Systemic Prostaglandin E_1 Infusion and Hepatic Aminonitrogen to Urea Nitrogen Conversion in Patients With Type 2 Diabetes in Poor Metabolic Control

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Amino acid catabolism and urea synthesis are increased in type 2 diabetes mellitus in poor metabolic control. In different catabolic conditions, prostaglandins (PGs) of the E series produced metabolic effects on nitrogen metabolism, decreasing urea formation. In 10 patients with type 2 diabetes in poor metabolic control, urea synthesis and amino acid to urea nitrogen exchange were measured in the basal state and during an alanine load (6 hours) with 2-hour superinfusion of a PGE_1 analog (30 μg/h) or saline in random order. The urea synthesis rate was calculated as the sum of urinary urea excretion and urea accumulation in total body water (TBW); total nitrogen exchange was calculated as the difference between infused amino acid-nitrogen and urea appearance. Plasma α-aminonitrogen (α-amino-N) increased 100% in response to alanine, to a steady-state without differences in relation to PG superinfusion. The urea synthesis rate (mean ± SD) was 34.0 ± 11.4 mmol/h in the basal period and increased to 161.2 ± 37.0 during alanine + saline and to 113.5 ± 34.6 during alanine + PG ($P < .001$). Nitrogen exchange was negative at baseline (−25.0 ± 9.0 mmol/h). It became moderately positive during alanine + saline (14.6 ± 25.1) and far more positive during alanine + PG (53.5 ± 21.4), with the difference due to reduced urea formation. The metabolic effects of PG were not related to differences in insulin and glucagon. We conclude that PGE_1 slows the high rate of hepatic urea-N synthesis in poorly controlled type 2 diabetes. Such metabolic effects have therapeutic implications.

THE LOCAL PRODUCTION of prostaglandins (PGs) in tissues is relevant for several processes. Their vasodilating properties have been extensively investigated and form the basis for their pharmacologic use in peripheral vascular disease,[1-3] frequently associated with type 2 diabetes, and renal disease of microvascular origin.[4-6]

Less is known about the metabolic effects of PGs. They have long been supposed to be mediators of accelerated muscle proteolysis during trauma and sepsis.[7,8] More recent studies support the hypothesis that PGs might be secreted to counteract catabolic mediators, acting synergically with insulin to stimulate tissue repair, stimulating muscle protein synthesis[9] and/or inhibiting muscle proteolysis.[10,11]

Experimental studies suggested that PGs may have a permissive influence on surgical stress–induced urea synthesis,[12,13] a key point in whole-body nitrogen economy. Only a few data have been obtained in humans. In patients with advanced liver disease, characterized by reduced urea formation because of the failure of the central organ, infusion of PGE_1 further reduced the hepatic urea-N synthesis rate (UNSR). The effect is independent of hormones and/or hepatic flow, and was probably mediated at the peripheral level of amino acid transport, thus reducing amino acid supply to the liver.[14] The resulting net nitrogen-sparing was suggested to be the biochemical basis for the beneficial effect of PGE_1 in clinical hepatology, namely in acute liver failure and liver transplantation.[15,16]

Such a mechanism might be particularly beneficial in conditions characterized by increased protein catabolism and urea formation. In patients with poorly controlled diabetes mellitus, both type 1 and type 2, protein/calorie malnutrition may be present as a result of protein and muscle wasting. In both conditions, the hepatic conversion of amino acids to urea, measured under standardized conditions of amino acid drive, was increased after an amino acid load[17] in relation to hyperglucagonemia.[18,19] Insulin treatment did not completely normalize hepatic nitrogen metabolism.[19]

In the present study, we assessed the effects of short-term infusion of a PGE_1 analog (Alprostadil-α-ciclodestrin; Schwarz Pharma, Monheim, Germany) on urea synthesis in a group of patients with poorly controlled type 2 diabetes, under stable conditions of substrate availability induced by continuous amino acid infusion.

