomerular filtration rate (eGFR) < 15 ml/min], acute infection, bronchial asthma (steroid dependent), rheumatoid arthritis requiring any type of therapy, any organ transplantation, congenital cardiac or renal anomalies, pregnant patients and patients on erythropoietin (EPO) therapy were excluded from the study.

Medical history and demographic data as well as concomitant therapy were recorded at the first patient visit. The following laboratory parameters were measured: HbA1c, full blood count, creatinine, lipids, high-sensitivity C-reactive protein (hsCRP), urine status and urinary albumin/creatinine ratio. eGFR was calculated according to the Cockcroft–Gault formula. All measurements were performed by the MVZ Lab Dr. Limbach u. Kollegen in Heidelberg, Germany. HbA1c was measured by high-pressure liquid chromatography, urine albumin by a turbidimetric immunoassay in a sample of first morning urine after exclusion of any urinary tract infection and menstruation. Other laboratory measurements were conducted using routine methods by an autoanalyser (Dimension, Siemens, Germany) using serum or lithium-heparinized plasma.

Diabetic patients were grouped according to the applied long-acting insulin (human insulin, insulin detemir, insulin glargine) and short-acting insulin (human insulin, insulin aspart, insulin glulisine, insulin lispro). In general, treatment with IA was initiated by patients' GP/diabetologist and the reasons for change from HI to IA were mostly hypoglycemic events during night, high postprandial glucose levels or insufficient glycemic control. The time of treatment with the specified type of IA was recorded according to patients' statements and/or information from the GP/diabetologists' practice.

Relative frequencies were used to describe ordinal scaled factors, the location and spread of ratio scaled variables were represented by medians with 5th and 95th percentiles. Differences between groups of patients treated with different insulin types were assessed by uncorrected chi-squared and Wilcoxon tests as appropriate. Univariate linear regression was used to identify patient characteristics related to hemoglobin levels and characteristics related to laboratory parameters reflecting renal function (eGFR and the urinary albumin/creatinine ratio, which was log-transformed to obtain a nearly normally distributed variable). The identified related patient characteristics were taken into account in multiple linear regression models which were applied to quantify differences among types of insulin in hemoglobin levels and renal function. Locally weighted scatter plot smoothing (LoWeSS) regression was used to visually depict the relationship between eGFR and hemoglobin levels in the investigated patient collective. Statistical analyses were conducted using the SAS system version 9.3 and the R language and environment for statistical computing.

The study was approved by the local ethics committee and written informed consent was obtained from all patients.

### Results

The baseline demographic and laboratory characteristics of the investigated patients, with their treatment modalities and concomitant diseases are summarized in Table 1. Most patients used an intensified insulin therapy (91%), 7.3% of them with an insulin pump. Exclusive treatment with an IA received 46.8% of patients, 31.4% received exclusively HI and 21.8% received a combination of HI and IA. Average treatment

**Table 1.** Main patient characteristics (median values with 5th and 95th percentiles) and probability values for no difference with patients treated with human insulin from Wilcoxon and chi-squared tests.

Characteristic	Unit	Type of insulin			<i>p</i>	Human + Analog			
		Human ( <i>n</i> = 160)		Analog ( <i>n</i> = 238)		Human + Analog ( <i>n</i> = 111)			
		Med	5th to 95th	Med	5th to 95th		Med	5th to 95th	<i>p</i>
Age	Years	51	28 to 71	51	25 to 72	0.63	48	24 to 71	<b>0.01</b>
Female gender	%	53		46		0.18	41		<b>0.04</b>
Diabetes duration	Years	19	3 to 44	20	5 to 45	0.25	18	3 to 44	0.44
BMI	kg/m <sup>2</sup>	24	20 to 30	25	20 to 33	0.16	25	20 to 31	0.53
HbA1c	mmol/mol	59.6	40.9 to 82.5	58.5	38.8 to 86.9	0.42	58.5	42.1 to 95.6	0.42
hsCRP	mg/l	1.7	0.0 to 8.0	1.4	0.2 to 9.9	0.71	1.0	0.2 to 8.3	0.26
Triglycerides	mg/dl	87	43 to 308	80	40 to 244	<b>0.01</b>	78	37 to 182	0.05
Cholesterol	mg/dl	198	136 to 263	192	139 to 262	<b>0.04</b>	188	127 to 241	<b>0.05</b>
HDL	mg/dl	58	34 to 110	63	39 to 95	0.33	62	38 to 95	0.47
LDL	mg/dl	115	65 to 169	107	63 to 170	0.18	103	64 to 160	0.14
eGFR	ml/min	83	34 to 128	94	45 to 134	<b>0.01</b>	101	40 to 137	<b>0.01</b>
U-albumin/creatinine	mg/g	7	2 to 205	6	1 to 383	<b>0.01</b>	6	1 to 233	0.10
Hemoglobin	g/dl	13.9	11.6 to 16.2	13.7	11.3 to 15.8	<b>0.02</b>	14.1	11.8 to 16.3	0.24
Ferritin	μl/l	90	7 to 148	108	10 to 360	0.11	92	43 to 514	0.45
Iron	μl/l	837	102 to 1320	820	470 to 1214	0.78	790	164 to 1484	0.83
Prevalence of retinopathy	%	39		31		0.15	35		0.45
Prevalence of hypertension	%	58		61		0.64	50		0.16
Systolic blood pressure	mmHg	134	104 to 180	132	110 to 169	0.95	130	108 to 163	0.12
Diastolic blood pressure	mmHg	78	60 to 93	78	61 to 95	0.47	76	63 to 92	0.63
Prevalence of treatment with RAS inhibitors*	%	43		47		0.37	39		0.56

