Technetium-99m MAG-3 Clearances After Captopril in Experimental Renovascular Hypertension

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Rats with one kidney clamped (2K1C), both kidneys clamped (2K2C), unilaterally nephrectomized with remaining kidney clamped (1K1C), and normals, were studied using ^{99m}Tc mercaptoacetyltriglycine ([^{99m}Tc]MAG-3) and ^{131}I orthoiodohippurate ([^{131}I]OIH). Clearances of [^{99m}Tc]MAG-3 and [^{131}I]OIH were performed after constricted rats became hypertensive. Clearances were repeated after i.v. Captopril. Clearances of [^{99m}Tc]MAG-3 and [^{131}I]OIH in normals didn't change significantly after Captopril. Clearances of [^{99m}Tc]MAG-3 and [^{131}I]OIH decreased insignificantly after Captopril in the 2K2C model. In the 2K1C group, normal kidney clearance increased ([^{99m}Tc]MAG-3 $p < 0.01$ and [^{131}I]OIH $p < 0.025$) and clamped kidney clearance decreased after inhibition ([^{99m}Tc]MAG-3, $p < 0.01$, [^{131}I]OIH $p < 0.02$). Clearances increased in the 1K1C group after Captopril ([^{99m}Tc]MAG-3 $p < 0.0025$ and [^{131}I]OIH, $p < 0.001$). The ratio of [^{99m}Tc]MAG-3 to [^{131}I]OIH before Captopril was 0.81 and 0.84 after Captopril. Changes in renal function after Captopril depend on the model of renovascular hypertension and possibly the dose administered. Technetium-99m MAG-3 clearance parallels [^{131}I]orthoiodohippurate in renovascular hypertension.

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The rationale for the initial use of angiotensin converting enzyme inhibition (ACE) in the differential diagnosis of renovascular hypertension was based largely on empirical observations in man. Subsequent experimental studies in animals and humans have provided a more rational basis for its use (*1,2*). These studies suggest that the short-term administration of the angiotensin converting enzyme inhibitor (Captopril) affects the filtration pressure, glomerular filtration rate, and renal plasma flow (RPF) in kidneys ipsilateral to renal arterial constriction but has little or no effect on kidneys with a normal blood supply (*3,4*). The specificity of these changes offers a potential to greatly increase the accuracy of radionuclide tests in renovascular hypertension (RVH, Captopril renography). One of the radiopharmaceuticals used to estimate the renal plasma flow in these studies was iodine-131 orthoiodohippurate ([^{131}I]OIH). Although [^{131}I]OIH has a high extraction ratio, its radioiodine labeling unfortunately results in a radiopharmaceutical with poor imaging characteristics (*5–7*). Iodine-123 OIH is an ideal substitute for [^{131}I]OIH with desirable imaging and dosimetry characteristics. Unfortunately its use is restricted because of its high cost and difficulties with ready availability. Recently technetium labeled mercaptoacetyltriglycine ([^{99m}Tc]MAG-3) has been introduced as a potential substitute for hippuran in renal imaging and function studies (*8–10*). Technetium-99m MAG-3 is more highly bound to plasma protein (77%) than [^{131}I] OIH (35%), but has a rapid plasma clearance and extraction efficiency (*8,10*). Its radiotechnetium label is potentially a major improvement over radioiodine for the study of renovascular hypertension.

The study reported here was designed to assess the potential use of [^{99m}Tc]MAG-3 in place of [^{131}I]orthoiodohippurate for the differential diagnosis of renovascular hypertension. The substitution of the ^{99m}Tc label would result in far more useful images and better count rate statistics. Three models of renovascular hypertension were used, each with different pathophysiology corresponding to the clinical situation.

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METHODS

Technetium-99m MAG-3 was prepared from a lyophilized kit supplied by Mallinckrodt Company. The lyophilized vials were stored at room temperature and reconstituted with 20 mCi ^{99m}Tc generator eluate contained in 4 ml. Excess stannous ion was oxidized by venting the vials with a hypodermic needle immediately following the introduction of the ^{99m}Tc generator eluate, and 2 ml of sterile air was introduced into the vial using a hypodermic syringe and an 0.22-μM Luer adaptor—fitted in line filter. Vials were then heated for 10 minutes in a boiling water bath and assayed for radiochemical purity by ascending paper chromatography (60:40 acetonitrile: water, (Rf=0.65)) immediately and 3 hr after reconstitution. Iodine-131 orthoiodohippurate (Mallinckrodt Company) was purchased as a commercial preparation supplied for routine clinical use.

