Supplementary material

Legend to supplementary tables and figures

Supplementary table

Table S1. Demographic and related data in the SONIA 2 showing all data in the nitisinone and no-nitisinone groups. The visits range from V1 (baseline), V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48).

Table S2. Dietary approach to sTYR in the United Kingdom National Alkaptonuria Centre.

Table S3. Derived metabolic data shown according to visits in the nitisinone receiving AKU patients in the SONIA 2. The visits range from V1 (baseline), V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48).

Supplementary figures

Figure S1. The PHE.TYR metabolic pathway is shown highlighting the site of the enzyme defect observed in AKU and the site of action of nitisinone, a reversible competitive inhibitor of 4-hydroxyphenylpyruvate dioxygenase. The pathway also highlights the dynamic relationships between HPPA, TYR and HPLA, a key relationship after introduction of nitisinone. (HPPR – 4- hydroxyphenylpyruvate reductase)

Figure S2. Changes in sHGA, $uHGA_{24}$, $_{TBW}$HGA, and $cHGA_{24}$ in the nitisinone group of the SONIA 2. (p values only indicated for between-visit comparisons where statistical significance was achieved). The visits range from V1 (baseline), V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48).

Figure S3. Changes in sHPPA, $uHPPA_{24}$, $_{TBW}$HPPA, and $cHPPA_{24}$ in the nitisinone group of the SONIA 2. (p values only indicated for between-visit comparisons where statistical significance was achieved). The visits range from V1 (baseline), V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48).

Figure S4. Changes in sHPLA, $uHPLA_{24}$, $_{TBW}$HPLA, and $cHPLA_{24}$ in the nitisinone group of the SONIA 2. (p values only indicated for between-visit comparisons where statistical significance was achieved). The visits range from V1 (baseline), V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48).

Figure S5. Changes in sHGA/sTYR, $uHGA_{24}/uTYR_{24}$, $_{TBW}$HGA/$_{TBW}$TYR, and $cHGA_{24}/cTYR_{24}$ in the nitisinone group of the SONIA 2. (p values only indicated for between-

visit comparisons where statistical significance was achieved). The visits range from V1 (baseline), V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48).

Figure S6. Changes in sHPPA/sTYR, $uHPPA_{24}/uTYR_{24}$, $_{TBW}HPPA/_{TBW}TYR$, and $cHPPA_{24}/cTYR_{24}$ in the nitisinone group of the SONIA 2. (p values only indicated for between-visit comparisons where statistical significance was achieved). The visits range from V1 (baseline), V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48).

Figure S7. Changes in sHPPA/sHPLA, $uHPPA_{24}/uHPLA_{24}$, $_{TBW}HPPA/_{TBW}HPLA$, and $cHPPA_{24}/cHPLA_{24}$ in the nitisinone group of the SONIA 2. (p values only indicated for between-visit comparisons where statistical significance was achieved). The visits range from V1 (baseline), V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48).

Figure S8. Changes in sHPLA/sTYR, $uHPLA_{24}/uTYR_{24}$, $_{TBW}HPLA/_{TBW}TYR$, and $cHPLA_{24}/cTYR_{24}$ in the nitisinone group of the SONIA 2. (p values only indicated for between-visit comparisons where statistical significance was achieved). The visits range from V1 (baseline), V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48).

Figure S9. Pathway adapted from KEGG (phenylalanine and tyrosine metabolism) showing alternative route of disposal of PHE to HPPD bypassing TYR. (KEGG - Kyoto Encyclopedia of Genes and Genomes)

Figure S10. Changes in in sHGA, $uHGA_{24}$, $_{TBW}HGA$, and $cHGA_{24}$ in the nitisinone group of the SONIA 2 excluding V1. (p values only indicated for between-visit comparisons where statistical significance was achieved). The visits range from V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48).

Figure S11. Changes in sHGA, $uHGA_{24}$, $_{TBW}HGA$, and $cHGA_{24}$ in the nitisinone group of the SONIA 2. (p values only indicated for between-visit comparisons where statistical significance was achieved). The visits range from V1 (baseline), V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48). The Y-axis is shown in log scale.

Figure S12. Changes in sHGA/sTYR, $uHGA_{24}/uTYR_{24}$, $_{TBW}HGA/_{TBW}TYR$, and $cHGA_{24}/cTYR_{24}$ in the nitisinone group of the SONIA 2. (p values only indicated for between-visit comparisons where statistical significance was achieved). The visits range from V1 (baseline), V2 (month 3), V3 (month 12), V4 (month 24), V5 (month 36) to V6 (month 48). The Y-axis is shown in log scale.

