Original Research Article

Caloric Substitution of Diets with Apple Pomace was Determined to be Safe for Renal and Bone Health Using a Growing Rat Model

Abstract

Aims: to determine the safety of caloric substitution with 10% apple pomace substitution (g/kg) to a healthy or Western diet.

Study design: Growing (age 22-29 days) female Sprague-Dawley rats were randomly assigned (n=8 rats/group) to consume a purified standard rodent diet (AIN-93G), AIN-93G/10% g/kg apple pomace (AIN/AP), Western diet, or Western/10% g/kg apple pomace (Western/AP) diets for 8 weeks.

Results: Histological evaluation showed renal interstitial hypercellularity in rats fed AIN/AP, Western, and Western/AP diets. However, there was no effects on renal expression of oxidative stress and inflammatory genes or serum measures of kidney damage and function among diet groups. Apple pomace is also high in calcium which can affect calcium balance. Dietary calcium consumption was highest (P < .001) in rats consuming Western/AP. However, there was no significant differences in calcium absorption and retention among diet groups. Further, there was no evidence of renal calcification. There were also impact on femoral calcium and total mineral content, size, and strength.

Conclusions: Based on the results, apple pomace consumption was safe for renal and bone health, regardless of diet quality and should be considered for repurposing for human consumption.

Keywords: apple pomace, safety, minerals, Western diet, bone, kidney, sustainability

1. Introduction

Apple processing generates waste, consisting of skin, stem, seeds, and calyx, collectively known as apple pomace. The environmental pollution and burden of waste disposal costs to apple farmers and producers can be decreased by re-purposing apple pomace as a product for human consumption [1-3]. However, among popular consumed fruits, apples had the highest fructose content [1]. Muir, et al. [4] reported apples to have 10.5 g of fructose/serving compared to 3.2 g/serving for bananas, 6.4 g/serving for blueberries, and 2.5 g/serving for oranges. Further, apple pomace contains 44.7% fructose compared to 5.8-6.0% fructose in whole apple [5]. This is a health concern because fructose overconsumption has been reported to contribute to renal disease and to produce deleterious effects on bone [6,7]. Apple pomace contains a higher mineral content than whole apples, particularly calcium which is required for bone health [1,8]. However, overconsumption of calcium can increase nephrocalcinosis and reduced kidney function [9,10]. In turn, renal dysfunction can lead to bone loss due to mineral imbalance, resulting in increased risk of osteoporosis and other bone-mineral disorders [11]. Diets typical of Western countries are characterized by high-fat and high-sucrose. Western diet consumption has been shown to increase the risk of chronic kidney disease by inducing renal steatosis, inflammation, and oxidative stress. Western diet consumption has also been reported to increase risk of kidney stones due to the high sugar content [12,13]. Additionally, consuming a Western diet can result in early onset of osteoporosis by promoting mineral balance and inflammation leading to decreased bone mineral density [14,15]. Dietary advice suggests replacing calories in the diet with healthier food choices instead of dietary supplementation with a purified isolated nutrient [16].

Previously, our laboratory reported caloric substitution of a Western diet with 10% g/kg apple pomace attenuating features of NAFLD [17]. However, the effects of apple pomace on renal and bone was not assessed in this study. To our knowledge no studies have evaluated the safety of apple pomace consumption on renal and bone health. Therefore, the objectives of this study were to determine the safety of apple pomace, due to its high fructose content and increased calcium content, in growing rats consuming a “healthy” and Western diet. Female rats were used due to their increased susceptibility to nephrocalcinosis, and growing rats because kidney disease has been shown to have more severe bone effects in a pediatric population [18,19]. We hypothesize apple pomace will not detriment kidney or bone health in growing female rats consuming “healthy” or Western diets.

2. Materials and Methods

2.1 Diets

Locally sourced apple pomace was provided by Swilled Dog Hard Cider Company (Franklin, WV). Apple pomace was freeze dried in equipment? Nutrient composition analysis of apple pomace was performed by Medallion Laboratories (Minneapolis, MN). Apple pomace contains 32.5% fructose compared to the published average of 5.9% fructose for whole apples. Dietary calcium and phosphorus were determined by inductively coupled plasma mass spectrometry (ICP) (model P400, Perkin Elmer, Shelton, CT). Freeze-dried apple pomace contained 1.47 mg/g calcium and 1.97 mg/g phosphorous (Supplementary Table 1) compared to respective published values of 0.06 mg/g and 0.11 mg/g in whole apples [17].

