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Renal effects of a high unsaturated fat diet in renal artery stenosis in rats

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Abstract. The renal effects of an unsaturated fat (UNSAT) diet in mild to moderate two-kidney, one-clip (2K1C) renovascular hypertension were evaluated. An UNSAT diet (37% by energy) prevented the development of hypertension compared to 2K1C rats fed a high saturated fat (SAT) (37% by energy) and a normal fat (CONTROL) (11% by energy) diet. Urinary sodium and fractional sodium excretion increased in 2K1C rats as compared to SHAM operated controls, regardless of the diet received. In the early weeks of the experiment (weeks 2–4 post-surgery to induce hypertension), an enhanced natriuresis occurred in the 2K1C UNSAT as compared to the 2K1C CONTROL and SAT diet groups. This resulted from an increase in the glomerular filtration rate (GFR in mls · min^{-1}) as measured using the single-injection [^{51}Cr] EDTA method (2K1C UNSAT; 1.99 ± 0.18 versus 2K1C SAT; 1.27 ± 0.09, $p < 0.02$; and versus SHAM CONTROL; 1.45×0.01; $p < 0.02$). The increased GFR was not associated with alterations in effective renal plasma flow (ERPF) as measured using the single-injection [^{125}I] Na hippurate method. No differences in sodium excretion; GFR; ERPF or renal blood flow (microsphere technique) were noted between the 2K1C UNSAT and SAT diet groups at weeks 6–8 post-surgery, despite a continued antihypertensive effect of the UNSAT diet. Hence, the antihypertensive effect of an unsaturated fat diet in 2K1C renovascular hypertension in rats is associated with transient glomerular changes leading to an enhanced natriuresis.
Key words. Unsaturated dietary fats; renal hypertension; natriuresis; glomerular filtration rate; renal blood flow.

Several studies in mild to moderate human and experimental hypertension have demonstrated that diets high in unsaturated fats have antihypertensive effects[1–3]. We have shown that a diet high in predominantly unsaturated fats prevents the development of mild to moderate hypertension in 2K1C rats[4,5]. Several mechanisms have been proposed to explain the antihypertensive effect of dietary unsaturates, including natriuretic renal responses[6,7]. Indeed, an increase in urinary sodium excretion occurs in 2K1C rats in response to feeding an unsaturated fat diet[4]. However, the renal mechanisms responsible for the natriuretic effect of an unsaturated fat diet have not been determined. We therefore compared sequential renal haemodynamic, glomerular and tubular function measurements over time in 2K1C rats receiving either unsaturated fat or control diets.

Materials and methods

The following experiments have been approved by the Animal Ethics Committee of the University of the Witwatersrand (AEC No. 90/65/4; 89/35/5; 89/67/4). Ninety one male Sprague Dawley rats (Olac, U.K.) weighing 250–300 g were used in this study. Between 28 and 32 rats per diet group were fed a high unsaturated fat (UNSAT), a high saturated fat (SAT) and a normal fat (CONTROL) diet. Both the SAT and CONTROL diets were designed to act as control diets. After 28 days of feeding, two-kidney, one-clip (2K1C) renovascular hypertension was induced in 52 rats and sham operations (SHAM) performed in 39 rats. The numbers of animals in each group were therefore: 2K1C UNSAT = 21; 2K1C SAT = 22; 2K1C CONTROL = 9; SHAM UNSAT = 10; SHAM SAT = 10; SHAM CONTROL = 19. The diets were then continued for eight weeks post-surgery.

Operations to induce 2K1C hypertension were performed on rats anaesthetised with fentanyl-droperidol (0.02 mg fentanyl and 1 mg droperidol intramuscularly – i.mi.; Janssen Pharmaceutica) as previously described[8]. The left renal artery was exposed and a 0.25 mm diameter silver clip was placed on the artery. The wound was closed in layers and 22,000 units of procaine penicillin administered i.mi. We have previously shown that a 0.25 mm renal artery clip produces mild to moderate renovascular hypertension[4]. We chose to determine the effect of an unsaturated fat diet on mild to moderate experimental hypertension, as antihypertensive effects produced by dietary unsaturated fats have been demonstrated in mild to moderate rather than severe human hypertension[1,3]. Sham operated rats were subjected to the same procedure, except that the clip was not applied.

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Systolic blood pressure was measured prior to surgery and once weekly for eight weeks in a sample of rats from each group (those rats terminated 7–8 weeks after surgery) as previously described[9]. The numbers of rats in each group were therefore: 2K1C UNSAT = 12; 2K1C SAT = 14; 2K1C CONTROL = 9; SHAM UNSAT = 10; SHAM SAT = 10; SHAM CONTROL = 10. Rats were trained in stocks for three days prior to the first measurement of systolic blood pressure in order to allow them to adapt to the procedure. Using a tail-cuff plethysmographic technique, measurements were taken on pre-warmed conscious rats. Each reading was taken at midday to avoid diurnal variation and the mean of four measurements was recorded.