Forty Sprague Dawley male rats weighing 300–350g were used for the studies. Four groups of rats (each with ten animals) were investigated: rats with two normal kidneys (2K2N); rats with two kidneys one clamped (2K1C) corresponding to unilateral renovascular disease; rats with two kidneys both clamped (2K2C), corresponding to bilateral disease, and rats previously nephrectomized with 1 kidney clamped (1K1C); corresponding roughly to renal transplants with stenosis. The renal artery was clamped with two millimeter lengths of polyethylene tubing (0.58 mm ID) cut longitudinally and placed over the main renal artery taking care not to interfere with smaller arterial branches. The tubing was tied in place with 4-0 surgical silk thread. The rats were maintained on a normal diet with standard laboratory Purina rat chow and were allowed water ad libitum until they recovered from surgery and became hypertensive (~1–2 wk). Femoral vein and femoral artery catheters (PE 50 silastic tubing) and ureteral (PE 10) catheters were inserted under light ether anesthesia at the time of the clearance study when the rats were hypertensive. Total clearance was measured in the normal rats using a bladder catheter. Clearances were performed after the animals awakened from anesthesia and were alert. Ten microcuries per ml of [^{99m}Tc]MAG-3 and [^{131}I] OIH were mixed in normal saline and a 0.3 ml priming dose was injected intravenously. A continuous infusion of 1.5 ml per hr was maintained using the SAGE automatic infusion pump. About 20 min after the priming dose, when a steady state of plasma concentration of radioactivity was reached, clearances were begun and were determined every 10 min for the subsequent 40 min. If the baseline studies were stable, Captopril was injected after they were completed using a dose of 1–1.2 mg per 100g BWt in water administered via the femoral vein catheter. After the blood pressure stabilized at the new level, clearance was again determined serially as previously described. Urine was collected from the ureters and 0.15 ml of blood (two heparinized hematocrit capillary tubes) from the femoral artery was obtained for clearance determination at the midpoint of each clearance period. The tubes were spun in a micro centrifuge and the plasma was used for clearance calculation. The blood pressure was measured using the femoral artery catheter just before and after each blood sample was collected. All plasma samples were counted in a well counter with correction for ^{131}I downscatter into the ^{99m}Tc window. The samples were recounted after ^{99m}Tc had been allowed to decay to background levels to confirm the accuracy of the count. The mean of the four clearance periods before and after Captopril were used for the analysis. All data were analyzed using a t-test of paired comparisons and by linear regression analysis.

An additional group of 14 animals received 5.5 mg/100 g BWt or 2.5 mg/100 g BWt of probenicid by injection to further evaluate the similarity of [^{99m}Tc]MAG-3 to [^{131}I]OIH in its renal excretion characteristics.

