

## Original Research

# Breeding Characteristics and Dose-dependent Blood Pressure Responses of Transgenic Cyp1a1–Ren2 Rats

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Hypertension is a leading risk factor for cardiovascular and chronic kidney disease. A new rodent model (transgenic male Cyp1a1–Ren2 rats) provides reversible induction of hypertension through the addition of indole-3-carbinol (I3C) to the diet, without the need for surgical intervention, thus giving researchers control over both the onset of hypertension and its magnitude (I3C dose-dependency). We here report the breeding performance and productivity of Cyp1a1–Ren2 rats. Despite being transgenic, these animals proved to be efficient breeders. In addition to confirming inducible and reversible dose-dependent hypertension (by using I3C doses of 0.125%, 0.167%, and 0.25% [w/w] in the diet for 14 d, followed by normal chow for 4 d), we demonstrated that hypertension can be sustained chronically (14 wk) by continuous dosing with I3C (0.167% [w/w]) in the diet. In chronically dosed male rats, systolic blood pressure continued to rise, from 173 ± 11 mm Hg after 1 mo to 196 ± 19 mm Hg after 3 mo, with no adverse phenotypic features observed. In conclusion, Cyp1a1–Ren2 rats are a useful animal model to investigate hypertension-induced end-organ damage and potential new therapeutic targets to manage hypertension.

**Abbreviations:** I3C, indole-3-carbinol; SBP, systolic blood pressure

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Hypertension has repeatedly been shown to be a leading risk factor contributing to global death rates.<sup>1,3,6,20,24,32</sup> As much as 44% of the world population older than 20 y is hypertensive<sup>19</sup> (that is, blood pressure of greater than 140/90 mm Hg). The sequelae of chronically elevated blood pressure manifest as cerebrovascular disease, cardiac disease, and chronic kidney diseases, imposing an ever-increasing fiscal load on the delivery of health care.<sup>1,2,35,36</sup> The American Heart Association reports that 69% of cases presenting with a myocardial infarction and 74% of cases with reported chronic heart failure have a history of hypertension.<sup>1</sup> In the United States alone, the prevalence of hypertension were anticipated to increase by more than 7% and the incidence of cardiovascular disease by 40.8% by the year 2030,<sup>12</sup> leading to a projected annual cost estimate for the treatment of hypertension alone of more than \$US340 billion.<sup>1</sup> However, recent estimates suggest that the prevalence of hypertension will rise more rapidly, with the hypertensive population now estimated to increase by more than 8% by the year 2030 (bringing the rate to almost 50% of the adult population).<sup>1</sup>

To investigate key regulatory components that influence blood pressure, several transgenic hypertensive rat models have been developed. One such model are transgenic Cyp1a1–Ren2 rats, in which hypertension can be induced reversibly by diet, without the need for surgical intervention.<sup>19</sup> In this line, the expression of mouse Ren2 cDNA is under the control of an

inducible cytochrome p450-1a1 promoter, which is integrated into the Y chromosome of Fischer 344 rats<sup>19,26</sup> and is therefore active only in males. Dietary administration of indole-3-carbinol (I3C) activates the promoter gene (Cyp1a1) and thus increases the production of Ren2.<sup>17,19</sup> I3C is a naturally occurring, nontoxic, xenobiotic found in cruciferous vegetables (such as broccoli) that acts as a benign inducer with a short half-life. The production of Ren2, primarily in the liver, on induction of the Cyp1a1 promoter through I3C,<sup>18,31</sup> leads to increased circulating renin levels, activation of the renin–angiotensin–aldosterone system and a consequent increase in blood pressure. After the withdrawal of I3C from the diet, the production of mouse renin falls, and blood pressure returns to previous, normotensive levels.<sup>19</sup> This model, therefore, allows inducible and reversible hypertension through modulation of the renin–angiotensin–aldosterone system. Importantly, the extent of hypertension is I3C dose-dependent,<sup>26,29</sup> allowing tight control of blood pressure. Thus, both the timing of the onset of hypertension and its magnitude can be controlled in this unique animal model, in which the effect of various antihypertensive treatments can be assessed.

