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Effect of Dietary Enrichment with Hempseed (*Cannabis sativa* L.) on Blood Pressure Changes in Growing Mice between Ages of 5 and 30 Weeks

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Abstract: Dietary hempseed (*Cannabis sativa* L.) reduces blood pressure in adults and animal models of hypertension; however, whether hempseed consumption throughout early life reduces adult blood pressure is not known. This study tested the hypothesis that hempseed enrichment versus a control diet modifies the age-dependent pattern of blood pressure changes in growing female C57BL/6 mice and results in lower adult blood pressure. From ages 5 to 30 weeks, early post-weaning to mid-adulthood, mice were fed either a control AIN-93G (0%), 50 g/kg (5%), or 150 g/kg (15%) hempseed-supplemented diet (n = 8 per group). Biweekly measurements of systolic, diastolic, and mean arterial pressure were collected using the tail-cuff method. Mice fed 5% or 15% hempseed versus the control diet exhibited no significant differences in systolic, diastolic, or mean arterial blood pressure (repeated measures ANOVA main effect of diet, $p > 0.05$). Blood pressure did not differ significantly between diet groups in adulthood ($p > 0.05$). However, mice fed a control or 5% hempseed, but not 15% hempseed, diet exhibited blood pressure changes across age marked by significant increases during early adulthood (weeks 11–17) versus early post-weaning (week 5) ($p < 0.05$). In conclusion, long-term dietary hempseed enrichment at 5% and 15% concentrations during development does not reduce adult blood pressure, but a 15% dose blunts the temporary increase in blood pressure during early adulthood seen in mice fed a control diet.

Keywords: hempseed; blood pressure; mice; C57BL/6

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1. Introduction

Dietary hempseed is shown to benefit cardiovascular health by reducing blood pressure, exerting antioxidant and antiplatelet activity, and improving serum lipid profiles [1,2]. The bioactive properties of hempseed can be traced to its content of essential fatty acids alpha-linolenic and linoleic acid, protein, fiber, vitamins A and E, multiple minerals, and polyphenols [3,4]. Knowledge of the beneficial health effects of seeds and societal trends toward plant-based diets support the expanding consumption of foods such as seeds [5–8]. The global hempseed market value continues to rise and is expected to reach USD 1634 million by 2027, a compound annual growth rate of 11.1% [9]. The United States is a leader in the hempseed market, and demand is rising in countries such as China and India [10]. Hempseed's value as a functional food and an important source of protein in vegetarian diets has contributed to the increase in hempseed consumption [1,2,8]. This expansion of hempseed as a dietary component has the potential to impact outcomes such as cardiovascular health.

Existing research findings on the cardiovascular effects of hempseed product consumption are primarily from relatively short-term animal feeding trials [11–17]. Many of these studies report on cardiovascular disease risk factor outcomes such as oxidative stress

level [11–13] and circulating lipid profile [12,13,17,18] rather than blood pressure. Majewski et al. showed that obese Zucker rats fed a 12% hempseed diet for 4 weeks displayed reduced lipid peroxidation in plasma and heart tissue and enhanced vasodilation [12]. In a rat model of high-fat diet-induced hypercholesterolemia, Kaushal et al. observed that 4 weeks of a 10% hempseed diet resulted in significant reductions in serum total cholesterol and triglycerides and improved redox status in aortic tissue [13]. In contrast, only two studies are known to have investigated blood pressure responses to dietary hempseed. For example, in spontaneously hypertensive rats, 8 weeks of dietary enrichment with hempseed protein reduced the rise in blood pressure during growth and 4 weeks of hempseed feeding lowered blood pressure during adulthood [15]. A recent human intervention trial demonstrated significant reductions in 24 h systolic and diastolic blood pressure in mildly hypertensive men and women fed hempseed protein or hempseed protein hydrolysate peptide for 6 weeks [19]. Thus, data on the effect of dietary hempseed on blood pressure, the strongest causal factor in cardiovascular disease development [20], are limited to short-term interventions and hypertensive models. No information is available on the impact of hempseed consumption on the changes in blood pressure that occur during early life [21] and blood pressure in adulthood.

To address the gap in information regarding the effects on blood pressure of long-term hempseed consumption during development, we measured systolic, diastolic, and mean arterial blood pressure in female C57BL/6 mice fed hempseed from the early post-weaning period to mid-adulthood. We hypothesized that hempseed enrichment at two concentrations (5% and 15%, representing low and high doses) versus a control diet would modify the age-dependent pattern of blood pressure development and result in lower adult blood pressure. This study was designed to contribute new information about the impact of dietary hempseed on the age-related changes in blood pressure during growth in a healthy mouse model. The results are intended to inform the research and health professional communities on the impact of hempseed consumption during development on blood pressure in order to guide the design of future studies and dietary recommendations.

2. Materials and Methods

2.1. Experimental Design

Prior to beginning the study, the study protocol was approved by Colorado State University—Pueblo, CO, USA Institutional Animal Care and Use Committee under protocol number 000-000A-022, and procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals composed by the National Institutes of Health. Female 3-week-old C57BL/6 mice obtained from Charles River Laboratories (Wilmington, MA, USA) were habituated for 2 weeks to the animal care facility and fed an AIN-93G pellet diet prior to initiation of the experiment. Mice were housed in pairs in one environmentally controlled room with lights on at 0600 and off at 1800 h. Body weight was measured weekly at the same time of day using a digital balance. To ensure accurate tracking of individual mice during the experiment, a programmable microchip (UID Identification Solutions, Lake Villa, IL, USA) was implanted into the subscapular region of each mouse at 4 weeks of age. At 5 weeks of age, mice were randomly separated into groups (n = 8 each) and fed ad libitum one of three diets [15% wt/wt hempseed (HS); 5% HS; or 0% HS (control)] for 6 months, until the age of 30 weeks. The age of 5 weeks represented early post-weaning, while the age of 30 weeks represented mid-adulthood [22]. Investigators were not blinded to diet group assignment. Animal health status was monitored daily and included physical examination for injury; sores; discharge; gastrointestinal problems, e.g., diarrhea; as well as physical activity level; physical appearance; body posture; and mobility. No animals in the study developed adverse reactions to either the experimental diet or biweekly blood pressure measurements. The Institutional Animal Care and Use Committee protocol included pre-established interventions for adverse events and humane endpoints. All animals completed the study without adverse events.

2.2. Diets

The AIN-93G diet [23] formed the basis of the study diets. Pellet AIN-93G was purchased from Dyets, Inc. (Bethlehem, PA, USA). The control diet was unaltered AIN-93G. Two hempseed diets were developed by including 5% or 15% by weight whole, ground, organic, toasted hempseed (CHII Naturally Pure Hemp—Naturally Splendid Enterprises, Ltd., Pitt Meadows, BC, Canada). The composition of the diets is shown in Table 1. Diets were formulated to provide ~16% of total kcals from fat. To create the hempseed-enriched diets, the AIN-93G diet was modified to balance nutrients naturally present in whole, unground hempseed. Cellulose, for example, was reduced from 50 g/kg diet (control) to 34.5 g/kg diet (5% HS) and 3.5 g/kg diet (15% HS) to balance the fiber contributed by the hempseed. Hempseed nutrition information is listed in Supplementary Table S1.