RESULTS

Table 1 shows the data for the [^{99m}Tc]MAG-3 and [^{131}I]OIH clearances. The actual clearance values for [^{99m}Tc]MAG-3 and [^{131}I]OIH are shown for each group before and after the administration of Captopril. Blood pressure values are shown also. The [^{99m}Tc]MAG-3 clearance is consistently less than the clearance of [^{131}I] OIH. The correspondence in the changes of the two compounds is consistent. The mean blood pressure in the normal control group of rats was significantly lower than in the animals whose renal arteries were clamped. The blood pressure decreased significantly after Captopril administration in all groups (normal, $p < 0.001$, 2K1C $p < 0.005$, 2K2C $p < 0.002$, and 1K1C $p < 0.015$) (Fig. 1). The control blood pressures and post Captopril blood pressures correlated significantly (2K2N; $r = 0.86$, 2K1C; $r = 0.90$, 2K2C; $r = 0.96$, and 1K1C; $r = 0.86$). In the control group and 2K2C group the clearance of [^{99m}Tc]MAG-3 and [^{131}I]OIH did not change significantly after captopril administration. In the 2K1C group, the normal kidney increased its clearance of both [^{99m}Tc]MAG-3 ($p < 0.01$) and [^{131}I]OIH ($p < 0.02$) significantly. The clamped kidney clearance of both [^{99m}Tc]MAG-3 and [^{131}I]OIH decreased significantly ($p < 0.01$ and < 0.02, respectively.) In the 1K1C group clearance increased significantly after Captopril ([^{99m}Tc]MAG-3 $p < 0.0025$ and [^{131}I]OIH $p < 0.001$) even though the blood pressure decreased significantly. A significant linear correlation of the clearances of all the groups was observed between [^{99m}Tc]MAG-3 and [^{131}I]OIH for both the control (Fig. 2) and the post-Captopril (Fig. 3) periods. The correlation of [^{99m}Tc] MAG-3 to [^{131}I]OIH clearance (control and after Captopril) in the 2K2N group was r=0.97 and r=0.94, respectively; in the 2K1C group the unclamped kidney correlation of [^{99m}Tc]MAG-3 to [^{131}I]OIH before and after Captopril was r=0.89 and r=0.94 and the correlations for the clamped kidney was r=0.97 and r=0.99. In the 2K2C model LK correlation was r=0.91 before and r=0.95 after Captopril. The RK was r=0.97 and r=0.99. In the 1K1C model r=0.86 and r=0.80 between [^{99m}Tc]MAG-3 and [^{131}I]OIH before and after Captopril. The average clearance ratio of the clearance of [^{99m}Tc]MAG-3/[^{131}I]OIH among all of the groups prior to Captopril was 0.81 ± 0.09 (n=60) and post-Captopril

TABLE 1
Renal Clearance (ml/min/100 BWt) Before and After Captopril Administration

		Control (Pre-Captopril)				Post-Captopril			
Model	Kidney	[^{99m}Tc]MAG-3	[^{131}I]OIH	MAG-3/OIH	BP (mmHg)	[^{99m}Tc]MAG-3	OIH	MAG-3/OIH	BP (mmHg)
Normal	2N	1.99 ± 0.41	2.50 ± 0.54	0.80 ± 0.05	105 ± 13	2.02 ± 0.58	2.33 ± 0.48	0.86 ± 0.10	95 ± 12‡
2K1C	(N)	0.94 ± 0.15	1.16 ± 0.22	0.83 ± 0.07	143 ± 23	1.05 ± 0.14*	1.28 ± 0.24†	0.83 ± 0.06	130 ± 18‡
	(C)	0.86 ± 0.15	1.03 ± 0.19	0.84 ± 0.05		0.52 ± 0.45*	0.64 ± 0.55†	0.87 ± 0.10	
2K2C	(LC)	0.79 ± 0.23	1.00 ± 0.23	0.79 ± 0.09	155 ± 32	0.66 ± 0.37	0.80 ± 0.44	0.82 ± 0.09*	138 ± 22‡
	(RC)	0.72 ± 0.33	0.95 ± 0.39	0.76 ± 0.08		0.59 ± 0.51	0.75 ± 0.64	0.81 ± 0.10*	
1K1C	(C)	1.26 ± 0.19	1.49 ± 0.20	0.84 ± 0.07	153 ± 11	1.43 ± 0.16*	1.68 ± 0.16*	0.85 ± 0.06	146 ± 13†

N = Unclamped kidney.
C = Clamped kidney.
* $p < 0.05$.
‡ $p < 0.02$.
† $p < 0.001$.
± = s.d.

was 0.84 ± 0.09 ($r=0.60$ $p < 0.001$) (Fig. 4). Although the ratio of clearance correlated very highly, the small difference in the ratio pre- and post-Captopril was statistically significant ($p < 0.025$). The radiochemical purity of the [^{99m}Tc]MAG-3 which was checked by ascending paper chromatography was 98.5% ± 0.84 (s.d. n=19).

The effect of probenecid on both [^{99m}Tc]MAG-3 and [^{131}I]OIH appeared quite similar regardless of the dose of probenecid administered. The percent change in [^{99m}Tc]MAG-3 clearance after probenecid correlated quite closely with the percent change in [^{131}I]OIH clearance ($r=0.95$, $p < 0.001$).