The high unsaturated and the high saturated fat diets used in these experiments have been previously described[5,10]. Briefly, the UNSAT fat diet was made up by the addition of 200 g sunflower seed oil to 1 kg of a 5% fat (by weight) normal rat chow diet (CONTROL diet) (Epol, South Africa). The SAT fat diet was made up in the same way except that 200 g of hydrogenated coconut oil was substituted for the sunflower seed oil. The CONTROL diet contained more protein; carbohydrate and fibre than the UNSAT or SAT diets[10]. The fat content of the SAT diet is thought to represent that of an average Western diet[11]. Fats contributed 37% of the total energy in the high unsaturated and high saturated fat diets (total energy = ± 4842 kcal · kg^{-1}) and 11% of the total energy in the control diet (total energy = 4115 kcal · kg^{-1}). The high fat diets were made up once weekly and stored at 4 °C. Adequate vitamin E was added to prevent oxidation of fats in the diets. Analysis of the fatty acids in sunflower seed oil and hydrogenated coconut oil added to the diets showed that no significant changes of the fat concentration occurred during storage. A homogeneous diet preparation was maintained during storage as mixing of the residual food was repeated on a daily basis. We have previously reported on the fatty acid content of the diets analysed after one week of storage at 4 °C[5]. The high saturated fat diet acted as a control for the lower protein, carbohydrate and fibre content of the unsaturated fat diet compared to the normal fat diet. The normal fat diet controlled for an increased total fat in both the high fat diets. We did not attempt to differentiate between the effects of specific unsaturates in this experiment.

Urinary sodium excretion and sodium balance. A random sample of the age and weight matched rats used to measure weekly SBP values were also used for the sequential weekly measurement of urine volume, urine Na^+ excretion and Na^+ balance measurements. The sample numbers are therefore: 2K1C UNSAT = 10; 2K1C SAT = 11; 2K1C CONTROL = 8; SHAM UNSAT = 10; SHAM SAT = 8; SHAM CONTROL = 6. Not all the rats were used due to the limited number of metabolic chambers available for this study. Once a week, for seven weeks following surgery, rats were placed for 72 h in individual metabolic chambers with free access to food and water. A 24 hour adjustment period was allowed before measurements were made. All urine and faeces were collected for the measurement of volume and sodium. Urine evaporation was prevented by adding a thin sodium free oil film over the urine. Cages were washed at regular intervals during the 48 hour collection period to prevent faecal contamination of urine. The amount of food eaten was measured by weighing food before and after the measurement period. Urine, food and faecal sodium concentrations were measured by flame photometry. Food and faecal matter were first dissolved in nitric acid prior to sodium measurement. The total sodium balance achieved for each rat was calculated as the difference between sodium intake and the urinary plus faecal sodium excretion.

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). At 4 and 7 weeks post-surgery, GFR and ERPF were measured in body weight and age matched 2K1C rats fed an UNSAT or SAT diet and SHAM rats fed a CONTROL diet, based on a single-injection technique as previously described[12,13]. Eight rats in each group were used for the measurement of GFR and ERPF at week 4. Five rats in each group were used for measurements at week 7. These measurements were made on the morning following collection of urine from metabolic chambers, in order that fractional Na^+ excretion ($FENa^+$) could be calculated using standard equations. The techniques used for the measurement of urine Na^+ excretion have been described above. The rats used at week 7, but not at week 4, were part of the group used to determine sequential weekly Na^+ balance as described above. We chose not to use inulin and PAH techniques that require constant plasma concentrations of the marker, together with exact urine collection, to estimate GFR and EFRP. We found that this latter technique required high doses of anaesthesia over a prolonged period of time due to the abdominal surgery that is required for urine collection in rats and the length of time required for adequate urine collection.