Although the transgenic Cyp1a1–Ren2 rat model has been used widely to study hypertension and associated organ damage during the 2 decades since its creation,<sup>10,14,19,26,28,30</sup> no information has been published regarding the line's breeding characteristics or reproductive performance (such as breeding efficiency and regularity, fertility, and litter size). Similarly, most studies using this model focus on acute hypertension, with few data in terms of chronic hypertension.<sup>13,15,30,31</sup> We therefore aimed to outline the breeding performance and productivity of transgenic Cyp1a1–Ren2 rats, demonstrate the inducible and

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reversible dose-dependent hypertension in this model, and describe the effect of chronic dosing with I3C on inducible hypertension.

## Materials and Methods

**Animals.** The initial transgenic Cyp1a1–Ren2 rat internal breeding stock was gifted by Professor JJ Mullins (Centre for Cardiovascular Science, University of Edinburgh, United Kingdom). The transgenic Cyp1a1–Ren2 rat colony was held at the University of Otago Animal Resource Unit, and animals were housed under controlled conditions of temperature (approximately 21 °C) and light (12:12-h light:dark cycle), with food (Meat-free Rat and Mouse Diet, Irradiated, Specialty Feeds, Glen Forest, Western Australia, Australia) and tap water provided without restriction. All animals were housed on corn-cob bedding in standard open-top caging, with ‘paper wool’ for nesting and a tunnel provided for enrichment.

The colony was monitored according to FELASA guidelines<sup>7</sup> through quarterly serologic testing for specific pathogens (Table 1). The testing was performed by Cerberus Labs (Cerberus Sciences, Melbourne, Victoria, Australia). Rats were negative for all agents except *Helicobacter* spp. and *Pneumocystis carinii*.

Colony breeding pairs were monitored regularly to assess and characterize breeding performance, including recording the number of litters, litter size, sex ratio, survival, overall health, and behavioral traits. Continuous, inbred (brother–sister) pairs were used for breeding. Randomly selected litters were weighed daily to obtain a standard weight curve for the colony.

All Cyp1a1–Ren2 rats used for experiments were obtained from internal breeding stock and were housed in pairs or in groups of 4 rats per cage. All experiments were approved by the Animal Ethics Committee of the University of Otago (approval no. AEC 51/13), in accordance with the guidelines of the New Zealand Animal Welfare Act.<sup>25</sup>

**Experimental protocol. I3C dose–response curve.** Standard pelleted rat chow (Specialty Feeds) was ground and blended to a fine powder. I3C (Chem-Impex International, Wood Dale, IL) was added to the powdered diet to produce final concentration (w/w) of 0.125%, 0.167%, and 0.25%. Cyp1a1–Ren2 rats housed in pairs or triplicates were randomly assigned to a dose group at 8 wk of age and allotted 50 g of powdered chow daily per rat. Similarly, 8-wk-old female Cyp1a1–Ren2 rats were assigned to receive either standard chow or 0.167% (w/w) I3C diet. Food was weighed and topped up every morning.

To demonstrate the dose-dependent effect of I3C on blood pressure, Cyp1a1–Ren2 rats experienced a 14-d dosage regimen using different percentages of I3C: the Cyp1a1 gene inducer. Male and female Cyp1a1–Ren2 rats (age, 8 wk;  $n = 29$ ) were randomly assigned to 1 of 3 groups and received I3C (0.125%, 0.167%, or 0.25% [w/w]) in the diet. Rats were weighed and food was replenished daily, with systolic blood pressure (SBP) measured every 3 to 4 d. At the end of the 14-d I3C dosing period, 6 female rats were euthanized by using halothane. At day 14 or 18 after SBP recordings, male rats from each group were euthanized by using halothane, and tissue (heart and kidneys) was harvested for further analyses.