Bold type denotes statistical significance at the 5% confidence level.  
 \*Including ACE inhibitors and AT1 blockers.  
 ACE, angiotensin-converting enzyme; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; RAS, renin-angiotensin system.

times with specific IA types were 4.9 (lispro), 4.6 (aspart), 5.1 (glargine) and 4.5 years (detemir). The majority of patients (78%) had been treated for more than 3 years before study measurements with the respective IA.

Age, gender, diabetes duration, body mass index (BMI), HbA1c, hsCRP, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol differences between patients treated with HI and IA did not reach statistical significance. Total cholesterol ( $p = 0.04$ ) and triglyceride levels ( $p = 0.01$ ) were slightly higher in the HI group. Prevalence of hypertension, antihypertensive therapy with angiotensin-converting enzyme (ACE)/AT1 blockers and the quality of blood pressure control were similar in the HI and IA groups.

The median unadjusted Hb concentration was 13.7 g/dl in the IA group, somewhat lower than in the HI group (13.9 g/dl;  $p = 0.02$ ). The kidney function (eGFR) was within the normal range (eGFR > 90 ml/min) for 49.3% of the patients. Among diabetes patients with decreased eGFR (<90 ml/min), 96.9% showed a renal function in chronic kidney disease (CKD) stages II and III (eGFR 30–89 ml/min), 3.1% in CKD stage IV (eGFR = 15–29 ml/min).

The kidney function in patients treated with IA was on average 13.3% higher ( $p = 0.01$ ), the urinary albumin/creatinine ratio (ACR) approximately 19% lower ( $p = 0.01$ ) than in the HI group. Patients treated with a combination of HI and IA presented with the highest eGFR in the three groups. However, ACR and hemoglobin

**Table 2.** Univariate analysis of possible associations with eGFR, urinary ACR and hemoglobin in the investigated collective.

Characteristic	eGFR		Log(ACR)		Hemoglobin	
	Parameter estimate	<i>p</i>	Parameter estimate	<i>p</i>	Parameter estimate	<i>p</i>
Age	-1.1671	<.0001	0.0087	<b>0.0111</b>	-0.0149	<b>0.001</b>
Female gender	-11.5398	<.0001	-0.2505	<b>0.0083</b>	-1.1563	<.0001
Diabetes duration	-0.7145	<.0001	0.0026	0.50	-0.0122	<b>0.02</b>
BMI	1.6321	<.0001	0.0189	0.16	-0.0178	0.32
HbA1c	-1.1798	0.23	0.1212	<b>0.0004</b>	-0.0628	0.17
hsCRP	-0.7160	0.18	0.0154	0.40	-0.0633	<b>0.01</b>
Hypertension	-23.0817	<.0001	0.4491	<.0001	-0.1783	0.18
ACE-inhibitors/AT1-blockers	-20.9838	<.0001	0.4192	<.0001	-0.3890	<b>0.003</b>
Systolic blood pressure	-0.2842	<.0001	0.0104	<.0001	0.0028	0.41
eGFR	-	-	-0.0082	<.0001	0.0173	<.0001

Bold type denotes statistical significance at the 5% confidence level.  
ACE, angiotensin-converting enzyme; ACR, albumin/creatinine ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein.

concentrations did not significantly differ from HI-treated patients (Table 1).

