Dietary factors that influence kidney calcinosis (KC) in weanling, female Sprague-Dawley rats

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BACKGROUND

- Kidney Calcinosis (KC) is an abnormal condition of the kidney where deposits of calcium (Ca) form in the filtering units. This may reduce kidney function and eventually lead to significant damage and kidney stone formation.
- Several dietary factors, including an imbalance of dietary Ca and phosphorus (P), (typically a Ca/P molar ratio less than 1) can promote KC in weanling, female SD rats (see Fig 1, Ritskes Hoitinga et al). The AIN-76A rodent diet, a purified-ingredient diet, promotes KC, and this may be due to its low Ca/P molar ratio (~0.75) (Cockell et al).
- Modification of the AIN-76A diet with elevated Ca/P ratio is expected to reduce KC, but other factors within this diet that may affect development of KC include carbohydrate, fiber, and sulfur containing amino acid (AA) supplement – specifically, fructose (Bergstra et al), insoluble fiber cellulose (Anderson et al), and DL-methionine (Reeves et al).
- The AIN-93G diet was a purified-ingredient diet established to address some of the concerns of the AIN-76A (Reeves et al), including an elevated Ca/P above 1, but at the expense of a P-deficient mineral mix dependent on contribution of P from casein.
- While grain-based chow diets are typically thought to provide adequate maintenance of growth and health in rodents, previous data have suggested that some chow diets also promote mild to moderate KC in weanling, female rats (Rao).
- Development of a purified-ingredient, Open Standard Diet (OSD) with a P-sufficient mineral mix is necessary to address concerns of the AIN-76A and AIN-93G diets.

OBJECTIVE

To study the influence of:

1.) a P-sufficient mineral mix with Ca/P molar ratio above 1 in the AIN-76A diet and the influence of an OSD on KC (Study 1).

2.) modifying components in an OSD (dietary carbohydrate, fiber, and supplemental AA, separately or all in combination) on KC (Study 2).

3.) different grain-based chow diets on KC and their Ca/P molar ratios (Study 2).

MATERIALS AND METHODS

Animals:

- 116 Weanling, female SD rats maintained at Taconic Biotechnology (Rensselaer, NY).

Procedure:

- At 23 days old, rats were separated into groups by body weight and then fed experimental diets ad-lib for 4 weeks.
- Body weights were collected every week throughout the study.
- At study termination on Day 28, kidneys were harvested.
- Kidney and Dietary Ca and P levels were measured with ICP-MS (Diagnostic Center for Population and Animal Health, Michigan State University)

Diets:

- Study 1 included 3 purified-ingredient diets (Research Diets, Inc.): AIN-76A, AIN-76A+RDI minerals (replaced AIN-76A mineral mix with RDI mineral mix), Open Standard Diet (OSD), and grain-based chow Purina 5002. (n=9 rats/group except OSD where n=8) (See Table 1 for AIN-76A and OSD formulas and Table 2 for Mineral Mixes)
- Study 2 included 7 purified-ingredient, Open Source diets (Research Diets, Inc.): OSD, OSD with cellulose only (OSD-C), DL-methionine in place of L-cystine (OSD-M), increased sucrose (OSD-S), or with combination of 3 factors (OSD-CMS), AIN-93G, and AIN-76A, and 2 grain-based chows, Purina 5002, and NIH-31M (Zeigler Bros) (See Table 1 for all Open Source diet formulas).

Statistics:

- Kidney Ca and P were analyzed with One-Way ANOVA, and when significant variation was observed, data was analyzed with non-parametric statistics using Kruskal-Walis test and post-hoc Dunn's Multiple Comparison Test when $p<0.05$.