Plasma from cardiocentesis of normotensive male Cyp1a1–Ren2 rats (maintained on standard chow) and hypertensive male Cyp1a1–Ren2 rats (maintained on diet containing 0.167% [w/w] I3C) was analyzed for renin by using a commercial in-house ELISA assay (EndoLabs, Christchurch Hospital, Christchurch, New Zealand) on day 14.

**Chronic elevation of blood pressure.** Male transgenic Cyp1a1–Ren2 rats were maintained on either pelleted standard chow

**Table 1.** Results of annual health testing of the Cyp1a1–Ren2 rat colony

Organism	Status
<b>Bacteria and fungi</b>	
Cilia-associated respiratory bacillus	Negative
<i>Citrobacter rodentium</i>	Negative
<i>Clostridium piliforme</i>	Negative
<i>Corynebacterium kutscheri</i>	Negative
<i>Helicobacter bilis</i>	Negative
<i>Helicobacter ganmani</i>	Negative
<i>Helicobacter hepaticus</i>	Negative
<i>Helicobacter mastomyrinus</i>	Negative
<i>Helicobacter rodentium</i>	Negative
<i>Helicobacter</i> spp.	Positive
<i>Helicobacter typhlonius</i>	Negative
<i>Mycoplasma pulmonis</i>	Negative
<i>Pasteurella pneumotropica</i>	Negative
Pasteurellaceae group	Negative
<i>Pneumocystis carinii</i>	Positive
<i>Salmonella</i> spp.	Negative
<i>Streptobacillus moniliformis</i>	Negative
<i>Streptococcus</i> group A ( $\beta$ hemolytic)	Negative
<i>Streptococcus</i> group B ( $\beta$ hemolytic)	Negative
<i>Streptococcus</i> group C ( $\beta$ hemolytic)	Negative
<i>Streptococcus</i> group G ( $\beta$ hemolytic)	Negative
<i>Streptococcus pneumoniae</i> ( $\alpha$ hemolytic)	Negative
<b>Ectoparasites</b>	
<i>Myobia musculi</i>	Negative
<i>Mycopetes musculinus</i>	Negative
<i>Radfordia affinis</i>	Negative
<b>Endoparasites</b>	
<i>Aspiculuris tetraptera</i> (pinworm)	Negative
<i>Giardia muris</i>	Negative
<i>Spiroplasma muris</i>	Negative
<i>Syphacia muris</i> (pinworm)	Negative
<b>Nonpathogenic protozoa</b>	
<i>Entamoeba muris</i>	Negative
<i>Tritrichomonas muris</i>	Negative
<b>Viruses</b>	
Adenovirus type 1	Negative
Adenovirus type 2	Negative
Kilham rat virus	Negative
Pneumonia virus of mice	Negative
Rat coronavirus	Negative
Rat minute virus	Negative
Rat parvovirus	Negative
Rat theilovirus	Negative
Reovirus type 3	Negative
Sendai virus	Negative
Toolan H1 virus	Negative

(Specialty Feeds) or pelleted standard chow with addition of 0.167% (w/w) I3C (SF13-086, Specialty Feeds) for 14 wk. All animals were housed under controlled conditions in groups of 4 and had free access to water and food. Rats were weighed and food was replenished weekly, with SBP measured every 4 wk. At the end of the 14-wk period, rats were euthanized by using halothane.

**Physiologic measurements.** All rats were acclimated through daily handling and were weighed (to the nearest gram) at 8 wk of age. Weights were calculated as the gain in weight from this start point to account for variability in initial weight. SBP was measured in conscious habituated rats by using tail-cuff plethysmography (NIBP controller, AD Instruments, New Zealand) and recorded (PowerLab 4SP, AD Instruments). Animals were given 30 min to acclimate prior to the blood pressure recording procedure, and a heat lamp was used to gently warm the tail prior to SBP readings. Data were captured and analyzed by using Chart (version 7, AD Instruments). A minimum of 10 clear recordings were obtained from each rat during each session.