Table S1. Baseline demographic and related variables for nitisinone-treated AKU patients in the SONIA 2 study						
SONIA 2						
	Control group			Nitisinone group		
	All	Male	Female	All	Male	Female
Numbers of patients	69	40	29	69	45	24
Age years	47·7 (10·2)	48·1 (9·9)	47 (10·7)	49 (11·3)	47·4 (11·9)	51·9 (9·6)
Weight kg	74·1 (15·6)	80·4 (13·3)	65·6 (14·6)	74·8 (14·8)	79·2 (12·6)	66·3 (15·1)
Body Mass Index (kg/M^2)	26·4 (4·6)	27 (4·1)	25·5 (5·2)	26·9 (4·4)	27·3 (4·2)	26·2 (4·7)
$uHGA_{24}$ (µmol/day)	35394 (13868)	38740 (12282)	30778 (14797)	35019 (13124)	37149 (12583)	31024 (13447)
sHGA (µmol/L)	28·3 (8·7)	29·1 (7·7)	27·1 (9·8)	30·3 (11)	31·7 (11·2)	27·9 (10·4)
sTYR (µmol/L)	64·5 (15·5)	69·4 (15·3)	57·8 (13·1)	65·3 (14·8)	67 (13·7)	62·2 (16·6)

Table S2.

Derived data						
	V1 (n=69)	V2 (n=69)	V3 (n=67)	V4 (n=65)	V5 (n=60)	V6 (n=56)
$_{TBW}$HGA µmol****	1376 (586)	33 (61)	35 (80)	98 (312)	105 (283)	108 (271)
$_{TBW}$TYR µmol****	2963 (940)	43094 (13146)	43218 (13021)	40952 (14454)	41830 (14846)	40370 (15913)
$_{TBW}$PHE µmol****	2574 (746)	2704 (847)	2774 (807)	3069 (938)	3163 (931)	3267 (1106)
$_{TBW}$HPPA µmol		1852 (1390)	1718 (531)	1852 (602)	1884 (665)	1939 (894)
$_{TBW}$HPLA µmol		4083 (1537)	4107 (1368)	4254 (1793)	4226 (1602)	4529 (2208)
cHGA_{24} µmol/day****	36361 (13330)	196 (194)	213 (427)	1088 (4178)	1745 (6639)	1675 (6481)
cTYR_{24} µmol/day****	3125 (977)	44827 (13451)	44490 (13257)	42132 (14768)	43101 (15271)	41482 (16225)
cPHE_{24} µmol/day***	2696 (901)	2808 (883)	2830 (818)	3135 (951)	3214 (940)	3356 (1161)
c$HPPA_{24}$ µmol/day****	45 (103)	22597 (13400)	17324 (5563)	16322 (5849)	16489 (7050)	16209 (7504)
c$HPLA_{24}$ µmol/day****	43 (66)	20017 (9534)	17163 (5041)	18810 (6400)	18250 (6705)	16724 (8130)
$_{TBW}$HGA/$_{TBW}$TYR****	0.48 (0.17)	0.0008 (0.002)	0.0008 (0.002)	0.0011 (0.001)	0.001 (0.001)	0.002 (0.002)
$_{TBW}$TYR/$_{TBW}$PHE****	1.2 (0.3)	16.7 (4.7)	16 (3.9)	14.2 (3.7)	14.3 (3.5)	13.3 (3.8)
$_{TBW}$HPPA/$_{TBW}$TYR		0.044 (0.04)	0.041 (0.01)	0.046 (0.01)	0.044 (0.01)	0.047 (0.01)
$_{TBW}$HPPA/$_{TBW}$HPLA		0.47 (0.32)	0.44 (0.13)	0.48 (0.18)	0.47 (0.15)	0.46 (0.14)
$_{TBW}$HPLA/$_{TBW}$TYR		0.095 (0.03)	0.097 (0.03)	0.10 (0.03)	0.097 (0.03)	0.11 (0.04)
cHGA/cTYR****	12.6 (6.0)	0.0043 (0.004)	0.0052 (0.01)	0.166 (0.92)	0.40 (1.8)	0.42 (2.2)
cTYR/cPHE****	1.18 (0.27)	16.7 (4.6)	16.2 (3.9)	13.9 (4.2)	13.9 (4.4)	12.9 (4.4)
cHPPA/cTYR****	0.014 (0.03)	0.53 (0.32)	0.42 (0.16)	0.4 (0.13)	0.38 (0.14)	0.4 (0.17)
cHPPA/cHPLA	0.99 (1.23)	1.11 (0.34)	1.03 (0.26)	1.03 (0.8)	0.94 (0.3)	1.22 (1.22)
cHPLA/cTYR****	0.015 (0.02)	0.46 (0.21)	0.4 (0.11)	0.45 (0.17)	0.41 (0.12)	0.4 (0.18)

Variation among column means is significantly greater than expected by chance with p<: *<0.05; **<0.01; ***<0.001; ****<0.0001; within sTYR group comparisons are shown in figures (main and supplementary).
TBW - total body water; c represents combined TBW plus 24-h urine values; HGA – homogentisic acid; TYR – tyrosine; PHE – phenylalanine; HPPA – 4-hydroxyphenylpyruvate; HPLA – 4-hydroxyphenyllactate;