Table 1. Composition of experimental diets.

Ingredient, g per kg Diet	Control Diet [a]	5% Hempseed	15% Hempseed
Casein, High Nitrogen	200	185	155
L-Cystine	3	3	3
Sucrose [b]	100	100	100
Cornstarch	397.486	395.99	392.997
Dyetrose	132	132	132
Soybean Oil	70	52	16
t-Butylhydroquinone	0.014	0.01	0.003
Cellulose	50	34.5	3.5
Mineral Mix #210025 [c]	35	35	35
Vitamin Mix # 310025 [d]	10	10	10
Choline Bitartrate	2.5	2.5	2.5
Hempseed [e]	0	50	150
Total	1000	1000	1000
Kilocalories per kg	3760	3814	3922

[a] AIN-93G. [b] Ninety percent tetrasaccharides and higher; [c] Composition (g/kg mineral mix): $CaCO_3$, 357.0; KH_2PO_4, 196.0; K Citrate·H_2O, 70.78; NaCl, 74.0; K_2SO_4, 46.6; MgO, 24.3; Fe citrate, 6.06; $ZnCO_3$, 1.65; $MnCO_3$, 0.63; $CuCO_3$, 0.31; KIO_3, 0.01; Na_2SeO_4, 0.01025; (NH_4) 6 Mo_7O_{24}·$4H_2O$, 0.00795; Na_2SiO_3·$9H_2O$, 1.45; $CrK(SO_4)$ 2·$12H_2O$, 0.275; LiCl, 0.0174; H_3BO_3, 0.0815; NaF, 0.0635; $2NiCO_3$·$3Ni(OH)$ 2·$4H_2O$, 0.0318; NH_4VO_3, 0.0066. [d] Composition (g/kg vitamin mix): thiamin HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7; nicotinic acid, 3.0; Ca pantothenate, 1.6; folic acid, 0.2; D-biotin, 0.02; vitamin B^{12} (0.1% in mannitol), 2.5; vitamin A palmitate (500,000 IU/g), 0.8; DL-α-tocopheryl acetate (500 IU/g), 15; vitamin D3 (400,000 IU/g), 0.25; vitamin K/dextrose 10 mg/g (phylloquinone), 7.5. [e] Whole, ground hempseed (nutrition information supplied in Supplementary Table S1).

2.3. Measurement of Blood Pressure and Area under the Curve (AUC) Analysis

Blood pressure at the mouse caudal artery was measured biweekly beginning at 5 weeks of age until 29 weeks of age. Measurements were consistently made between 0900 and 1200 h on the day of the experiment. The 24 mice were divided into two groups of 12 mice each for testing on two consecutive week days, specifically, Monday, group one of n = 12 mice; Tuesday, group two of n = 12 mice. Within each group of 12 mice, there were n = 4 mice from each of the three diet groups. The order of testing was as follows: Group 1: CON-1, 5HS-1, 15HS-1, etc., ending with CON-12, 5HS-12, 15HS-12; Group 2: CON-13, 5HS-13, 15HS-13, etc., ending with CON-24, 5HS-24, 15HS-24. This protocol ensured that each mouse was tested at the same time of day to minimize variation in their daily routine and that the three diet groups were evenly distributed over the 3 h test period. To minimize stress, precautions described in the protocols literature for mice [24–26] were strictly followed. This included maintaining a calm, quiet, dimly lit, and warm environment, with no extraneous odors and no perfumes when performing experiments. The mouse holding tube, tail cuffs, and work area were cleaned with 70% ethanol after each animal test. The same two researchers, with prior training and following the same protocol, performed blood pressure measurements. Each person was assigned one of the two groups of n=12 mice for the entire duration of the study. They were not blinded

to the animal identification. Mice were weighed prior to blood pressure measurement collection as part of the weekly weighing and recording procedure.

Blood pressure was measured using the CODA Noninvasive Blood Pressure System and accompanying CODA Software, v. 4.1 (Kent Scientific Corporation, Torrington, CT, USA) following the manufacturer's methods and protocol for mice as described previously in the literature [24–26]. This method does not allow for continuous monitoring of arterial pressure as compared to the direct indwelling catheter method but has the main advantages of not requiring invasive surgery (especially difficult for small, young mice) and allowing for repeated measurements over a long time period. The CODA system directly measures systolic and diastolic blood pressures and calculates mean arterial pressure for individual readings. An illustration of the experimental setup is shown in Figure 1. Mice were placed into a polycarbonate cylindrical holding tube consisting of holes for air, a cone where the nose was placed, and a rear gate. The tail extended outside of the rear gate of the tube to allow the occlusion cuff and volume pressure recording (VPR) cuff to be placed around the tail base. The mice were secured into the tube at both ends to minimize movement and stress during the collection period. The holding tube was placed over a heating pad to maintain the mouse's core body temperature and increase blood flow to the caudal artery [25]. Tail base temperature was maintained in the range of 32–35 °C and monitored frequently using an infrared thermometer (General Tools & Instruments LLC, Secaucus, NJ, USA) aimed at the tail base. Measurement collection began when the mouse's tail base temperature reached at least 32 °C, indicating that the caudal artery was dilated and well perfused with blood.

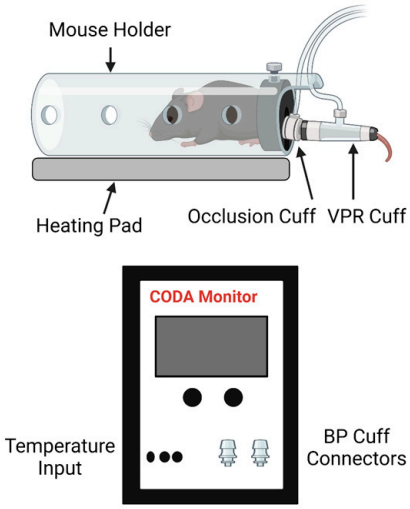


Figure 1. Blood pressure measurement using the CODA Noninvasive Blood Pressure System (Kent Scientific Corporation). A mouse was placed into a cylinder fitted with a rear gate and front gate to minimize movement, placed on a heating pad. An occlusion cuff and volume pressure recording (VRP) cuff were secured around the tail base and lines were connected to the CODA Monitor.

The mice were allowed 5 min to acclimate after placement in the holding tube before caudal artery blood pressure measurement collection began. After the 5 min rest period and attainment of an adequate tail base temperature, 20 cycles of blood pressure measurements were recorded (5 initial acclimation measurements and 15 experimental measurements). When the experiment was ended, mice were removed from the holder and immediately returned to their home cage. The data were exported from CODA to an Excel file for processing. A minimum of 15 valid cycles were deemed acceptable, and these cycles were used to calculate the average of replicate measurements for each blood pressure parameter (systolic, diastolic, and mean) for each mouse. For each diet group, a grand average and SEM (n = 8) was then calculated using the individual mouse average values.