Statistical significance (that is,  $P < 0.05$ ) was assessed by using 2-way ANOVA with Bonferroni posthoc analysis (KaleidaGraph, Synergy Software, Reading, PA).

## Results

**Transgenic Cyp1a1–Ren2 rat colony.** Compared with outbred stocks, inbred strains typically show lower productivity—not only in frequency of productive matings but also smaller litters and fewer litters during a dam's lifetime. However, despite being an inbred strain, many of the reproductive features observed in the Cyp1a1–Ren2 rat colony were typical of those in outbred strains. Sexual maturity occurred similarly to other common rat strains, and first litters typically were born within 1 mo after initial pairing of breeders. Decreased breeding performance in the Cyp1a1–Ren2 rat colony was due to a slower reproductive cycle (average, 28 to 35 d) compared with the typical duration (20 to 24 d). However, Cyp1a1–Ren2 rats displayed a normal gestational period (20 to 24 d).

Fertility in the colony varied widely during the first few generations, with some pairs never producing a litter. However, careful colony maintenance (for example, culling of nonproductive pairs) eliminated this infertility within a few generations. In addition, Cyp1a1–Ren2 rats in this colony frequently produced the first litter within expected timeframes (that is, within 30 d of pairing) but then had a long period with no visible conception (around 50 to 70 d before visible pregnancy), followed by regular and efficient production after the birth of the second (that is, delayed) litter. This pattern was not restricted to specific breeding lines within the colony or associated with any noted excessive stress or unusual behavior. This characteristic appears to be strain-specific and not one that has been reported previously for Cyp1a1–Ren2 rats. Despite this reproductive behavior, the generation time of these rats was normal, with gestation of approximately 3 wk followed by 3 wk to weaning. Surprisingly, this colony of inbred transgenic Cyp1a1–Ren2 rats produced litter sizes comparable to those of outbred, nontransgenic strains, with average litters of  $12 \pm 4$  pups.

To date, the colony of Cyp1a1–Ren2 rats at the University of Otago has produced only 2 cases of birthing defects. In both cases, pups were born with microphthalmia (microeye), and their lineages were removed from the breeding colony. No strain-specific behaviors have been distinguished within the Cyp1a1–Ren2 rat colony, nor are any specific behaviors documented for this transgenic strain. No data available suggest that Cyp1a1–Ren2 rats experience any adverse effects due to the genetic manipulation itself.

**Standard growth curve of Cyp1a1–Ren2 rats.** Prior to weaning, pups showed a steady and rapid daily weight gain. All pups were weaned at 21 d of age, and a small reduction in the rate of weight gain was noted during the 2 to 3 d after weaning but quickly reverted to a steady gain. Weight records were

continued in male Cyp1a1–Ren2 rats maintained on normal pelleted chow until 24 wk of age (Figure 1). Rats maintained steady and strong growth until 8 wk of age (mean  $\pm$  1 SD,  $232 \pm 19$  g), which then continued at a slower rate until 24 wk of age ( $443 \pm 22$  g).

**I3C dose curves. Weight.** Female Cyp1a1–Ren2 rats, which lack the transgene, showed a steady weight gain on standard powdered chow from day 0. Female Cyp1a1–Ren2 rats placed onto a diet containing 0.167% (w/w) I3C showed an initial delay in weight gain when compared with females on standard chow, and this delay continued until day 14 ( $16 \pm 5$  g compared with  $22 \pm 4$  g, respectively; Figure 2). Male Cyp1a1–Ren2 rats maintained on chow containing either 0.125% or 0.167% (w/w) I3C showed a weight gain from day 0, similar to that of male rats fed standard chow (Figure 3). In contrast, male Cyp1a1–Ren2 rats fed the highest dose (0.25% w/w I3C), failed to show a gain in weight over the 14-d dosing period ( $-2.8 \pm 9.5$ g, Figure 2). Following removal of the I3C from the diet on day 14 and returning to normal chow, rats increased weight.