SUBJECTS AND METHODS

Subjects

Ten patients (8 males) with type 2 diabetes mellitus in poor metabolic control were studied. They had an age range of 53 to 71 years (median, 65), and their body mass index (mean ± SD) was 25.1 ± 3.1 kg/m^2 (range, 20.1 to 29.6). The mean waist to hip ratio was 0.93 (range, 0.84 to 1.12), but only 2 male patients had a waist to hip ratio exceeding 1.00. The duration of the disease was 1 to 240 months (median, 75). All subjects had normal liver and kidney function tests at routine biochemistry, including normal creatinine levels (mean, 0.91 mg/dL; range, 0.73 to 1.04). Two had mild peripheral vascular disease (stage 1 to 2); 4 had nonproliferative retinopathy. All were treated with oral hypoglycemic agents (glibenclamide, metformin, or a combination of the two). They were selected because of poor metabolic control (fasting glucose > 11 mmol/L) and entered the study protocol within 2 days of selection. They continued their standard diet to provide 30 kcal and 0.8 to 1.0 g protein/kg body weight. Oral hypoglycemic agents were discontinued the day before the study.

The experimental study was performed during day-hospital admission. On the day of the study, fasting glucose was 11.8 to 16.9 mmol/L,

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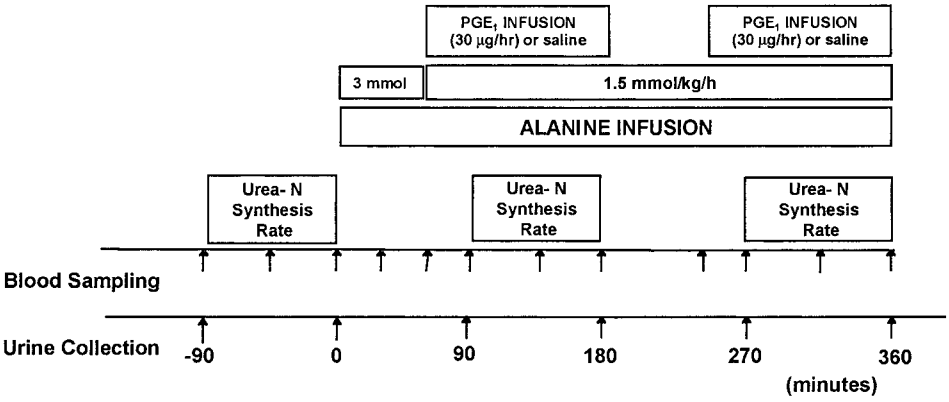

Fig 1. Experimental protocol for the study of the urea-N synthesis rate in response to alanine infusion with superimposed saline or PGE_1 infusion in patients with poorly controlled type 2 diabetes.

fructosamine 288 to 545 mg/dL, glycosylated hemoglobin 6.8% to 12.5%, and fasting triglycerides 61 to 379 mg/dL.

Subjects provided informed consent to participate in the study, which was approved by the Ethical Committee for Human Studies in our hospital.

Experimental Protocol

The UNSR was measured using a simplified procedure[20] in the course of 3 90-minute periods, including the fasting postabsorptive state and 2 periods of steady-state α-amino-N concentrations attained by a primed-continuous intravenous alanine infusion as described by Vilstrup[21] (Fig 1). Alanine (Ajinomoto, Tokyo, Japan; 10% wt/vol water solution) was infused at a rate of 2.5 mmol/kg/h for 1 hour (from time 0 to 60 minutes), followed by a constant infusion at 1.5 mmol/kg/h from 60 to 360 minutes. Blood samples were obtained from a vein of the contralateral arm at timed intervals (Fig 1) starting 90 minutes before alanine infusion (time -90) to 360 minutes. Urine was collected quantitatively by voiding during 3 90-minute periods. Subjects were not fed in the course of the test.

From time 60 minutes, a superinfusion of saline or a PGE_1 analog (Alprostadil-α-ciclodestrin) was started. The infusion lasted 2 hours, followed by a 1-hour washout period and a new superinfusion lasting 2 hours more. The two superinfusion periods were rotated in random order, with 5 patients receiving saline first and 5 receiving PGE_1 first. PGE_1 was infused at a constant rate of 30 μg/h in isotonic solution throughout the experimental period. The infusion rate was calibrated to yield plasma concentrations of 5 to 6 pg/mL, ie, approximately 3 times the basal values (normal plasma level, 1.2 to 1.8 pg/mL).[22]

In the last 90 minutes of the two superinfusion periods, urine was collected for the measurement of urea-N excretion. Urine flow was stimulated by peroral water or saline infusion to keep diuresis above 2 mL/min. This was attained in all subjects (mean diuresis, 2.5 $\pm$ 0.6 mL/min). The total amount of water or saline administered in paired experiments was approximately the same, ie, about 1,800 mL. Mild fluid retention was observed in a few experiments, but it never exceeded 0.6 L ($<$1.5% of body water).