The ‘healthy’ diet was the standard purified diet American Institute of Nutrition (AIN-93G) for growing rats [20] while a Western diet consisting of 45% fat and 34% sucrose was used to typify the high-fat, high-sugar diet consumed by Western countries [21,22]. AIN-93G and Western diet were calorically substituted with 10% g/kg freeze-dried apple pomace. AIN diets were adjusted to be isocaloric (3.7-3.8 kcal/g) and Western diets were adjusted to be isocaloric (4.7 kcal/g). The complete ingredient composition of experimental diets is provided as Supplementary Table 2. Diets were stored at -20°C until fed to animals.

2.2 Animals

Weanling (age 22-29 days) female Sprague-Dawley rats (n=32) were purchased from Harlan-Tekald (Indianapolis, IN). All animal procedures were approved by the Animal Care and Use Committee at West Virginia University and conducted in accordance with the guidelines of the National Research Council for the Care and Use of Laboratory Animals [23]. Rats were individually housed and kept in a room at constant temperature of 21+2°C with a 12 h light/dark cycle throughout the study duration. Following a 7-days acclimation, rats were randomly assigned (n=8 rats/group) to four dietary groups consisting of: 1) AIN-93G, a standard purified rodent diet, 2) AIN-93G with 10% weight (g/kg) substituted with apple pomace (AIN/AP), 3) Western diet (45% fat, 33% sucrose by kcals), or 4) Western diet with10% of weight (g/kg) substituted with apple pomace (Western/AP). Rats were provided ad libitum access to their assigned diets and deionized distilled water (ddH2O) throughout the eight weeks study duration. Food intake was measured and assigned diets replaced every other day while ddH2O was replaced weekly. At the end of the study, rats were fasted overnight then euthanized by carbon dioxide inhalation. The kidney was excised, weighed, and then flash frozen in liquid nitrogen and stored at -80°C until analyzed. Both femurs were removed, cleaned, and stored at -20°C.

2.3 Kidney histology

The left kidney was removed, weighed, flash frozen in liquid nitrogen, and stored at -80°C until analysis. A center sagittal section was cut from each frozen tissue (n=6-8) and stored in 10% neutral buffered formalin for 48 hours (fixation). After fixation, samples underwent a dehydration protocol consisting of 10-15 minutes incubation in increasing ethanol concentrations (50-to-100%) followed by two 20-minute incubations in xylenes. Following xylene incubation, samples were incubated in molten paraffin wax for 20 minutes (infiltration) and embedded into blocks. 5-7μm sections were cut and mounted on charged slides and sections stained with hematoxylin and eosin. Histological evaluation included gross morphological assessment which included the following: glomerular hypercellularity and matrix deposition, interstitial hypercellularity, tubulointerstitial calcification, inflammation, and fibrosis. All slides were analyzed using a Nikon Labophot 2 microscope (Nikon Instruments, New York, NY) at magnification 10X by a trained investigator blinded to the identity of the groups. Images were captured using a LCL-500-LHD digital camera with a PC Method Capture Imaging software (Ludesco, Parkville, MD).

2.4 Renal RNA isolation and inflammatory gene expression

Total RNA was extracted from frozen kidney tissue (50 mg) using the Zymo Research Direct-zol RNA Miniprep Plus Isolation Kit (Irvine, CA, catalog #R2071) according to the manufacturer's instruction for total RNA isolation. Isolated RNA integrity was visualized on a 1.5% agarose gel and quantified by spectrophotometry (NanoDrop 100; Thermo Fisher Scientific, Waltham, MA). Following DNase I treatment with TURBO DNA-free kit (Thermo Fisher Scientific), total mRNA was amplified using the Superscript IV First-Strand Synthesis System with oligo dT primers (Thermo Fisher Scientific).

Real-time quantitative polymerase chain reaction (RT-qPCR) consisted of 2.5 µl of SYBR Green Master Mix (Thermo Fisher Scientific), 1 µL of cDNA (diluted 1:10), 1 µL of respective forward and reverse primers and 0.5 µl of deionized distilled water for a total reaction volume of 5 µl. The reactions were performed in a 7500 ABI Real-Time PCR System (Thermo Fisher Scientific). The thermal profile consisted of 50°C for 2 min, 95°C for 10 min then 40 cycles of 95°C for 15 sec and 60°C for 1 min. A melt curve analysis was applied at the end of cycling. Primers that were designed for transcription factors, nuclear factor kappa-light chain enhancer of B cells (NFκB) and NADPH oxidase 4 (NOX4) and for inflammatory cytokines, tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) as well as for housekeeping genes, β-actin and glyceraldehyde 2-phosphate dehydrogenase (GAPDH) using the Primer3 program (Howard Hughes Medical Institute) and respective mRNA sequences obtained by NCBI. Forward and reverse primers for genes of interest are listed in Supplementary Table 3:

2.5 Serum and urinary measures of renal function and health

Serum measures of kidney function included: blood urea nitrogen (BUN), creatinine, total protein, calcium, phosphorous, alanine aminotransferase (ALT). Additionally, serum glucose and amylase were measured. Values were determined enzymatically using a commercially available Vet-16 rotor and quantified by a Hemagen Analyst automated spectrophotometer (Hemagen Diagnostics Inc., Columbia, MD).

Serum and urine uric acid was determined by commercially available enzymatic assay (Cayman Chemical). Briefly, serum and urine samples were aliquoted onto a 96-well plate and incubated for 15 minutes. Reaction was initiated by adding 15 µl of uricase and horseradish peroxidase enzyme mixture, and read at an excitation of 535 nm and an emission of 590 nm using a BioTek Synergy H1 microplate reader (Winooski, VT). Inter-assay coefficient of variation was 32.1% for both serum and urine.

2.6 Calcium balance and retention

Rats were fasted overnight and euthanized by carbon dioxide inhalation. Blood was collected by aorta puncture. Collected blood was centrifuged at 1,500 g for 10 min at 4°C to obtain serum. Serum samples were stored at -80°C until analyzed. Serum calcium was determined enzymatically using a commercially available Vet-16 rotor and quantified by a Hemagen Analyst automated spectrophotometer.

During the initial and final weeks of the feeding study, rats were individually housed in metabolic cages to collect urine and feces for 24 h. Initial and final day urine samples were collected, centrifuged at 1,500 g for 10 min at 4°C, filtered through Whatman no. 1 paper, and then diluted 1:10 in dd H2O. Initial and final feces were collected and dried for 48 h, then ashed in a muffle furnace (model CP18210, Thermolyne, Dubuque, IA) at 550°C for 24 h. Fecal samples were then acidified in 70% nitric acid, neutralized in ddH2O, filtered through Whatman no. 1 paper, and further diluted (1:50 v/v) in ddH2O. Ca content of feces and urine was determined by ICP.

Measures of Ca excretion, absorption, and retention were performed according to Maditz, et al. [24]. Briefly, urinary calcium excretion was calculated as urinary Ca concentration/urine volume. Ca apparent absorption was calculated as [(Ca intake-fecal Ca excretion)/(Ca intake)] x 100. Calcium retention was calculated as [(Ca intake – (fecal Ca excretion + urinary Ca excretion)].

2.7 Femur morphometry and mineralization

Following CO2 inhalation, the left and right femur were collected, and then defleshed. After no bilateral differences were determined using a t-test with significance set at $P < .05$ left femurs were used for all analyses. Femoral morphometry measurements of depth, width, and length were determined using a Vernier caliper (Bel-Art Products, Pequannock, NJ, USA). Length was measured from the medial condyle to the greater trochanter. Femurs were weighed using an analytical balance (Mettler Toledo, Columbus, OH, USA).

Total bone mineral was determined by ashing in a muffle furnace at 600°C for 24 hours, then weighed. To measure specific minerals, ash was dissolved in 2 mL of 70% nitric acid. Acidified samples were filtered through Whatman no. 1 paper and diluted (1:50 v/v) to volume with ddH2O and Ca determined using ICP.

2.8 Femur biomechanical strength

Femoral strength indices were assessed using a TA,XT2i Texture Analyzer (Texture Technologies, Scarsdale, NY, USA) fitted with a three point bending apparatus. Femora were placed on supports and force applied to the midshaft marked at a position halfway between the greater trochanter and the distal medical condyle. Bone was broken by lowering a centrally placed blade (1 mm width) at a constant crosshead speed (0.1 mm/s). The load cell was 250 kg.

The load-deflection data were collected by a PC interfaced with the TA,XT2i. Sample test distance was set at 10 mm with a signal collection rate of 100 points per second. Peak force, ultimate stiffness, ultimate bending stress and Young's modulus were calculated according to Yuan and Kitts [25].