DISCUSSION

The effects of a Captopril dose of 1–1.2 mg per 100g BWt on the renal clearance and blood pressure in various models of renovascular hypertension and in normal control animals in this study was dependent on the pathophysiologic characteristics of each model. The mean blood pressure decreased significantly in all four groups after Captopril administration (Table 1). However, the percentage reduction of the blood pressure in each group was significantly lower than that observed in a previous study which used 1.7mg of Captopril per 100g BWt in all groups (*1*) except for the 2K2C model (Table 2). Table 2 compiles data from this study (Captopril dose 1–1.2 mg/100 g BWt) and a previous report (Captopril dose 1.7 mg/100 g BWt) for purposes of comparison (*1*). All values are expressed as percent change to make comparison easier. There appear to be significant differences between models which are further accentuated by differences in the dose of Captopril used. The relative contribution of the reduction in blood pressure and the inhibition of converting enzyme on the renal functional changes observed after Captopril is an important subject which warrants further study.

Acute low dose Captopril administration in this study which was chosen to minimize the blood pressure effect did not affect [^{131}I]OIH clearance in the 2K2N model. The contralateral kidney of the 2K1C model increased its clearance significantly. The higher doses of Captopril which were used in a previous study increased clearance in normal rats, and in the contralateral kidney in 2K1C (*1*). The relative increase of clearance in the normal two kidney model was less than the increase in the contralateral normal kidney in the 2K1C model. The clearance in the clamped kidney in the 2K1C model decreased to the same level in both the low dose and high dose studies.

These observations on a dose dependent effect are similar to previous reports of results in patients with renal artery stenosis. When patients acutely received 25 mg Captopril (*2*), the function of the contralateral kidney did not change, but the kidney ipsilateral to the stenosis decreased its clearance significantly. The acute response to 50 mg Captopril in patients with unilateral RAS resulted in a decrease in extraction efficiency of [^{131}I]OIH significantly in both kidneys with or without RAS (*4,11*).

Captopril in this study affected the 1K1C group differently than the other animal models. In the 1K1C group low dose Captopril administration increased [^{131}I] orthoiodohippurate clearance even though the blood

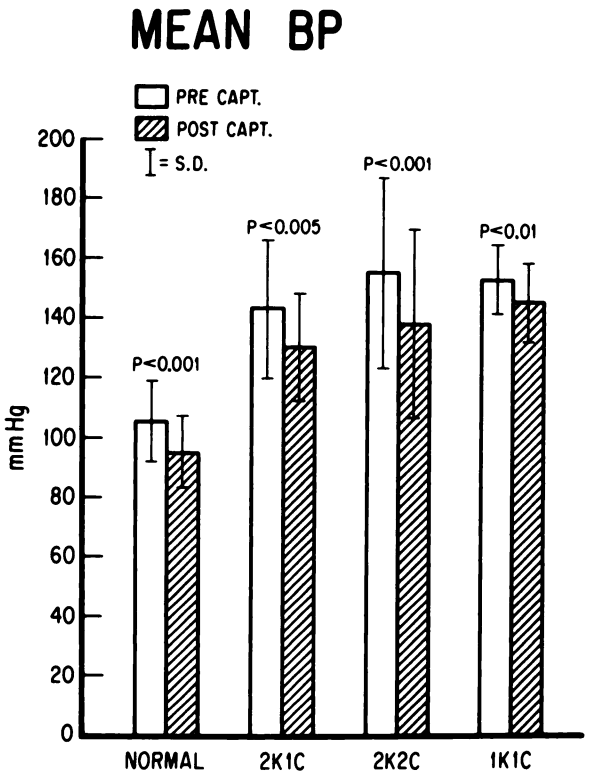

FIGURE 1
The mean blood pressure before and after the administration of Captopril in each of the animal models studied is shown on the bar graph. Although the degree of change varied, it was significant in each model. The blood pressures in the animals with renal artery stenosis are all significantly higher than in the control animals.