Table 2 shows the results of univariate linear regression models examining the possible relationship between patient characteristics, and eGFR, ACR and Hb. For example, a 1-year increase in the patient age translated into an eGFR decrease of 1.2 ( $p < 0.0001$ ). In the subsequent multiple regression analyses, differences in eGFR, ACR and hemoglobin by type of insulin treatment were investigated taking into account the identified confounders in univariate analyses.

### Differences in kidney function and urinary albumin excretion by type of insulin treatment

Table 3 and Figure 1(a) show the mean differences in eGFR between patients treated with IA or HI and IA *versus* HI-treated patients as the reference group adjusted for age, sex, diabetes duration, BMI, prevalent hypertension, concomitant therapy with ACE inhibitors or AT1 blockers, and systolic blood pressure. Multiple regression analysis revealed that the average eGFR of patients in the IA group was approximately 7.1 ml/min higher ( $p = 0.0002$ ) and in the HI and IA group approximately 4.7 ml/min higher ( $p = 0.06$ ) than in patients treated with human insulin. The goodness of the fitted multiple regression model was  $R^2 = 0.43$ .

Stratified analyses by IA type showed the largest mean difference eGFR for the 'lispro' ( $p = 0.02$ ) and the 'aspart/glargine' ( $p = 0.006$ ) groups. However, differences among particular IA types did not reach statistical significance (overlapping confidence intervals). In the 'glargine-based' therapy cluster, the eGFR was about 6.7 ml/min higher ( $p = 0.003$ ) than in the HI group. For the cluster 'detemir-based', it was about 5.8 ml/min higher with borderline significance ( $p = 0.05$ ). Seventeen patients treated with insulin glulisine (glulisine/glargine  $n = 11$ ; glulisine/detemir  $n = 6$ ) were not considered in this and further analyses due to the low number of observations.

In order to examine whether these differences are present both in patients with normal (eGFR >90 ml/min) and reduced (eGFR <90 ml/min) kidney function, statistical analyses were accordingly stratified. For the patient group with normal kidney function, differences between insulin types were not statistically significant (data not shown). Multiple regression results for patients with an eGFR <90 ml/min are shown in Table 3. Patients treated with aspart/glargine and 'glargine-based' presented with a higher eGFR than HI-treated patients [by 7.9 ml/min ( $p = 0.03$ ) and 5.5 ml/min ( $p = 0.04$ ), respectively].

Table 4 and Figure 1(b) show mean differences in the logarithm of the ACR between patients treated with IA or HI and IA compared with

**Table 3.** Results from multiple linear regression analyses of the effect of insulin type on eGFR for all patients and those with eGFR lower than 90 ml/min. Results are provided as mean differences between named IA- and HI-treated patients as reference. Estimates are adjusted for identified confounders.

Patients	Insulin type	Subtype	Patients (n)	Mean difference	95% CI		p	
Any	HI		160	Reference				
	IA		238	7.1369	3.3351	10.9387	<b>0.0002</b>	
	HI and IA		111	4.6973	-0.0172	9.4117	0.06	
	IA		Lispro	34	9.3943	1.5514	17.2371	<b>0.02</b>
			Lispro glargine	76	5.7618	0.6467	10.8768	<b>0.03</b>
			Lispro detemir	23	6.0301	-2.4029	14.4631	0.16
			Aspart glargine	47	8.8780	2.5101	15.2459	<b>0.006</b>
			Aspart detemir	41	5.9424	-1.3433	13.2282	0.10
			Glargine based	123	6.6890	2.3417	11.0363	<b>0.003</b>
			Detemir based	64	5.8006	0.0272	11.5740	<b>0.05</b>
eGFR <90 ml/min	IA	Lispro	12	4.4565	-5.8492	14.7621	0.40	
		Lispro glargine	34	3.5690	-2.9863	10.1243	0.29	
		Lispro detemir	9	1.5344	-9.4584	12.5272	0.78	
		Aspart glargine	21	7.9266	0.7134	15.1397	<b>0.03</b>	
		Aspart detemir	19	-2.6071	-11.9392	6.7249	0.58	
		Glargine based	55	5.4526	0.2646	10.6406	<b>0.04</b>	
		Detemir based	28	-0.4824	-7.9455	6.9806	0.90	

Bold type denotes statistical significance at the 5% confidence level.  
CI, confidence interval; eGFR, estimated glomerular filtration rate; HI, human insulin; IA, insulin analog.