Figure S1. Tyrosine pathway

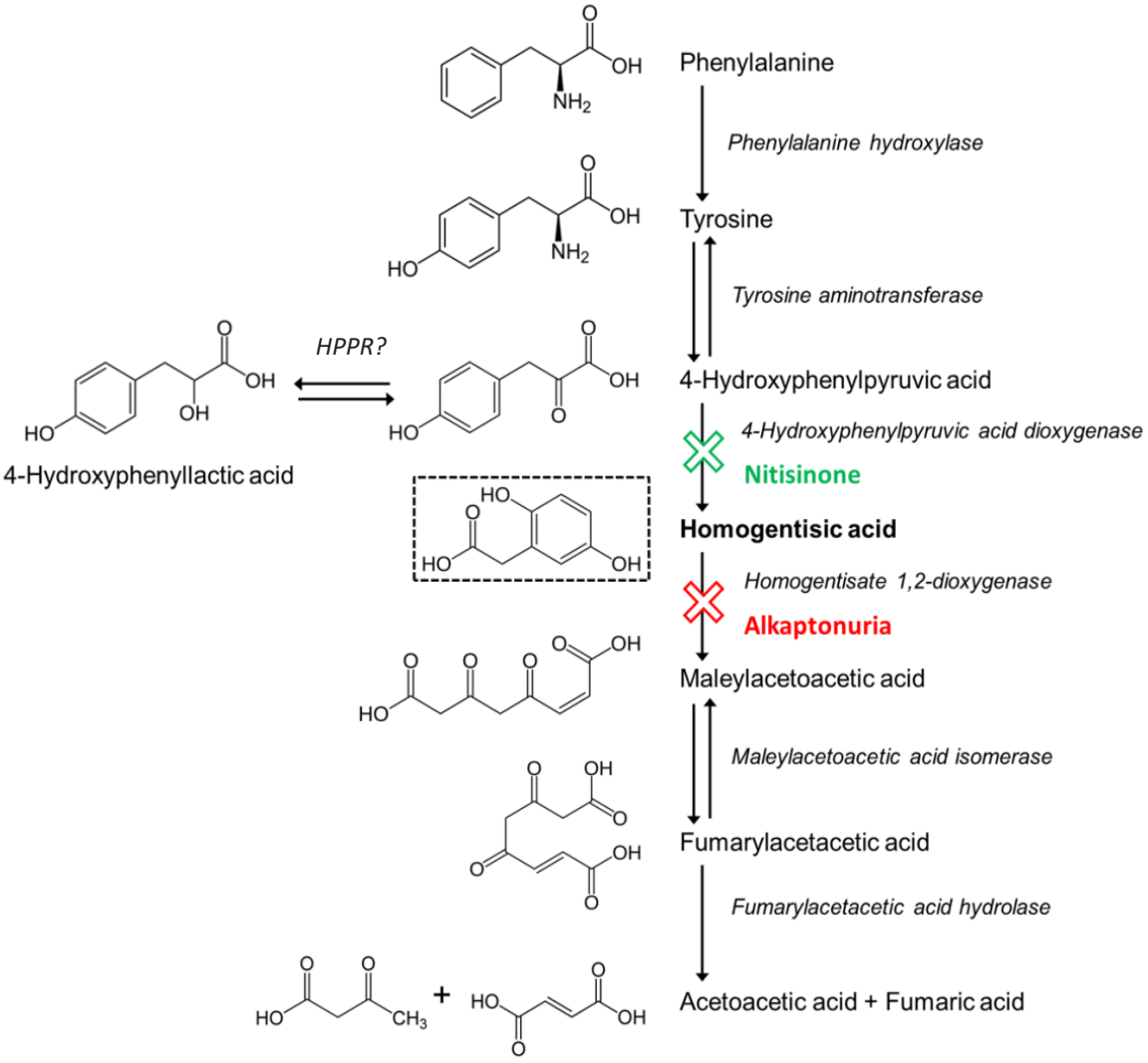


Figure S2.