Rats were anaesthetised with fentanyl-droperidol and the right carotid and left external jugular vein catheterised with PP50 and PP25 polyethylene tubing respectively. At time 0, approximately 10 µc of [^{51}Cr] EDTA (Amersham) and 10 µc of [^{125}I] Na hippurate (Amersham) were injected simultaneously into the venous catheter. At 5, 10, 20, 30, 40, 60, 90 and 120 minutes, 250 µl of blood was collected from the arterial catheter in capillary tubes which were centrifuged in order to separate the plasma from cells. 80 µl of plasma from each timed sample was counted in a Packard Auto Gamma Scintillation Spectrometer (Model 500C) and the concentration of isotope in the plasma determined

from a standard preparation of stock isotopes which were counted in duplicate. These stock solutions were also used to determine the total amount of radioactivity injected which was calculated as the difference between the counts measured from 10 μc of stock solution and the counts remaining in the syringe and venous catheter used to inject the isotope. During the experiment, blood sampling was followed by an injection of the same volume of normal saline. Blood pressures monitored through the arterial catheter were not significantly altered by blood withdrawal. Mean arterial blood pressure (MAP) recorded on a Beckman dynograph recorder (type R511A) coupled to a Statham (P23AA) pressure transducer.

GFR and ERPF were determined from calculations of the rate of clearance of [^{51}Cr] EDTA and [^{125}I] Na hippurate respectively, as previously described for other markers of renal clearance[12]. Clearance curves were obtained by plotting the counts/minute · ml obtained against time. A typical example of the clearance curves obtained is provided in figure 1. The calculations used to determine the renal clearance of the isotopes (C) are as follows and are based on an analysis of the total area under the plasma radioactivity-time curve[12].

$$C = \frac{c(i)}{A_1 + A_2}$$

$c(i)$ = counts of isotope injected.
A_1 = area under the plasma radioactivity-time curve from 0 to 120 min.
A_2 = area under the plasma radioactivity-time curve from 120 min–time = ∞.
where $A_2 = c(t_1)/k$.
$c(t_1)$ = plasma counts at 120 min.
k = rate constant of the monoexponential part of the plasma radioactivity-time curve.

Renal haemodynamics. At eight weeks, RBF was measured using a technique previously described[14], in the remaining rats in the three 2K1C diet groups and the SHAM CONTROL group. Catheterisation of the left ventricle (via the right carotid artery) and the left femoral artery was performed on fentanyl-droperidol anaesthetised rats, using PP25 polyethylene tubing. The left ventricular catheter was shown to be in place by recording an end diastolic pressure of 0–5 mmHg. Saline and Tween (0.4 ml) containing 30000–40000 radioactive microspheres (^{46}Sc, 15 ± 3 μm, 2M Corp, New England Nuclear) were dispersed with a vortex shaker for 10 minutes, then injected into the left ventricle of rats over 15 s. As the microspheres were injected, blood was withdrawn from the femoral artery at a rate of 2 ml · min^{-1} for 80 s. Left ventricular and arterial blood pressure were measured as described above. Total renal blood flow (RBF) was calculated by dividing the product of the blood withdrawal rate and counts per minute (CPM) in the right kidney, by the CPM in the femoral blood sample. Before counting the kidneys, they were decapsulated, weighed and dissolved in nitric acid. Renal arterial resistance was calculated from standard equations.

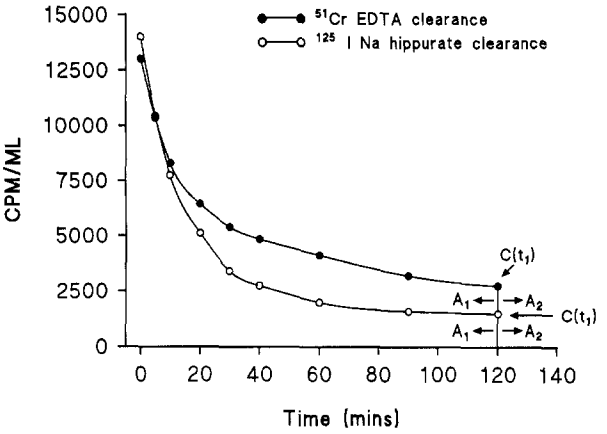

Figure 1. Line graphs illustrate examples of the clearance curves of plasma radioactivity (counts · min^{-1} · ml – CPM/ml) versus time obtained after a single, simultaneous injection of a marker of GFR ([^{51}Cr] EDTA) and a marker of ERPF ([^{125}I] Na hippurate). A_1 and A_2 refer to the area under the curves from 0 to 120 min and from 120 min–time = ∞ respectively. $C(t_1)$ refers to the counts at 120 min. Calculations used to determine GFR and ERPF are provided in the text.

Data analysis. Differences in systolic blood pressure levels between the 2K1C UNSAT diet and the 2K1C CONTROL and SAT diet fed groups, as well as the 2K1C and the SHAM groups were established by a repeated measures ANOVA followed by Kruskal-Wallis statistics. The same statistical tests were applied to determine differences in sodium balance, urine volume, and sodium excretion between diet groups. Differences in MAP; GFR; ERPF; RBF; $FENa^+$ and renal vascular resistance, between the dietary groups and between the 2K1C and SHAM groups were established using ANOVA followed by Kruskal-Wallis statistics. P values < 0.05 are considered to be statistically significant. Results are expressed as mean ± SEM.

