How do dietary sodium and potassium regulate blood pressure?

1. Project Description.

What is the scientific question to be addressed and why is it important?

Hypertension (affecting 1 in 3) , chronic kidney disease (affecting 1 in 8) and metabolic syndrome (affecting 1 in 4) are the most common and arguably the most expensive diseases in the United States. These diseases are not easily controlled with medication, they are the most common causes of mortality, and the numbers of people afflicted by these epidemics continue to escalate.

Interestingly, these diseases, notably hypertension, are found mainly in industrialized societies, with very low prevalence in isolated societies. This is a key observation that has driven investigations into the cause of the changes. Anthropologic and epidemiological studies suggest the decrease in the dietary K:Na ratio that occurs as people move from isolated to industrialized societies is a key culprit. The daily intake of K:Na is 150 mmol K: 30 mmol Na in isolated people (K:Na = 5) and 50 mmol K : 250 mmol Na (K:Na = 0.2) in Western societies that eat high Na prepared foods and drastically less high K fruits and vegetables (Fig 1).

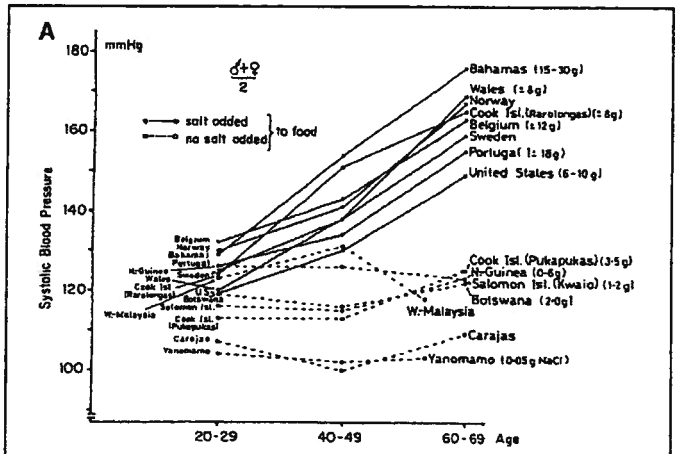

Fig 1. Blood pressure change with age is a function of habitual daily salt intake. From Meneton, P. et al. Physiol. Rev. 85: 679-715 2005

Two interventional studies have varied dietary Na and K in humans and measured blood pressure and cardiovascular disease endpoints and found remarkable beneficial effects of increasing the K:Na ratio. The DASH diet study fed non-hypertensive adults control (K~ 50 mmol/day) or the DASH diet rich in fruits and vegetables and low fat dairy (K~120 mmol/day) and varied Na intake in both groups (65, 107 or 142 mmol/day). The results (Fig 2) demonstrate that a rise in BP for a given increase in Na intake is significantly blunted in the setting of high K intake. This effect is seen after just a couple of weeks. The second study, in elderly Taiwanese Veterans (PMID: 16762939), replaced 50% of the NaCl in the kitchens with KCl and found that after 31 months cardiovascular disease mortality was reduced 41%. Based on these limited studies, the American Heart Association (AHA) and Institute of Medicine (IOM) recommend lowering dietary Na to no more than 100 mmol/day and while the AHA states that "the dearth of dose-response trials precludes a firm recommendation for a specific level of K to lower BP" (PMID: 16434724) the IOM recommends raising K to 120 mmol/day based on what was consumed in the DASH diet. However, the IOM also concluded that American women consume only half this recommended amount, and men only moderately more. Compliance to these guidelines is very poor because the recommendations are vague, low Na diets are difficult because of the high salt load in the prepared food, and there are no phenotypic markers of too much Na or not enough K as plasma levels of these cations remain in the normal level. This last point is demonstrated in our own studies in rats fed a diet chronically reduced fro 1% K to 0.33% K. We detected no change in plasma levels of Na or K but measured a dramatic decrease in insulin stimulation of muscle K uptake and inactivation of renal K channels leading us to conclude that the animals were actively responding to low K stress (PMID: 16354756).

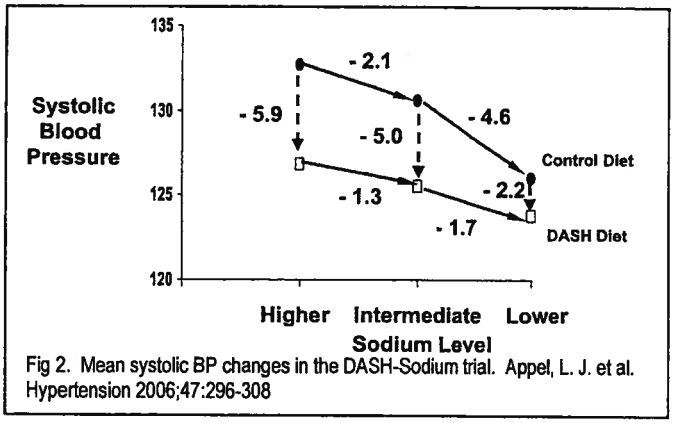

Fig 2. Mean systolic BP changes in the DASH-Sodium trial. Appel, L. J. et al. Hypertension 2006;47:296-308

If simply changing the dietary K:Na ratio has such a remarkable beneficial effect on these common, debilitating and expensive diseases why aren't we all talking about it and researching the mechanisms? We

recently reviewed the literature for a review on K homeostasis, including cardiovascular benefits (PMID: 18759636) and came to the conclusion that most of the basic research in this area has focused on the effects of completely removing K from the diet and provoking frank hypokalemia, or the effects of raising Na to very high levels. Little attention has been paid to examining the ratio of K:Na except for a couple of studies in hypertension prone rats where dramatic effects were observed: Dahl (PMID: 5043414) reported that when salt sensitive rats were fed a 5% NaCl diet with little K BP increased to 205 mmHg while if K was supplemented to 5% BP was reduced to 160 mmHg. Tobian (PMID: 9022555) reported that mortality of stroke prone spontaneously hypertensive rats fed 4% NaCl diet was reduced by 93% when K was raised from 0.75% to 2%.