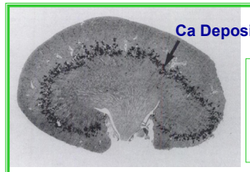


Figure 1. Kidney section of weanling, female Wistar rat fed Ca/P molar ratio = 0.64 (Ritskes-Hoitinga et al)

Table 1. Composition of Purified-Ingredient, Open Source Diets

	OSD	OSD-C	OSD-M	OSD-S	OSD-CMS	AIN-76A	AIN-93G
Macronutrient (kcal%)							
Protein	20	20	20	20	20	21	20
Carbohydrate	65	65	65	65	65	68	64
Fat	15	15	15	15	15	12	16
kcal/gm	3.80	3.78	3.80	3.80	3.78	3.90	4.00
Ingredient (gm)							
Casein	200	200	200	200	200	200	200
L-Cystine	3	3	0	3	0	0	3
DL-Methionine	0	0	3	0	3	3	0
Corn Starch	356	362	356	141	147	150	397.486
Maltodextrin 10	35	35	35	0	0	0	132
Dextrose	250	250	250	0	0	0	0
Sucrose	0	0	0	500	500	500	100
Cellulose	75	100	75	75	100	50	50
Inulin	25	0	25	25	0	0	0
Soybean Oil	70	70	70	70	70	0	70
Corn Oil	0	0	0	0	0	50	0
t-Butylhydroquinone	0	0	0	0	0	0	0.014
RDI Mineral Mix	45	45	45	45	45	0	0
AIN-76A Mineral Mix	0	0	0	0	0	35	0
AIN-93G Mineral Mix	0	0	0	0	0	0	35
AIN-76A Vitamin Mix	10	10	10	10	10	10	0
AIN-93 Vitamin Mix	0	0	0	0	0	0	10
Choline Bitartrate	2	2	2	2	2	2	2.5
Total (gm)	1071	1077	1071	1071	1077	1000	1000
Total (kcal)	4071	4070	4071	4071	4070	3902	4000
Calcium (gm)	6.0	6.0	6.0	6.0	6.0	5.2	5.0
Phosphorus (gm)	4.4	4.4	4.4	4.4	4.4	5.4	3.0
Ca/P Molar Ratio	1.07	1.07	1.07	1.07	1.07	0.75	1.32

Table 2. Mineral Levels Provided by Mineral Mixes in AIN-76A, AIN-93G, and OSD

	AIN-76A per 35 gm	AIN-93G per 35 gm	RDI per 45 gm	NRC* Recommendation Rat per kg	Mouse per kg
Calcium (gm)	5.2	5	6	5	5
Phosphorus (gm)	4	1.56	3	3	3
Magnesium (gm)	0.5	0.5	0.5	0.5	0.5
Potassium (gm)	3.6	3.6	6	3.6	2
Sulfur (gm)	0.33	0.3	0.33	N/A**	N/A
Sodium (gm)	1	1	1	0.5	0.5
Chloride (gm)	1.6	1.6	1.6	0.5	0.5
Chromium (mg)	2	1	2	N/A	N/A
Copper (mg)	6	6	6	5	6
Iodide (mg)	0.2	0.2	0.2	0.15	0.15
Iron (mg)	45	45	45	35	35
Manganese (mg)	59	10	59	10	10
Selenium (mg)	0.16	0.16	0.16	0.15	0.15
Zinc (mg)	29	29	29	12	10
Molybdenum (mg)		0.15	1.6	0.15	0.15
Fluoride (mg)		0.9	0.9	N/A	N/A
Silica (mg)		11.7		N/A	N/A
Lithium (mg)		5.74		N/A	N/A
Boron (mg)		0.5		N/A	N/A
Nickel (mg)		17.3		N/A	N/A
Vanadium (mg)		0.11		N/A	N/A

