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The impact of dietary salt on the development of hypertension and gut microbiome dysbiosis in captive-bred vervet monkeys (*Chlorocebus aethiops*)

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Abstract

Background The study was designed to establish a hypertensive nonhuman primate model to evaluate the role of dietary salt intake on blood pressure levels and gut microbiome regulation. Sixteen adult vervet monkeys were selected and assigned into two groups (control and experimental). The control group was given a maintenance diet (100 g), whereas the diet of the experimental group was supplemented with 1.5 g/day of dietary salt in the mornings for six months (T_0-T_6), thereafter, the dose was increased to 2 g/day for additional six months (T_9-T_{12}). Blood and stool samples were collected for biochemical and 16 S ribosomal RNA gene sequencing.

Results The control group was borderline hypertensive (134.7/62.9 mmHg), whereas elevated blood pressure levels (171.3/81.3 mmHg) were observed at T_{12} indicating the experimental group to be salt sensitive. Furthermore, gut microbiome analysis showed two main phyla, Bacteroidetes and Firmicutes. However, there was no significant difference for alpha and beta diversity for both groups.

Conclusion These findings suggested that dietary salt intake (1.5–2 g/day) caused alterations in systolic blood pressure levels, chloride and alkaline phosphatase (ALP). However, these changes were not associated with gut microbiome dysbiosis even though significant changes were observed over time for the individual groups.

Keywords Blood pressure, Bacteroidetes, And Firmicutes, Microbiome diversity, Salt sensitivity, Nonhuman primates

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Background

In South Africa, the burden of hypertension is reported to be on the rise despite considerable progress in prevention, diagnosis, and treatment [1, 2]. Among the contributing factors, high dietary salt intake is known to be directly linked to hypertension [3, 4]. However, the underlying mechanism of salt sensitivity and elevated high blood pressure (BP) requires further elucidation. In addition to the genetic and epigenetic factors, gut microbiome dysbiosis is also associated with the development of hypertension [5, 6]. The elevated ratio of Firmicutes-to-Bacteroidetes (F/B) is observed in hypertensive individuals [6, 7], and can also be potentially used as a biomarker for other metabolic syndromes such as obesity, and diabetes mellitus [8–11]. The non-human primates (NHPs) such as the baboon (*Papio hamadryas*) and chimpanzee (*Pan Troglodytes*) have been extensively used in scientific research and are known to develop spontaneous hypertension (wild and in captivity) in a manner similar to humans [12]. Furthermore, the association between the features of hypertension and gut microbiome dysbiosis has been reported in animal models [5].

In the present study, the captive-bred vervet monkey model (*Chlorocebus aethiops*) was used as a preferred NHP model to assess the relationship between high salt intake and hypertension. This NHP model was selected based on the knowledge that *Chlorocebus aethiops* also develop spontaneous hypertension in captivity (≥ 140/90 mmHg) [13, 14]. These vervet monkeys have been used extensively at the SAMRC/Primate Unit and Delft Animal Centre (PUDAC) for non-communicable disease studies [15–21]. Research focusing on the role of gut microbiota in regulating BP in the Sub-Saharan African regions is still limited due to resource constraints and expertise. Therefore, developing the induced-hypertensive vervet model will provide insight into the regulation of BP in response to a high salt diet and the possible interaction with the gut microbiome.

Table 1 Baseline lipogram screening and group allocation

Groups	Control ($n=8$; 4M & 4 F)	Experimental ($n=8$; 4M & 4 F)	*P*-value*
Age	6.88 ± 1.64	7.25 ± 2.25	0.74
Total cholesterol (mmol/L)	3.48 ± 0.79	4.18 ± 0.47	0.04
Triglycerides (mmol/L)	0.40 ± 0.09	0.52 ± 0.30	0.31
LDL-C (mmol/L)	1.35 ± 0.61	1.70 ± 0.35	0.18
HDL-C (mmol/L)	1.75 ± 0.30	2.25 ± 0.35	0.01
Non-HDL-C (mmol/L)	1.73 ± 0.57	1.93 ± 0.43	0.44

*p value for unpaired t-test; Values are mean ± standard deviation; Females (F); Males (M); Low-density lipoprotein cholesterol (LDL-C); High-density lipoprotein cholesterol (HDL-C)

Materials and methods

The captive-bred Vervet colony (housing, diet and animal welfare)

