Epideiological studies demonstrating an increase in cardiovascular disease in adult children born of women who suffered nutritional privation during World War II led to many subsequent studies, demonstrating that diverse conditions leading to intrauterine growth retardation had similar effects on cardiovascular disease including hypertension.\(^1\) Relatively mild maternal protein deficiency leads to abnormal nephron structure and dysregulation of the renin–angiotensin II–aldosterone system.\(^2,3\) The report in this issue, by Geurts et al\(^4\) builds on the finding that a protein-sufficient casein-based diet significantly increases the degree of salt-induced hypertension and renal damage in the inbred Dahl Salt-Sensitive rat strains SS/Mcw when compared with SS/Crl fed a grain-based diet with similar proportions of protein, fat, and carbohydrates.\(^5\) SS/Mcw rats were derived from Dahl SS/Jr rats purchased from Harlan Sprague Dawley Laboratories and maintained by brother-sister mating at the Medical College of Wisconsin since 1991, where they were fed a purified diet using casein as the protein source. SS/Crl rats were derived from SS/Mcw given to Charles River Laboratories in 2001 and fed a grain-based diet. In the present study, progeny of 4-way embryo transfers between SS/Mcw and SS/Crl dams fed their usual diets, progeny of parental strains on their usual diets, and SS/Crl rats after 1 generation of the purified casein-based diet were studied with or without 3 weeks on a high-salt diet. Blood pressure, albuminuria, renal histopathology, and transcriptome analyses of the outer renal cortex were compared. The degree of pathology induced by high-salt consumption in the progeny of embryo transfers correlated with the diet of the recipient dam, thus the gestational and lactational environment, rather than genetic background. Rats of both strains experiencing the gestational and lactational environment of SS/Mcw dams fed the casein-based diet had similar hypertension and renal pathology on salt challenge as the SS/Mcw controls, which was significantly greater than that of rats of either strain transferred to SS/McwCrl dams fed the grain-based chow. Similarly, the response of SS/Crl fed the casein-based diet for one generation to high salt was the same as that of SS/Mcw rats fed the diet for many generations.

The complex comparisons of transcriptomes is an admirable tour de force. The most significant differences in the transcriptomes of the outer renal medulla between the different groups, with and without salt challenge, were attributable to the protein source in the different diets and were similar to those obtained in the previous simpler study. Many of the genes fell within pathways known to be involved in hypertension and renal pathology. The genetic differences noted between the strains did not correlate with sodium sensitivity.

Discovery of epigenetic phenomena has provided mechanisms for the maternal programming of metabolic and hemodynamic derangements, including those that affect kidney function, the renin–angiotensin–aldosterone system, hypothalamic–pituitary–adrenal axis, and blood pressure.\(^1,5,7\) Epigenetic changes occur most frequently, but not exclusively, in early life and are crucial mediators of adaptive gene transcription responses to environmental challenges that in some instances may be inherited and can be reversed.\(^6,7\) Although not addressed in the current study, epigenetic changes probably had a significant role in the differences in gene transcription in response to the sodium challenge in SS/Crl and SS/Mcw rats fed the different diets. Ironically, the purified diet used for the SS/Mcw colony may have been chosen to decrease seasonal variation in protein sources, phyto- and xenosteroids, other micronutrients, and contaminants, which could exert epigenetic changes. A difference of just 3 weeks of the casein or grain diet from weeks 3 to 6 made a significant difference in the blood pressure and renal phenotype. The implications of the effect of diet during relatively short periods later in ontogeny are sobering if this work done in rats translates to humans; it also offers an opportunity. Although this may be difficult to swallow, it may explain widely discrepant results from laboratories ostensibly using the same rodent models.

Differences in maternal and neonatal diet and intestinal microflora are associated with epigenetic changes that modulate the risk for metabolic diseases, including the predisposition to hypertension and renal disease.\(^8,9\) The substrates available to intestinal microorganisms determine their metabolic byproducts absorbed by the host, as well as alter their complex demographics. The identity of gut microflora of the progeny in this study\(^4\) would be expected to reflect that of the gestational dams maintained on their original diets; however, the different dietary substrates after weaning may alter the proportions of specific microbial populations related to the whole, as well as enzyme expression by the bacteria.

The authors surmise that the grain-based diet suppressed salt-induced pathology; however, it is more appropriate to assume that casein as the sole source of protein exacerbates the
effect of salt in a species that evolved consuming a low sodium diet and protein from a wide variety of sources, primarily vegetable, except as a neonate. For example, cysteine is essential for the synthesis of the crucial antioxidant glutathione. Absorption of cysteine by intestinal epithelial cells and neurons is regulated. Activating the μ opioid receptor inhibits cysteine uptake. Intestinal microbes convert casein to μ opioid receptor peptide agonists; bovine, compared to human, casein-derived peptides have relatively high affinity for the μ opioid receptor. Inhibition of cysteine uptake, thus decreasing glutathione synthesis may explain the greater inflammation and reactive oxygen species generation in the casein-fed rats. Increased reactive oxygen species would decrease the availability of S-adenosyl methionine available as a methyl donor for CpG methylation, possibly failing to suppress transcription of many of the genes found to be upregulated in this study.10

The transcriptome analysis in this study clearly demonstrates hazards of comparing animals from different sources. Contradictory information derived from diverse inbred Dahl SS colonies resulting from genetic differences is but one example.11 Although the genetic differences identified between the SS/Mcw and SS/Crl in this study did not affect salt sensitivity of the blood pressure or renal pathology, they may well be germane to others processes. The SS/Mcw genome has been sequenced and numerous congenic and knockout strains have been developed on its background. The value of these important resources would be greatly diminished if the parental strain were no longer available to the scientific community. Therefore, the SS/Crl (SS/McwCrl) commercial breeding colony should be rederived to reflect the genetics of the current SS/Mcw strain before its continued use as the wild type for studies of salt-sensitive hypertension.2

The results presented by Geurts et al. also demonstrate that it is imperative to consider the environment throughout the lives of animals to be compared. Because of space constraints and colony maintenance costs, it is not uncommon to purchase wild-type animals. We now have ample evidence that comparing genetically modified animals from an institutional colony to commercially obtained parental stock fed different diets and subjected to the stress of shipping, particularly at the vulnerable weaning period, introduces variables, many of which may be indelible.

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References
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