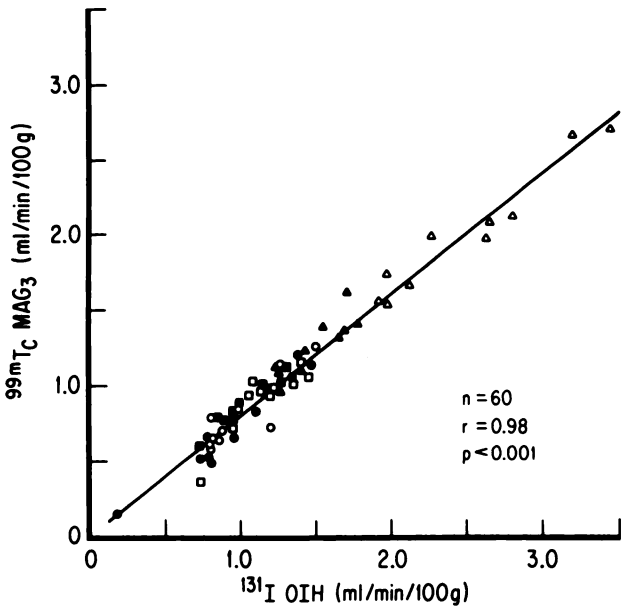

FIGURE 2
The control clearance of [^{99m}Tc]MAG-3 is plotted against the clearance of [^{131}I]OIH for each group. There does not appear to be any difference between any of the groups in the correlation which is highly significant. This supports the similar behavior of [^{99m}Tc]MAG-3 and [^{131}I]OIH in a wide variety of pathophysiologic situations. (△) Normal, (□) 2K1C (N), (■) 2K1C (C), (○) 2K2C (L), (●) 2K2C (R), (▲) 1K1C.

pressure decreased. The higher dose used previously decreased clearance significantly.

These data suggest that the results obtained after Captopril administration may vary with the dose of ACE used and the model of hypertension studied.

Technetium-99m MAG-3 clearance correlated well with [^{131}I]OIH clearance in all models in the present study. The control values of [^{99m}Tc]MAG-3 and [^{131}I] OIH in all groups correlated very closely (r =0.98) (Fig. 2) and after Captopril administration the clearance of the two compounds maintained this close correlation (r=0.98) (Fig. 3). The ratio of [^{99m}Tc]MAG-3 to [^{131}I] OIH was increased from 0.81 ± 0.07 (s.d. n=60) to 0.84 ± 0.09 ($p < 0.001$) for Captopril administration. This very small change, although statistically significant, is probably of little if any physiologic or clinical significance. The close correlation suggests that although the degree of change in [^{99m}Tc]MAG-3 or [^{131}I]OIH clearance may differ slightly, both change in the same direction, yielding similar information. Further proof of the similar behavior of [^{99m}Tc]MAG-3 and of [^{131}I]OIH is provided by the close correlations of the clearance before and after Captopril. The blockade of [^{99m}Tc] MAG-3 transport by probenecid has been observed previously (*12*). Technetium-99m MAG-3 has shown considerable promise in recent animal and patient volunteer studies as a ^{99m}Tc replacement for [^{131}I]OIH although the [^{99m}Tc]MAG-3 excretion is not only a

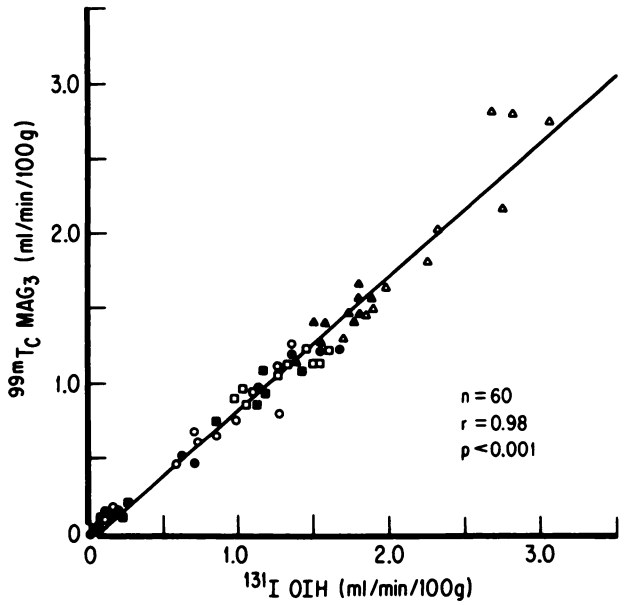

FIGURE 3
The clearance of [^{99m}Tc]MAG-3 after Captopril is plotted against the clearance of [^{131}I]OIH for each group. The correlation is highly significant and holds for all groups. The symbols are defined in the legend for Figure 2.