HI-treated patients as the reference group adjusted for the identified confounders, i.e. age, sex, HbA1c, prevalent hypertension, concomitant therapy with ACE inhibitors or AT1 blockers, and systolic blood pressure (Table 2). Based on multiple linear regression analyses, the ACR was significantly lower under IA treatment than with HI therapy ( $p = 0.0003$ ). No statistical difference was found in patients treated with a combination of HI and IA. The goodness of fit of the multiple regression model was poor ( $R^2 = 0.07$ ). Stratification of patients according to particular IA type revealed that treatment with insulin lispro, lispro/glargine and aspart/detemir as well as the cluster 'glargine-based' insulin therapy were associated with a significantly lower ACR.

After patient stratification according to kidney function, both patients groups with an eGFR higher and lower than 90 ml/min showed a lower ACR under IA treatment ( $p = 0.03$  and  $p = 0.04$ , respectively). The group treated with HI and IA did not show a significant difference in comparison to the reference group (Table 4).

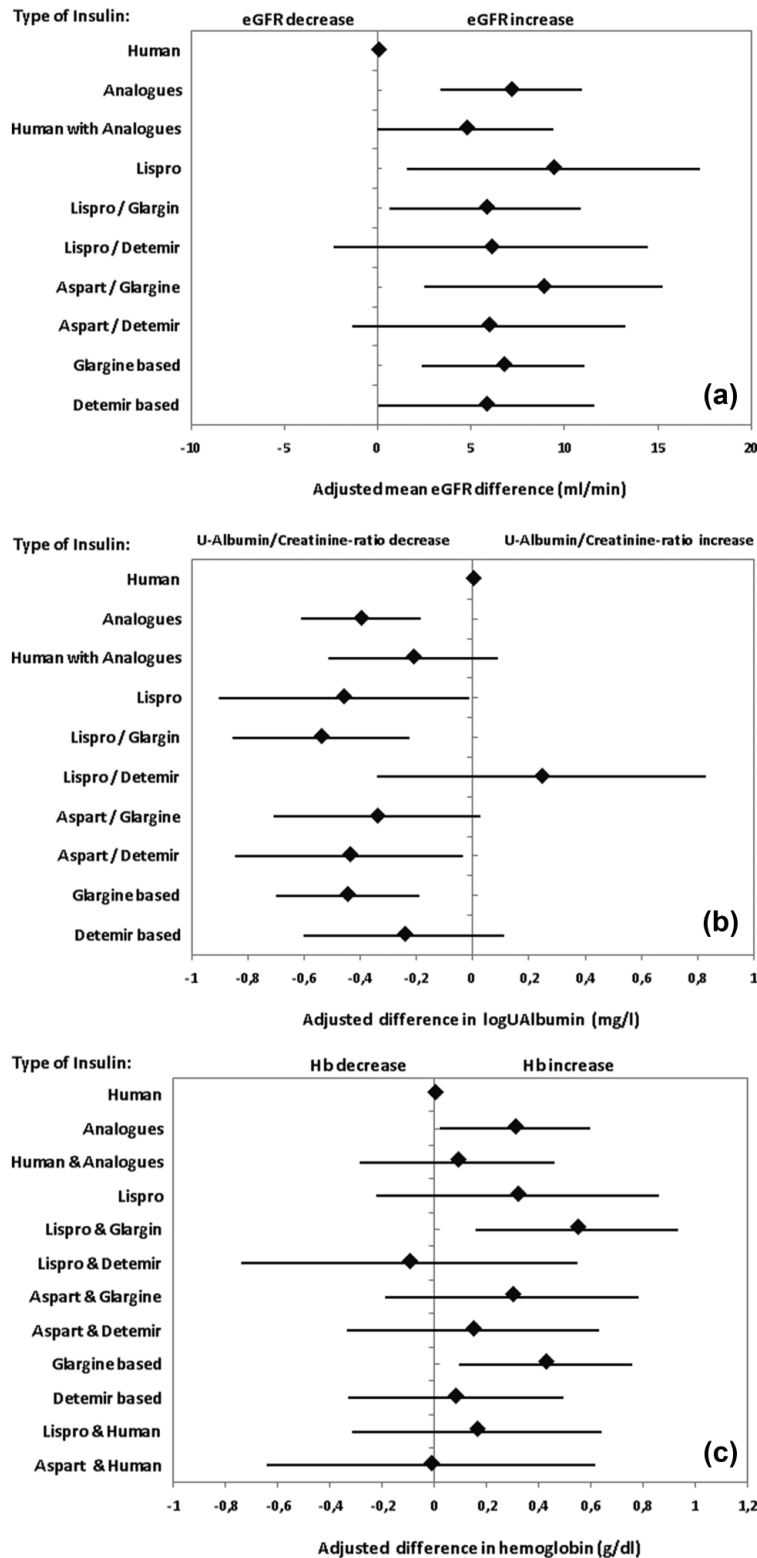
After stratification according to the particular type of IAs, the ACR in the group with normal kidney function was only lower for the combination

lispro/glargine than in the reference group ( $p = 0.04$ ). Patients with reduced kidney function under lispro/glargine administration and 'glargine-based' insulin therapy presented with a decreased ACR than the reference group ( $p = 0.008$  and  $p = 0.004$ , respectively; Table 4).