The selected vervet monkeys were captive-bred at the Primate Unit and Delft Animal Centre (PUDAC) of the South African Medical Research Council (SAMRC). The animals were housed and maintained at PUDAC according to the revised South African National Standard for the Care and Use of Animals for Scientific Purposes (South African Bureau of Standards, SANS 10386, 2021). All individuals were housed indoors, and their housing environment was maintained as previously reported [17, 22]: temperature (24–26°C), humidity (40–70%), ventilation rate (15–20 air changes per hour, fresh air 100%), lighting (12-hour photoperiod), smell (< 20 ppm ammonia), noise (< 60 dB). For the duration of the study, animals were housed in single cages fitted with full perches, foraging pans, and grooming panels to allow for social interaction with adjacent cage companions. All cages were supplied with enrichment devices regularly as per PUDAC standard operating procedures (SOP). The animal diets were prepared at PUDAC, and all subjects were fed three times a day. Since the monkeys had no access to direct sunlight, their diet was supplemented with vitamin C and D3. In addition to their standard diet, they received a variety of fruits and vegetables (apples, oranges, mandarins, cucumber, sweet potato, etc.), and water was provided *ad lib* through an automated watering device. As per PUDAC vervet SOP, all animals were observed daily by a South African Veterinary Council (SAVC) authorized animal technologist for any signs of illness, discomfort, and any other unusual behavior. These observations were recorded on the animal and welfare log sheet daily.

Animal selection

Based on the availability of the experimental animals in the colony, 16 young adult vervet monkeys were randomly selected. After baseline screening, the monkeys were assigned into two groups (control and experimental) based on their lipid content. Each group consisted of eight animals (4 females and 4 males, aged (> 3 years–10 years) (Table 1). There were no animals that were excluded after baseline analysis. The sample size ($n=8$ per group) was selected based on other NHP studies consisting of 6 to 16 animals [23, 24] and NHP validated testing protocols conducted at PUDAC which involved 4–5 animals per group and these studies reported reliable data [17, 25]. Since many NHP studies are not terminal in nature [26], it is therefore common to use a small sample size [17, 27]. The descriptive information (mean ± standard deviation) of the lipogram levels for the control and experimental groups is reported in Table 1.

Formulations and administration of dietary salt

The control group was given a maintenance diet (30 g) of pre-cooked maize meal (Avi products) once in the morning, and the experimental diet was supplemented with 1.5 g/day of salt mixed with the maintenance diet (30 g) once in the morning for six months. Thereafter, the dose was adjusted to 2 g/day for an extended period of six months. The treatment duration was extended based on the fact that it takes several months to obtain significant changes in BP levels [28, 29]. After the consumption of the initial 30 g portion of food, the groups received the remaining portion of the food (70 g), which amounts to 100 g of food bolus per day. Food consumption was monitored and recorded daily by weighing food wastage. Animals had access to water through an automated water system and were monitored daily for any signs of discomfort, illness and significant reduction of body weight (5%), and observations were recorded on a daily log sheet by PUDAC's animal technicians.

Sample collection

Animals were handled after chemical restraint with Ketamine (Kyron laboratories, South Africa) at 10 mg/kg, which was administered by intramuscular injection via the squeeze back mechanism. Once an animal was fully unconscious, body weight (kg) and BP were measured and recorded for each animal. The BP readings with 2–3 consecutive repeats were non-invasively recorded using the Dinamap blood pressure monitor (Johnson and Johnson, USA). Blood samples (2–4 ml) were obtained via femoral venipuncture at baseline and once every four weeks for six months (T_0-T_6). Thereafter, the sampling interval changed to every three months for an additional six months (T_9-$_{12}$), followed by a washout period to monitor biochemistry (liver and kidney) and lipogram levels. Moreover, fresh stool samples (2 g) for gut microbiome analysis were collected at baseline (T_0) and after six months (T_6) of dietary salt intervention. The samples were immediately stored at -80 °C for downstream analysis.

Biochemical analysis for efficacy

The collected blood (2 ml) was used for biochemical analysis for sodium, potassium, chloride, bicarbonate, urea, creatinine, anion gap, calcium, magnesium, aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT) and alanine aminotransferase (ALT). The blood samples were immediately sent to PathCare Laboratories (South Africa) for analysis.

Gut Microbiome analysis

Stool sample and 16 S ribosomal RNA gene sequencing data

The frozen stool samples collected at T_0 and T_6 were used to extract DNA using Quick-DNA™ Fecal /Soil Microbe Miniprep Kit (Zymoresearch, USA). The purified DNA samples were subjected to Ion Reporter™ software next-generation sequencing at Central Analytical Facilities (CAF), Stellenbosch University. The Ion 16 Metagenomics kit included two primer sets corresponding to the seven-hypervariable regions (V2-9) of the 16 S region in the bacteria mixed population. The library preparation (sample names, barcode associations and library concentrations) was performed using the Ion Plus Fragment Library Kit (ThermoFisher Scientific, USA) as per Ion universal library quantitation kit instructions. The OTUs with sequencing reads of less than 10 copies and those with low quality were excluded. However, the genus and species cut-off were set at 97% and 99%, respectively. The generated raw data files were mapped to Curated Greengenes v13.5 and MicroSEQ® 16 S reference library as 16 S rRNA gene reference databases. Thereafter, the Quantitative Insight In Microbial Ecology pipeline (QIIME -version 5.12.2) was used for microbial classification. Further analysis included the alpha diversity analysis to evaluate species richness (Chao1 index), evenness and diversity (Shannon and Simpson). The number of operational taxonomic units (OTUs) was determined at phylum, family, and genus level. Beta diversity was analyzed using the three-dimensional principal coordinate analysis (PCoA) with the Bray-Curtis distance matrix.