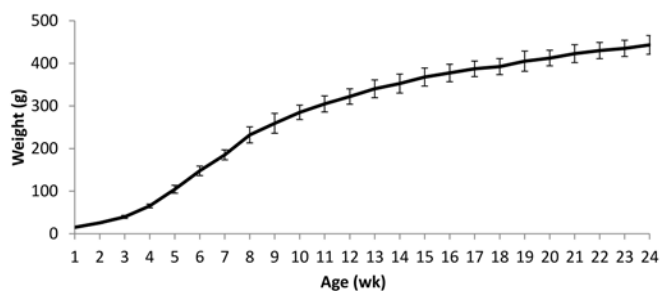
**Blood pressure.** Female and male Cyp1a1–Ren2 rats maintained on the standard diet were shown to have a SBP of  $111 \pm 7$  mm Hg. Similarly, female rats maintained on an I3C dose of 0.167% were recorded to have a mean SBP of  $109 \pm 7$  mm Hg (Figure 3). After 14 d on a dietary dose of 0.125% I3C, male Cyp1a1–Ren2 rats had an increase in SBP to  $140 \pm 6$  mm Hg, which was significantly ( $P > 0.01$ ) higher than in normotensive males. After 14 d on a diet containing 0.167% I3C, male rats had a SBP of  $165 \pm 15$  mm Hg, which was significantly ( $P > 0.01$ ) higher than both normotensive male rats and male rats on 0.125% I3C. Animals maintained on the highest dose (0.25% I3C) had a SBP of  $202 \pm 20$  mm Hg (Figure 3), again significantly ( $P > 0.001$ ) higher than all other groups. By 4 d after the return to standard rat chow, SBP in all groups had returned to the normotensive range ( $107 \pm 5$  mm Hg; Figure 3).

Plasma renin in hypertensive animals (maintained on 0.167% [w/w] I3C diet) was significantly ( $P < 0.01$ ) elevated after 14 d compared with normotensive animals ( $22 \pm 4$  and  $11 \pm 3$  nmol/L, respectively).

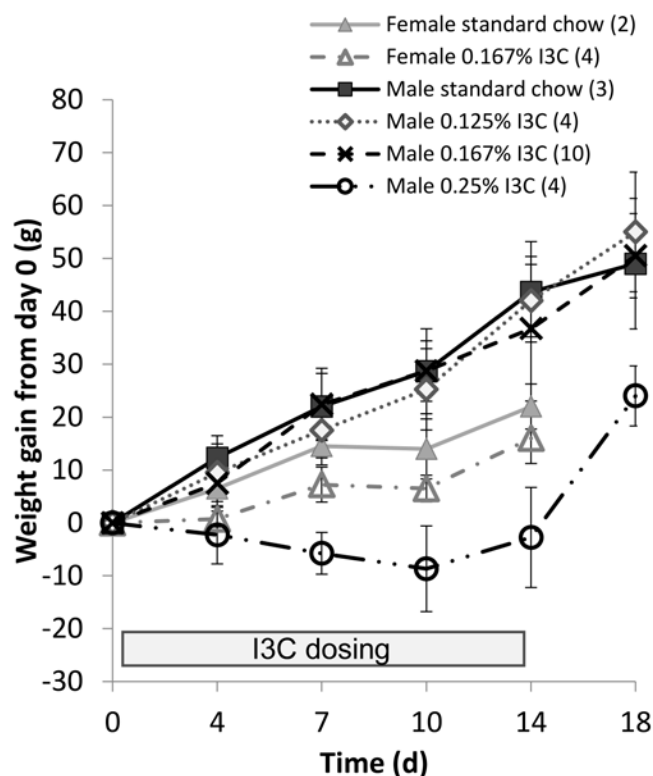
**Chronic elevation of blood pressure in Cyp1a1–Ren2 rats.** Feeding I3C to male transgenic Cyp1a1–Ren2 rats led to a steady increase in SBP over time (Figure 4). In contrast, male Cyp1a1–Ren2 rats maintained on normal chow showed no increase in SBP over the 14 wk. After the initial 2 wk of dietary dosing with 0.167% (w/w) I3C, SBP rose to  $159 \pm 10$  mm Hg (giving similar results to the I3C dose curve, Figure 3). Furthermore, SBP rose to  $173 \pm 11$  mm Hg after an additional 4 wk and to  $196 \pm 19$  mm Hg after 3 mo (Figure 4).