Results

Blood pressure. The effect of an UNSAT diet on systolic blood pressure (as measured using an indirect technique) in awake 2K1C rats is illustrated in figure 2 (top panel). These values do not include measurements made on rats terminated at week 4. Systolic blood pressures were significantly lower in the 2K1C UNSAT group compared to both the 2K1C SAT and the 2K1C CONTROL group from 3 to 7 weeks post-surgery. Seven weeks after surgery to induce hypertension, the 2K1C UNSAT group had failed to develop hypertension compared to SHAM operated controls (not illustrated). In contrast, both the 2K1C SAT and 2K1C CONTROL diet groups developed significant hypertension from 3 weeks after surgery to induce hypertension,

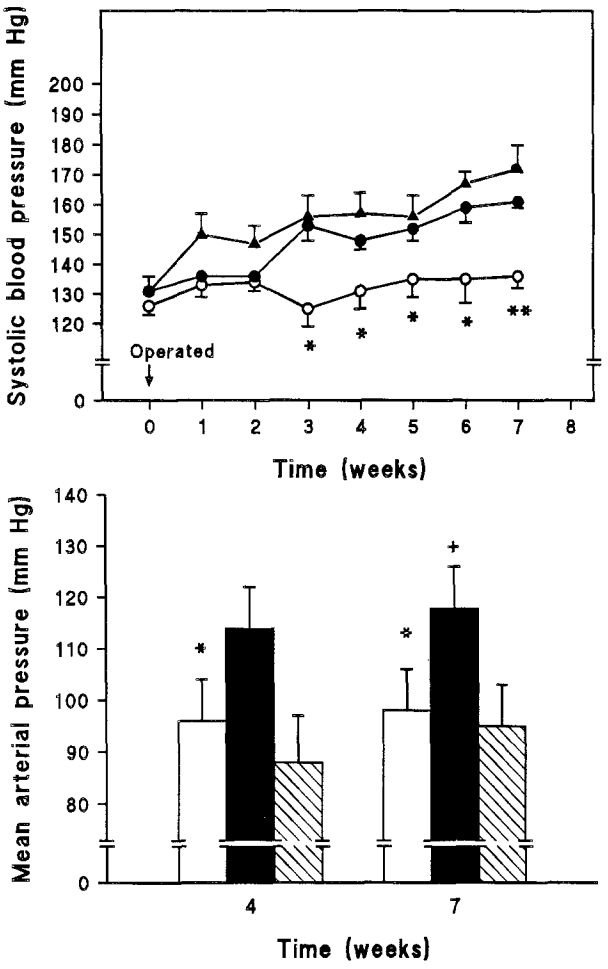

Figure 2. The effect of a high unsaturated fat (UNSAT, ○, n = 12) compared to a high saturated fat (SAT, ●, n = 14) and a normal fat (CONTROL, ▲, n = 9) diet on systolic blood pressures measured using an indirect tail-cuff technique (top panel) and on mean arterial blood pressure using a direct invasive technique (lower panel) in 2-kidney, 1-clip (2K1C) rats. Comparisons are between 2K1C UNSAT (□) versus 2K1C SAT (■) and 2K1C CONTROL (▨). *, +$p < 0.05$; ** $p < 0.005$.

compared to the SHAM control rats ($p < 0.01$) (not illustrated). Similar values for SBP (mmHg) as compared to the values indicated in figure 2 (top panel) were noted in the 2K1C rats terminated at week 4 (2K1C UNSAT = 131 ± 4; 2K1C SAT = 152 ± 6; $p < 0.05$). Sham operated rats showed no differences in SBP over the 7-week period between diet groups (SBP in mmHg at 7 weeks: SHAM UNSAT = 130 ± 4; SHAM SAT = 126 ± 4; SHAM CONTROL = 129 ± 2). Consistent with what we have previously demonstrated[4,5] there were no differences in heart rate or body weight between the dietary groups.

The effect of an UNSAT diet on mean arterial blood pressure (as measured using a direct technique) is anaesthetised 2K1C and SHAM operated rats at 4 and 7 weeks post-surgery is illustrated in figure 2 (lower panel). The week 4 and 7 measurements were obtained from the rats used to measure GFR and ERPF. These data confirm the indirect blood pressure measurements obtained in awake rats. The 2K1C SAT group had increased blood pressures as compared to the SHAM CONTROL group at weeks 4 and 7. However, the 2K1C UNSAT group had blood pressures that were not different from the SHAM CONTROL group, but less than the 2K1C SAT group at both 4 and 7 weeks post-surgery.