We could not find in the literature any studies that compared a typical *western* diet with a K: Na ratio of 0.5 (1% KCl, 2% NaCl) (PMID: 9022555) to an *optimal* diet as recommended by AHA and IOM with a ratio of 4 (4%KCl, 0.74% NaCl). The central goal of this proposal is to establish the protocols and platforms to rigorously investigate the effect of various Na and K containing diets, including these diets, on BP, renal disease and metabolic syndrome. Rat models have proven very valuable for the study of BP regulation - salt sensitivity and genetic transmission were first defined by Dahl's studies in rats – and they are much easier to study than mouse models. We has accumulated extensive experience with measuring BP, insulin sensitivity, reactive oxygen species, sodium transporter regulation, hormonal and sympathetic nervous system regulation in rats. We are experts in cell biology as well as whole animal physiology. A classic table of the vasculoprotective propeties of potassium is provided in Fig 3 and we have experience measuring most of these parameters. While most of these findings were collected in radically altered diets or *in vitro*, it is a useful summary of what has been proposed.

Fig 3. Vasculoprotective properties of potassium
Coca SG etal, AJKD 45:233-247, 2005.
Decrease blood pressure
Natriuresis
- Decrease proximal tubule Na reabsorption
- Decrease renin release
- Increase GFR

Vasodilator
- Stimulate Na,K-ATPase
- Decrease AngII
- Increase NO production

Decrease ROS production
Decrease VSMC proliferation
Decrease platelet aggregation
Decrease endothelial dysfunction
Decrease macrophage adherence

We will begin with the following questions: 1)Will feeding young SHRs (spontaneously hypertensive rats) from weaning an *optimal* diet decrease the rise in BP compared to feeding them a *western* diet? 2) Does feeding adult SHRs an *optimal* vs *western* diet lower BP? 3) Does an *optimal* diet lower BP in normotensive rats (from weaning or in adults). We will then explore variations in the amount and ratios of the cations providing the needed dose response information.

Physiological measurements including intake and output measurements, ambulatory BP, will be collected and recorded *in vivo* before anesthesia in which regional GFR, RBF, and regional Na transport (proximal vs. distal nephron) will be measured. Then tissue and blood sample sets will be generated for assessment of renal pathology, determinants of metabolic syndrome (high BP, elevated blood glucose and triglycerides, insulin resistance of glucose and K uptake) and renal sodium and potassium transporter distribution and activity. In addition, we will measure the parameters that emerge as candidates for the beneficial effects of K. For example, Huang recently reviewed studies suggesting that dietary K can change expression of WNK kinases in a manner that will differentially regulate Na reabsorption vs K secretion in the renal distal nephron(PMID: 17957).

To accomplish these aims we will need to innovate high throughput methods for animal and tissue studies. In the end we believe that these studies will provide a factual and compelling analysis of the benefits of one diet over another and set a standard for asking similar question regarding other nutrients. Ideally, the results will provoke the public to demand healthier diets from the food industry and in schools. A change to healthier salts in our diets has the potential for reversing these epidemic disease trends and help people function better and longer with less health costs.

What are the pioneering, high risk approaches that might lead to groundbreaking paradigm shifting results? We plan to assay many parameters in each rat in order to figure out the cardiovascular benefit of high K-low Na intake. The list of parameters to assess is based on the reported effects of altered sodium and potassium diets (Fig 3) even though these were often collected on radical diets of zero K and low Na or very high Na and low K. Relevant to this issue, we have recently determined that most of the reported effects of a nominally K free diet, some of which we ourselves reported (including slower weight gain, renal hypertrophy, 8 fold increase in renal NHE3, decreased expression of NCC and NKCC, increase renin expression), are <u>not</u> observed when the K deficient diet includes 2% NaCl, the salt content of a typical *western* diet. This is an example of a clinically relevant true paradigm shift. We would anticipate many more in the execution of this project. These findings lead us to the new hypothesis that the previously reported K deficient effects were due to low total cation content of the diet rather than low K per se. We can now test this hypothesis by varying total cation content as well as the Na/K ratio.

We expect that dietary sodium and potassium will change many things in tandem. Some changes will depend on Na and the extracellular fluid volume, others will depend on K independent of Na and the effects of the Na:K ratio *per se* remain to be established. The high risk aspect is that the plan may appear diffuse and unfocussed, analogous to how gene chip and proteomics approaches were viewed before their utility was established. We will take advantage of established methods and innovate new ones in order to: 1) inventory and store many tissue, blood and urine samples from each animal, 2) assay many samples at a time, for example by executing protocols for using one immunoblot to probe for many protein by using secondary antibodies tagged with different colored probes, 3) record the data set using computer, back-up and web based approaches that can be accessed from multiple sites by our lab members and collaborators.