## Discussion

A persistent difficulty in the investigation of hypertension is the availability of appropriate models that reproduce the clinical situation. In humans, elevation of blood pressure occurs slowly and insidiously, whereas most animal models of hypertension involve an intervention that produces a rapid elevation in blood pressure. The development of Cyp1a1–Ren2 transgenic rats in which blood pressure can be manipulated by a dietary additive has made possible the investigation of hypertension under controlled settings.<sup>19</sup> In contrast to other genetic and transgenic hypertensive rat models (including Spontaneously Hypertensive Rats,<sup>4,9,21</sup> Dahl salt-sensitive rats,<sup>11,21</sup> and transgenic m(Ren2)27 rats),<sup>5,23</sup> hypertension in Cyp1a1–Ren2 transgenic rats can be induced at any chosen age or time, allowing tailoring of experimental design and exploration of the effect of hypertension over time and on animals of different ages. In addition, this model



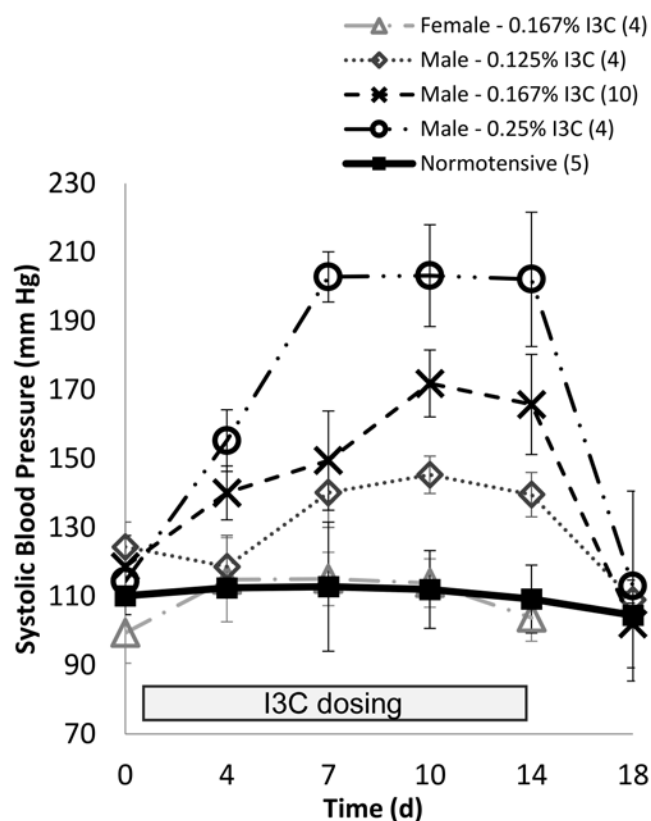
**Figure 1.** Standard weight curve for normotensive male Cyp1a1–Ren2 rats maintained on normal chow until 24 wk of age. Data are shown as mean  $\pm$  1 SD ( $n = 43$ ).



**Figure 2.** Weight gain from day 0 of transgenic Cyp1a1–Ren2 rats maintained on different doses of dietary indole-3-carbinol (I3C). At day 14, dietary I3C was removed and normal chow administered. The number of animals in each experimental group is given in parentheses. Data are shown as mean  $\pm$  1 SD.

permits dose-dependent titration of hypertension, which enables the investigation of a broad range of elevated blood pressures, from prehypertension to malignant hypertension, simply by adjusting the level of I3C in the diet. In contrast to other hypertensive rat models, Cyp1a1–Ren2 rats have a relatively slow ramping of blood pressure, which is more closely related to the development of hypertension in the clinical setting. This incremental development of hypertension consequently provides a more realistic model in which to study the effects of increased pressure on vascular structure and function.