* NRC = National Research Council ** N/A = Not Available

RESULTS

Figure 2. Kidney calcium and phosphorus levels of rats in Study 1

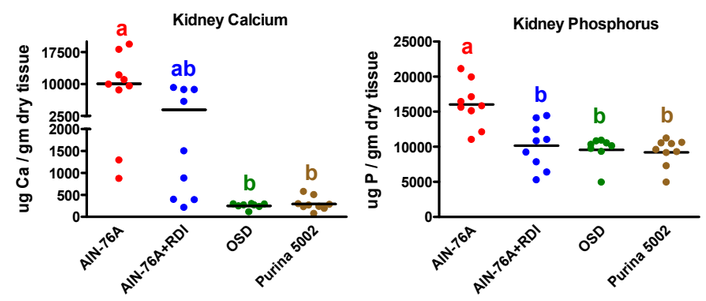


Figure 3. Kidney calcium and phosphorus levels of rats in Study 2

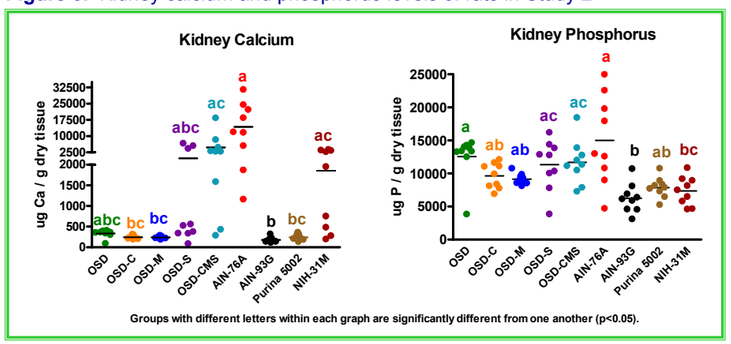
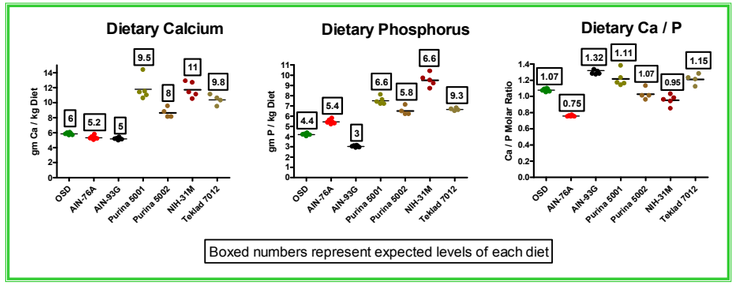


Figure 4. Calcium and phosphorus levels and Ca/P molar ratios of diets used in Study 2 and other manufactured lots from these and other diets



RESULTS SUMMARY

- The AIN-76A diet promoted significant KC. Replacing AIN-76A mineral mix with RDI minerals containing higher Ca / P molar ratio tended to have lower kidney Ca relative to AIN-76A, while both OSD and Purina 5002 had significantly lower kidney Ca than AIN-76A ($p<0.001$, Fig 2).
- Replacing sucrose with corn starch and dextrose within OSD tended to decrease kidney Ca levels. The combined influence of modifying fiber type and supplemental sulfur-containing amino acid tended to increase kidney Ca when sucrose was high. Modification of either fiber type or sulfur-containing amino acid alone had no influence on kidney Ca (Fig 3).
- Rats fed NIH-31M had higher kidney Ca (6 – 10 fold) relative to those fed Purina 5002, OSD, and AIN-93G (Fig 3); Dietary Ca / P molar ratio was less than 1 (0.85) in NIH-31M while Purina 5002, OSD and AIN-93G had ratios above 1 (1.04 – 1.27) (Fig 4), perhaps accounting for some of the elevation in kidney Ca in rats fed NIH-31M.
- Dietary Ca, P, and Ca/P molar ratios in different grain-based chow diets, as well as among different manufacturing lots of the same diets, can vary widely while less variability existed for these factors among purified-ingredient diets (Fig 4).

CONCLUSIONS

- Rats fed OSD with mineral mix sufficient in P and a Ca/P molar ratio above 1 had kidney Ca similar to AIN-93G.
- Carbohydrate type influences KC in weanling female rats, even when dietary Ca/P molar ratio >1.
- A grain-based chow diet may promote moderate KC, which was perhaps due to a low Ca/P molar ratio.
- Purified-ingredient diets may allow for more consistency in Ca, P, and Ca/P molar ratios compared to grain-based chow diets. Variability in these diet factors may alter KC across different studies.

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