Urinary sodium excretion. The results of our studies on urinary sodium excretion confirm findings previously published regarding the effect of renovascular hypertension[15]. Renovascular hypertension resulted in an increased urinary sodium excretion in the SAT and CONTROL groups. At week 4, urinary sodium excretion (mmol · 24 h^{-1} · 100 g^{-1} body weight) was: 2K1C SAT = 0.25 ± 0.02; SHAM SAT = 0.19 ± 0.02; 2K1C CONTROL = 0.23 ± 0.02; SHAM CONTROL = 0.17 ± 0.02 (2K1C SAT and 2K1C CONTROL versus SHAM groups; $p < 0.05$). An increased urinary sodium excretion in 2K1C renovascular hypertension is thought to be the result of a pressure natriuresis[15]. The natriuresis persisted until week 7 (2K1C SAT = 0.45 ± 0.09; SHAM SAT = 0.20 ± 0.03; $p < 0.05$; 2K1C CONTROL = 0.47 ± 0.03; SHAM CONTROL = 0.17 ± 0.02; $p < 0.02$). No significant differences in sodium excretion were noted between the 2K1C SAT and 2K1C CONTROL groups.

A comparison of sodium balance, urine volume, and urine sodium excretion between the 2K1C UNSAT and 2K1C SAT groups is made in figure 3. We have previously published data demonstrating an increased urinary sodium excretion in 2K1C UNSAT as compared to 2K1C CONTROL diet fed rats[4]. We therefore only report on results comparing 2K1C UNSAT versus 2K1C SAT rats. At weeks 3, 4 and 5 the 2K1C UNSAT group retained significantly less sodium than the 2K1C SAT group. The sodium balance values are comparable to those reported in the literature[16]. The positive sodium balance noted in both groups reflects the sodium required for the high growth rate of rats. The decreased positive sodium balance in the 2K1C UNSAT group in the early weeks of the experiment was mainly due to an increased urinary sodium excretion compared to the 2K1C SAT group. The natriuresis normally observed in 2K1C renovascular hypertension was thus enhanced in 2K1C rats fed an UNSAT diet during the early weeks of the experiment. As similar values for urinary sodium excretion were noted in the 2K1C CONTROL rats as compared to the 2K1C SAT group, the differences between the 2K1C UNSAT group and the 2K1C SAT group cannot be attributed to a decreased sodium excretion produced by the SAT diet. The differences in sodium balance were not as a result of alterations in sodium intake (mmol · 24 h^{-1} · 100 g^{-1}) (week 4: 2K1C UNSAT; 0.51 ± 0.05: 2K1C SAT; 0.59 ± 0.04: 2K1C