### Effect of the type of insulin on the hemoglobin level

Figure 2 represents the relationship between hemoglobin level and kidney function under treatment with HI and IA. The hemoglobin level was higher for IA- than for HI-treated patients for any eGFR value, with particularly large differences in the hemoglobin level for eGFR values in the upper and lower ranges.

Table 5 and Figure 1 (c) shows the mean differences in hemoglobin levels between patients treated with IA and patients treated with a combination of HI and IA compared with HI-treated patients as a reference group. Multiple linear regression taking into account possible confounders (age, sex, diabetes duration, hsCRP, concomitant therapy with ACE inhibitors and AT1 blockers) indicated that the hemoglobin level of patients treated with IA was 0.31 g/dl higher



**Figure 1.** Results from a multiple linear regression model of the effect of insulin type on: (a) estimated glomerular filtration rate (eGFR) summarized by mean eGFR differences between specified insulin analog (IA)- and human insulin (HI)-treated patients as the reference, (b) on the logarithm of the urinary albumin/creatinine ratio summarized by mean log(ACR) differences between specified IA- and HI-treated patients and (c) on hemoglobin concentration differences between specified IA- and HI-treated patients.

**Table 4.** Results from multiple linear regression analyses of the effect of insulin type on the logarithm of the urinary albumin/creatinine ratio. Results are provided as mean differences between named IA and HI treated patients as reference. Estimates are adjusted for identified confounders.

Patients	Insulin type	Subtype	Patients (n)	Mean difference	95% CI	p	
Any	HI		160	Reference			
	IA		238	-0.3971	-0.6098 -0.1845	<b>0.0003</b>	
	HI and IA		111	-0.2131	-0.5150 0.0889	0.17	
	IA	Lispro	34	-0.4603	-0.9061 -0.0145	<b>0.04</b>	
		Lispro glargine	76	-0.5406	-0.8555 -0.2256	<b>0.0008</b>	
		Lispro detemir	23	0.2437	-0.3423 0.8296	0.42	
		Aspart glargine	47	-0.3416	-0.7087 0.0256	0.07	
		Aspart detemir	41	-0.4388	-0.8445 -0.0331	<b>0.03</b>	
		Glargine based	123	-0.4457	-0.7011 -0.1904	<b>0.0006</b>	
		Detemir based	64	-0.2446	-0.6020 0.1128	0.18	
	eGFR <90 ml/min	IA	Lispro	12	-0.5161	-1.4235 0.3914	0.27
			Lispro glargine	34	-0.7756	-1.3444 -0.2068	<b>0.008</b>
			Lispro detemir	9	0.5154	-0.4889 1.5196	0.32
		Aspart glargine	21	-0.4874	-1.1524 0.1776	0.15	
		Aspart detemir	19	-0.2834	-1.0429 0.4760	0.47	
		Glargine based	55	-0.6660	-1.1185 -0.2134	<b>0.004</b>	
		Detemir based	28	0.0288	-0.6116 0.6691	0.93	
eGFR > 90 ml/min	IA	Lispro	22	-0.3282	-0.7970 0.1405	0.17	
		Lispro glargine	42	-0.3442	-0.6813 -0.0071	<b>0.04</b>	
		Lispro detemir	14	-0.0873	-0.7626 0.5881	0.80	
		Aspart glargine	26	-0.1530	-0.5508 0.2448	0.45	
		Aspart detemir	22	-0.4040	-0.8252 0.0172	0.06	
		Glargine based	68	-0.2548	-0.5524 0.0428	0.09	
		Detemir based	36	-0.3270	-0.7256 0.0716	0.10	

Bold type denotes statistical significance at the 5% confidence level.  
CI, confidence interval; eGFR, estimated glomerular filtration rate; HI, human insulin; IA, insulin analog.