Area under the curve (AUC) analysis is useful in capturing the overall level of blood pressure across time and has been used previously to summarize interval measurements of blood pressure in longitudinal studies in humans [27] and acute blood pressure measurements in mice over several minutes of continuous recording using implanted blood pressure radio transmitters [28]. Here, we used AUC analysis to summarize overall levels of systolic, diastolic, and mean arterial blood pressure in single mice using the data obtained from biweekly measurements between the ages of 5 and 29 weeks. Specific intervals of interest for AUC analysis included (1) age of 5–15 weeks (AUC1), (2) age of 15–25 weeks (AUC2), and (3) age of 5–29 weeks for the entire study duration (AUC3). Additionally, the difference in AUC value was calculated for AUC2 (15–25 weeks) and AUC1 (5–15 weeks) to compare overall blood pressure between "adulthood" versus "adolescence", respectively.

AUC values were calculated using IgorPro, version 9.0 (WaveMetrics). Figure 2 illustrates the basic steps involved in processing data for the age intervals of interest. The basic steps are as follows: STEP 1 (Figure 2A): Copy data for a single mouse into IgorPro and make an XY-waveform pair. In this step, the raw data are interpolated, i.e., additional points are inserted between raw data points. A raw data set for each mouse contains 13 points of biweekly measurements from ages 5 to 29 weeks and the interpolation value is set to 168 points, corresponding to the total number of days in the study. STEP 2 (Figure 2B): Integrate the interpolated data set for each single mouse in the group. Here, a rectangular function is used. STEP 3 (Figure 2C): Mark the intervals of interest on the integrated waves and obtain AUC values.

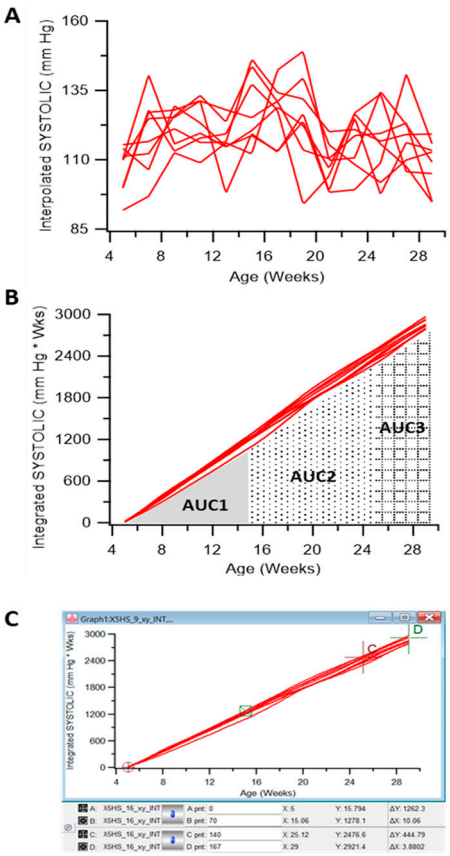


Figure 2. Area under the curve (AUC) analysis performed in IgorPro 9.0 (WaveMet-rics). Representative figures illustrate the basic steps. Data shown are for AUC analysis of systolic blood pressure in n = 8 mice in the 5% hempseed diet group. (**A**) Interpolated waves. (**B**) Integrated waves with intervals marked: AUC1 (age 5–15 weeks), AUC2 (age 15–29 weeks), and AUC3 (age 5–29 weeks). (**C**) Placement of cursors A, B, C, and D to mark intervals of interest for AUC analysis.

2.4. Measurement of Body Composition (DXA Scan)

Body composition was measured every month by dual-energy X-ray absorptiometry (DXA) using a Lunar PIXImus scanner (GE Lunar, Madison, WI, USA). A quality control procedure was performed with a calibration phantom at the start of each imaging session. Mice were anesthetized with 2.5% isoflurane inhalant anesthetic and placed in the prone position for imaging; the total scan time per mouse was about 5 min. Measurements included fat mass (g) and lean mass (g). The skull was excluded from analysis, and the region of interest included the whole body and tail. Body length was measured manually while mice were anesthetized from the tip of the snout to the base of the tail using a ruler.

2.5. Calculations of BS, BMI, LMI, and FMI

Body surface area was calculated using Meeh's formula [29] as follows:

(i) Body surface area (BS, cm^2) = k Mb2/3, where Mb = body mass (g), and k = constant.

The literature reports various k values for rats and mice, for example, C57BL6 mice, k = 9.82 [30]; C57BL6 mice, k = 9.617 (8 AM) and k = 9.802 (4 PM); Wistar rats, k = 9.83 [31]. Here, we used k = 9.82 [30].

From the body composition parameters measured with DXA, several derivative values were calculated, as described in Gargiulo et al. [32], to investigate correlations with blood pressure during development in the mice, as follows:

(i) Body mass index (BMI, g/cm^2): Body weight (g)/Body surface area (cm^2).
(ii) Lean mass index (LMI, g/cm^2): Lean (g)/Body surface area (cm^2).
(iii) Fat mass index (FMI, g/cm^2): Fat (g)/Body surface area (cm^2).

2.6. Statistical Analysis

Data analysis was conducted using JMP 17.0 software (Cary, NC, USA). Data were evaluated for normal distribution and equality of variances using the Shapiro–Wilk goodness-of-fit test and Levine's test, respectively. ANOVA with repeated measures was used to test the effect of diet group, age, and their interaction on systolic, diastolic, and mean arterial pressure. ANOVA was used to test the effect of diet group, age, and their interaction on AUC values. The interaction of age and diet group was not significant for all dependent variables; thus, the interaction was removed from ANOVA in final analyses of main effects. Tukey's post hoc analysis was performed when ANOVA detected significant main effects. Regression analysis of the relationship between blood pressure and body composition measures was performed using Spearman's rank correlation coefficients. Estimating a difference between groups of 10 mm Hg in systolic blood pressure and a within-group variance of 140 mm Hg, a sample size of 8 per diet group provided >80% power to detect a statistically significant difference between groups. Data are reported as means with standard error of the mean (SEM). No data points were excluded from analysis. A p value of <0.05 was deemed statistically significant.

3. Results

3.1. Effect of Diet and Age on Body Weight, Length, Surface Area, BMI, LMI, and FMI

At the experimental endpoint of the study, all animals were maintaining body weight and intakes of feed and fluid. Body weight, length, surface area, BMI, LMI, and FMI increased with advancing age in all mice ($p < 0.0001$) (Figure 3A–F). These increases were not affected by diet ($p > 0.05$), with the exception of FMI. Fat mass index was significantly higher in mice consuming the 5% HS versus control and 15% HS diets ($p = 0.004$ main effect of group, $p < 0.05$ post hoc test 5% HS versus control and 15% HS); see Figure 3F.

