# Inhibition of renal ornithine decarboxylase activity prevents kidney hypertrophy in experimental diabetes

STEEN B. PEDERSEN, ALLAN FLYVBJERG, AND BJØRN RICHELSEN
*Division of Endocrinology and Metabolism, University Clinic of Internal Medicine, Aarhus Amtssygehus, and Institute of Experimental Clinical Research, Aarhus Kommunehospital, DK-8000 Aarhus C, Denmark*

**Pedersen, Steen B., Allan Flyvbjerg, and Bjørn Richelsen.** Inhibition of renal ornithine decarboxylase activity prevents kidney hypertrophy in experimental diabetes. *Am. J. Physiol.* 264 (*Cell Physiol.* 33): C453–C456, 1993.—The selective ornithine decarboxylase (ODC) inhibitor difluoromethyl ornithine (DFMO) was used to investigate the role of polyamines in initial diabetic renal enlargement. ODC activity in kidneys from diabetic animals was increased (fivefold) 24 h after diabetes induction ($P < 0.05$), and throughout the study (7 days) the activity remained 2- to 3-fold elevated ($P < 0.05$). Insulin treatment normalized renal ODC activity, whereas DFMO treatment totally inhibited the kidney ODC activity. The kidney weight in diabetic rats was 21% higher than that of control rats (1,074 ± 35 mg and 889 ± 16 mg, $P < 0.001$). Insulin treatment normalized kidney weight (847 ± 13 mg). Despite unaltered diabetic metabolic aberrations the kidney weight in DFMO-treated diabetic rats was normalized (911 ± 7 mg). In conclusion, the ODC activity in diabetic kidneys undergoing hypertrophy was increased. Insulin treatment normalized both kidney weight and kidney ODC activity. Finally, selective inhibition of ODC activity by DFMO resulted in kidneys of normal size, despite unaltered diabetic metabolic aberrations. These findings support the hypothesis that polyamines play an important role in initial diabetic renal enlargement.

difluoromethyl ornithine; polyamines; growth

EXPERIMENTAL DIABETES is associated with kidney hypertrophy and hyperfiltration (16, 27). However, the exact mechanism behind these alterations is still not fully clarified. Various hormones, including growth hormone (GH) (6), insulin-like growth factor-1 (IGF-1) (4–7), and glucagon (3) have been suggested as initiators of diabetic renal enlargement. However, the cellular events leading to growth are still unknown. We have previously suggested that the polyamine pathway is involved in diabetic kidney growth (20). It has previously been demonstrated that the intracellular enzyme ornithine decarboxylase (ODC) was maximally elevated shortly after diabetes induction (13, 14, 20). The ODC activity remained 2.5-fold elevated for at least 2 wk compared with control kidneys. Furthermore, insulin treatment normalized ODC activity as well as kidney weight in diabetic rats. It was therefore suggested that the ODC enzyme may act as a messenger in initiating the kidney growth.

The present study investigates this in more details by selectively blocking the ODC enzyme with difluoromethyl ornithine (DFMO) in untreated and insulin treated diabetic animals. Kidney weight and ODC activity were measured. To asses a possible toxic effect of the DFMO treatment, body weight, food intake, and metabolic control of the animals were measured.

## MATERIALS AND METHODS

*Materials.* DL-[1-$^{14}$C]ornithine (0.1 mCi/ml) was from New England Nuclear. All other reagents were purchased from Sigma Chemical, St. Louis, MO. DFMO was a generous gift from Dr. P. S. Mamont, Merrell Dow Research Institute, Strasbourg, France. A very long-acting, heat-treated Ultralente insulin was donated by Novo-Nordisk, Bagsvaerd, Denmark.

*Animals.* Male Wistar rats (Møllegaards Avlslab, Eiby, Denmark) with a mean body weight of 225 g were used in the study. The animals had free access to standard rat chow and water and were exposed to a 12:12-h light-dark cycle throughout the experiment. The animals were randomized into five groups: *1*) nondiabetic control animals, *2*) untreated diabetic animals, *3*) DFMO-treated diabetic animals, *4*) insulin-treated diabetic animals, and *5*) insulin- and DFMO-treated diabetic animals. Diabetes was induced on *day 0* as previously described by intravenous injection of streptozotocin (STZ, 55 mg/kg body wt) in acidic NaCl (0.154 M, pH 4.5) after 12 h of food deprivation (4).