2.9 Statistics

Results are expressed as mean ± standard error of the mean (SEM). Gene expression was determined as a function of mRNA abundance (A), where A=1/(gene of interest primer efficiency x ΔCT (g.o.i.) – (average housekeeping primer efficiency x ΔCT (h.k.)), where the product of efficiency and average of expression of β-actin was averaged with the product of efficiency and average of expression of GAPDH to determine the overall expression of the two housekeeping gene [17,26,27]. Gene expression data for each treatment group were log-transformed prior to statistical analysis. One-way ANOVA was used to determine differences among dietary groups. Post hoc multiple comparison tests were performed using Tukey's test with treatment differences considered significant at P = .05 and a tendency at P = .08. All statistical analyses were performed using JMP 12.2 statistical software package (SAS Institute, Cary, NC).

3. Results and Discussion

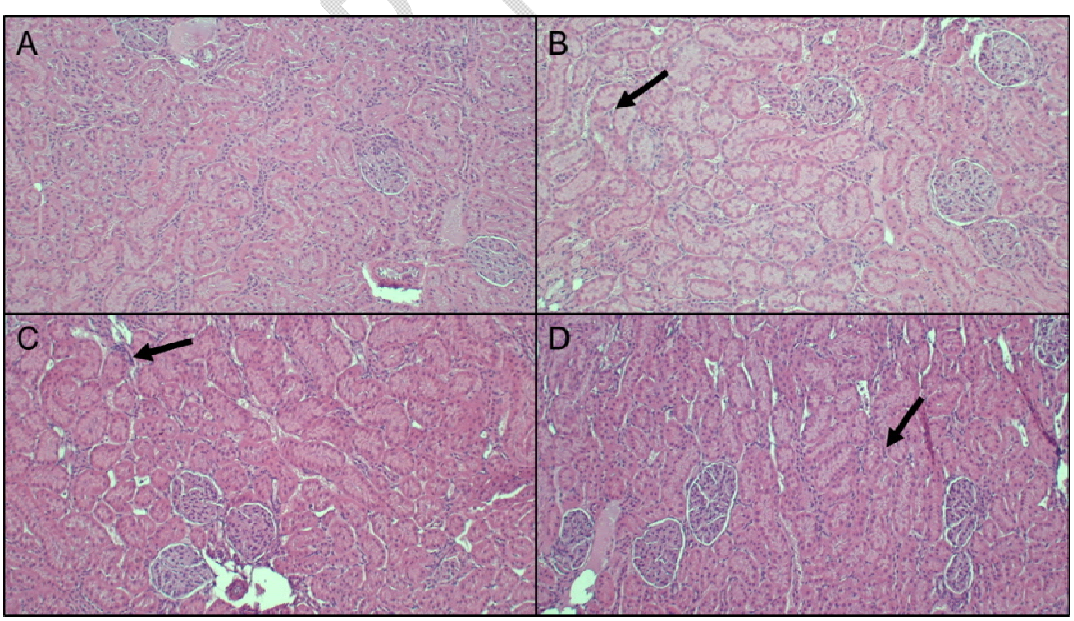
Rats are susceptible to renal disease and diets high in fructose and high in calcium have been shown to be detrimental to renal health, and high-fructose diets can detriment bone health [7,28,29]. In the current study, no differences were observed in body or organ weights (Table 1), but histological analysis of the kidneys showed no evidence of fibrosis, glomerular hypercellularity, glomerular matrix deposition, or amyloidosis.

Table 1. Weekly caloric and macronutrient intake, weekly body weight gain, and kidney and bone weights of growing female rats consuming different diets substituted with apple pomace (10% g/kg) for 8 weeks.

Measurements	Treatments [1]				
	AIN	**AIN/AP**	**Western**	**Western/AP**	***P*-Value**
Caloric intake (kcal/week)	368 ± 11[b]	345 ± 8[b]	422 ± 9[a]	430 ± 17[a]	<.001
Initial bwt (g)	95 ± 3	92 ± 3	95 ± 3	95 ± 3	0.80
Final bwt (g)	216 ± 4	216 ± 8	229 ± 5	234 ± 5	0.08
Average weekly bwt gain (g)	16 ± 3	16 ± 3	18 ± 3	18 ± 3	0.94
Average mineral intake (mg/d)	304.0 ± 9.3[b]	318.8 ± 7.3[b]	368.9 ± 7.8[a]	374.7 ± 15.0[a]	<.001
Right kidney weight (g)	0.69 ± 0.02	0.68 ± 0.02	0.71 ± 0.02	0.73 ± 0.02	0.28
Left kidney weight (g)	0.69 ± 0.02	0.67 ± 0.02	0.74 ± 0.03	0.74 ± 0.02	0.07
Relative right kidney weight (mg/g)	0.32 ± 0.01	0.31 ± 0.01	0.32 ± 0.01	0.31 ± 0.01	0.86
Relative left kidney weight (mg/g)	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.32 ± 0.00	0.70
Left kidney ash (mg/g)	9.86 ± 0.56	10.07 ± 0.54	9.14 ± 1.09	10.34 ± 0.67	0.71