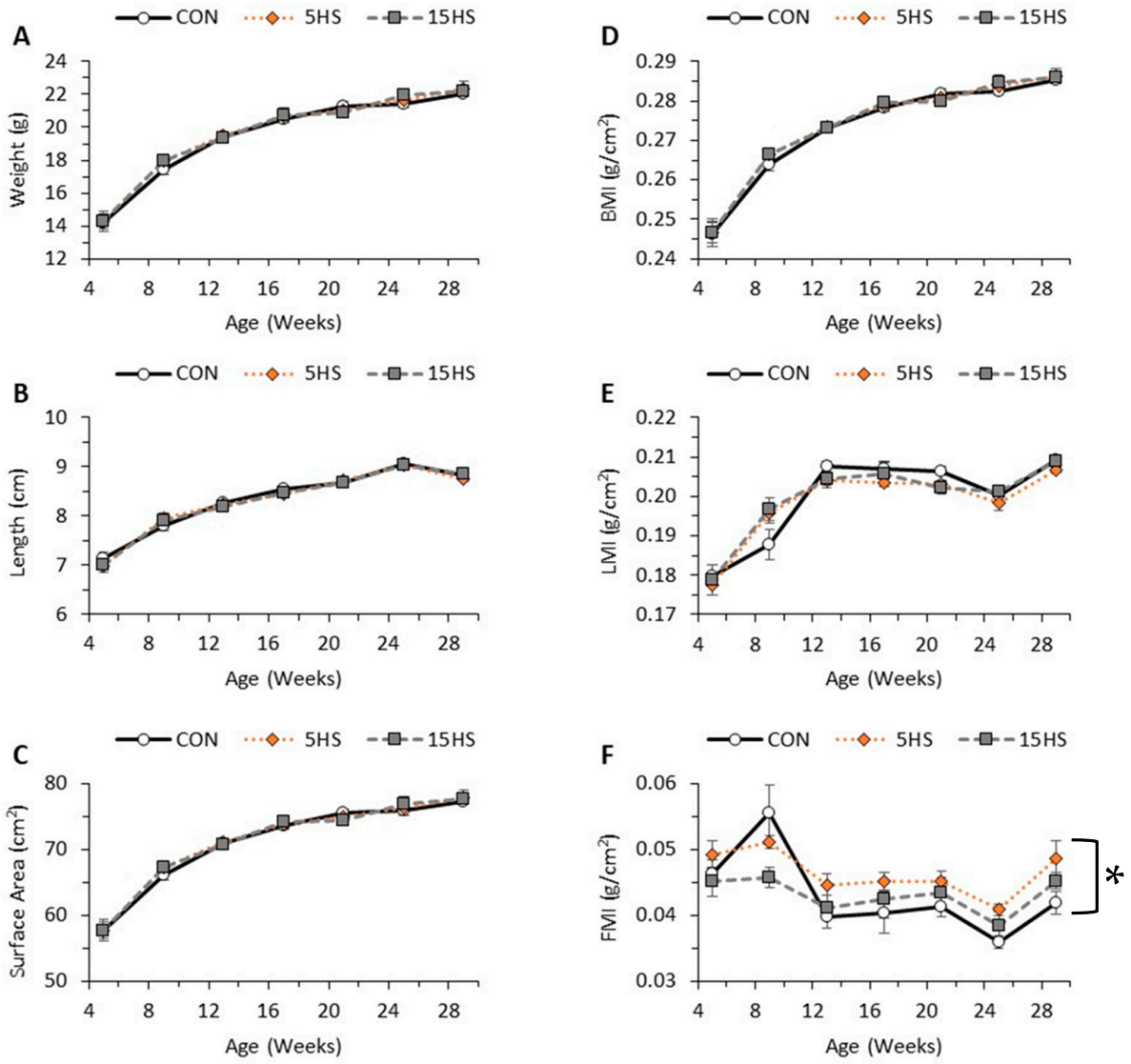


Figure 3. Effect of diet and age on body weight, length, surface area, BMI, LMI, and FMI. (**A**–**F**) Age exerted a main effect on all parameters ($p < 0.0001$). (**F**) Diet showed a main effect only on FMI, with 5% HS having higher values than CON and 15% HS (* $p = 0.004$ main effect of group; $p < 0.05$ 5% HS versus CON and 15% HS). $n = 8$ per diet group.

3.2. Effect of Diet and Age on Systolic, Diastolic, and Mean Arterial Blood Pressure

Hempseed enrichment did not alter systolic, diastolic, or mean arterial blood pressure across the 25-week experiment ($p > 0.05$). However, age exerted a significant main effect on systolic, diastolic, and mean arterial blood pressure ($p \leq 0.01$ for all three diet groups); see Figure 4A–C.

The pattern of change across age for systolic, diastolic, and mean arterial pressure for the three diet groups followed a similar trend, starting at roughly the same initial pressure and increasing until the age of 10–15 weeks, where pressure began to stabilize or slowly decrease throughout the remainder of the study; see Figure 4A–C. When the effect of age on blood pressure measurements was analyzed for each diet group separately, the post hoc pairwise comparisons (age, individual weeks 7–29 versus starting age, week 5) showed different patterns of age-related change in blood pressure between diet groups. Specifically, for the control diet group, systolic, diastolic, and mean arterial blood pressure increased after the age of 5 weeks and were significantly greater at weeks 11 and 15 versus week 5. For the 5% HS diet group, the significant rise in blood pressure was delayed: systolic, diastolic, and mean arterial pressure was significantly higher at weeks 15 and 17 versus

week 5. For the 15% HS diet group, no significant differences in comparisons of blood pressure between pairs of weeks were seen.