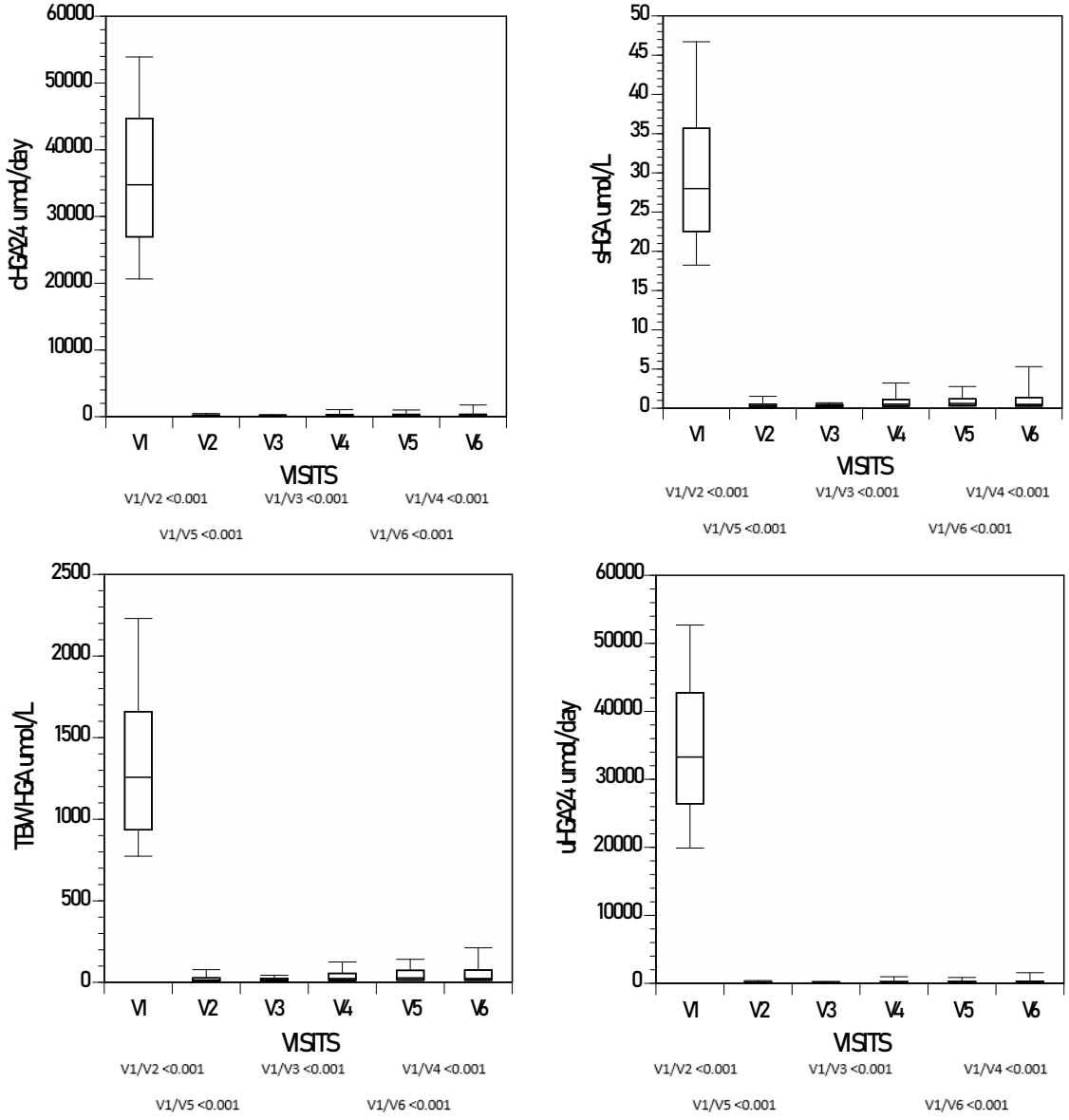

cHGA24 umol/day
60000
50000
40000
30000
20000
10000
0
V1
V2
V3
V4
V5
V6
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001
sHGA umol/L
50
45
40
35
30
25
20
15
10
5
0
V1
V2
V3
V4
V5
V6
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001
TBWHGA umol/L
2500
2000
1500
1000
500
0
V1
V2
V3
V4
V5
V6
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001
uHGA24 umol/day
60000
50000
40000
30000
20000
10000
0
V1
V2
V3
V4
V5
V6
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001

Figure S3.