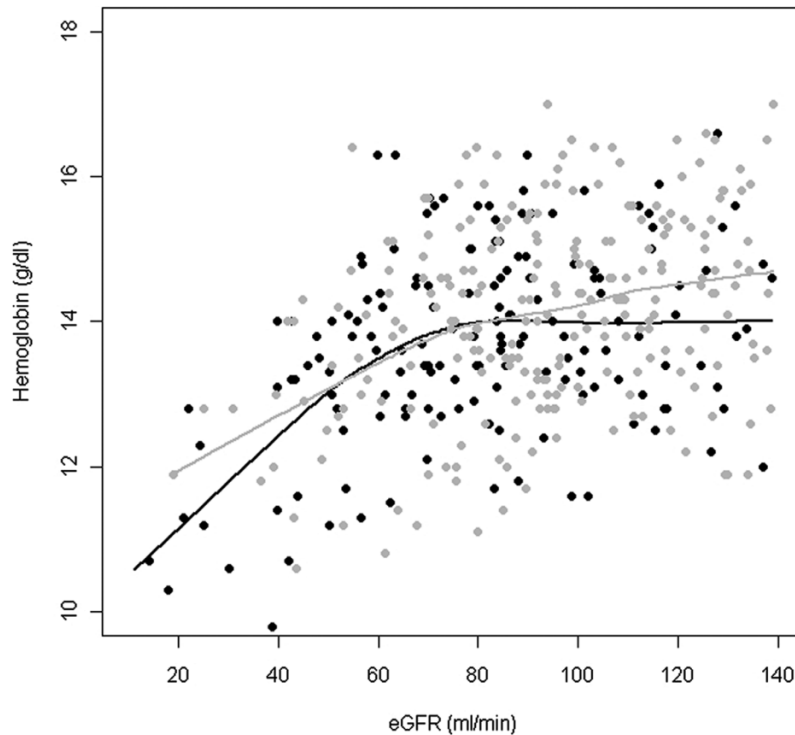
( $p = 0.04$ ) than in HI-treated patients. The difference between patients treated with HI and IA and HI-treated patients did not reach statistical significance. The goodness of the fitted multiple regression model was in this case  $R^2 = 0.18$ . After stratification according to particular type of IA, increased hemoglobin levels were only found for the combination lispro/glargine (0.54 g/dl,  $p = 0.006$ ) and for the cluster 'glargine-based' insulin therapy (0.42 g/dl,  $p = 0.01$ ).

After patient stratification according to normal and reduced kidney function (eGFR  $\geq 90$  ml/min and  $< 90$  ml/min, respectively), Hb levels were significantly higher in patients treated with lispro/glargine than in HI-treated patients in both groups (eGFR  $> 90$  ml/min: +0.42 g/dl,  $p = 0.05$ ; eGFR  $< 90$  ml/min: +0.68 g/dl,  $p = 0.03$ ). Differences for other types of insulin treatment did not reach statistical significance.

## Discussion

The study shows for the first time that treatment of type 1 diabetes patients with IA, especially with insulin glargine and lispro, is associated with a better kidney function and higher hemoglobin levels in early CKD stages when compared with HI therapy.

IA are widely used in the treatment of insulin-requiring diabetic patients, especially in cases with transient glucose peaks or risk of hypoglycemia. IA cannot just improve the glycemic control of diabetes but may also lower the risk of cardiovascular events, as reported in several studies in Type 2 diabetes patients [Rhoads *et al.* 2009; Scholz and Dippel, 2012; Rathmann and Kostev, 2013]. Their effects on development of diabetic nephropathy, a further diabetes-related complication with poor renal and cardiovascular prognosis, are unknown as of yet. In our study on a



**Figure 2.** Relationship between hemoglobin concentration and estimated glomerular filtration rate (eGFR) without consideration of additional covariates. Individual data and fitted locally weighted scatter plot smoothing (LoWeSS) curves are plotted in gray for analog insulin and in black for human insulin treated patients.

high number of Type 1 diabetic patients with normal or impaired kidney function we found that patients treated with IA showed on average a 7.2 ml/min higher eGFR and lower urinary albumin excretion than patients treated with HI. After stratification according to kidney function, higher eGFR values were only detected in IA treated patients with an eGFR < 90 ml/min, a lower urinary albumin excretion in both groups, i.e. eGFR > 90 ml/min and < 90 ml/min. Stratified analyses of particular IA types revealed that the above-described effects were consistently found under administration of insulin glargine and/or lispro, rarely under administration of aspart or detemir. For all comparisons, potential confounders were taken into account.