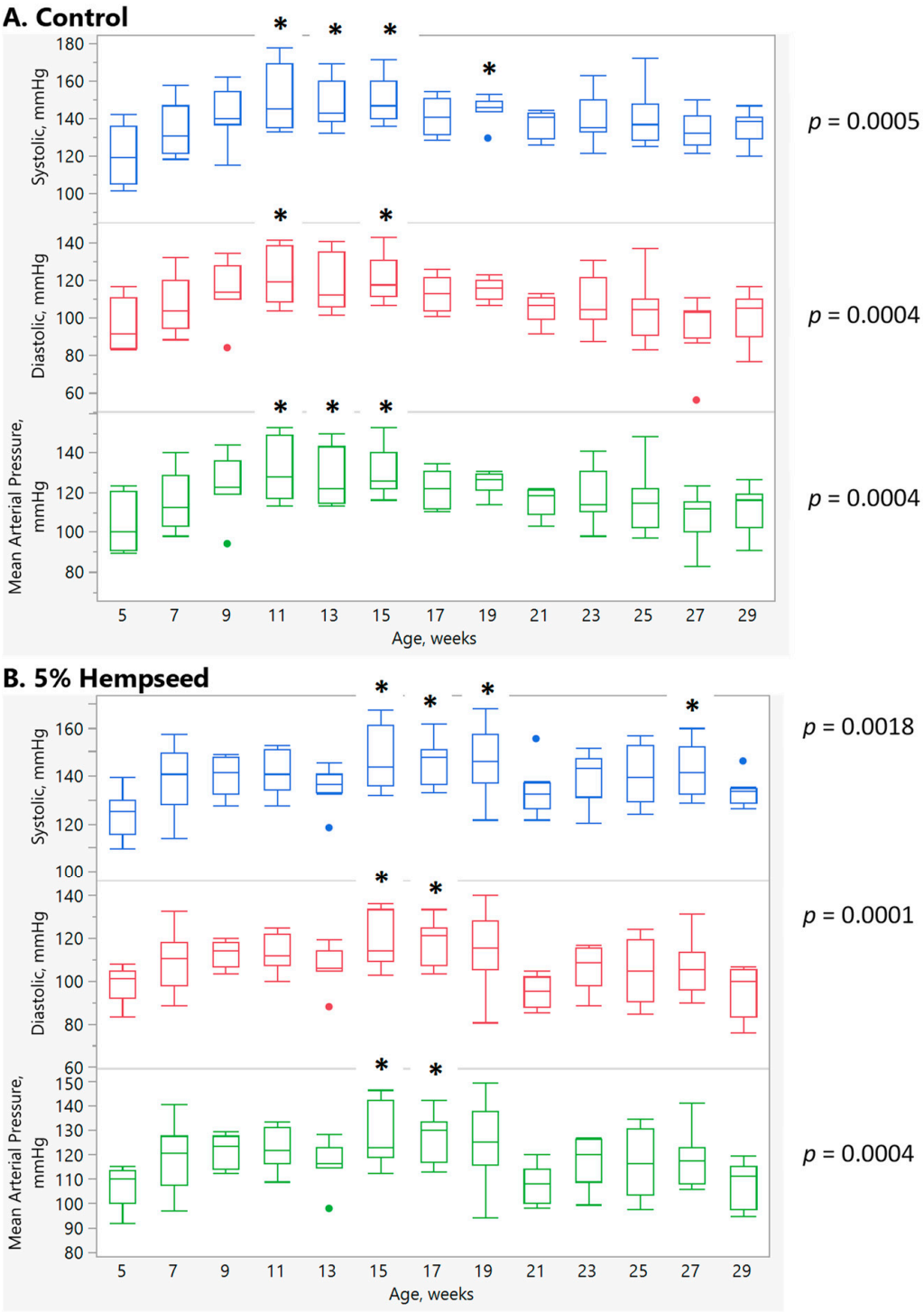


Figure 4. *Cont.*

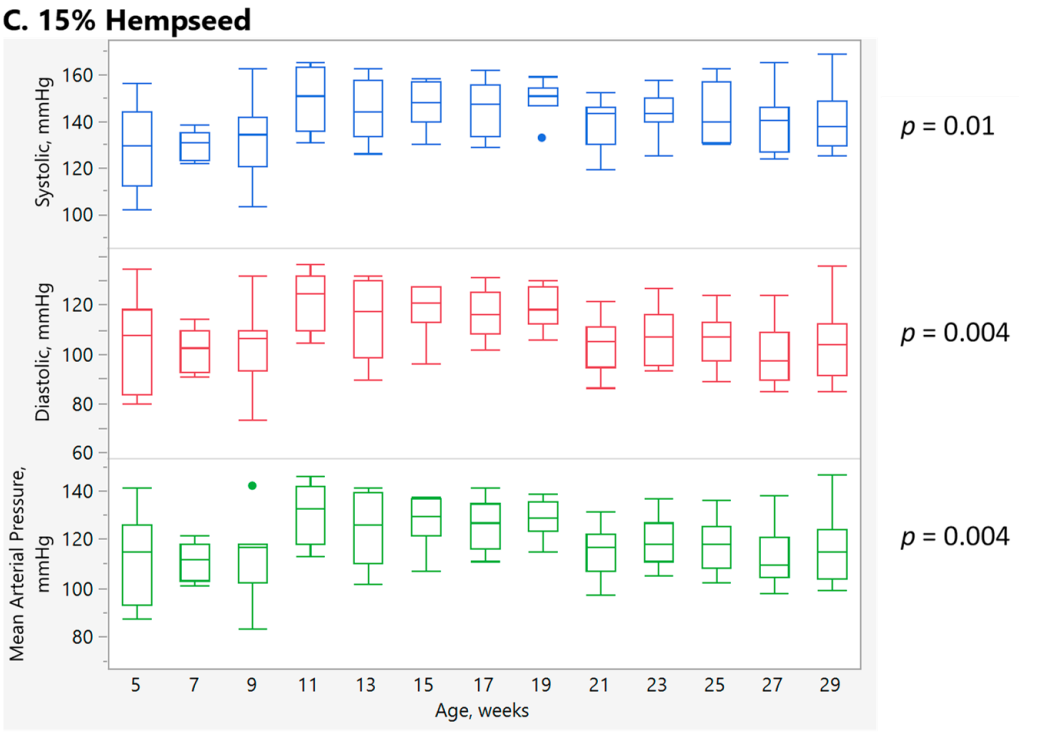


Figure 4. Box plots for systolic (blue), diastolic (red), and mean arterial (green) blood pressure. The line within the box indicates the median value; the upper edge of the box indicates the upper quartile; the lower edge of the box indicates the lower quartile; the bars extending beyond the box indicate the maximum and minimum values; the single points indicate outliers. (**A**) Control diet; (**B**) 5% hempseed; (**C**) 15% hempseed. $n = 8$ per diet group. p values are for the ANOVA main effect of age on blood pressure parameter within diet group. * indicates significant difference ($p < 0.05$) compared to age of 5 weeks by Tukey's post hoc analysis.

Blood pressure area under the curve calculations of AUC1 (age 5–15 weeks, adolescence), AUC2 (age 15–29 weeks, adulthood), AUC3 (age 5–29 weeks), and the difference between AUC2 and AUC1 showed no effect of diet on systolic, diastolic, or mean arterial blood pressure ($p > 0.05$).

3.3. Relationship between Body Mass and Body Composition and Systolic, Diastolic, and Mean Arterial Blood Pressure

A weak but significant positive relationship between systolic, but not diastolic or mean arterial, blood pressure and BMI was found for both the combined and individual diet groups (all mice combined, $r^2 = 0.095$, $p < 0.0001$; each diet group, $p < 0.05$). Lean mass index also showed a significant positive relationship with systolic, but not diastolic, blood pressure: all mice combined ($r^2 = 0.096$, $p < 0.0001$; each diet group, $p < 0.05$). Lean mass index was positively related to mean arterial blood pressure for the combined group ($r^2 = 0.035$, $p = 0.017$) but for none of the individual diet groups ($p > 0.05$). Fat mass index was significantly negatively related to systolic blood pressure for the combined group ($r^2 = 0.037$, $p = 0.015$) but for none of the individual diet groups ($p > 0.05$). Fat mass index showed no relationship with diastolic or mean arterial blood pressure.