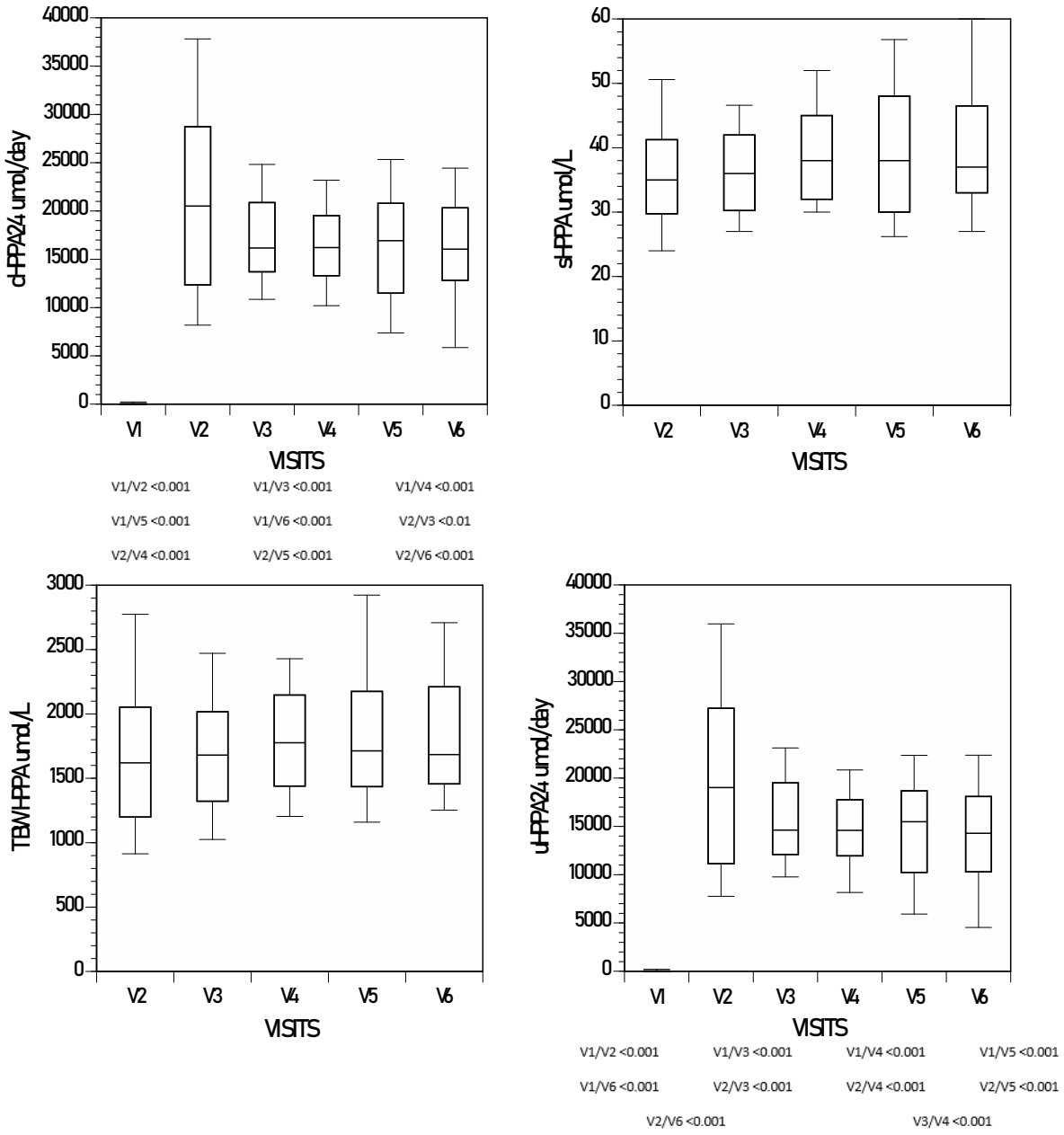

d-PPA24 umol/day
40000
35000
30000
25000
20000
15000
10000
5000
0
V1
V2
V3
V4
V5
V6
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001
V2/V3 <0.01
V2/V4 <0.001
V2/V5 <0.001
V2/V6 <0.001
s-PPA umol/L
60
50
40
30
20
10
0
V2
V3
V4
V5
V6
VISITS
TBVI-PPA umol/L
3000
2500
2000
1500
1000
500
0
V2
V3
V4
V5
V6
VISITS
u-PPA24 umol/day
40000
35000
30000
25000
20000
15000
10000
5000
0
V1
V2
V3
V4
V5
V6
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001
V2/V3 <0.001
V2/V4 <0.001
V2/V5 <0.001
V2/V6 <0.001
V3/V4 <0.001

Figure S4.