DFMO treatment (200 mg/kg twice daily intraperitoneal throughout the study) was initiated immediately after STZ administration. Eighteen hours after administration of STZ and daily thereafter, the animals were weighed, urinanalysis was performed for glucose and ketone using Neostix 4 (Ames Limited, UK), and tail vein blood glucose was determined by Haemoglucotest 1–44 and a Reflolux II reflectance meter (Boehringer-Mannheim, Mannheim, FRG). Insulin was given in an initial dose of 4–6 U, (18 h after administration of STZ, when all animals had blood glucose levels >15 mmol/l) followed by 1–2 U daily, depending on blood glucose values. Twenty-four-hour food consumption was measured in all animals on *days 2* and *7*. On *days 1*, *2*, *3*, *4*, and *7*, eight rats from each of the four diabetic groups were studied, and six control rats on *days 0*, *2*, *4*, and *7*. The left kidney was removed under barbital sodium anesthesia (50 mg/kg body wt), rapidly cleaned, weighed, and immediately frozen in liquid nitrogen for later determination of ODC activity.

*ODC activity.* The activity of ODC was determined essentially as previously described (26). Briefly, the tissue was homogenized in a Polytron homogenizer three times for 10 s in a buffer containing 2.5 mM dithiothreitol, 0.1 mM pyridoxal 5′-phosphate, 0.1 M tris(hydroxymethyl)aminomethane (Tris), and 0.1 mM EDTA, pH 7.2. The homogenate was centrifuged for 60 min at 40,000 *g*, at 4°C. The supernatant (below the lipids, 250 μl) was incubated with cold ornithine (final concentration, 0.33 mM) and [$^{14}$C]ornithine (0.25 μCi) for 60 min at 37°C in a closed tube equipped with filterpaper wetted in phenylethylamine to trap released $^{14}CO_2$. The incubation was terminated by injecting 0.5 ml 5 M $H_2SO_4$; then the tubes were incubated for further 45 min at 37°C to release $^{14}CO_2$ from the incubation buffer. To estimate the nonspecific $^{14}CO_2$ release during the incubation, blank tubes were set up in which sulfuric acid was added before the supernatant, but otherwise the tubes were treated similarly, the nonspecific release was subtracted from the sample release. The filter papers were removed, and the trapped $^{14}CO_2$ was counted by liquid scintillation. The protein content was determined, and the specific ODC activity was

expressed as counts per minute (cpm) per milligram protein per hour.

*Statistical analysis.* Data are given as means ± SE. Significance was determined by one-way analysis of variance followed by Duncan's procedure performed in the SPSS statistical package.

## RESULTS

*Metabolic events.* As shown in Fig. 1*A*, the blood glucose increased to ~26 mmol/l 24 h after STZ injection and was normalized 24–48 h after initiation of insulin treatment. Blood glucose levels in untreated diabetic and DFMO-treated diabetic animals stabilized at ~26 mmol/l and were not significantly different. The body weight (Fig. 1*B*) in control rats increased from 223 ± 6 g at *day 0* to 267 ± 8 g at *day 7*. Induction of diabetes was accompanied by reduced body weight gain. However, insulin-treated diabetic animals regained weight from *day 2* and onward, and on *day 7* they had a weight comparable to control animals. DFMO-insulin-treated diabetic animals and insulin-treated diabetic animals had similar body weights. Untreated diabetic and DFMO-treated diabetic animals started to regain weight on *day 5*, and on *day 7* they had regained their initial weights. On *day 7* their body weights were significantly lower than DFMO-insulin-treated, insulin-treated diabetic, and control rats ($P < 0.05$).

No differences were found in 24-h food consumption on *day 2*, but on *day 7* a significant increase was seen in untreated diabetic rats and DFMO-treated diabetic rats (Fig. 2). Nondiabetic control rats, insulin-treated diabetic rats, and DFMO-insulin-treated diabetic rats had similar 24 h food intakes on *days 2* and *7* (Fig. 2).