Statistical data analysis

Clinical and biochemical statistical parameters were analyzed using SAS software 9.4. A mixed model was used to assess the difference between the control and experimental group over time (T_0, T_6, T_{12}), allowing for the repeated measures for each animal using the Toeplitz covariance structure. Global p-values indicate significance of the overall model and the individual p values (t-tests) indicate differences at each time point. Gut microbiome statistical data was analyzed using R Studio (version 3.6.3) and STATA (version 16). Alpha diversity data was summarized as median and interquartile ranges and Wilcoxon signed rank test was performed to determine a statistically significant difference in median sequence per sample before (T_0) and after 6 months (T_6). Principal component analysis (PCA) was performed for the OTUs (phylum, family, and genus) to reduce the dimensionality of the data between T_0 and T_6. The first principal component (PC1) was selected for further analysis as it represented the most significant variation of the data. To evaluate the differences between controls and experimental groups, a paired t-test was conducted using PC1 values. The results were considered statistically

significant if the mean difference between the two groups did not overlap. Furthermore, the principal coordinate analysis (PCoA) was conducted to visualize beta-diversity patterns using the vegan package R studio. Microsoft Excel® 2010 (Microsoft Office) and GraphPad Prism version 6.00 (La Jolla California USA) was used for graphical representation. A p-value of ≤ 0.05 was considered statistically significant.

Results

Clinical and biochemical analysis

The selected animals ($n = 16$) consumed 100% of their food during the first six months, however, when the dosage was increased to 2 g/day, food intake declined slightly. Body weight comparison at T_0 and T_{12} indicated that there was not a significant difference in the weight increases over the 12 months period (average increase = 0.09; $p = 0.14$). The descriptive statistics of the biochemical characteristics versus time comparisons for each characteristic is provided in Table 2. These results showed that salt administration in the experimental group significantly elevated BP levels from 125.5/56.2 mmHg ($p = 0.57$) to 171.3/81.3 mmHg at T_{12} ($p = 0.003$) (Fig. 1). Additionally, at T_{12} 14% of the control group and 87.5% of the experimental group were hypertensive (Systolic BP > = 140; $p = 0.01$) and this suggested that the experimental group is salt sensitive. Significant differences between the control and experimental groups were observed for chloride (T_{12}: $p = 0.03$) and ALP (T6: $p = 0.05$). Although the other biochemical parameters were not statistically significant between the control and experimental group, mean differences were observed after salt administration (Table 2).

Gut microbiome estimation

Gut microbial data showed a total of 4 870 107 reads at T_0 and 4 843 950 reads at T_6 that were mapped to Greengenes v13.5 and MicroSEQ v2013.1 databases. This resulted in a total of 2 714 451 mapped reads at T_0 and 2 688 001 at T_6.

Alpha diversity analysis

Alpha diversity was quantified by the total number of observed species per sample, richness (Chao1), evenness and diversity (Shannon and Simpson) OTU indexes. The rarefaction curve showed that the microbial communities in the vervet stool samples were well represented, and the findings showed no statistical difference between the control and experimental group (T_0: $p = 0.13$; T_6: $p = 0.72$) (Fig. 2). However, an increase in the number of observed species in both groups was observed at T_6 compared to T_0 (control: $p = 0.001$; experimental: $p = 0.01$) (Fig. 2). The validity of these findings was further confirmed using Chao1 indices which also showed no statistical difference between the control and experimental group for Chao1 (T_0: $p = 0.76$; T_6: $p = 0.70$), Shannon (T_0: $p = 0.07$; T_6: $p = 0.69$) and Simpson (T_0: $p = 0.06$; T_6: $p = 0.64$). However, the median microbial abundance differed significantly between T_0 and T_6 for both groups for Chao1 (control: $p < 0.0001$; experimental: $p < 0.0001$), Shannon (control: $p < 0.0001$; experimental: $p < 0.0001$) and Simpson (control: $p < 0.0001$; experimental: $p < 0.0001$) (Fig. 3).

Beta diversity analysis

Beta diversity was determined using three-dimension (3D) scatterplots to visualize whether the control and experimental group had different microbial distributions (Fig. 4). The principal coordinate analysis (PCoA) confirmed that there were no significant differences in the distribution of the gut microbial communities between the control and experimental group at T_0 and T_6. However, the distribution of the samples was clustered on the first principal coordinate at T_0, which explained 37.4% of variations, while at T_6 they were clustered more on the second principal coordinate which explained 19.8% of the variation (Fig. 4).