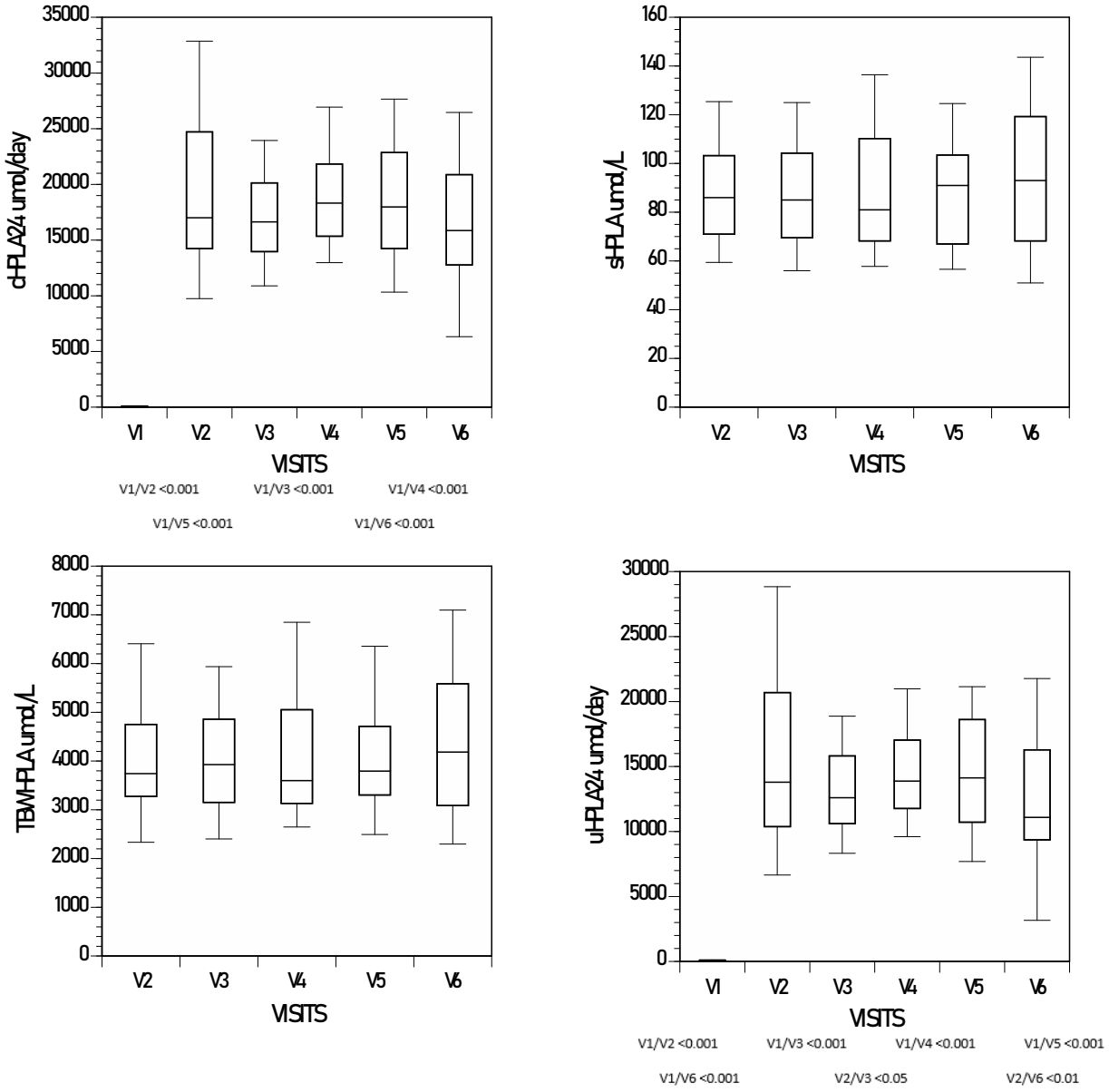

cHPLA24 umol/day
35000
30000
25000
20000
15000
10000
5000
0
V1
V2
V3
V4
V5
V6
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001
sHPLA umol/L
160
140
120
100
80
60
40
20
0
V2
V3
V4
V5
V6
VISITS
TBWHPLA umol/L
8000
7000
6000
5000
4000
3000
2000
1000
0
V2
V3
V4
V5
V6
VISITS
uHPLA24 umol/day
30000
25000
20000
15000
10000
5000
0
V1
V2
V3
V4
V5
V6
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001
V2/V3 <0.05
V2/V6 <0.01

Figure S5.