[1]Values expressed as mean ± SEM (*n* = 6–8 rats/group). Different superscript letters a and b within the same row. Indicate significant difference at *P* < .05 by one-way ANOVA followed by Tukey's test. Abbreviations: Bwt, body weight; CHO, carbohydrate.

However, rats consuming Western diet and diets containing apple pomace showed renal interstitial hypercellularity (Figure 1), suggesting renal inflammation.



Histological changes	AIN	AIN/AP	Western	Western/AP
Inflammation	0	0	0	0
Fibrosis	0	0	0	0
Glomerular hypercellularity	0	0	0	0
Glomerular matrix deposition	0	0	0	0
Amyloidosis	0	0	0	0
Interstitial Calcification	0	0	0	0
Interstitial hypercellularity	0	2	1	2

Figure 1. Representative histological staining images of the kidney of growing female rats consuming (A) AIN, (B) AIN/AP, (C) Western, or (D) Western/AP following 8 weeks of feeding.

To further investigate, gene expression of inflammatory transcription factor, NFκB and proinflammatory cytokines, TNF-α and IL-6 as well as NOX4, a highly expressed enzyme regulating generation of reactive oxygen species, were measured in the kidneys. No significant differences were found in renal expression of any of the genes of interest among diet groups (Figure 2).

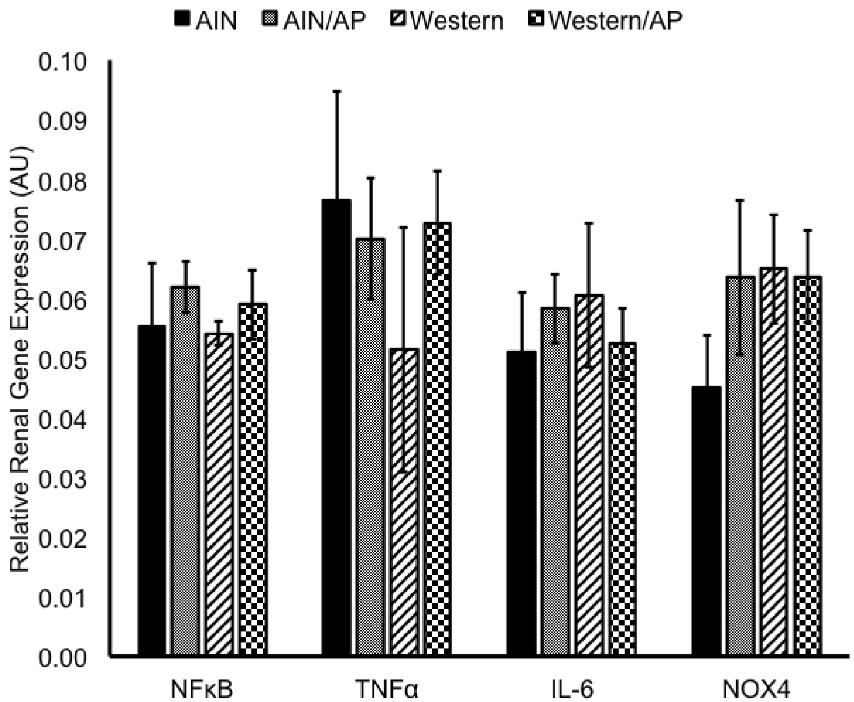


Figure 2. Renal expression of genes involved in inflammation and oxidative stress in rats consuming different diets substituted with 10% apple pomace (g/kg). Values expressed as mean ± SEM (n=5-7 animals/group). Different superscript letters a and b within the same figure indicates significant difference at *P* < .05 by one-way ANOVA followed by Tukey's test. Abbreviations: AU, arbitrary units; IL-6, interleukin-6; NOX4, NADPH oxidase 4; NFκB, nuclear factor kappa-light enhancer of B cells; TNFα, tumor necrosis factor alpha.

Serum creatinine, BUN, ALT, and total protein also showed no significant differences among diet groups, collectively indicating absence of inflammation and oxidative stress (Table 4).