The microbial distribution at the phylum level

The bacterial composition was assessed at the phylum, family, and genus level. At the phylum level, Firmicutes and Bacteroidetes were dominant in the gut microbiota of the selected vervet monkeys for both groups. This was followed by Proteobacteria with a small proportion of Actinobacteria (Table 3). However, there was a slight non-significant decrease in the F/B ratio in the experimental group at T_6 ($p = 0.40$) (Table S1).

The microbial distribution at the family and genus level

A paired t-test was used to determine whether there was a statistically significant mean difference between PCA components at T_0 and T_6 (Table 4). The PCA analysis revealed statistically significant differences in PC1 for specific OTUs within the control and experimental groups when comparing T_0 and T_6. At the family level, both Bacteroidetes ($p = 0.01$) and Proteobacteria ($p = 0.01$) showed significant differences in PC1 between T_0 and T_6 for the control group (Table 4 and S2). For the experimental group, Proteobacteria also showed a significant difference in PC1 ($p = 0.01$) between T_0 and T_6 (Table 4). However, when comparing the control and experimental groups, no statistically significant mean difference was observed, as indicated by the overlapping 95% confidence intervals (CIs) for the mean differences between the groups.

Out of the ten bacterial families that were prevalent in both groups (Table S4), *Prevotellaceae*, *Enterobacteriaceae*, *Desulfovibrionaceae* and *Hyphomicrobiaceae* were higher in the control compared to the experimental

Table 2 Biochemistry characteristics during salt intervention period between the control and experimental group

Parameters	Groups	T_0[$]	T_6[$]	T_{12}[$]	Global *P*-Value*
Weight (Kg)	Control	5.23 ± 1.53	5.24 ± 1.47	5.40 ± 1.47	0.50
	Experimental	4.72 ± 1.32	4.67 ± 1.46	4.73 ± 1.37	
	P-value**	*0.48*	*0.44*	*0.36*	
	Estimates (SE = 0.72)	-0.52	-0.57	-0.67	
Systolic BP (mmHg)	Control	134.7 ± 26.8	130.5 ± 15.2	120.4 ± 38.8	0.09
	Experimental	125.5 ± 41.9	157.8 ± 37.2	171.3 ± 35.4	
	P-value**	0.57	0.16	0.0034	
	Estimates (SE = 16.1)	-9.21	23.53	50.89	
Diastolic BP (mmHg)	Control	62.9 ± 25.2	70.0 ± 17.3	68.8 ± 35.6	0.69
	Experimental	56.2 ± 29.1	76.1 ± 34.6	81.3 ± 26.6	
	P-value**	0.65	1.00	0.28	
	Estimates (SE = 14.8)	-6.75	0.07	16.72	
Sodium (mmol/L)	Control	149.88 ± 2.42	130.50 ± 52.75	149.88 ± 2.42	0.34
	Experimental	150.13 ± 2.42	151.00 ± 1.51	151.25 ± 1.49	
	P-value**	0.81	0.07	0.20	
	Estimates (SE = 1.0)	0.25	2.04	1.38	
Potassium (mmol/L)	Control	3.55 ± 0.59	3.71 ± 0.69	3.35 ± 0.21	0.05
	Experimental	3.55 ± 0.46	3.40 ± 0.33	3.56 ± 0.53	
	P-value**	1.00	0.20	0.38	
	Estimates (SE = 0.24)	0.00	-0.32	0.21	
Chloride (mmol/L)	Control	110.38 ± 2.67	108.43 ± 1.99	107.75 ± 2.55	0.004
	Experimental	108.75 ± 2.25	108.25 ± 1.83	110.38 ± 1.92	
	P-value**	0.21	1.00	0.05	
	Estimates (SE = 1.26)	-1.63	0.00	2.63	
Bicarbonate (mmol/L)	Control	28.63 ± 4.52	29.51 ± 2.60	33.11 ± 2.35	0.91
	Experimental	27.85 ± 6.76	28.59 ± 6.03	31.41 ± 3.01	
	P-value**	0.73	0.64	0.45	
	Estimates (SE = 2.20)	-0.78	-1.04	-1.70	
Urea (mmol/L)	Control	6.20 ± 1.42	6.46 ± 1.64	5.11 ± 1.83	0.39
	Experimental	6.63 ± 0.98	7.29 ± 1.39	5.64 ± 0.82	
	P-value**	0.55	0.14	0.46	
	Estimates (SE = 0.70)	0.43	1.08	0.53	
Creatinine (mmol/L)	Control	53.88 ± 12.90	61.71 ± 14.80	59.25 ± 10.93	0.98
	Experimental	54.00 ± 12.40	63.50 ± 13.47	62.13 ± 9.51	
	P-value**	0.98	0.80	0.63	
	Estimates (SE = 5.90)	0.13	1.51	2.88	
Anion GAP (mmol/L)	Control	9.29 ± 2.69	11.43 ± 2.88	9.13 ± 1.64	0.42
	Experimental	10.29 ± 1.60	11.57 ± 2.07	9.63 ± 2.26	
	P-value**	0.53	0.80	0.66	
	Estimates (SE = 1.20)	0.76	0.30	0.50	
Calcium (mmol/L)	Control	2.16 ± 0.07	2.17 ± 0.08	2.26 ± 0.05	0.20
	Experimental	2.24 ± 0.09	2.25 ± 0.08	2.26 ± 0.06	
	P-value**	0.08	0.10	1.00	
	Estimates (SE = 0.04)	0.08	0.08	0.00	
Magnesium (mmol/L)	Control	0.62 ± 0.05	0.65 ± 0.07	0.64 ± 0.05	0.36
	Experimental	0.60 ± 0.06	0.64 ± 0.05	0.63 ± 0.06	
	P-value**	0.63	0.89	0.78	
	Estimates (SE = 0.03)	-0.02	0.00	-0.01	
AST (IU/L)	Control	61.75 ± 14.89	67.71 ± 18.41	57.00 ± 19.39	0.20
	Experimental	64.25 ± 14.49	57.88 ± 16.80	53.38 ± 14.16	
	P-value**	0.83	0.24	0.75	
	Estimates (SE = 11.22)	2.50	-14.29	-3.63	