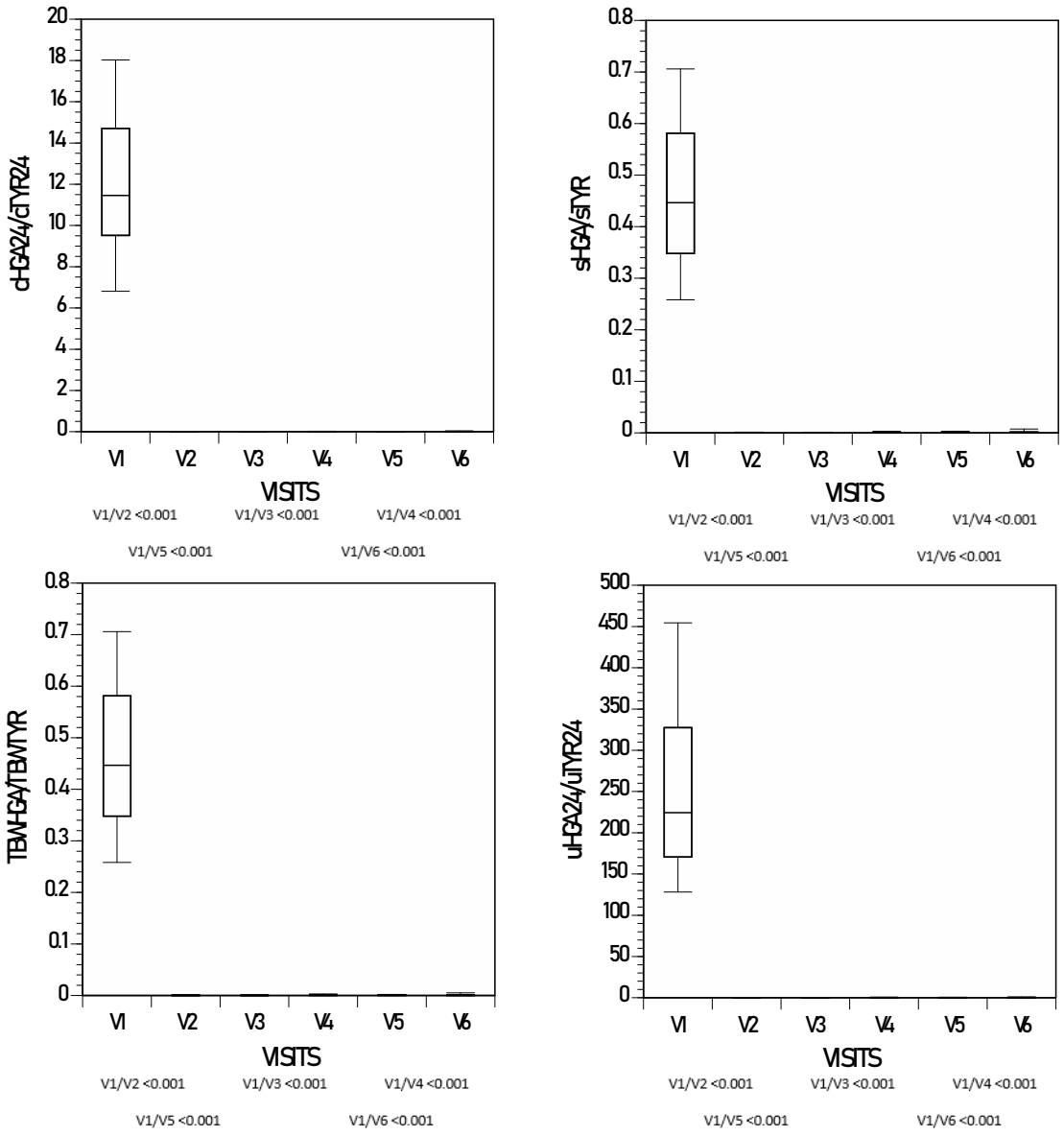

dHGA24/dTYR24
sHGA/sTYR
TBWHGA/TBWTYR
uHGA24/uTYR24
V1
V2
V3
V4
V5
V6
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001

Figure S6.