The heart rate and arterial blood pressure were measured at the end of the basal preinfusion period and the end of the two paired experimental periods of superinfusion with saline or PGE_1. Blood pressure values used in the analysis are the average of the two measurements read at the nearest 5 mm Hg.

There were no side effects or complications in relation to the infusion of alanine. During PGE_1 infusion, one patient showed a transient erythema over the infused vein, but the experiment was not stopped.

Calculations

The UNSR during each 90-minute period was measured as the sum of the urea-N excretion rate in urine and urea-N accumulation in the urea space, assumed to equal total body water (TBW), as UNSR = (E + A)/(1 $-$ L), where E is urine flow (L/h) $\times$ urinary urea-N (mmol/L), A is change in blood urea-N (mmol/L/h) $\times$ TBW (L), and L is the fractional loss of newly formed urea in the gut.[21] TBW was calculated by means of a nomogram based on age, height, and weight.[23] Intestinal loss of urea-N due to bacterial hydrolysis was also derived from the literature.[19]

The ratio of the UNSR to the α-amino-N concentration during steady-state conditions has the dimension of a clearance (L/min). It measures the amount of blood flowing through the liver, which is cleared from α-amino-N to form urea-N in the unit of time.[21] It was calculated both in the basal condition and during alanine infusion with or without PGE_1 superinfusion.

The stoichiometric balance between the infused amino acid-N and urea-N appearance rate (nitrogen exchange in millimoles per hour) was calculated as the difference between the alanine-N infusion rate, corrected for urinary α-amino-N excretion and α-amino-N accumulation, and urea-N appearance (urea-N excretion + urea-N accumulation in TBW, not corrected for gut hydrolysis). The volume of distribution of α-amino-N was assumed to equal TBW.

Laboratory Procedures

The urea-N concentration in plasma and urine was measured by the urease Berthelot method.[24] Total α-amino-N was analyzed by the dinitrofluorobenzene method.[25] All analyses were performed in batches in duplicate or triplicate to minimize the analytic error. The intraassay coefficient of variation is $\pm$1.5% for urea, and $\pm$2% for α-amino-N.

The plasma insulin level was measured by an immunoenzymometric assay (AIA-PACK IRI, AIA-1200 system; Tosoh, Tokyo, Japan). Plasma glucagon was analyzed by radioimmunoassay (Glucagon kit; Biodata-Serono, Guidonia, Italy). Glucose was determined enzymatically.

Statistical Analysis

All analyses were performed on a personal computer using the StatView II program (Abacus Concepts, Berkeley, CA). Differences between paired data were analyzed by paired *t* test. Since 5 sets of data were analyzed (urea-N in blood and urine, plasma α-amino-N, plasma glucose, plasma hormones [insulin and glucagon], and hemodynamic parameters), the significance limit was set at $P' = 1 - \sqrt[(n-1)]{(1-P)}$, where P is .05 and n is 5.[26] The final critical value of significance was therefore .012. Data in the text and tables are shown as the mean $\pm$ SD, whereas in the figures, they are the mean $\pm$ 2 SE.

RESULTS

Plasma levels of α-amino-N, glucose, and urea-N slightly decreased in the course of the fasting preinfusion period (Fig

