

Supplementary Materials

Gut microbiota-derived caproic acid attenuates aging-associated arteriosclerosis

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Supplementary Table 1. Baseline characteristics of the study population(Young vs. Aged). This study compared 97 Young (≤ 65 years) and 30 Aged (≥ 66 years) participants. Sex distribution was well balanced between groups (49.5% vs. 50.0% female, $P=0.964$), fulfilling the design requirement. As expected, age differed significantly ($P<0.0001$). The Aged group showed markedly lower Caproic acid levels (0.258 ± 0.140 vs. 0.410 ± 0.240 , $P<0.0001$), higher systolic blood pressure and pulse pressure (both $P<0.01$), and lower diastolic pressure ($P=0.008$), indicating age-related vascular stiffening. BMI did not differ ($P=0.591$).

	Young	Aged	P value
Number of Cases	97	30	-
Age (years)	52.3 ± 7.2	69.1 ± 3.2	****
Sex (Female%)	49.5%	50.0%	0.9637 [#]
Caproic acid	0.410 ± 0.240	0.258 ± 0.140	****
SBP (mmHg)	121.5 ± 10.1	128.7 ± 10.5	**
DBP (mmHg)	72.3 ± 12.0	65.8 ± 9.2	**
PP (mmHg)	49.2 ± 9.8	62.9 ± 8.7	****
BMI(kg/m ²)	21.2 ± 1.9	21.0 ± 1.7	0.5912 [#]

*** $P < 0.001$, **** $P < 0.0001$, [#] $P \geq 0.05$. SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; BMI: body mass index.

Supplementary Table 2. Human data grouping and baseline description (stratified by sex). Two-sample t-test showed no significant difference between the means ($P=0.96$); age ranges were broadly comparable.

Gender	Sample size (N)	Age range	Caproic acid
Male	64	40-76	0.374 ± 0.218
Female	63	40-75	0.376 ± 0.221

Supplementary Table 3. Sex-specific mean Caproic acid levels stratified by age group. Two-way ANOVA (age group $\times$ sex) interaction term: $F=0.01$, $P=0.92$, indicating that sex does not moderate the age-Caproic acid relationship.

Group	Male mean	Female mean
Young (≤ 65 years)	0.411	0.410
Aged (>66 years)	0.259	0.257

Supplementary Table 4. Sex-stratified partial correlation coefficients between Caproic acid and vascular parameters (adjusted for age). All correlation coefficients were small in magnitude ($|r| < 0.25$), and none of the sex differences reached statistical significance (all Fisher's Z $P > 0.05$), indicating that the associations between Caproic acid and vascular parameters do not differ by sex..

Vascular parameter	Male (N=64) r(P-value)	Female (N=63) r(P-value)	Fisher's Z test for difference between correlations (P-value)
SBP	-0.19 (0.13)	-0.17 (0.18)	0.87
DBP	-0.08 (0.05)	-0.11 (0.39)	0.79
PP	-0.23 (0.07)	-0.20 (0.11)	0.82

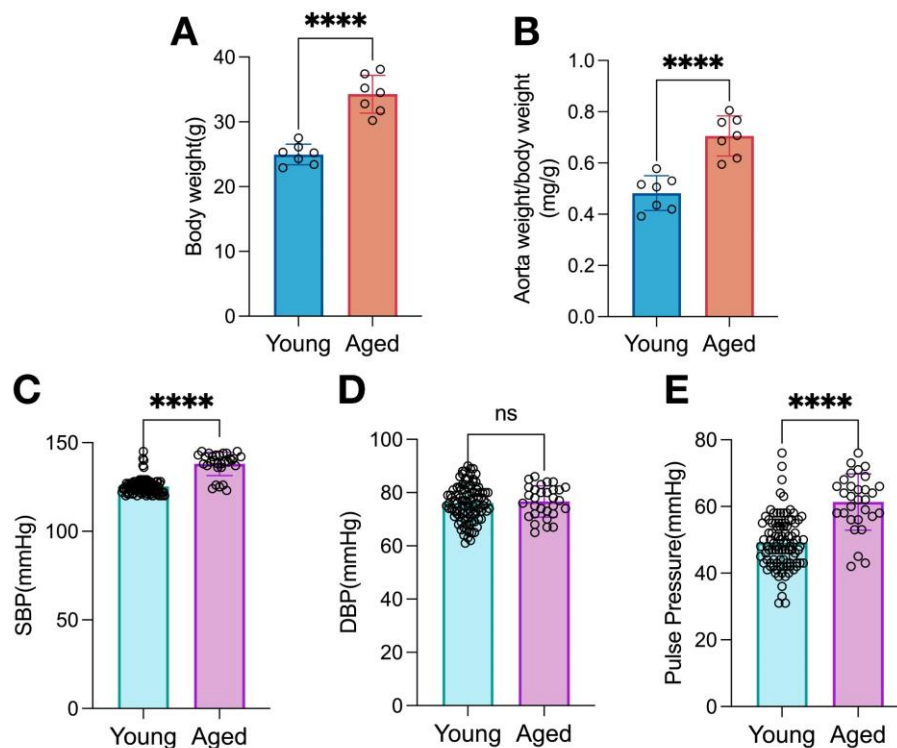
SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure.