*Kidney growth.* The kidney weight in diabetic rats increased by 21% on *day 7* compared with control rats (1074 ± 35 mg compared with 889 ± 16 mg $P < 0.001$) (Fig. 3*A*). The kidney weight in control rats increased from 803 ± 13 mg to 889 ± 16 mg during the study period. DFMO or insulin treatment of diabetic animals completely abolished the excessive kidney growth, resulting in a kidney weight at *day 7* of 847 ± 13 mg and 911 ± 7 mg (not statistically significant from nondiabetic control rats). Diabetic rats treated with DFMO and insulin had kidneys of similar size (849 ± 19 mg at *day 7*).

*ODC measurements.* The ODC activity from diabetic animals was increased fivefold on *day 1*, and from *day 2* and throughout the study the ODC activity was about two- to threefold higher than in control rats ($P < 0.05$) (Fig. 3*B*). Six hours after the first insulin injection, ODC activity tended to be reduced (from 2,101 ± 699 cpm·mg protein$^{-1}$·h$^{-1}$ to 1,583 ± 338 cpm·mg protein$^{-1}$·h$^{-1}$, $P > 0.15$). During the rest of the study insulin-treated diabetic rats had normal kidney ODC activity. DFMO treatment of untreated and insulin-treated diabetic rats rapidly decreased the ODC activity to almost undetectable levels (Fig. 3*B*).

*Effects of ODC inhibition on normal kidney growth.* To evaluate the effects of DFMO treatment on normal kidney growth, normal rats were treated for 7 days with either saline or DFMO (200 mg/kg twice daily). After 7 days the body weight, kidney weight, and ODC activity were studied. However, despite total inhibition of the ODC activity in the DFMO-treated rats, their weight gain and kidney growth were equal to that in control rats (Table 1).

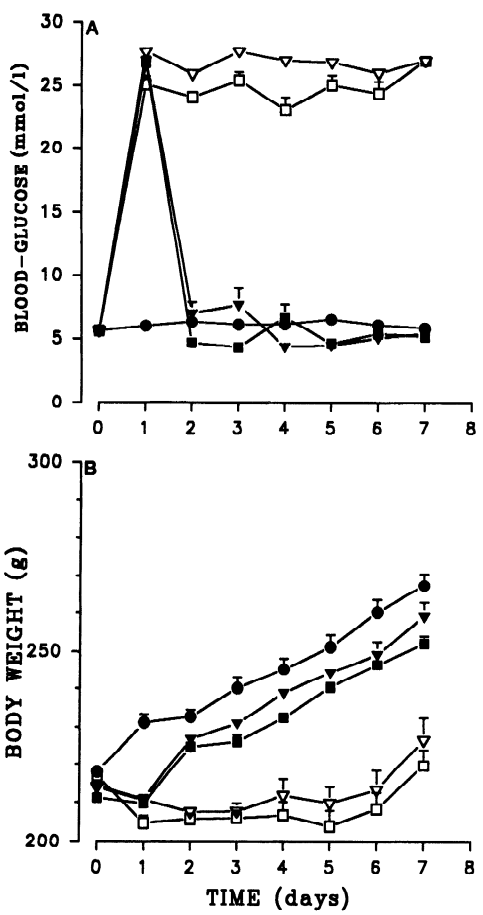


Fig. 1. Time course changes in blood glucose levels (*A*) and body weight (*B*) in 5 different groups studied. Difluoromethyl ornithine (DFMO) treatment (200 mg/kg twice daily) was given immediately after streptozotocin (STZ) administration, and insulin treatment was initiated 18 h later. •, Nondiabetic control rats; ▽, untreated diabetic rats; ▼, insulin-treated diabetic rats; □, DFMO-treated diabetic rats; and ■, insulin- and DFMO-treated diabetic rats. Values are means ± SE ($n$ = 6–8).