Table 2 (continued)

Parameters	Groups	T_0[$]	T_6[$]	T_{12}[$]	Global *P*-Value*
ALP (IU/L)	Control	117.13 ± 38.77	128.57 ± 47.67	112.38 ± 38.91	0.39
	Experimental	158.00 ± 52.15	174.25 ± 45.68	148.25 ± 35.98	
	P-value**	0.08	0.04	0.12	
	Estimates (SE = 21.59)	40.88	49.62	35.88	
GGT (IU/L)	Control	49.38 ± 17.94	49.29 ± 22.90	51.00 ± 20.96	0.90
	Experimental	55.25 ± 19.83	58.00 ± 13.33	57.50 ± 17.25	
	P-value**	0.55	0.61	0.51	
	Estimates (SE = 9.73)	5.88	5.02	6.50	
ALT (IU/L)	Control	45.63 ± 17.41	43.29 ± 16.71	43.50 ± 19.45	0.62
	Experimental	47.13 ± 24.41	59.50 ± 25.17	44.63 ± 15.92	
	P-value**	0.90	0.33	0.92	
	Estimates (SE = 11.67)	1.50	11.64	1.13	

$ Descriptive mean ± standard deviation; $n = 16$ (8 control and 8 experimental)

** *P-value* for differences at individual time points

- Global *P* values for T_0 to T_{12}; $P < 0.05$ is considered significant
- Alanine Aminotransferase (ALT); alkaline phosphatase (ALP); aspartate Aminotransferase (AST); gamma-glutamyl transpeptidase (GGT)
- Estimates average difference experimental-control group