Increased fructose consumption and elevated uric acid may play a role in renal inflammation [30-32]. Elevations in uric acid levels have been shown to change the fundamental architecture of renal histology and has been implicated in acute and chronic renal failure [33]. The current study results showed no significant difference in serum or urine uric acid among diet groups (Table 2).

Table 2. Effect of consumption of different diets substituted with apple pomace (10% g/kg) by growing female rats on serum and urine measurements of liver function enzymes, and uric acid following 8 weeks of feeding.

	Treatments[1]				
Measurements	**AIN**	**AIN/AP**	**Western**	**Western/AP**	***P*-Value**
Serum Creatinine (U/L)	1.46 ± 0.08	1.45 ± 0.11	1.38 ± 0.09	1.43 ± 0.04	0.90
Serum BUN (mg/dl)	17.84 ± 1.59	19.63 ± 1.41	20.25 ± 2.32	16.00 ± 0.94	0.27
Serum ALT (U/L)	107.63 ± 19.59	118.71 ± 43.60	94.5 ± 12.58	133.5 ± 30.59	0.78
Serum Total Protein (g/dl)	3.9 ± 0.25	4.62 ± 0.34	4.08 ± 0.67	4.19 ± 0.34	0.79
Serum Phosphorous (mg/dl)	14.18 ± 0.54	13.46 ± 1.72	15.68 ± 0.53	13.09 ± 1.02	0.35
Serum Ca (mg/dl)	9.56 ± 0.80	11.10 ± 1.09	11.49 ± 0.54	10.51 ± 1.00	0.48
Serum Uric Acid (μM)	7.24 ± 0.31	6.27 ± 1.61	7.19 ± 0.86	7.57 ± 1.25	0.86
Urine Uric Acid (μM)	5.94 ± 2.26	10.35 ± 2.11	10.40 ± 1.12	6.79 ± 1.41	0.23

[1]Values expressed as mean ± SEM (n=4-8 animals/group). Different superscript letters a and b within the same figure indicates significant difference at *P* < .05 by one-way ANOVA followed by Tukey's test. Abbreviations: ALT, alanine aminotransferase; BUN, blood urea nitrogen.

Interstitial hypercellularity was observed in 13-29% of animals, but there were no significant differences in oxidative stress and inflammatory gene expression or serum and urine measurements of renal dysfunction and injury were observed among diet groups. These results indicate renal interstitial hypercellularity was unlikely to be of biological significance. Collectively, the results indicate the fructose content of apple pomace was not a risk for renal injury and development of chronic kidney disease in either 'healthy' or Western diet.

In our study, Western diets were high in calcium with Western/AP diet having the highest calcium content (Table 2). Differences in calcium content in diets can have significant effects on calcium excretion, absorption, and retention [34]. Increased calcium excretion can induce nephrocalcinosis [35]. Initial urinary and fecal calcium excretion, calcium retention, and calcium absorption showed no significant differences among diet groups (Table 3). At final week, no differences were observed in rats urinary calcium excretion among all groups, but an increase (*P* = .04) in fecal calcium excretion by rats consuming a Western/AP compared to AIN was observed. This was also likely due to a combination of the high insoluble dietary fiber content in apple pomace possibly binding to calcium and the increased dietary calcium in Western/AP diets. This also explains the lack of change in apparent calcium absorption among all diet groups. No differences were observed in calcium retention among all diet groups.

Table 3. Calcium balance of rats fed different diets substituted with 10% (g/kg) apple pomace.

Calcium Balance	Treatments [1]				
	AIN	**AIN/AP**	**Western**	**Western/AP**	***P*-Value**
Ca Intake (mg/d)	135.6 ± 4.2^{c}	140.1 ± 3.2^{c}	162.4 ± 3.5^{b}	184.9 ± 7.4^{a}	<0.001
Initial					
Urine Ca excretion (mg/dl)	0.16 ± 0.04	0.19 ± 0.04	0.17 ± 0.04	0.18 ± 0.04	0.96
Fecal Ca excretion (mg/d)	25.9 ± 3.6	22.9 ± 3.5	31.3 ± 3.7	34.7 ± 2.7	0.12
Ca retention (mg/d)	89.3 ± 9.4	94.9 ± 5.9	96.4 ± 5.8	109.8 ± 6.2	0.32
Ca absorption (%)	62.5 ± 4.6	68.0 ± 4.7	61.4 ± 4.2	63.3 ± 3.0	0.70
Final					
Urine Ca excretion (mg/ml)	0.15 ± 0.02	0.16 ± 0.04	0.16 ± 0.04	0.10 ± 0.01	0.25
Fecal Ca excretion (mg/d)	60.9 ± 2.9^{b}	79.4 ± 11.6^{ab}	81.2 ± 3.9^{ab}	99.3 ± 7.1^{a}	0.04
Ca retention (mg/d)	77.7 ± 5.3	66.7 ± 5.3	80.8 ± 5.0	78.9 ± 5.3	0.25
Ca absorption (%)	54.2 ± 4.1	41.8 ± 11.8	49.7 ± 3.2	46.3 ± 5.3	0.65