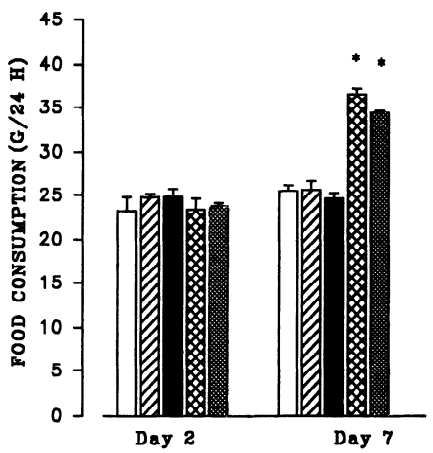


Fig. 2. Twenty-four hour food consumption on *days 2* and *7* in nondiabetic control rats (open bars); untreated diabetic rats (cross-hatched bars); insulin-treated diabetic rats (solid bars), DFMO-treated diabetic rats (stippled bars), and insulin- and DFMO-treated diabetic rats (hatched bars). * $P < 0.05$ compared with nondiabetic control rats. Values are means ± SE ($n$ = 6–8).

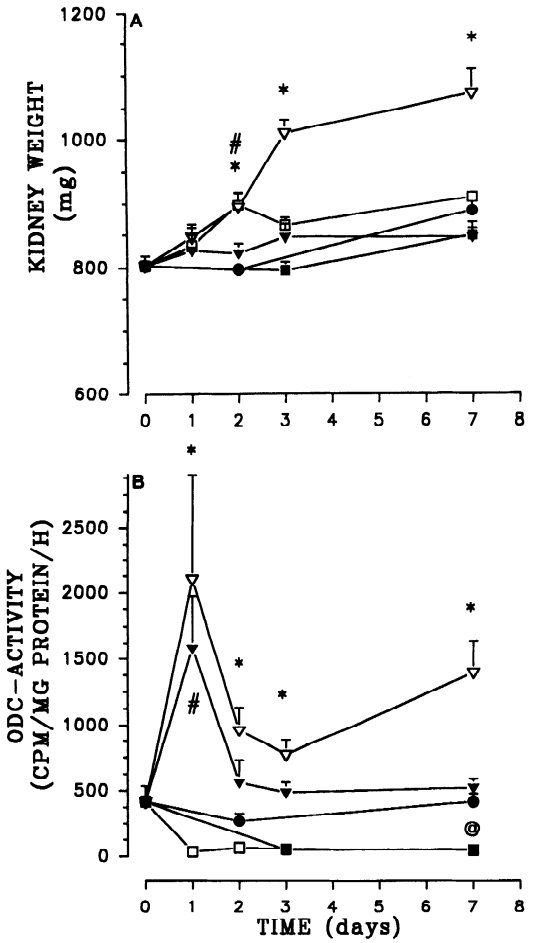


Fig. 3. Time course changes in left kidney weight (*A*) and kidney ODC activity (*B*) in nondiabetic control rats (●), untreated diabetic rats (▽), insulin-treated diabetic rats (▼), DFMO-treated diabetic rats (□), and insulin- and DFMO-treated diabetic rats (■). * $P < 0.05$ between nondiabetic control rats and untreated diabetic rats. # $P < 0.05$ between nondiabetic control rats and insulin treated diabetic rats. @ $P < 0.05$ between nondiabetic control rats and DFMO-treated diabetic and DFMO-insulin treated diabetic rats. Values are mean ± SE ($n$ = 6-8).

Table 1. *Effects of DFMO treatment on normal kidney growth*

| | *Day 0* | *Day 7* | |
|---|---|---|---|
| | | Control rats | DFMO-treated rats |
| Body weight, g | 261.3±3.0 | 307.5±4.3 | 304.3±4.0 |
| Kidney weight, mg | 931.8±17.1 | 1051.4±29.5 | 1036.0±30.3 |
| ODC activity, cpm·mg protein$^{-1}$·h$^{-1}$ | 687±86 | 506±66 | 3±1* |

Data are means ± SE. Body weight as well as kidney weight were measured before treatment was initiated. Rats were randomized into 2 groups: 1 group received intraperitoneal injections with isotonic saline (1 ml twice a day for 7 days) and other group received difluoromethylornithine (DFMO) treatment (200 mg/kg twice a day for 7 days). After 7 days rats were killed, and body weight, kidney weight, and kidney ornithine decarboxylase (ODC) activity were measured. * $P < 0.001$ between control and DFMO-treated rats. No. of rats = 7 for DFMO-treated group and 8 for Control and *Day 0*.