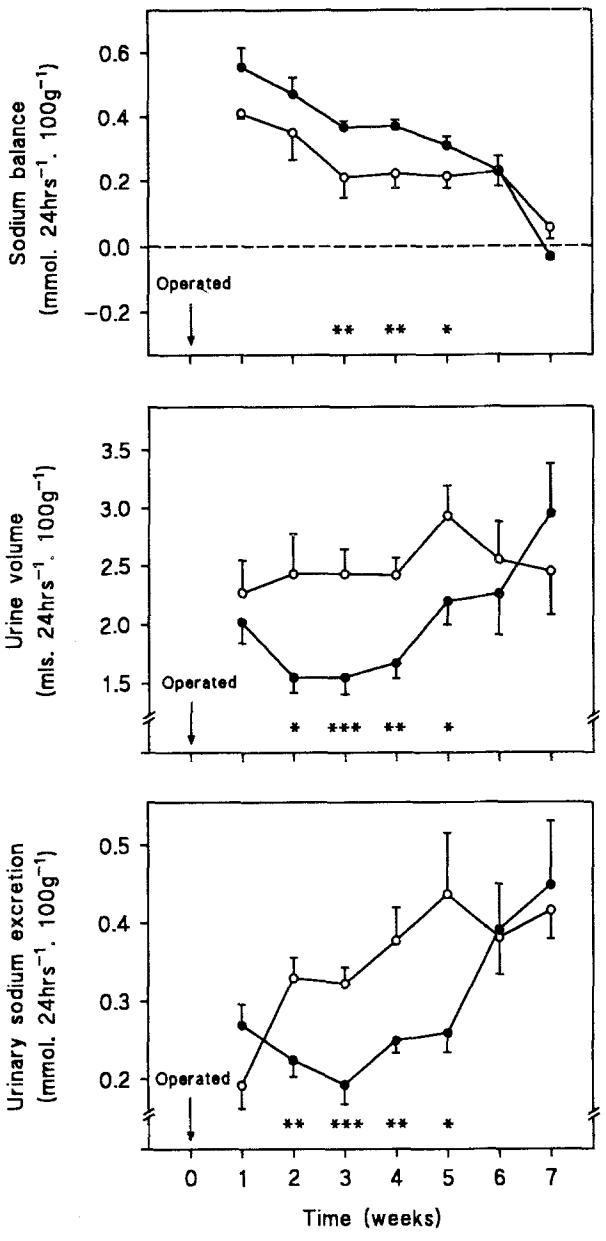

Figure 3. The effect of a high unsaturated fat (UNSAT, ○, n = 10) compared to a high saturated fat (SAT, ●, n = 11) diet on sodium and water excretion and sodium balance in 2-kidney, 1-clip (2K1C) rats. *, $p < 0.05$; **, $p < 0.02$; ***, $p < 0.005$.

CONTROL; 0.53 ± 0.06: week 7: 2K1C UNSAT; 0.41 ± 0.05: 2K1C SAT; 0.43 ± 0.04; 2K1C CONTROL; 0.45 ± 0.05).

The differences in urinary sodium excretion as noted during the early weeks of the experiment between 2K1C UNSAT and 2K1C SAT diet groups (fig. 3) was transient. At weeks 6 and 7, no differences in urinary sodium excretion were noted between the 2K1C dietary groups (fig. 3).

GFR and ERPF. GFR, ERPF and calculated $FENa^+$ in anaesthetised 2K1C UNSAT, 2K1C SAT and SHAM CONTROL groups are illustrated in figure 4. Urinary sodium excretion (mmol · 24 h^{-1} · 100 g^{-1}) was increased in the 2K1C UNSAT rats used to measure GFR and ERPF at week 4 (2K1C UNSAT = 0.325 ± 0.02; 2K1C SAT = 0.024; $p < 0.02$), but not at week 7 (fig. 3). The increased urinary sodium excretion at week 4 in 2K1C UNSAT as compared to 2K1C SAT rats is attributed to an increased GFR. The differences in GFR were not as a result of the SAT diet as GFR's were similar between 2K1C SAT and SHAM CONTROL rats. The high GFR in the 2K1C UNSAT group was not as a result of an increased ERPF which was similar between the three groups. Differences in urinary sodium excretion between the 2K1C UNSAT and the SHAM CONTROL group at week 4 may be attributed to both an increase in GFR as well as an increase in $FENa^+$. The increased urinary sodium excretion in 2K1C SAT as compared to SHAM CONTROL rats at week 4, may only be attributed to an increased $FENa^+$. At week 7, no differences in GFR were noted between the groups. $FENa^+$, however, remained elevated in the 2K1C UNSAT and SAT groups compared to the SHAM group.

Renal blood flow. The different groups of rats were able to maintain a fairly constant renal blood flow in spite of significant differences in mean arterial pressure (table).

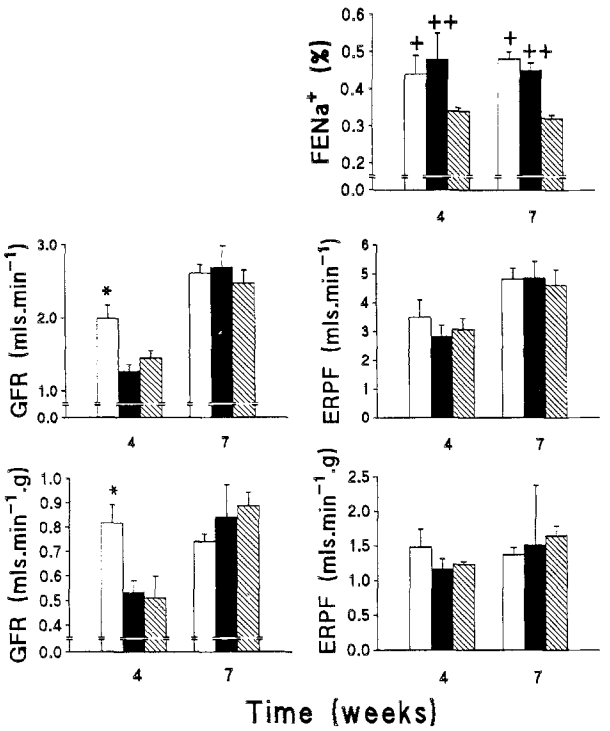

Figure 4. Glomerular and tubular function in 2-kidney, 1-clip (2K1C) and SHAM operated rats fed a high unsaturated (UNSAT, □), saturated (SAT, ■) and control fat diet (▨). GFR = glomerular filtration rate (mls · min^{-1} · g^{-1} = per g of kidney weight); $FENa^+$ = fractional sodium excretion; ERPF = effective renal plasma flow (mls · min^{-1} · g = per g of kidney weight); *, $p < 0.02$ 2K1C UNSAT versus 2K1C SAT and SHAM control; +, $p < 0.05$ 2K1C UNSAT versus SHAM CONTROL; ++, $p < 0.002$ 2K1C SAT versus SHAM control; n = 8 for each group at week 4; n = 5 for each group at week 7.