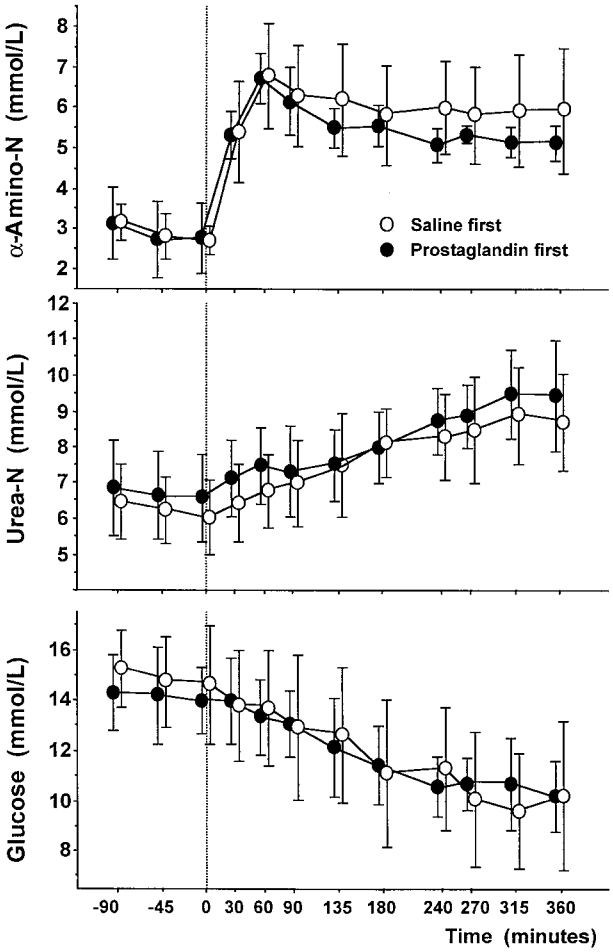

Fig 2. Time course of plasma α-amino-N, urea-N, and glucose concentration in the basal state and during alanine infusion (from time 0 to 360) according to the random order of superimposed saline or PGE_1 infusion. PGE_1 was infused from 60 to 180 minutes in subjects receiving PGE_1 first (●) and from 240 to 360 minutes in subjects receiving saline first (○). Data are presented as the mean and 95% confidence interval.

2). The mean α-amino-N was in the normal range, whereas glucose was nearly 3 times higher than normal. Insulin and glucagon were increased as well (by approximately 100%) in comparison to values measured in our laboratory in healthy subjects (Table 1). Alanine infusion caused a 4-fold increase in α-amino-N, to a peak level of 6.8 ± 1.1 mmol/L after 60 minutes. When the alanine infusion rate was reduced to 1.5 mmol/kg/h, plasma α-amino-N decreased to steady-state levels in the range of 5.5 to 6.0 mmol/L and remained stable during the whole experimental period.

Alanine produced a remarkable decrease of plasma glucose to a mean value of about 10 mmol/L in the last 2 hours of the experimental period. By contrast, plasma urea-N increased progressively, reaching a maximum value of 18.3 ± 4.0 mmol/L at the end of alanine infusion (Fig 2). Also, urinary urea-N excretion increased from 71 ± 27 mmol/h in the fasting preinfusion period to 143 ± 56 from 90 to 180 minutes and 164 ± 54 in the last 90 minutes of the experimental period.

There were no differences in the plasma analyte concentration in relation to the superinfusion of saline or PGE_1 (Table 1).

The fasting UNSR was higher than normal in our patients (37.9 ± 9.4 mmol/min) as compared with normal values measured in healthy subjects in our laboratory (<30 mmol/min; Fig 3). Following alanine infusion, urea synthesis was stimulated by increased substrate availability and the UNSR increased 3 to 4 times in all subjects. This was the result of both increased plasma urea-N and elevated urinary urea-N excretion. However, during PGE_1 superinfusion, the UNSR (113.5 ± 34.8) was, on average, 48 mmol/h lower than that measured during saline (161.2 ± 37.0, $P < .001$). The UNSR was reduced in all subjects in response to PGE_1 superinfusion (Fig 3).

The functional hepatic nitrogen clearance was, on average, 13.5 ± 4.1 L/min in the basal period. In response to alanine, it increased to 29.4 ± 9.0 during saline superinfusion. PGE_1 reduced the hepatic clearance of urea-N by 9.1 L/h compared with saline superinfusion (20.3 ± 7.2, $P < .001$ *v* saline).