The causes of the described results are not clear. The patients treated with HI and IA did not differ according to essential factors that could affect kidney function such as age, duration of diabetes, gender distribution, control of diabetes and blood pressure, treatment with renin-angiotensin (RAS) inhibitors. Transient glucose peaks and hypoglycemia, which are observed more rarely under IA

therapy than under HI administration, are not known to be typical risk factors for the occurrence of a nephropathy. Kulozik and Hasslacher [Kulozik and Hasslacher, 2015] recently showed that low 1,5-AG levels as indicator of transient hyperglycemia are not associated with an increased prevalence of nephropathy. Ruggenenti and colleagues [Ruggenenti *et al.* 2003] found in a study on the renal hemodynamics that postprandial hyperperfusion of the glomeruli, which is detectable under HI therapy, does not occur under treatment with insulin lispro. It is not known to what extent this effect on glomerular hemodynamics can also be found with other IA, and whether this effect has a protective influence on the course of nephropathy.

Impairment of oxygen metabolism in kidney tissue has been discussed as a possible partial cause for the development of nephropathy [Nangaku and Eckardt, 2007; Miyata van Ypersele de Strihou, 2010]. Damage to the arterial and/or glomerular blood flow or lower hemoglobin levels may decrease the oxygen supply especially to the tubular structures and may lead to various



**Table 5.** Results from multiple linear regression analyses of the effect of hemoglobin levels. Results are provided as mean differences between named IA and HI treated patients as reference. Estimates are adjusted for identified confounder.

Patients	Insulin type	Subtype	Patients (n)	Mean difference	95% CI	p
Any	HI		160	Reference		
	IA		238	0.3081	0.0212 0.5949	<b>0.04</b>
	HI and IA		111	0.0872	-0.2853 0.4597	0.65
	IA	Lispro	34	0.3185	-0.2229 0.8599	0.25
		Lispro glargine	76	0.5458	0.1571 0.9346	<b>0.006</b>
		Lispro detemir	23	-0.0972	-0.7429 0.5484	0.77
		Aspart glargine	47	0.2969	-0.1882 0.7820	0.23
		Aspart detemir	41	0.1454	-0.3378 0.6286	0.56
		Glargine based	123	0.4251	0.0919 0.7583	<b>0.01</b>
		Detemir based	64	0.0808	-0.3322 0.4937	0.70
eGFR <90 ml/min	IA	Lispro	12	0.0124	-1.0505 1.0752	0.98
		Lispro glargine	34	0.6847	0.0592 1.3102	<b>0.03</b>
		Lispro detemir	9	0.5486	-0.4815 1.5787	0.30
		Aspart glargine	21	-0.1202	-0.8832 0.6428	0.76
		Aspart detemir	19	-0.1703	-0.9697 0.6292	0.68
		Glargine based	55	0.3811	-0.1632 0.9254	0.17
		Detemir based	28	0.1268	-0.5386 0.7923	0.71
eGFR >90 ml/min	IA	Lispro	22	0.1785	-0.3081 0.6652	0.47
		Lispro glargine	42	0.4199	0.0033 0.8365	<b>0.05</b>
		Lispro detemir	14	-0.3877	-1.0871 0.3117	0.28
		Aspart glargine	26	0.2961	-0.2945 0.8867	0.33
		Aspart detemir	22	0.5220	-0.0631 1.1070	0.08
		Glargine based	68	0.3551	-0.0371 0.7473	0.08
	Detemir based	36	0.2155	-0.3202 0.7512	0.43	

Bold type denotes statistical significance at the 5% confidence level.  
CI, confidence interval; eGFR, estimated glomerular filtration rate; HI, human insulin; IA, insulin analog.

reactions with formation of fibrosis in the renal medulla. Several studies on diabetic patients with earlier CKD stages have shown an association between hemoglobin concentration and loss of kidney function [Breyer *et al.* 1996; Babazono *et al.* 2006; Oh *et al.* 2012]. In the present study, we could confirm that the hemoglobin level already starts to be lower in the early stages of nephropathy (Figure 2) [Bosmann *et al.* 2002; El-Achkar *et al.* 2005]. However, under administration of insulin glargine and lispro or the combination of both, the hemoglobin levels of patients with an eGFR < 90 ml/min were 0.69 g/dl higher than under treatment with HI, even when numerous confounders were considered. Significant differences could not be detected with administration of insulin detemir or aspart or combined administration of IA and HI when compared to human insulin. Patients with normal kidney function also showed slightly higher hemoglobin values

with glargine/lispro administration than with HI, and the difference bordered on statistical significance. These results confirm overall the findings of our earlier study in a smaller patient collective and extend them by the identification of two IAs that are essentially responsible for the hemoglobin-stabilizing effect [Hasslacher *et al.* 2010].