Table. Renal haemodynamics in 2-kidney, 1-clip (2K1C) and SHAM operated rats fed different fat diets. n = sample number.

Group	n	MAP (mmHg)	RBF ($mls \cdot min^{-1} \cdot g^{-1}$)	Renal vascular resistance ($mmHg \cdot ml^{-1} \cdot min \cdot g^{-1}$)
2K1C UNSAT	7	87 ± 5*	3.2 ± 0.4	29 ± 2*
2K1C SAT	9	116 ± 3	3.02 ± 0.5	41 ± 6
2K1C CONTROL	6	$114 \pm 3^{+}$	2.86 ± 0.5	$40 \pm 10^{+}$
SHAM CONTROL	6	76 ± 3*	3.8 ± 0.7	24 ± 4*

MAP = mean arterial pressure; RBF = renal blood flow; UNSAT = unsaturated fat diet; SAT = saturated fat diet; CONTROL = normal fat diet: 2K1C UNSAT and SHAM CONTROL versus 2K1C SAT; *$p < 0.05$; 2K1C CONTROL versus 2K1C UNSAT and SHAM CONTROL: $^{+}p < 0.05$.

The differences in renal resistance in 2K1C rats fed the UNSAT versus the SAT and CONTROL diets may reflect generalised alterations in systemic resistance as a result of the UNSAT diet, or a normal autoregulatory renal vascular resonse. Renal blood flow values using microspheres are similar to those previously published[14]. As we have not used the microsphere technique to measure intrarenal blood flow distribution, our results are not subject to the inaccuracies of streaming artifacts.

Discussion

We have previously shown that the antihypertensive effect of an unsaturated fat diet in 2K1C renovascular hypertension in rats is associated with a natriuresis[4]. Our current results demonstrate that the natriuresis produced by renovascular hypertension (a pressure-natriuresis) is enhanced as a result of a high unsaturated fat diet. The augmented natriuresis is however transient and occurs as a consequence of glomerular rather than tubular changes. The transient glomerular changes are not the result of aterations in renal plasma flow.

Antihypertensive responses to high unsaturated fat diets have been reported in normotensive, mildly hypertensive humans[1,3] and in rat models of hypertension[1,2]. Our results differ from others who have demonstrated an antihypertensive effect of a diet deficient in essential fatty acids in 2K1C rats[17]. The constituents of the unsaturated fat diet which may be responsible for the blood pressure-lowering effect in our experiment have previously been discussed[5].

To date, we are the only authors that have reported a natriuretic together with a diuretic effect of an unsaturated fat diet in hypertension. Studies in other models of hypertension in rats failed to show changes in sodium excretion using this diet[18,19]. Differences in the models of hypertension used, the timing of renal measurements and the quantity and quality of fat in the diets may explain this discrepancy. The transient glomerular changes noted in our present experiment suggest that these discrepancies are as a result of differences in the timing of renal measurements. However, our results are consistent with the findings of other authors[6,7]. A natriuresis has been demonstrated in unsaturated fatty acid supplemented as compared to essential fatty acid deficient rats[7]. A modest natriuresis, but without a concomitant diuresis has been demonstrated in normotensive humans[6].

A transient as opposed to a sustained increase in GFR and urine sodium excretion in 2K1C rats fed an unsaturated fat diet as compared to those fed a control diet is not surprising. A decrease in plasma volume produced by an unsaturated fat diet in 2K1C rats[4] may produce a number of compensatory glomerular changes including alterations in renal arteriolar tone and glomerular permeability, designed to oppose the natriuretic effect of the unsaturated fat diet. As renal vascular resistance was decreased at week 8 post-surgery in 2K1C UNSAT rats, it is likely that compensatory changes in glomerular permeability resulted in the GFR returning to normal values. Possible glomerular permeability changes as a result of plasma volume depletion may have resulted from an enhanced sympathetic tone (although this is unlikely as heart rates were unchanged) or an increased circulating plasma angiotensin II concentration producing an increased mesangial cell contraction.