The apparent N exchange, measured in the fasting preinfusion period in the absence of any N supply, was markedly negative (Table 2 and Fig 3). During continuous alanine infusion, at steady-state α-amino-N concentrations, the amount of urea produced in the liver (sum of plasma urea-N accumulation and urinary urea-N excretion) was lower than the alanine-N infusion rate, resulting in a net positive N exchange (Table 2). However, significant differences were observed in relation to the superinfusion of saline or PGE_1. PGE_1 superinfusion decreased plasma urea-N accumulation by nearly 50% and urinary urea-N excretion by over 30%, resulting in a net N exchange that was significantly more positive ($P < .001$ *v* saline). All subjects had a more positive N exchange during PGE_1 superinfusion (Fig 3).

The heart rate was, on average, 79.4 ± 7.9 bpm in the basal period and 77.6 ± 5.0 at the end of saline infusion; mean arterial pressure was 106.8 ± 6.3 and 107.5 ± 6.0 mm Hg, respectively. PGE_1 infusion produced a mild increase in the heart rate (84.4 ± 6.9, $P < .05$ *v* basal and saline infusion) and a decrease in mean arterial pressure (101.3 ± 5.5, $P < .01$ *v* basal and saline infusion).

Table 1. Substrate and Hormone Levels and Urea-N Synthesis Rate in the Fasting Preinfusion Period (−90 to 0 minutes) and During Steady-State α-Amino-N Concentrations Obtained by Primed-Continuous Alanine Infusion With and Without PGE_1 Superinfusion (mean ± SD)

Parameter	Basal Period	Alanine Infusion*	
		Saline Infusion	PGE_1 Infusion
α-Amino-N	2.94 ± 0.76	5.64 ± 1.09	5.80 ± 1.12
Urea-N	12.9 ± 3.8	16.1 ± 4.0	16.0 ± 3.9
Glucose	14.5 ± 2.0	11.4 ± 2.6	10.9 ± 2.5
Insulin	227 ± 111	308 ± 171	258 ± 150
Glucagon	44.9 ± 15.8	99.7 ± 30.9	95.7 ± 16.7

NOTE. Glucose, urea-N, and α-amino-N concentrations are in mmol/L; insulin and glucagon are in pmol/L. Control values in our laboratory are as follows: α-amino-N, 2.5-3.5 mmol/L; insulin, 36-108 pmol/L; and glucagon, 15-50 pmol/L.

* All values are significantly different *v* basal.

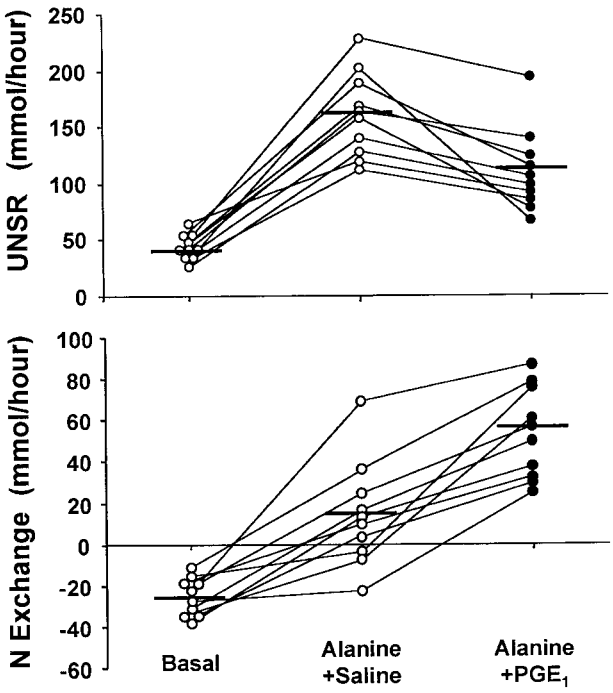

Fig 3. UNSR and apparent nitrogen exchange in the basal period and during alanine infusion with superimposed saline (○) or PGE_1 (●) infusion. Data for individual patients are connected by a line. Bold horizontal lines represent mean values. All values measured during the alanine load are significantly different *v* baseline; the difference between alanine + saline and alanine + PGE_1 is also significant ($P < .001$).

DISCUSSION

The present study shows that PGE_1 infusion significantly influences amino acid-N economy in patients with type 2 diabetes in poor metabolic control, leading to a significant nitrogen sparing. This effect does not depend on changes in the glucoregulatory hormones, insulin and glucagon, also responsible for hormone-mediated control of urea synthesis.