[1]Values expressed as mean ± SEM (n=4-8 animals/group). Different superscript letters a and b within the same figure indicates significant difference at $P < .05$ by one-way ANOVA followed by Tukey's test.

Further, renal histological evaluation showed no evidence of calcium deposition in any of the diet groups, further indicating apple pomace consumption to be safe (Figure 2).

While Western diet (high fat and high sugar) and fructose consumption have also been reported to detriment bone health, whole apples have been shown to favorably alter bone health, through increased bone mineral density, decreased calcium loss, and decreased inflammation due to antioxidants present in apples [36-39]. Apple pomace has been shown to contain more calcium than apples [5]. Increasing dietary calcium has been shown to prevent osteoporosis and to lower the risk of bone fractures [40,41]. Further, children with adequate calcium consumption had increased bone mineral density [42,43]. The present study showed no significant differences in femoral calcium content among diet groups. Additionally, there were no significant differences in femur size or bone strength measurements including: peak force, ultimate stiffness, ultimate bending stress, and Young's modulus among diet groups (Table 4).

Table 4. Femoral morphometry and strength measurements of rats fed different diets substituted with 10% (g/kg) apple pomace.

Measurement	Treatments [1]				
	AIN	AIN/AP	Western	Western/AP	*P*-value
Femur morphometry					
Length (mm)	29.71 ± 0.53	29.09 ± 0.78	30.52 ± 0.56	29.36 ± 0.78	0.09
Medial lateral width (mm)	2.98 ± 0.04	3.12 ± 0.12	3.06 ± 0.08	3.15 ± 0.10	0.13
Depth (mm)	2.78 ± 0.07	2.73 ± 0.12	2.60 ± 0.09	3.06 ± 0.17	0.43
Wet wt (g)	0.77 ± 0.02	0.74 ± .05	0.73 ± 0.03	0.74 ± 0.04	0.89
Dry wt (g)	0.48 ± 0.01	0.46 ± 0.03	0.45 ± 0.02	0.47 ± 0.02	0.77
Femur mineralization					
Ash (mg/g of bone)	407.92 ± 11.42	407.75 ± 9.26	399.66 ± 7.40	396.94 ± 6.46	0.80
Calcium (mg/g of bone)	37.99 ± 0.78	39.09 ± 4.41	40.09 ± 2.26	38.28 ± 2.08	0.75
Femur biomechanical strength					
Peak force (N)	1.74 ± 0.18	1.99 ± 0.25	1.55 ± 0.11	1.23 ± 0.23	0.07
Ultimate stiffness (N/S)	382.03 ± 16.28	399.49 ± 27.07	397.55 ± 38.73	347.15 ± 14.01	0.60
Ultimate bending stress (N/S)	42.32 ± 1.57	38.21 ± 2.19	40.12 ± 3.46	42.19 ± 2.59	0.48
Young's Modulus (N/mm^2)	1604.92 ± 76.18	1484.85 ± 284.92	1549.57 ± 90.13	1275.92 ± 200.17	0.75

[1]Values expressed as mean ± SEM (n=6-8 animals/group). Different superscript letters a and b within the same figure indicates significant difference at $P \leq .05$ by one-way ANOVA followed by Tukey's test.

Another concern is rats consuming Western/AP diets had significantly increased gonadal fat pad weights than rats consuming AIN diets (Table 1). Obesity and diabetes have been reported to be causal factors in diet-induced kidney disease progression [6,44]. In our study, despite higher adiposity in rats fed Western/AP there were no significant differences in fasting serum glucose or amylase among diet groups (data now shown). Our study provides evidence that high fructose and high calcium content of apple pomace was not sufficient to effect renal or bone health in rats, regardless of diet. Studies on apple pomace have reported numerous health benefits including decreases in body weight, as well improvements in serum lipid, insulin, glucose, antioxidant status, and digestion [45-51]. Yet, few studies have evaluated the safety of apple pomace consumption. Devrajan, et al. [52] fed rats unfermented or fermented apple pomace for 2 weeks showed a nonsignificant increase serum BUN, but found no indication of kidney damage [53]. Additionally, histology was not used to evaluate kidney health.