The natriuretic glomerular changes produced by the unsaturated fat diet may have contributed toward the antihypertensive effect of the diet. A normal renal response to a high blood pressure is a pressure-induced natriuresis which occurs as a consequence of alterations in tubular function. The quantity of Na^{+} excreted via this effect is diminished in 2K1C rats[20] as compared to that excreted in normal rats[21] exposed to an equivalent increase in blood pressure. This shifted pressure-natriuresis curve has been postulated to contribute to some extent toward the development of 2K1C hypertension. In our study, the natriuretic-glomerular changes produced by the high unsaturated fat diet may have opposed the abnormal pressure-natriuresis curve found in 2K1C rats. This effect may in turn have contributed toward preventing the development of hypertension.

Despite the enhanced natriuretic effect produced by an unsaturated fat diet in 2K1C rats, a positive Na^{+} balance due to the high growth rate of rats was noted. It may be argued that a negative Na^{+} balance is required for plasma volume contraction and a subsequent drop

in blood pressure to occur. However, the lower positive Na^+ balance in 2K1C rats fed the unsaturated fat diet as compared to the 2K1C control diet fed rats, may have been sufficient to prevent Na^+ accumulation keeping pace with the demand for growth. Growth was not affected by the unsaturated fat diet. We may therefore speculate that the growth rate outstripped positive Na^+ balance in the 2K1C rats fed the unsaturated fat diet, the consequence of which was a contracted plasma volume as previously demonstrated by us[4].

A transient as opposed to a sustained increase in GFR in the unsaturated fat fed 2K1C rats suggests that renal alterations produced by the diet contribute toward the early but not the sustained antihypertensive effect of the diet. As discussed above, compensatory glomerular changes designed to oppose fluid loss will be the consequence of a contracted plasma volume produced by the natriuretic effect of the unsaturated fat diet. These compensatory alterations may prevent a further decrease in plasma volume, rather than normalise the plasma volume. That is, an equilibrium between the glomerular effects of the unsaturated fat diet and the opposing glomerular effects produced by renal compensation in response to plasma volume contraction may be reached at a lower plasma volume. Indeed, in support of this we have previously demonstrated a decreased plasma volume at 7 weeks post-surgery in 2K1C rats fed a diet high in unsaturated fats[4].

Salt depletion in renovascular hypertension is usually compensated for by an increased plasma renin activity[22] and hence systemic resistance in this model. We have previously demonstrated a high plasma renin activity with a normal total peripheral resistance in 2K1C rats fed a high unsaturated fat diet[4]. This suggests an attenuated responsiveness to angiotensin II in animals fed diets high in unsaturated fats. Recent data from our laboratory confirm this idea[5]. We suggest that vascular alterations are likely to contribute toward the sustained antihypertensive effects of an unsaturated fat diet in renovascular hypertension.

The mechanism of the enhanced natriuretic effect of unsaturated dietary fat in 2K1C rats is an increased GFR. We have previously shown that creatinine clearance is unaltered by an unsaturated fat diet in 2K1C rats[4]. Hence, either creatinine clearance does not reflect a measure of GFR in 2K1C rats fed an unsaturated fat diet, or our measures of GFR using plasma clearance techniques are inaccurate. We have used a technique that has been well correlated with the more acceptable GFR measures made using plasma and urine marker measurements[13]. Indeed our data correlate well with previous measurements made using similar measures of GFR (single timed blood samples) in rats[23]. It is likely that the differences between our current results and our previous estimates of GFR made using creatinine clearance techniques reflect inaccuracies that are known to exist in the creatinine clearance measures.

The increase in GFR that occurred in the 2K1C unsaturated fat fed rats was not a consequence of changes in renal plasma flow. Changes in colloid osmotic pressures are unlikely to have produced any change in filtration fraction as no protein differences exist between the UNSAT and SAT diets. Altered afferent or efferent arteriolar vascular tone might have resulted in hydrostatic pressure changes leading to an increased GFR. Increased concentrations of PFI_2 may have produced changes in renal arteriolar tone, as an increased production of metabolites of this substance have been found in renal homogenates and urine of rats, before and after renal artery clipping, after being fed an unsaturated fat diet with the same fatty acid content as that used in our current experiment[24]. Furthermore, an altered renal vascular responsiveness to angiotensin II occurs in 2K1C rats fed an unsaturated fat diet[5].

In conclusion we have demonstrated that the enhanced natriuresis produced by an unsaturated fat diet in 2K1C rats is the result of a transient increase in the glomerular filtration rate. This effect is not a consequence of alterations in renal plasma flow. This change may contribute toward the antihypertensive effect of dietary unsaturated fats in the development of mild renovascular hypertension.

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