These conclusions are based on changes in the plasma level and urinary excretion of urea-N and on total-body N exchange, suggesting that during PGE_1 infusion, the production of urea in the liver is reduced. Were the block at the hepatic level, amino acids would accumulate in the plasma during alanine infusion and PGE_1 superinfusion. Since no significant accumulation of α-amino-N was observed, PGE_1 is more likely to act via a block in amino acid outflow from both the liver and peripheral tissues and/or via an increased amino acid uptake for protein synthesis.

The methodology of the present study, ie, measurement of the dynamics of hepatic urea synthesis during standardized conditions of substrate availability,[21] is similar to that previously used to measure the effects of hormones or medications in several physiologic[27-29] and pathologic conditions.[17-19,30-32] The assumptions underlying the technique have been covered extensively in a review article.[21] In the calculation of the UNSR, intestinal hydrolysis was considered a fixed fraction of total urea-N excretion on the basis of the mean values derived from the literature,[19] and urea space was assumed to equal TBW indirectly estimated from a nomogram. Any error introduced by these assumptions is not relevant for the final results, since paired data are expected to be similarly affected and there is no evidence for a direct effect of PGE_1 on intestinal hydrolysis and/or TBW.

In comparison to previous experiments,[14,30,31] the standard procedure for UNSR measurement in humans was modified to measure the response of urea-N synthesis to infusion of PGE_1 and saline in a single experimental setting. Accordingly, the alanine infusion rate was calibrated to obtain steady-state α-amino-N concentrations for a period sufficient to measure the UNSR in both the presence and the absence of PGE_1. The extremely short half-life of the agent (only a few seconds) guarantees no carryover effects after infusion. Anyway, to avoid differences in hormone and substrate levels, the superinfusion periods were rotated, with 5 subjects having PGE_1 infusion first and 5 having saline first. Irrespective of which superinfusion was administered first, PGE_1 significantly decreased the UNSR. In patients receiving PGE_1 first, the UNSR was 125.4 ± 42.3 mmol/h during PGE_1 and increased to 153.8 ± 45.8 during saline ($P < .005$); in patients receiving saline first, the UNSR was 101.6 ± 23.6 and 168.5 ± 29.0 during PGE_1 and saline, respectively ($P < .01$). The small nonsignificant difference in the UNSR according to the random procedure may derive from the slightly higher α-amino-N levels observed soon after priming the alanine infusion.

PGs were reported to increase renal blood flow in experimental animals.[33] Also, in man, there is evidence that both PGE_1 infusion[34] and peroral misoprostol[35] may increase renal plasma flow. Renal flow was not measured in the present study, but increased renal flow would be expected to increase urinary urea-N excretion. This did not happen in the present study; urine flow was not different in paired alanine-stimulated conditions, and urinary urea-N excretion was, on average, reduced by 30% by PGE_1.

The process of urea synthesis is linearly dependent on substrate availability and hormone concentrations.[21] In a previous study, the dynamics of α-amino-N to urea-N formation in

Table 2. Calculation of the Apparent Nitrogen Exchange in the Fasting State Before Alanine Infusion and During Continuous Alanine Infusion in Paired Periods With and Without PGE_1 Superinfusion (mean ± SD in mmol/h)

Parameter	Basal Period	Alanine Infusion	
		Saline Infusion	PGE_1 Infusion
N infusion rate	—	146 ± 21	
Plasma α-amino-N accumulation	−6.1 ± 6.0	−0.3 ± 0.3*	−0.2 ± 0.6*
Plasma urea-N accumulation	−12.9 ± 9.8	46.5 ± 29.8*	25.1 ± 23.0*
Urinary urea-N excretion	44.0 ± 11.9	85.6 ± 29.8*	68.0 ± 26.7*†
Urea-N production	31.1 ± 7.7	132.2 ± 30.3*	93.1 ± 28.4*†
Apparent N exchange	−25.0 ± 9.0	14.6 ± 25.1*	53.5 ± 21.4*†

* Significantly different *v* basal.