4. Conclusions

Caloric substitution of a healthy or Western diet with 10% apple pomace had no impact on renal or bone health in growing female rodents. Based on our results apple pomace is safe for consumption, despite its high fructose content combined with a high calcium content, regardless of diet quality in rodents. The study provides evidence for apple pomace, a “waste” byproduct of apple processing has a favorable nutritional profile and is safe and therefore has potential to be repurposed as a sustainable food source for human consumption. Still, human clinical trials should be conducted to further determine the efficacy and safety of apple pomace consumption.

Competing Interests

Authors have declared that no competing interests exist.

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Supplemental Material

Supplementary Table 1. Composition of locally sourced freeze-dried apple pomace.

Macronutrients (%)	
Protein	3.56
Fat	1.3
Carbohydrates	68.1
Sugars (%)	
Fructose	32.5
Glucose	9.77
Sucrose	13.9
Maltose	<0.1
Lactose	<0.1
Dietary Fiber (%)	
Insoluble Dietary Fiber	22.2
Soluble Dietary Fiber	11.0
Polyphenols (g/kg)	**0.029**
Minerals (mg/g)	
Total Minerals	15.5
Calcium	1.47
Phosphorous	1.97
Calories (kcal/100 g)	**387**

Supplementary Table 2. Composition of rodent diets substituted with apple pomace (10% g/kg) fed to growing female rats.

	Diet Groups *			
	AIN	**AIN/AP**	**Western**	**Western/AP**
Ingredients (g/kg) *				
Apple pomace	0.0	100.0	0.0	100.0
Corn Starch	397.486	392.086	63.36	57.96
Maltodextrin	132.0	132.0	60.0	60.0
Sucrose	100.0	43.9	340.0	283.9
Fructose	50	54.45	170	174.45
Total Dietary Fiber	50.0	50.0	50.0	50.0
Insoluble Fiber †	50.0	39.0	50.0	39.0
Soluble Fiber ‡	0.0	11.0	0.0	11.0
Anhydrous Milkfat	0.0	0.0	210.0	210.0
Soybean Oil	70.0	68.7	20.0	18.7
Casein	200.0	196.0	195.0	191.0
L-Cystine	3.0	3.0	3.0	3.0
Vitamin Mix	10.0	10.0	12.5	12.5
Mineral Mix	35.0	35.0	43.0	43.0
Total Minerals	22.1	24.2	26.4	28.0
Calcium	10.4	10.8	12.8	14.6
Phosphorous	7.2	7.5	7.6	7.5
Choline Bitartrate	2.5	2.5	3.1	3.1
TBHQ, antioxidant	0.014	0.014	0.04	0.04
Polyphenols	0.0015	0.0029	0.0008	0.0032
Macronutrients (% kcal)				
Protein	18.8	18.9	14.8	14.8
Fat	17.2	17.3	44.6	44.8
Carbohydrate	63.9	63.7	40.6	40.4
Calories (kcal/g)	3.8	3.7	4.7	4.7

* Abbreviations: AIN, the American Institute of Nutrition; AP, apple pomace; TBHQ, tert-butylhydroquinone. † Insoluble fiber is cellulose. ‡ Soluble fiber is mainly pectin [1].

Supplementary Table 3. Forward and reverse primers for genes of interest in study.

Gene	NCBI Gene ID	Forward Primer	Reverse Primer
NFκB	81736	5’ TTATGGGCAGGATGGACCTA 3’	5’ CCTTTCAGGGCTTTGGTTTA 3’
TNFα	24835	5’ CACAAGGCTGCTGAAGATGT 3’	5’ GAGGGAAGGAAGGAAGGAAG 3’
IL-6	24498	5’ TGGCTAAGGACCAAGACCAT 3’	5’ TTGCCGAGTAGACCTCATAGTG 3’
NOX4	85431	5’ CCTCCATCAAGCCAAGATTC 3’	5’ CTCCAGCCACACACAGACTAAC 3’
β-actin	81822	5’ TTGCTGACAGGATGCACAAG 3’	5’ CAGTGAGGCCAGGATAGAGC 3’
GAPDH	24383	5’ TCAAGAAGGTGGTGAAGCAG 3’	5’ CCTCAGTGTAGCCCAGGATG 3’