4. Discussion

The current investigation demonstrated that long-term dietary hempseed enrichment at 5% and 15% concentrations during early life does not reduce adult blood pressure, but a 15% dose may impact the pattern of blood pressure changes seen in growing mice fed a control diet. The findings presented here comprise the first report on the effect of habitual hempseed consumption on changes in blood pressure during growth and development.

The mice in this study were fed a hempseed-enriched diet from shortly past weaning (age 5 weeks) to mid-adulthood (age 30 weeks), a period encompassing blood pressure maturation and stabilization prior to late adulthood.

Young developing mice showed a pattern of change in systolic, diastolic, and mean arterial blood pressure across the 25 weeks of this study characterized by a gradual rise from age 5 to ~11–17 weeks followed by a slow decline until age 30 weeks, the end of the experiment. Our findings are consistent with those of Tieman et al., who reported developmental increases in systolic and diastolic blood pressure in C57BL/6 mice from post-natal ages of 21–50 days followed by relatively constant levels [21]. Our study and Tieman et al.'s study collected blood pressures measurements at different ages, rendering the results not directly comparable, but the pattern of blood pressure maturation was similar between the experiments. Similarly, Gros et al. reported significantly higher systolic blood pressure in C57BL/6 mice at age 24 weeks compared to 8 weeks [33]. Wiesmann et al. [34] observed increases in left ventricular mass in C57BL/6 mice between the ages of 3 days to 4 months, which could be expected to parallel increases in systolic blood pressure [35].

Hempseeds contain molecules such as polyphenols, peptides, fatty acids, and micronutrients that exert biological activity in the body [36,37]. Mechanisms underlying the effect of hempseed on blood pressure might involve the renin–angiotensin–aldosterone system and antioxidant defense and endocrine networks. Hempseed products, such as hempseed protein hydrolysate, demonstrate inhibitory actions on renin and angiotensin-converting enzyme activity in in vitro studies and cause reductions in systolic blood pressure in rats [15,38]. Hempseed protein short- and medium-chain peptides have also been shown to inhibit dipeptidyl peptidase IV (DPP-IV), which degrades glucagon-like peptide-1 (GLP-1) and thereby modulates the renin–angiotensin system that regulates blood pressure [39–42]. Hempseed peptides and lipids also support antioxidant defenses by scavenging free radicals [37,43,44], which modulate nitric oxide activity and vascular function [45,46]. Hempseed-enriched diets also affect estradiol levels in rats [47], and estradiol modulates blood pressure in women [48]. Moreover, during puberty in humans, multiple physiological pathways impact the development of adult blood pressure [49], and these may be affected by bioactive components in hempseed.

The apparent blunting of blood pressure changes across age in the 15% HS diet group relative to the control and 5% HS groups, and the delay in peak blood pressure in the 5% HS versus control diet group, might be worthy of further investigation. While no main effect of diet group or a diet x age interaction was seen, analysis of each diet group independently revealed these differences in time to reach peak blood pressure. This finding may indicate a dose–response effect of hempseed consumption on blood pressure in that the increase in blood pressure during development was delayed at lower HS levels and flattened at higher HS intakes. In previous reports generated from these same diet groups of mice, we showed that the 15% HS versus control group displayed reduced femur maximum load and whole-body bone mineral content [50,51].

The findings of this study may be applied to the development of hempseed-containing functional foods. Hempseed-enriched products such as meat analogs, baked foods, and beverages have been developed for purposes of improved nutrition and product stability [1,2,8]. Our results can inform investigations of the cardiovascular effects of habitual hempseed consumption in children and youth by calling attention to a possible alteration in blood pressure development at higher versus lower doses of hempseed. Whether this hempseed-associated alteration in blood pressure maturation pattern impacts cardiovascular health in later life is not known but may merit investigation.

Strengths of this study are the long duration (6 months) of the feeding experiment over the developmental period wherein biweekly blood pressure measurements were collected. Further, the diets were formulated to create similar profiles of nutrient composition across the 0%, 5%, and 15% hempseed enrichments. Limitations include the use of only female

mice, which renders application to male mice uncertain, and the absence of blood markers that would have informed our identification of possible mechanisms.

5. Conclusions

In healthy, growing female mice, long-term feeding of a hempseed-enriched diet does not reduce systolic, diastolic, or mean arterial blood pressure in adulthood; however, the higher-dose (15% HS) versus lower-dose (5% HS) diet appears to blunt the peaking of blood pressure during early adulthood seen in mice fed a control diet. These findings can direct future research to determine whether blood pressure in later life, when hypertension commonly develops, is impacted by a disrupted pattern of blood pressure changes during earlier life. The results may also inform the determination of the concentration of hempseed used in functional foods associated with optimal health outcomes across life stages.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app14178006/s1: Supplementary Table S1. Nutritional information regarding hempseed.

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References

1. Tănase Apetroaei, V.; Pricop, E.M.; Istrati, D.I.; Vizireanu, C. Hemp Seeds (*Cannabis sativa* L.) as a Valuable Source of Natural Ingredients for Functional Foods—A Review. *Molecules* **2024**, *29*, 2097. [CrossRef] [PubMed]
2. Farinon, B.; Molinari, R.; Costantini, L.; Merendino, N. The Seed of Industrial Hemp (*Cannabis sativa* L.): Nutritional Quality and Potential Functionality for Human Health and Nutrition. *Nutrients* **2020**, *12*, 1935. [CrossRef]
3. Montero, L.; Ballesteros-Vivas, D.; Gonzalez-Barrios, A.F.; Sánchez-Camargo, A.D.P. Hemp Seeds: Nutritional Value, Associated Bioactivities and the Potential Food Applications in the Colombian Context. *Front. Nutr.* **2022**, *9*, 1039180. [CrossRef] [PubMed]
4. Rodriguez-Leyva, D.; Pierce, G.N. The Cardiac and Haemostatic Effects of Dietary Hempseed. *Nutr. Metab.* **2010**, *7*, 32. [CrossRef]
5. Kakkar, S.; Tandon, R.; Tandon, N. The Rising Status of Edible Seeds in Lifestyle Related Diseases: A Review. *Food Chem.* **2023**, *402*, 134220. [CrossRef] [PubMed]
6. Aidoo, R.; Abe-Inge, V.; Kwofie, E.M.; Baum, J.I.; Kubow, S. Sustainable Healthy Diet Modeling for a Plant-Based Dietary Transitioning in the United States. *npj Sci. Food* **2023**, *7*, 61. [CrossRef]
7. Mylan, J.; Andrews, J.; Maye, D. The Big Business of Sustainable Food Production and Consumption: Exploring the Transition to Alternative Proteins. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2207782120. [CrossRef]
8. Rizzo, G.; Storz, M.A.; Calapai, G. The Role of Hemp (*Cannabis sativa* L.) as a Functional Food in Vegetarian Nutrition. *Foods* **2023**, *12*, 3505. [CrossRef]