Supplementary Table 5. Effect sizes and 95% confidence intervals for intergroup comparisons in both Young vs Aged and AngII-mice models.

Vascular parameter	Parameter	Group 1 (Mean $\pm$ SD)	Group 2 (Mean $\pm$ SD)	Mean Difference (Group 1 - Group 2)	95% CI of Difference	Cohen's d
YA (Young vs Aged, n=7)	Aortic	1.25 $\pm$	2.86 $\pm$	-1.61	-2.35 ~	-2.53
	Arch PWV	0.48	0.76		-0.87	
	(m/s)					
	SBP	111.5 $\pm$	130.5 $\pm$	-19.0	-23.0 ~	- 5.29
	(mmHg)	3.7	3.4		-15.0	

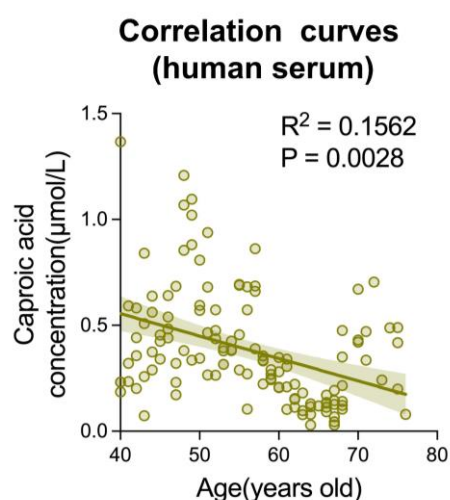
AngII (AngII vs AngII+Na- C6, n=5)	DBP (mmHg)	61.1±2.8	62.2±5.9	-1.1	-6.2~4.0	- 0.23
	Pulse Pressure (mmHg)	50.3±4.9	68.2±4.7	-17.9	-23.1~ -12.7	- 3.74
	Caproic acid (μ mol/L)	0.554± 0.064	0.387± 0.054	0.167	0.098~ 0.236	2.82
	Body weight (g)	24.96± 1.58	34.27± 2.91	-9.31	-12.04~ -6.59	-3.98
	Aorta weight/Bod y weight (mg/g)	0.482± 0.068	0.705± 0.078	-0.223	-0.308~ -0.138	-3.05
	Medial thickness (μ m)	53.74± 5.23	67.89± 6.52	-14.15	-21.03~ -7.27	-2.39
	Fibrosis level (fold change)	1.000± 0.209	1.301± 0.264	-0.301	-0.578~ -0.024	-1.26
	SBP (mmHg)	136.52 ±4.24	124.74± 2.47	11.78	6.72~ 16.84	3.4
	DBP (mmHg)	73.98± 3.84	71.64± 5.68	2.34	-4.73~9.41	0.48
	Pulse Pressure (mmHg)	62.56± 3.55	53.10± 5.67	9.46	2.56~ 16.36	2
	PWV (m/s)	3.73± 0.45	2.85± 0.21	0.88	0.37~1.38	2.53
Body	26.32±	25.76±	0.56	-2.93~4.05	0.23	

weight (g)	2.28	2.50			
Aorta					
weight /					
Body	1.075 ±	0.837 ±	0.238	0.073 ~	2.1
weight	0.085	0.136		0.404	
(mg/g)					
Medial					
thickness	69.11 ±	57.78 ±	11.33	8.02 ~	4.99
(μ m)	1.77	2.68		14.64	
Fibrosis					
level (fold	1.000 ±	0.780 ±	0.22	0.029 ~	1.68
change)	0.048	0.179		0.411	
Elastin					
breaks	6.80 ±	4.00 ±	2.8	1.11 ~ 4.49	2.41
(count)	1.48	0.71			