The experiments reported here verified the absence of any effect of I3C (inducer) on blood pressure in female rats lacking the *Ren2* gene. When male Cyp1a1–Ren2 rats received I3C in the diet for 2 wk, SBP was elevated in a dose-dependent fashion, with higher doses of I3C leading to higher levels of SBP (Figure 3). These results are similar to previous findings,<sup>29</sup> although, in contrast, our animals fed a diet containing I3C at 0.167% (w/w)

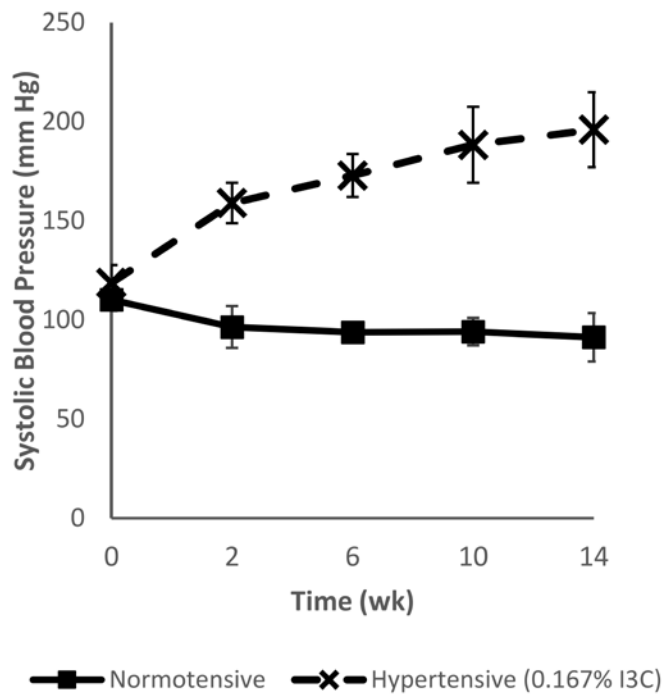


**Figure 3.** Systolic blood pressure (SBP) in transgenic 8-wk old Cyp1a1–Ren2 rats maintained on different dietary doses of indole-3-carbinol (I3C) for 14 d. On day 14, rats were returned to standard chow. Data are shown as mean  $\pm$  1 SD. The number of animals in each experimental group is shown in parentheses.

showed no growth retardation. When I3C-treated male Cyp1a1–Ren2 rats returned to normal chow, their SBP returned to normotensive values within 48 h, consistent with other published studies.<sup>13,18,30</sup>

A previous study<sup>29</sup> showed that the addition of 0.125% (w/w) I3C in the diet, male Cyp1a1–Ren2 rats achieved a stable mean arterial pressure of approximately  $170 \pm 5$  mm Hg after 6 wk on the diet. Similarly, a later study<sup>30</sup> showed a continued rise in mean arterial blood pressure up to 28 d after the commencement of I3C, although this progression was far slower than that seen in the first 2 wk. In contrast an earlier study,<sup>29</sup> we noted no plateau in blood pressure during the 14-wk time frame, and SBP continued to rise from  $173 \pm 11$  mm Hg after 1 mo to  $196 \pm 19$  mm Hg after 3 mo on the I3C diet (Figure 4). Interestingly, the diet-manipulated animals (maintained on 0.167% [w/w] I3C) showed no adverse phenotypic features related to their elevated blood pressure, such as lethargy, hunched posture, piloerection, and lower body weight gain, as have previously been reported, albeit associated with an I3C dose of 0.3% (w/w).<sup>18,27,29</sup>

Correct establishment and management of a rodent colony are essential, and knowledge of commonalities and deficiencies of a strain can be critical to maintaining a good colony and therefore also good research stock. Generally, laboratory rats become sexually mature between 5 and 10 wk of age;<sup>22,33</sup> consequently, breeding animals are often paired at approximately 10 wk of age to obtain the maximal reproductive performance.<sup>33,34</sup> Typically laboratory rats breed for 5 to 8 mo, producing 5 to 6 litters,<sup>16</sup> but this number can be reduced or extended due to strain-specific characteristics or mutant phenotypes that affect