9. PR Newswire. Global $544.93 Million Hemp Seeds Markets to 2027. *PR Newswire US*. 2021. Available online: https://www.prnewswire.com/news-releases/global-544-93-million-hemp-seeds-markets-to-2027--301284430.html (accessed on 28 August 2024).
10. Databridge Market Research. Global Hemp Seed Market—Industry Trends and Forecast to 2030. 2023. Available online: https://www.databridgemarketresearch.com/reports/global-hemp-seed-market (accessed on 28 August 2024).
11. Opyd, P.M.; Jurgoński, A.; Fotschki, B.; Juśkiewicz, J. Dietary Hemp Seeds More Effectively Attenuate Disorders in Genetically Obese Rats than Their Lipid Fraction. *J. Nutr.* **2020**, *150*, 1425–1433. [CrossRef]
12. Majewski, M.; Jurgoński, A. The Effect of Hemp (*Cannabis sativa* L.) Seeds and Hemp Seed Oil on Vascular Dysfunction in Obese Male Zucker Rats. *Nutrients* **2021**, *13*, 2575. [CrossRef]
13. Kaushal, N.; Dhadwal, S.; Kaur, P. Ameliorative Effects of Hempseed (Cannabis Sativa) against Hypercholesterolemia Associated Cardiovascular Changes. *Nutr. Metab. Cardiovasc. Dis.* **2020**, *30*, 330–338. [CrossRef] [PubMed]
14. Prociuk, M.A.; Edel, A.L.; Richard, M.N.; Gavel, N.T.; Ander, B.P.; Dupasquier, C.M.C.; Pierce, G.N. Cholesterol-Induced Stimulation of Platelet Aggregation Is Prevented by a Hempseed-Enriched Diet. *Can. J. Physiol. Pharmacol.* **2008**, *86*, 153–159. [CrossRef]
15. Girgih, A.T.; Alashi, A.; He, R.; Malomo, S.; Aluko, R.E. Preventive and Treatment Effects of a Hemp Seed (*Cannabis sativa* L.) Meal Protein Hydrolysate against High Blood Pressure in Spontaneously Hypertensive Rats. *Eur. J. Nutr.* **2014**, *53*, 1237–1246. [CrossRef]
16. Al-Khalifa, A.; Maddaford, T.G.; Chahine, M.N.; Austria, J.A.; Edel, A.L.; Richard, M.N.; Ander, B.P.; Gavel, N.; Kopilas, M.; Ganguly, R.; et al. Effect of Dietary Hempseed Intake on Cardiac Ischemia-Reperfusion Injury. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2007**, *292*, R1198–R1203. [CrossRef]
17. Saberivand, A.; Karimi, I.; Becker, L.A.; Moghaddam, A.; Azizi-Mahmoodjigh, S.; Yousefi, M.; Zavareh, S. The Effects of *Cannabis sativa* L. Seed (Hempseed) in the Ovariectomized Rat Model of Menopause. *Methods Find. Exp. Clin. Pharmacol.* **2010**, *32*, 467–473. [CrossRef] [PubMed]
18. Munteanu, C.; Mihai, M.; Dulf, F.; Ona, A.; Muntean, L.; Ranga, F.; Urdă, C.; Pop, D.; Mihaiescu, T.; Mârza, S.M.; et al. Biochemical Changes Induced by the Administration of Cannabis Sativa Seeds in Diabetic Wistar Rats. *Nutrients* **2023**, *15*, 2944. [CrossRef] [PubMed]
19. Samsamikor, M.; Mackay, D.S.; Mollard, R.C.; Alashi, A.M.; Aluko, R.E. Hemp Seed Protein and Its Hydrolysate Compared with Casein Protein Consumption in Adults with Hypertension: A Double-Blind Crossover Study. *Am. J. Clin. Nutr.* **2024**, *120*, 56–65. [CrossRef]
20. Fuchs, F.D.; Whelton, P.K. High Blood Pressure and Cardiovascular Disease. *Hypertension* **2020**, *75*, 285–292. [CrossRef]
21. Tiemann, K.; Weyer, D.; Djoufack, P.C.; Ghanem, A.; Lewalter, T.; Dreiner, U.; Meyer, R.; Grohe, C.; Fink, K.B. Increasing Myocardial Contraction and Blood Pressure in C57BL/6 Mice during Early Postnatal Development. *Am. J. Physiol. Heart Circ. Physiol.* **2003**, *284*, H464–H474. [CrossRef]
22. Dutta, S.; Sengupta, P. Men and Mice: Relating Their Ages. *Life Sci.* **2016**, *152*, 244–248. [CrossRef]
23. Reeves, P.G. Components of the AIN-93 Diets as Improvements in the AIN-76A Diet. *J. Nutr.* **1997**, *127*, 838S–841S. [CrossRef]
24. Wang, Y.; Thatcher, S.E.; Cassis, L.A. Measuring Blood Pressure Using a Noninvasive Tail Cuff Method in Mice. *Methods Mol. Biol.* **2017**, *1614*, 69–73. [CrossRef]
25. Daugherty, A.; Rateri, D.; Hong, L.; Balakrishnan, A. Measuring Blood Pressure in Mice Using Volume Pressure Recording, a Tail-Cuff Method. *J. Vis.* **2009**, *27*, e1291. [CrossRef]
26. Feng, M.; DiPetrillo, K. Non-Invasive Blood Pressure Measurement in Mice. *Methods Mol. Biol.* **2009**, *573*, 45–55. [CrossRef] [PubMed]
27. Cook, N.R.; Rosner, B.A.; Chen, W.; Srinivasan, S.R.; Berenson, G.S. Using the Area under the Curve to Reduce Measurement Error in Predicting Young Adult Blood Pressure from Childhood Measures. *Stat. Med.* **2004**, *23*, 3421–3435. [CrossRef] [PubMed]
28. Burgess, K.; Jovanović, S.; Sudhir, R.; Jovanović, A. Area under the Curve Analysis of Blood Pressure Reveals Increased Spontaneous Locomotor Activity in SPAK Knock-in Mice: Relevance for Hypotension Induced by SPAK Inhibition? *Physiol. Rep.* **2019**, *7*, e13997. [CrossRef] [PubMed]
29. Jungers, W.L. Scaling. Why Is Animal Size so Important? By K. Schmidt-Nielsen. New York: Cambridge University Press. 1984. Xi + 241 pp., Figures, Tables, Appendices, References, Index. $29.95 (Cloth), $9.95 (Paper). *Am. J. Phys. Anthropol.* **1986**, *69*, 129–130. [CrossRef]
30. Cheung, M.C.; Spalding, P.B.; Gutierrez, J.C.; Balkan, W.; Namias, N.; Koniaris, L.G.; Zimmers, T.A. Body Surface Area Prediction in Normal, Hypermuscular, and Obese Mice. *J. Surg. Res.* **2009**, *153*, 326–331. [CrossRef]
31. Gouma, E.; Simos, Y.; Verginadis, I.; Lykoudis, E.; Evangelou, A.; Karkabounas, S. A Simple Procedure for Estimation of Total Body Surface Area and Determination of a New Value of Meeh's Constant in Rats. *Lab. Anim.* **2012**, *46*, 40–45. [CrossRef]
32. Gargiulo, S.; Gramanzini, M.; Megna, R.; Greco, A.; Albanese, S.; Manfredi, C.; Brunetti, A. Evaluation of Growth Patterns and Body Composition in C57Bl/6J Mice Using Dual Energy X-Ray Absorptiometry. *Biomed. Res. Int.* **2014**, *2014*, 253067. [CrossRef]
33. Gros, R.; Van Wert, R.; You, X.; Thorin, E.; Husain, M. Effects of Age, Gender, and Blood Pressure on Myogenic Responses of Mesenteric Arteries from C57BL/6 Mice. *Am. J. Physiol. Heart Circ. Physiol.* **2002**, *282*, H380–H388. [CrossRef] [PubMed]