SE standard error of estimate

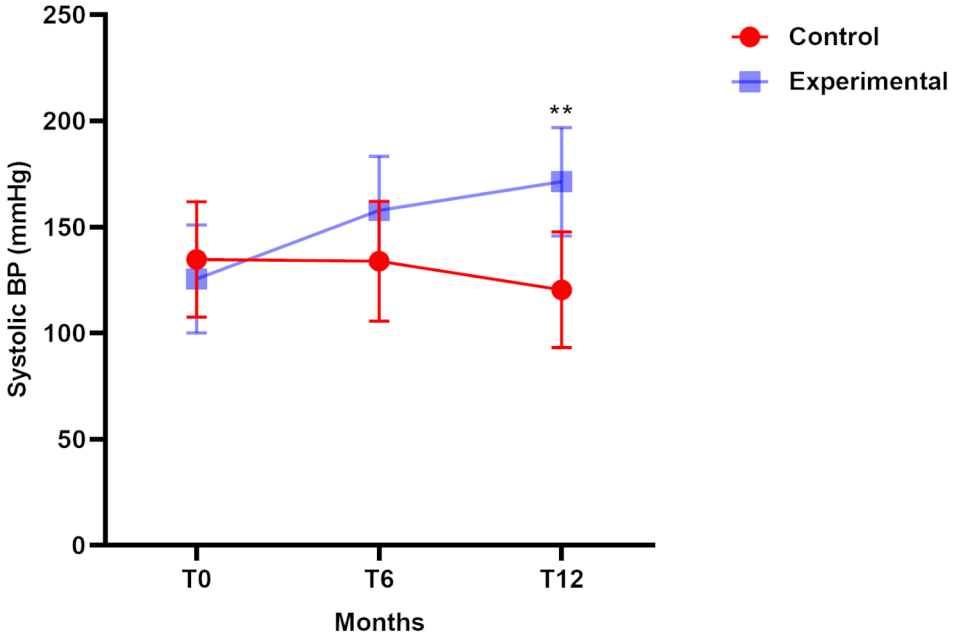

Fig. 1 Least square means (95%CI) for systolic blood pressure (BP) at T_0, T_6 and T_{12}. Salt administration in the experimental group significantly elevated BP levels from 125.50/56.19 mmHg to 171.25/81.29 mmHg at T_{12}, and this suggested that the group was salt sensitive compared to the control group. Statistical significance is depicted as ** $P \leq 0.01$

group at T_0 (Fig. S1). However, a decrease in abundance for most of these families was observed at T_6 except for *Succinivibrionaceae*, *Cytophagaceae*, *Sutterellaceae*, *Cryomorphaceae* and *Sphingobacteriaceae* which were elevated at T_6 (Fig. S1). At the genus level, Firmicutes ($p = 0.003$) and Proteobacteria ($p = 0.003$) showed significant differences in PC1 for the control group, while the experimental group was significant for Proteobacteria ($p = 0.004$) between T_0 and T_6 (Table 4 and S3).

* Mean difference between PCA variables at T_0 and T_6; ** *P*-value for paired t-test for each group (T_0-T_6); (-) not highly expressed.

Discussion

This study aimed at determining the impact of dietary salt intake on BP regulation, and gut microbiome using 16 captive-bred vervet monkeys. The baseline findings indicated that the control group was borderline hypertensive

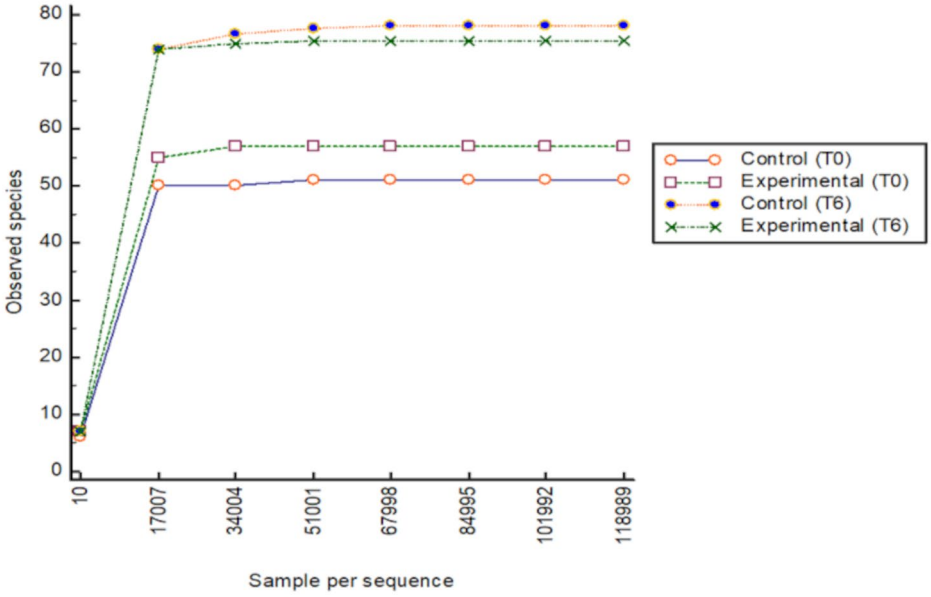

Fig. 2 Rarefaction curves of observed species for all samples between the control and experimental group at T_0 and T_6. The ending of the curves on the x-axis indicates the number of sequences obtained respectively