**Figure 4.** Chronic elevation (3 mo) of systolic blood pressure in male Cyp1a1–Ren2 transgenic rats maintained on a diet containing 0.167% (w/w) indole-3-carbinol (I3C; hypertensive, dashed line) or standard rat chow (normotensive, solid line). Hypertensive animals were given an initial 14-d ramping period on a diet containing 0.167% I3C to establish hypertension, whereas normotensive animals remained on normal chow. Data are shown as mean  $\pm$  1 SD ( $n = 8$ ).

fertility. In addition, interstrain differences can influence litter size, although 10 to 14 pups is common and expected.<sup>22</sup>

The performance (or productivity) of a colony is traditionally defined according to the fecundity of the breeder females (although reproductive failures may be due to the female, the male, or both). Because mating usually occurs within 24 h of placing sexually mature rats together,<sup>34,37</sup> with pregnancy visible and fetuses palpable often by day 14 and litters typically born by day 23 (ranging from 21 to 25 d),<sup>34,37</sup> a general rule is that if no visible signs of pregnancy are noted within 60 d from pairing or the birth of the previous litter, the breeding pair is considered nonproductive and is removed from the colony.<sup>22,33,34</sup> Assessment of breeding performance also considers hybrid vigor, litter size, strain-specific genetic mutations or transgenes (such as reduced fertility, effects of mammary gland function, or lethal embryonic effects) as well as behaviors (such as high aggression or poor mothering instincts). This practice helps to ensure that peak breeding efficiency is sustained, expected reproductive characteristics of the strain are maintained, and breeders are assessed sufficiently to select appropriate replacement stock. This continuous evaluation will identify reproductive problems before the colony is in reproductive crisis.<sup>22,33,34</sup>

Cyp1a1–Ren2 rats proved to be efficient and good breeders, comparable to common outbred laboratory rat strains. However, we noticed several characteristics while breeding this unique transgenic strain. First, despite being an inbred transgenic rat model, Cyp1a1–Ren2 rats produced large litters (that is, 10 to 16 pups). These litter sizes were much larger than reported for Fischer 344 rats (the background strain for Cyp1a1–Ren2 rats) from both Envigo (worldwide) and Janvier Laboratories (France, Europe), which both report an average litter of fewer than 8 pups. Although this characteristic is not

undesirable, it is unusual and therefore should be noted. Second (and more important to the management of the colony) was a prolonged reproductive cycle (that is, 28 to 35 d) compared with other laboratory rat strains. Furthermore, we observed repeatedly that, after the birth of the first litter, dams seemed to ‘skip’ either the second or third litter, resulting in periods of 60 to 70 d with no visible pregnancy. However, after this litter-free spell, rats produced efficiently from then on. Despite all efforts to remove this lag period, it appears to be a strain characteristic of the transgenic Cyp1a1–Ren2 rat. Further investigation is required to document these findings; regardless, breeding pairs of Cyp1a1–Ren2 rats should be afforded more than 60 d before being removed from the colony.

The development of transgenic Cyp1a1–Ren2 rats<sup>19</sup> has allowed increased control of the study of hypertension. Because the breeding characteristics of these transgenic animals have not been published previously, here we report that these rats breed surprisingly well despite being an inbred and transgenic strain. In addition, we showed that Cyp1a1–Ren2 rats maintained on decreased titrated doses of dietary I3C can tolerate prolonged periods of sustained hypertension but without the ill effects reported by others.<sup>15,29</sup> Given that hypertension repeatedly has been shown to be a major risk factor contributing to global death rates,<sup>1,8,20,24</sup> animal models such as Cyp1a1–Ren2 rats may hold key components to therapeutic developments targeting hypertension, and further research to clarify the dose-dependent response, especially long-term, in this model is needed.

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