34. Wiesmann, F.; Ruff, J.; Hiller, K.H.; Rommel, E.; Haase, A.; Neubauer, S. Developmental Changes of Cardiac Function and Mass Assessed with MRI in Neonatal, Juvenile, and Adult Mice. *Am. J. Physiol. Heart Circ. Physiol.* **2000**, *278*, H652–H657. [CrossRef] [PubMed]
35. Urbina, E.M.; Mendizábal, B.; Becker, R.C.; Daniels, S.R.; Falkner, B.E.; Hamdani, G.; Hanevold, C.; Hooper, S.R.; Ingelfinger, J.R.; Lanade, M.; et al. Association of Blood Pressure Level With Left Ventricular Mass in Adolescents. *Hypertension* **2019**, *74*, 590–596. [CrossRef]
36. Frazzini, S.; Torresani, M.C.; Roda, G.; Dell'Anno, M.; Ruffo, G.; Rossi, L. Chemical and Functional Characterization of the Main Bioactive Molecules Contained in Hulled *Cannabis sativa* L. Seeds for Use as Functional Ingredients. *J. Agric. Food Res.* **2024**, *16*, 101084. [CrossRef]
37. Senila, L.; Neag, E.; Cadar, O.; Kovacs, M.H.; Becze, A.; Senila, M. Chemical, Nutritional and Antioxidant Characteristics of Different Food Seeds. *Appl. Sci.* **2020**, *10*, 1589. [CrossRef]
38. Malomo, S.A.; Onuh, J.O.; Girgih, A.T.; Aluko, R.E. Structural and Antihypertensive Properties of Enzymatic Hemp Seed Protein Hydrolysates. *Nutrients* **2015**, *7*, 7616–7632. [CrossRef] [PubMed]
39. Cerrato, A.; Lammi, C.; Laura Capriotti, A.; Bollati, C.; Cavaliere, C.; Maria Montone, C.; Bartolomei, M.; Boschin, G.; Li, J.; Piovesana, S.; et al. Isolation and Functional Characterization of Hemp Seed Protein-Derived Short- and Medium-Chain Peptide Mixtures with Multifunctional Properties for Metabolic Syndrome Prevention. *Food Res. Int.* **2023**, *163*, 112219. [CrossRef] [PubMed]
40. Gupta, S.; Sen, U. More than Just an Enzyme: Dipeptidyl Peptidase-4 (DPP-4) and Its Association with Diabetic Kidney Remodelling. *Pharmacol. Res.* **2019**, *147*, 104391. [CrossRef]
41. Chen, H.-H.; Li, W.; Wang, Y.; Xu, B.; Hu, X.; Li, X.-B.; Liu, J.-Y.; Zhang, C.; Zhang, C.-Y.; Xing, X.-H. Mining and Validation of Novel Hemp Seed-Derived DPP-IV-Inhibiting Peptides Using a Combination of Multi-Omics and Molecular Docking. *J. Agric. Food Chem.* **2023**, *71*, 9164–9174. [CrossRef]
42. Aita, S.E.; Montone, C.M.; Taglioni, E.; Capriotti, A.L. Chapter Six—Hempseed Protein-Derived Short- and Medium-Chain Peptides and Their Multifunctional Properties. In *Advances in Food and Nutrition Research*; Toldrá, F., Ed.; Academic Press: Cambridge, MA, USA, 2024; Volume 110, pp. 275–325. ISBN 1043-4526.
43. Chen, H.; Xu, B.; Wang, Y.; Li, W.; He, D.; Zhang, Y.; Zhang, X.; Xing, X. Emerging Natural Hemp Seed Proteins and Their Functions for Nutraceutical Applications. *Food Sci. Hum. Wellness* **2023**, *12*, 929–941. [CrossRef]
44. Aiello, G.; Lammi, C.; Boschin, G.; Zanoni, C.; Arnoldi, A. Exploration of Potentially Bioactive Peptides Generated from the Enzymatic Hydrolysis of Hempseed Proteins. *J. Agric. Food Chem.* **2017**, *65*, 10174–10184. [CrossRef] [PubMed]
45. Amponsah-Offeh, M.; Diaba-Nuhoho, P.; Speier, S.; Morawietz, H. Oxidative Stress, Antioxidants and Hypertension. *Antioxidants* **2023**, *12*, 281. [CrossRef] [PubMed]
46. Griendling, K.K.; Camargo, L.L.; Rios, F.J.; Alves-Lopes, R.; Montezano, A.C.; Touyz, R.M. Oxidative Stress and Hypertension. *Circ. Res.* **2021**, *128*, 993–1020. [CrossRef] [PubMed]
47. Ma, J.; Guo, C.-Y.; Li, H.-B.; Wu, S.-H.; Li, G.-L. Prophylactic Effects of Hemp Seed Oil on Perimenopausal Depression: A Role of HPA Axis. *J. Oleo Sci.* **2023**, *72*, 939–955. [CrossRef] [PubMed]
48. Seely, E.W.; Walsh, B.W.; Gerhard, M.D.; Williams, G.H. Estradiol With or Without Progesterone and Ambulatory Blood Pressure in Postmenopausal Women. *Hypertension* **1999**, *33*, 1190–1194. [CrossRef]
49. Wojcik, M.; Krawczyk, M.; Wojcik, P.; Cypryk, K.; Wozniak, L.A. Molecular Mechanisms Underlying Curcumin-Mediated Therapeutic Effects in Type 2 Diabetes and Cancer. *Oxid. Med. Cell. Longev.* **2018**, *2018*, 9698258. [CrossRef]
50. Sparks, C.A.; Streff, H.M.; Williams, D.W.; Blanton, C.A.; Gabaldón, A.M. Dietary Hempseed Decreases Femur Maximum Load in a Young Female C57BL/6 Mouse Model but Does Not Influence Bone Mineral Density or Micro-Architecture. *Nutrients* **2022**, *14*, 4224. [CrossRef]
51. Blanton, C.A.; Barrott, J.J.; Kunz, K.; Bunde, E.; Streff, H.M.; Sparks, C.A.; Williams, D.W.; Gabaldón, A.M. The Impact of Hempseed Consumption on Bone Parameters and Body Composition in Growing Female C57BL/6 Mice. *Int. J. Environ. Res. Public Health* **2022**, *19*, 5839. [CrossRef]

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