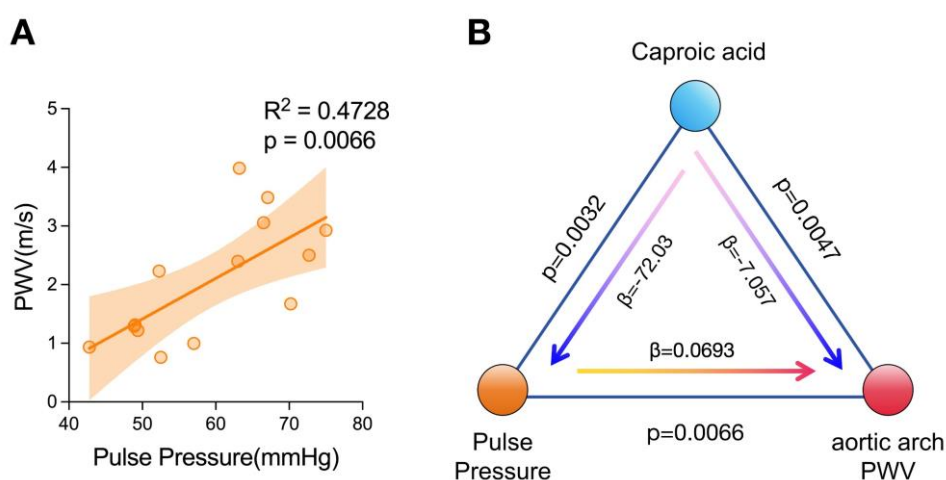


Supplementary Figure 1. Body weight, aorta index and hemodynamic characteristics in young and aged mice and human subjects. A: Body weight in young (blue, n=7) and aged (red, n=7) mice; B: Aorta weight normalized to body

weight in young (blue, n=7) and aged (red, n=7) mice; C-E: (C) SBP, (D) DBP, and (E) pulse pressure in young (cyan, n=97) and aged (purple, n=30) human subjects. SBP:Systolic blood pressure; DBP:diastolic blood pressure; Data are presented as mean $\pm$ SD. ****P<0.0001 vs. young group; ns, not significant (unpaired two-tailed Student's t-test).

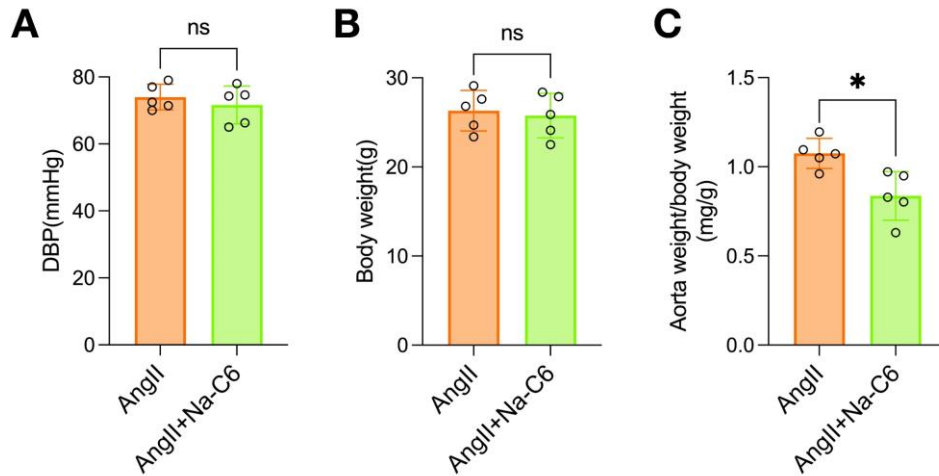


Supplementary Figure 2. Linear regression analysis of serum caproic acid concentration versus age in the human cohort (n=127). The solid line represents the linear fit, and the shaded area indicates the 95% confidence interval. R^2 (0.1562) and P values (0.0028) are shown.



Supplementary Figure 3. Relationships among caproic acid, pulse pressure and aortic arch PWV. (A) Linear regression analysis of aortic arch PWV versus pulse

pressure in aged mice (n=7). The solid line represents the linear fit, and the shaded area indicates the 95% confidence interval. R^2 and P values are shown; (B) Mediation analysis model illustrating the directional relationships among caproic acid, pulse pressure, and aortic arch PWV. Blue/purple arrows denote significant inverse (negative) associations, while the orange/red arrow denotes a significant positive association. All β values represent the slope coefficients from linear regression analyses for each path.



Supplementary Figure 4. Effects of sodium caproate on DBP, body weight and aortic index in AngII-infused mice. (A) DBP, (B) body weight, and (C) aorta weight normalized to body weight in AngII-infused mice treated with vehicle (AngII, orange) or sodium caproate (AngII+Na-C6, green). n=5/group for hemodynamic analyses, body weight and aorta weight-to-body weight ratio.

DBP: Diastolic blood pressure; Na-C6: sodium caproate. Data are presented as mean $\pm$ SD. *P<0.05 vs. AngII group; ns, not significant (unpaired two-tailed Student's t-test).