## DISCUSSION

Polyamine formation is important for growth of different cell lines and organs and inhibition of the ODC activity reduces or prevents growth in various tissues (10, 15, 22, 24). In the present study the effect of DFMO on renal hypertrophy was investigated in the initial kidney growth phase, taking place within the first week after induction of diabetes. The rationale for examining a possible effect of a specific ODC inhibitor on renal growth in diabetic rats was determined by the previous finding that a pronounced increase in kidney ODC occurs in diabetic kidneys within the first days after induction of diabetes before measurable growth, suggesting that the ODC enzyme is implicated in the initial growth phase (13, 14, 20). Our present results demonstrate that DFMO administration initiated at onset of diabetes totally abolishes the rise in kidney ODC and diabetic kidney growth, thus supporting the hypothesis that polyamines are involved in diabetic renal enlargement.

In accord with previous studies (6), untreated diabetic control animals exhibited a marked growth. In contrast, no kidney growth was seen in diabetic animals treated with DFMO. This difference might be due to other mechanisms than a specific inhibition of the ODC enzyme by DFMO. However, whereas the renal hypertrophy was abolished in DFMO-treated diabetic rats, there was no effect of DFMO on the severity of diabetes as indicated by comparable blood glucose levels, body weight changes, and food consumption. These findings indicate that the observed difference is not attributable to differences in metabolic control or to toxic effects of the drug (e.g., anorexia). In addition, we didn't find any inhibitory effect of DFMO administration on the slower normal renal growth despite total inhibition of the ODC enzyme activity, suggesting that polyamines play an important role for the diabetic kidney growth but have no role in the normal physiological kidney growth.

Polyamines have previously been shown to play a very important role in cellular division and hyperplasia, and inhibition of polyamine formation from ornithine by treating the cells with DFMO halts cellular division (8, 15, 21). However, the first 2 days after diabetes induction, the DFMO-treated diabetic rats had a kidney growth similar to untreated diabetic rats. An explanation for this finding might be that the initial kidney growth (i.e., the first 2 days) is due to primarily hypertrophy, whereas later on hyperplasia is the major event. Another possibility could be that the polyamine pool in the cells is sufficient for one or two cellular divisions as suggested by Heby (9).

Various hormonal factors have been proposed in the hypertrophy-hyperfiltration syndrome of diabetes. Glucagon received interest because imperfectly controlled experimental (1) and human diabetes (23) are characterized by elevated circulating glucagon levels. Furthermore, glucagon administration to normal and diabetic subjects (18, 19) and rats (11) is followed by increased renal function. In addition, a relationship between the increase in plasma glucagon and renal size in diabetic rats has been suggested (3). However, Almdal et al. (2) have demonstrated that glucagon administration in well-controlled diabetic rats does not induce kidney growth. GH and IGF-I have been increasingly implicated as possible mediators of renal hypertrophy and function. Diabetic dwarf rats with isolated GH and IGF-I deficiency exhibit slower and lesser initial

renal and glomerular hypertrophy (7), and IGF-I infusion to diabetic rats accelerates diabetic renal hypertrophy (5). Future research should be directed at elucidating possible effects of various hormones, including glucagon, GH, and IGF-I on kidney ODC activity. Such stimulatory effect may indicate that the ODC enzyme acts as a second messenger system for various hormones. At present it is already well established that testosterone (12, 25) and estrogen (17) both stimulate ODC activity in the kidney; however, these hormones have not been implicated as mediators for diabetic renal growth.

In the present study, DFMO administration was initiated at onset of diabetes. To evaluate a possible involvement of polyamines in long-term diabetic kidney disease and to know whether DFMO treatment is applicable in future clinical trials, it is necessary to investigate whether DFMO has reversible effects on established experimental diabetic kidney abnormalities.

In conclusion, the present study confirmed the previous finding that diabetic kidney hypertrophy is associated with increased kidney ODC activity and that insulin treatment, in doses inducing euglycaemia, normalizes both kidney ODC and renal size. Inhibition of the kidney ODC activity by DFMO has an equally inhibitory effect on kidney growth without affecting the metabolic control, thereby supporting the hypothesis that polyamines play an important role in the pathophysiological diabetic renal enlargement.

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Address for reprint requests: S. B. Pedersen, University Clinic of Internal Medicine, Div. of Endocrinology and Metabolism, Aarhus Amtssygehus, DK-8000 Aarhus C, Denmark.

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