† Significantly different *v* saline infusion.

poorly controlled type 2 diabetes was shown to be characterized by increased hepatic amino-N disposal,[19] and maintenance of N sparing at low amino-N levels but N losses after the load.[17] These results were confirmed in the present setting; the basal UNSR was only moderately increased in comparison to normal values in our laboratory, whereas the UNSR increased markedly during alanine infusion in the absence of PGE_1. Accordingly, the hepatic clearance of amino acids was markedly stimulated, but the different methodology of the study does not allow any comparison. As for the effects of hormones on the dynamics of the process, both insulin and glucagon were stimulated by alanine, in keeping with the well-known activity of alanine on endocrine pancreatic secretion. However, no differences were observed in relation to PGE_1. This rules out a potential role of the glucoregulatory hormones in the metabolic effects of PGE_1.

The metabolic effects of PGE_1 were confirmed by computing the apparent N exchange, a measure of the total amount of nitrogen irreversibly transferred from the α-amino-N pool to the urea-N pool, where nitrogen can no longer be reused. This calculation is quantitatively feasible during steady-state conditions, when nitrogen accumulation in the α-amino-N pool does not occur. As expected, basal N exchange, measured in the period before alanine infusion, was negative since no extra nitrogen was administered to meet urinary urea-N losses. During alanine infusion, the apparent N exchange was only moderately positive and was not significantly different from 0 (Fig 3), in keeping with the failure to retain nitrogen after the load.[17] This is indicative of the increased amino acid consumption and nitrogen wasting of poorly controlled type 2 diabetes, where amino acids are used for gluconeogenesis under glucagon control.[36] During PGE_1 infusion, the apparent N exchange was markedly positive due to the combined effects of a modest plasma urea-N deaccumulation (expressed by a reduction of the plasma urea-N concentration) and lower than normal urinary urea-N excretion. It is concluded that PGE_1 infusion promptly reduces urea formation during alanine infusion.

As for the mechanism responsible for PGE_1-mediated nitrogen sparing, only hypotheses may be derived from the present experiment. Stiegler et al[11] measured the arterial–deep venous difference in plasma amino acids during arterial infusion of PGE_1 in fasting healthy subjects and found that amino acid balance became significantly positive during PGE_1 infusion, in keeping with an inhibition of proteolysis or a stimulation of protein synthesis. Experimental data showed that PGE_2 increases the intracellular transport of glutamine by activation of the hepatic system N, as well as the transport of small neutral amino acids, eg, alanine, by activation of the ubiquitous system A.[37] Similar data were also obtained on system y(+)-mediated L-arginine transport.[38] These results strongly support our finding of positive apparent nitrogen exchange. Determining whether this is due to decreased amino acid efflux or, more likely, increased amino acid uptake and nonoxidative disposal and whether the primary site is the liver and/or peripheral tissues requires further investigation.

Any hepatic effect of PGE_1 might be mediated by changes in splanchnic hemodynamics. According to the kinetic characteristics of urea formation, it can be calculated that any changes in splanchnic perfusion may only affect the functional hepatic nitrogen clearance by less than 5%.[39] In a previous study in a different experimental condition, systemic PGE_1 infusion significantly affected the systemic circulation,[14] in keeping with the well-documented activity of PGE_1 at similar doses,[40] but no significant effects on splanchnic blood flow were detected. More interestingly, the large effect on the systemic circulation (30% increase in femoral artery flow),[14] associated with a positive amino acid balance,[11] might be an additional argument supporting the hypothesis that the metabolic effects of PGE_1 on nitrogen metabolism may be mediated at the peripheral level.

In a variety of pathologic conditions, PGs have been used as ancillary therapy in critically ill patients. In addition to renal effects, they have been shown to improve hepatic graft function and to reduce primary nonfunction after orthotopic liver transplantation,[16,41] because of favorable effects on the microcirculatory system and an accelerated liver regeneration process. Such regenerative processes might be the result of the nitrogen sparing observed in the present study. Along this line are also studies showing that PGEs have an insulinomimetic activity, blocking hepatic glycogenolysis and glucose production induced by stress hormones in rats[42] and improving insulin sensitivity and glucose disposal in diabetes.[43] The favorable effects on nitrogen economy in patients with poorly controlled type 2 diabetes presented in this study have potential clinical relevance.

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