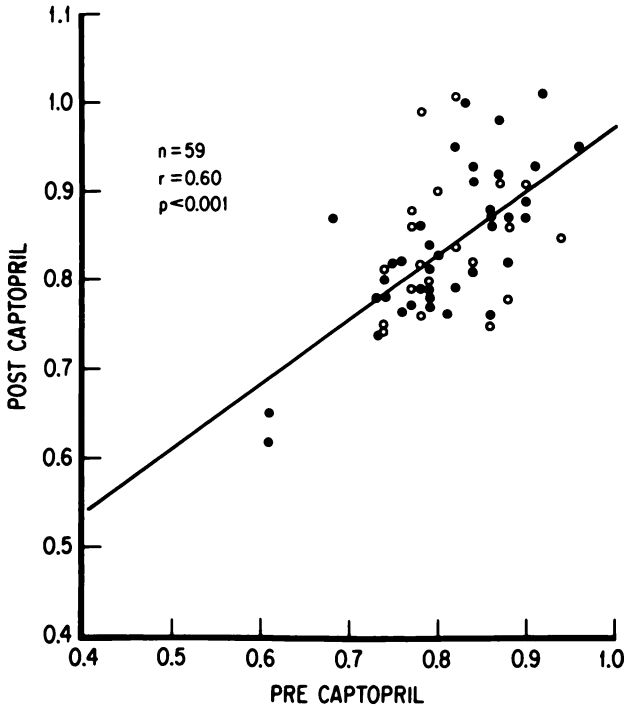

FIGURE 4
The correlation of the ratio of [^{99m}Tc]MAG-3/I-131 OIH before and after Captopril is plotted. The relationship is highly significant, although more variable than the absolute clearance value. (○) Normal K, (●) Clamped K.

function of plasma clearance by the kidney, but is also a function of the distribution of the radiocompound throughout the body other than kidneys (*12–14*). Some patients with impaired renal function showed minimal gallbladder activity after receiving [^{99m}Tc]MAG-3 (*13*). This drawback of [^{99m}Tc]MAG-3 is offset by the fact that [^{131}I]OIH has an unfavorable gamma energy for the gamma camera (*15*). A radiopharmaceutical labeled with ^{99m}Tc and a renal clearance similar to OIH would more closely approach the ideal agent for the study of effective renal plasma flow in RVH. The relative value of a renal plasma flow agent and of a GFR agent has not been adequately studied to determine if only one type of agent or both are necessary for accurate diagnosis.

It appears that both [^{99m}Tc]MAG-3 and [^{131}I]OIH plasma clearance adequately differentiate the contralateral normal kidney and renal artery clamped kidney in the 2K1C model after the administration of 1–1.2 mg Captopril per 100g BWt. Technetium-99m MAG-3 clearance parallels [^{131}I]OIH clearances in all of the models employed in this study, suggesting that [^{99m}Tc]MAG-3 is a potential substitute for [^{131}I]OIH in Captopril studies of RVH.

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TABLE 2
Relation of Captopril Dose to Changes in Blood Pressure and Clearance

	Δ Blood pressure (%)		Δ [^{131}I]OIH clearance (%)	
	Captopril dose 1–1.2mg/100g BWt	Captopril dose 1.7mg/100g BWt	Captopril dose 1–1.2mg/100g BWt	Captopril dose 1.7mg/100g BWt
Normal	−7.5 ± 1.6	−11.8 ± 1.7*	−5.6 ± 5.4	11.43 ± 12.06
2K1C (N)	−8.1 ± 2.0	−16.3 ± 1.9†	12.19 ± 4.8	21.46 ± 5.09
(C)			−46.48 ± 15.23	−46.48 ± 7.13
2K2C (L)	−10.2 ± 1.9	−14.7 ± 2.8	−17.65 ± 13.46	−19.34 ± 14.03
(R)			−34.18 ± 15.13	−4.63 ± 13.77
1K1C	−4.3 ± 1.4	−12.8 ± 1.4‡	12.88 ± 2.96	−30.49 ± 9.85§

*$p < 0.05$.
†$p < 0.01$.
‡$p < 0.005$.
§$p < 0.0025$.

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