As a cause of the observed effects on hemoglobin concentration, primarily potential differences in the mitogenic potency between HI and the individual IA are to be discussed, which are mediated by insulin and IGF-1 receptors. Insulin receptors primarily of the subtype A were detected in the cells of the hematopoietic system, which mediate mitogenic effects in addition to metabolic effects [Dainiak and Kreczko, 1985; Benecke *et al.* 1992; Belfiore *et al.* 2009]. The cells of the bone marrow further have IGF-1 as well as hybrid

receptors, which mediate both IGF-1 and insulin effects [Miyagawa *et al.* 2000; Bailyes *et al.* 1997; Federici *et al.* 1997]. Various *in vitro* studies have already shown that insulin and IGF-1 stimulate proliferation and growth of late erythroid progenitors [Aoki *et al.* 1994; Sawada *et al.* 1989; Shih *et al.* 1999].

The affinity of HI and IA to the insulin and/or IGF-1 receptors differs [Kurtzhals *et al.* 2000]. Thus, when compared with HI, insulin glargine was shown to have a several times higher affinity for the IGF-1 receptor and stronger mitogenic effects in different cell systems with high IGF-1 receptor density [Kurtzhals *et al.* 2000; Mayer *et al.* 2008; Sommerfeld *et al.* 2010; Varewijck *et al.* 2013]. Insulin glargine also stimulated the IR/IGF1 hybrid receptor *in vitro* significantly more strongly than HI [Pierre-Eugene *et al.* 2012]. Insulin lispro also has an increased affinity for the IGF-1 receptor, the hybrid receptor IR/IGF1R is slightly more stimulated than with HI as well [Kurtzhals *et al.* 2000; Pierre-Eugene *et al.* 2012]. Insulin aspart showed in a tumor cell line a slightly increased mitogenic potency that, however, was not confirmed in other studies [Pierre-Eugene *et al.* 2012; Gammeltoft *et al.* 1999; Eckhardt *et al.* 2007]. Analyses regarding binding and dissociation of aspart regarding the IGF1-1 or the hybrid receptor did not show any differences in comparison with HI [Kurtzhals *et al.* 2000; Pierre-Eugene *et al.* 2012]. Insulin detemir shows substantially weaker binding to the IGF-1 receptor and a higher dissociation velocity than HI [Kurtzhals *et al.* 2000], corresponding results were also found for the hybrid receptor [Pierre-Eugene *et al.* 2012]. The mitogenic potency of detemir is lower when compared with HI, or is not different [Kurtzhals *et al.* 2000; Mayer *et al.* 2008; Sommerfeld *et al.* 2010; Shukla *et al.* 2009]. These results obtained *in vitro* on the mitogenic potency of the different IA are in concordance with our clinical findings of higher hemoglobin levels during treatment with the IA glargine and lispro. It should be emphasized that the administered insulin doses were within the usual clinical range, as Kulozik and Hasslacher [Kulozik and Hasslacher, 2013] could recently show in a subgroup analysis of this patient collective. Glargine and lispro doses were not higher in patients with normal kidney function than HI doses and slightly lower in patients with reduced kidney function.

The main limitation of our study is its cross-sectional design. Therefore, a 'selection bias' cannot

be excluded with certainty. This disadvantage should be ameliorated by the large number of patients examined, performance of the study at a center according to fixed criteria and requests to the pretreating family physicians for the confirmation of information given by patients. Another limitation is that the time of exposure to specific types of IA could not be precisely determined in all patients. The majority of patients (78%) had been treated for more than 3 years before the time of the study with the respective IA. This time span may be long enough for the detection of effects on kidney function and hemoglobin concentration. A further limitation may be that other factors that may influence kidney function or hemoglobin levels such as nonsteroidal anti-inflammatory drug (NSAID) use, smoking habits, EPO levels could not be considered due to information unavailability or large proportions of missing values in our dataset. Further studies with repeated longitudinal follow up of diabetic patients or with intervention should be conducted to validate our findings.

In summary, our results suggest that treatment of type 1 diabetic patients with IAs, especially insulin glargine and lispro, associates with a better renal status and a reduced decrease in hemoglobin levels with impaired kidney function. If confirmed in future prospective studies, this may present an opportunity in delaying progression of diabetic nephropathy and the occurrence of anemia, with the well-known unfavorable effects on the renal and cardiovascular prognosis.

### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was kindly supported by an unrestricted grant of Manfred Lautenschläger Stiftung Diabetes, Wiesloch, Germany.

### Conflict of interest statement

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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