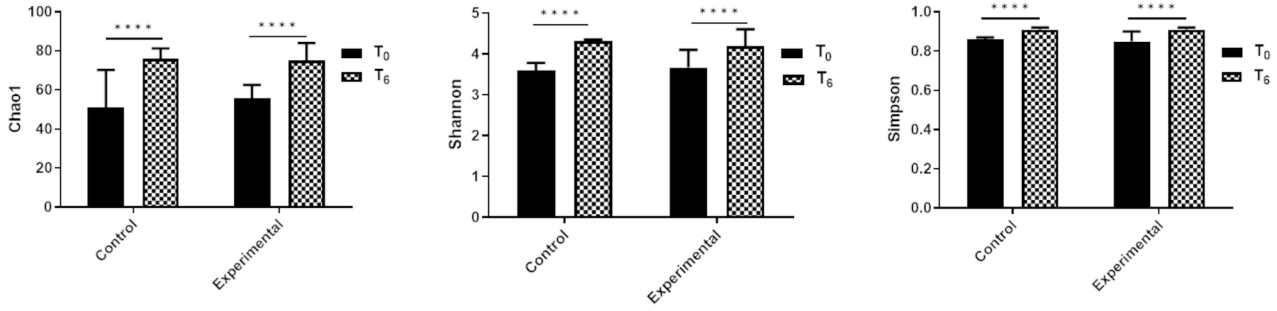

Fig. 3 Gut microbiota structural diversity in captive-bred vervet monkeys. The bar graphs (median and interquartile range) represent Chao1, Shannon and Simpson indexes of alpha diversity analysis which were used to evaluate richness, evenness and diversity. Statistical significance is depicted as ****$p \leq 0.0001$

with a maximum of 134.7/ 62.9 mmHg when compared to the experimental group (125.5/56.2 mmHg). However, the administration of dietary salt indicated that the experimental group was salt-sensitive as their BP levels were elevated throughout the treatment period (Table 2). A significant increase in microbial richness, evenness, and diversity was observed in both groups at T_0 and T_6. However, these changes were not associated with the fact that hypertension has an impact on gut microbiome composition especially since the control group also showed a significant increase at T_6 compared to T_0.

As observed in normal human gut microbiota, two major phyla (Firmicutes and Bacteroidetes) were dominant in the vervet stool samples. Bacteroidetes are considered beneficial due to the role they play in degrading polysaccharides and regulation of calorie absorption, while Firmicutes are capable of producing several short-chain fatty acids (SCFAs) which possess a protective role in cardiovascular diseases (CVDs) by controlling BP and glucose homeostasis [30, 31]. At a family level, a greater abundance of the *Prevotellaceae* family was observed, which is under the order *Bacteroidales* and class *Bacteroidia*. This was followed by *Oxalobacteraceae*, *Enterobacteriaceae*, and *Desulfovibrionaceae*.

Since the cause of hypertension is still not clear, the need will always exist to embark on metagenomics, identification of new biomarkers as well as development of animal models of close resemblance to the diverse pathophysiology of human hypertension. To the best of our knowledge, this is the first study to screen the gut microbiome of the captive-bred vervet monkeys in relation to salt sensitivity and hypertension. Although there were no adverse physical effects or signs of distress in the animals that were administered salt throughout the study,

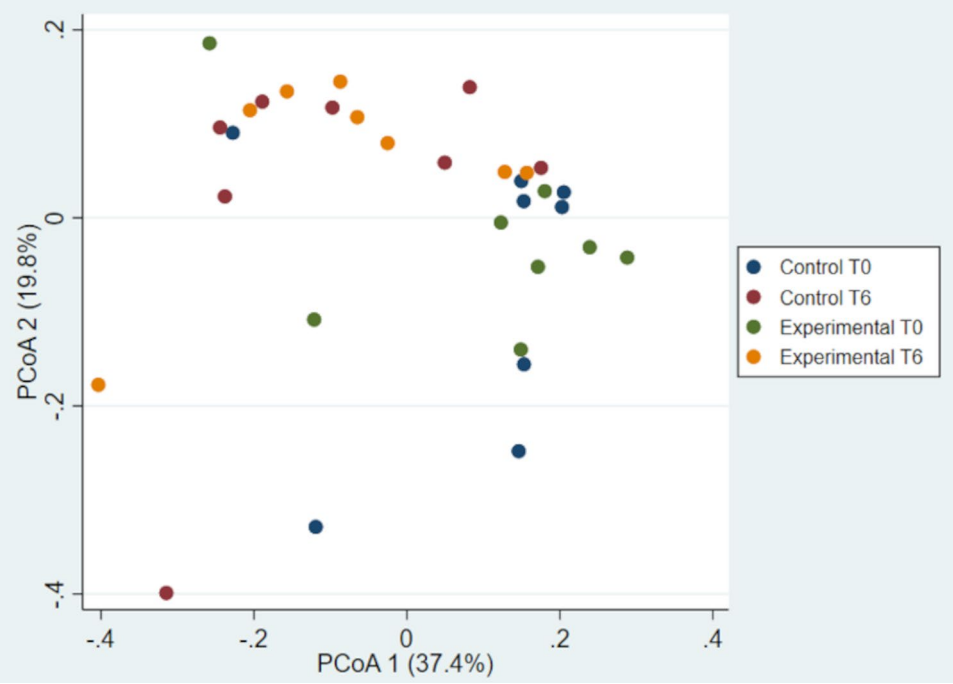

Fig. 4 Beta diversity in captive-bred vervet monkeys. The three-dimension principal coordinate analysis (PCoA) with Bray-Curtis dissimilarity was used to compare sample variation at T_0 and T_6. The first component represented the highest proportion at 37.4% followed by the second component at 19.8%

Table 3 Gut microbial composition at the phylum level in captive-bred Vervet monkeys

Parameters	Groups	T_0^	T_6^	*P*-Value*
Bacteroidetes	Control	31.63 ± 6.09	30.25 ± 8.92	0.61
	Experimental	33.13 ± 6.77	31.88 ± 6.13	0.73
Firmicutes	Control	44.00 ± 8.77	40.63 ± 7.60	0.10
	Experimental	47.63 ± 10.18	38.50 ± 9.50	0.11
Proteobacteria	Control	20.00 ± 14.68	17.25 ± 10.98	0.38
	Experimental	13.13 ± 6.79	16.00 ± 10.14	0.36
Actinobacteria	Control	2.25 ± 1.58	1.63 ± 1.19	0.42
	Experimental	2.25 ± 1.16	2.25 ± 1.58	1.00

**P*-value for paired t-test; ^ Mean (%) ± standard deviation

it is however acknowledged that the use of non-invasive techniques was one of the limitations for this study as the exact extent of the damage at a tissue level could not be ascertained. Additionally, the sample size and the selection of the borderline hypertensive individuals might have affected the outcomes of the study.

Conclusion

In this study, it was shown that dietary salt intake (1.5–2 g/day) elevated BP levels, resulting in salt sensitivity. Significant statistical changes were observed for chloride and ALP between the control group and experimental group. Although most of the biochemistry and clinical parameters were not significant, different responses to salt administration were observed. Moreover, gut microbiome analysis showed no significant differences between the control and experimental group after salt administration for microbial richness, evenness and diversity, however, significant increase for each group was observed between T_0 and T_6. Based on these findings, it can be concluded that salt administration (1.5–2 g/day) did not cause gut microbiome dysbiosis in captive-bred vervet monkeys. Therefore, future studies must explore the possibility of spontaneous hypertension in the captive-bred vervet model, select older

Table 4 Principal component (PC1) analysis for the control and experimental group at family and genus level

Group	Phylum	Family		Genus	
		Mean difference* (CI 95%)	*P*-value**	Mean difference* (CI 95%)	*P*-value**
Control	Bacteroidetes	2.36 (0.67; 4.05)	0.01	-	-
	Firmicutes	1.78 (-0.08; 3.64)	0.06	-1.67 (-2.54; -0.80)	0.003
	Proteobacteria	1.36 (0.47; 2.26)	0.01	1.99 (0.96; 3.02)	0.003
Experimental	Bacteroidetes	0.93 (-1.20; 3.07)	0.34	-	-
	Firmicutes	1.85 (-0.19; 3.89)	0.07	-1.09 (-2.42; 0.24)	0.10
	Proteobacteria	1.73 (0.61; 2.86)	0.01	2.07 (0.92; 3.23)	0.004

animals (16–25 years), increase the dose of salt (> 3–5 g/day) and sample size and shorten the treatment period to at least 3–6 months. The study must also consider using third generation sequencing platforms such as PacBio with 16 S rRNA gene sequencing for species level identification.

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aminotransferase
BP	Blood pressure
CVDs	Cardiovascular diseases
GGT	Gamma glutamyl transpeptidase
HDL-C	High-density lipoprotein
IFN-γ	Interferon-gamma
IL	Interleukin
LDL-C	Low-density lipoprotein cholesterol
NHP	Nonhuman primate
OTUs	Operational taxonomic units
PC	Principal component
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PUDAC	Primate Unit and Delft Animal Centre
QIIME	Quantitative Insight In Microbial Ecology pipeline
SAMRC	South African Medical Research Council
SCFAs	Short chain fatty acids
SRA	Sequence Read Archive
3D	Three dimension

Supplementary Information

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Supplementary Material 1

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Author contributions

ZEM, SK, CGC, and MG were responsible for the conceptualization, study design, and ethical submissions. ZEM and SK were further involved in conducting laboratory work and completion of the project (sample collection, laboratory experiments and analysis, and report preparation). EJ and MM provided bioinformatics and statistical analysis of biochemistry (EJ) and gut microbiome (MM) and EJ was also supervising the entire statistical analysis process. Therefore, the listed authors contributed to data interpretation, and editing of the final draft, and they all approved the final copy of the manuscript.

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Data availability

Supplementary data is available in a separate document and the raw read data was deposited to the NCBI Sequence Read Archive (SRA) under accession number PRJNA903662.

Declarations

Ethics approval and consent to participate

The study was approved by the animal ethics committee of the South African Medical Research Council (SAMRC) (Ref 10/18) and all the procedures were carried out in compliance with SANS guidelines. In addition to the National guidelines, PUDAC is guided by the Veterinary and Para-veterinary Professions Act (Notice 1445 of 1997), and the Animals Protection Act. All these National guidelines aligns with the ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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