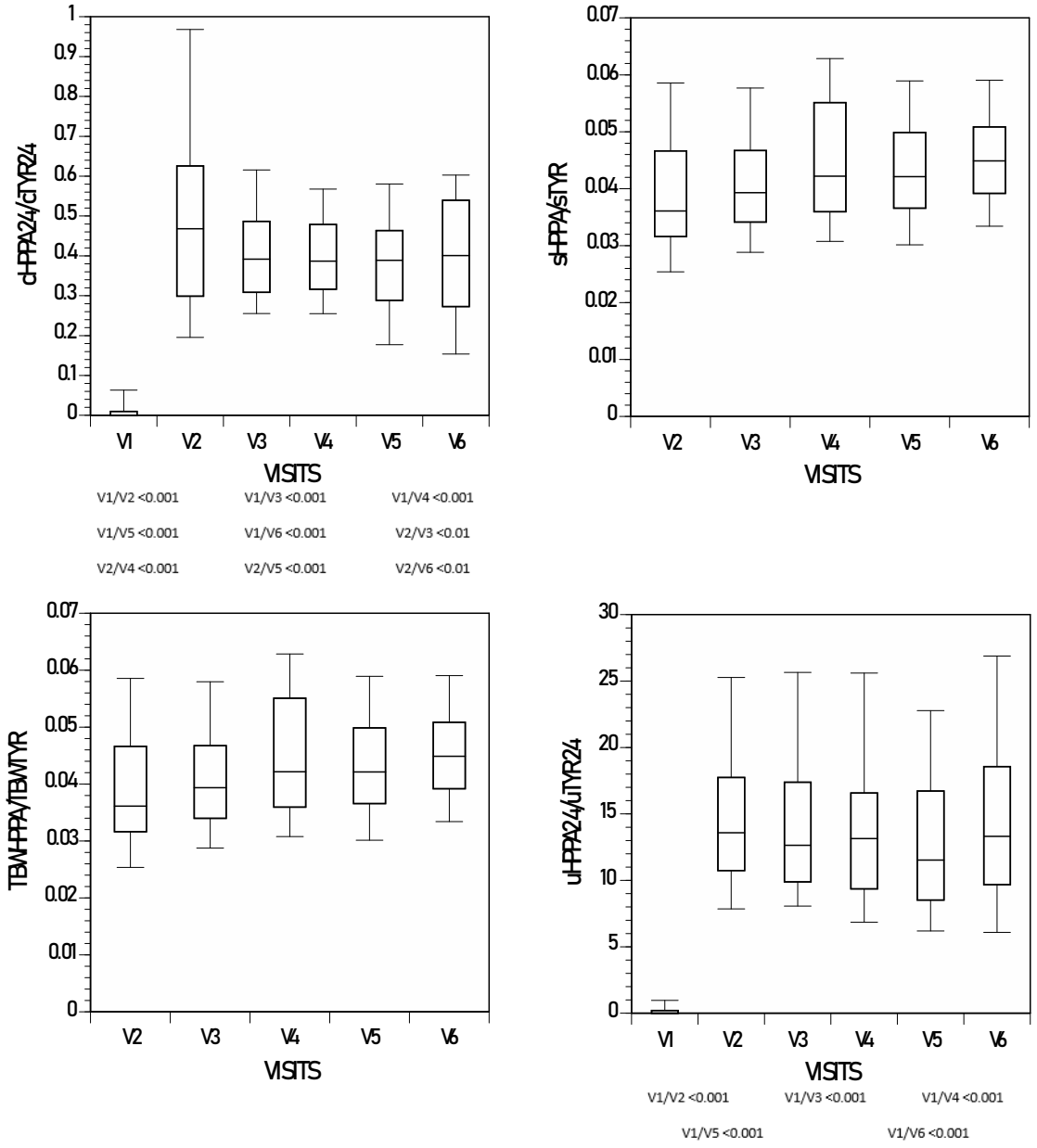

dHPPA24/dTYR24
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001
V2/V3 <0.01
V2/V4 <0.001
V2/V5 <0.001
V2/V6 <0.01
sHPPA/sTYR
VISITS
TBWHPPA/TBWTYR
VISITS
uHPPA24/uTYR24
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001

Figure S7.

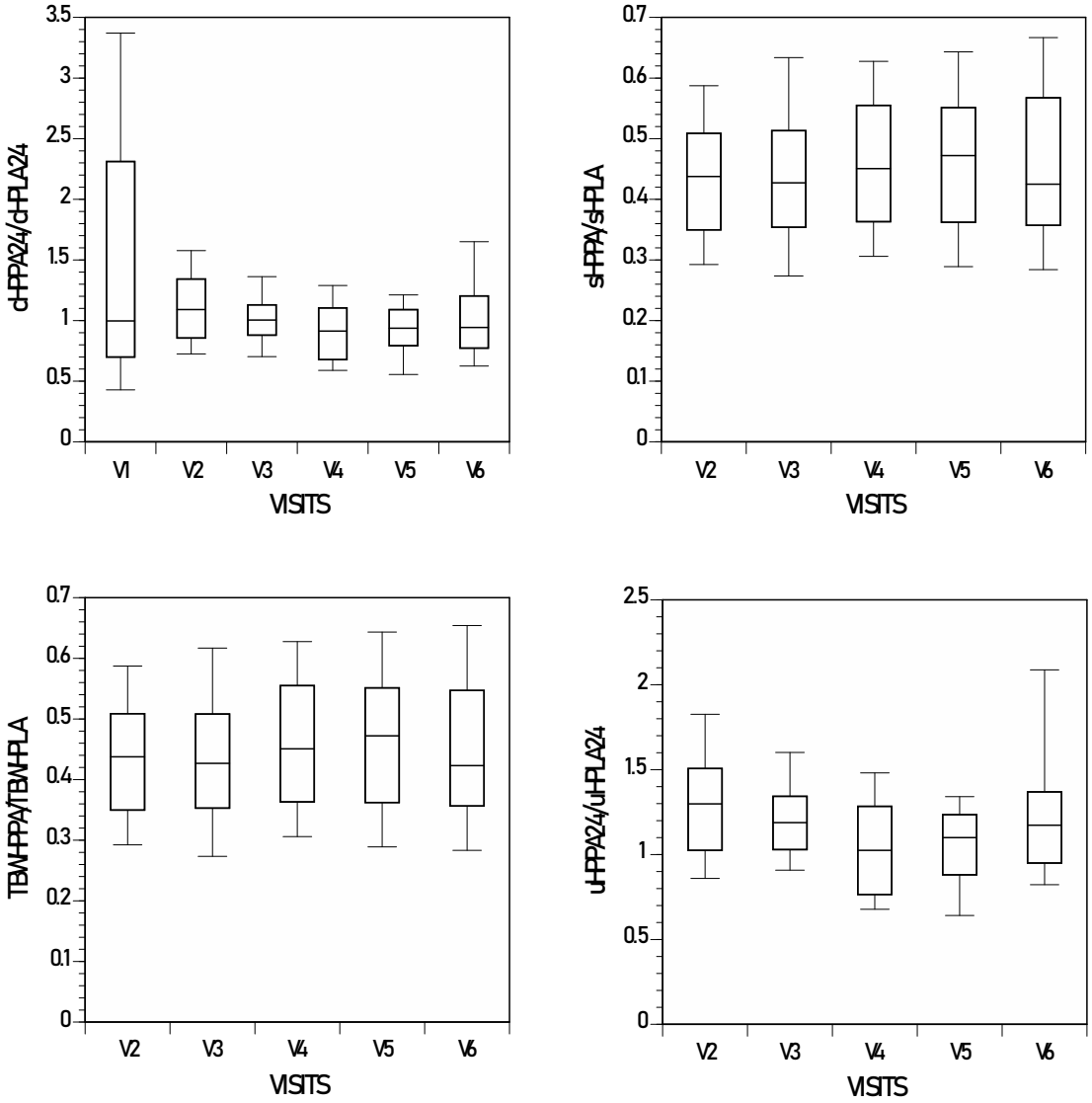

dHPPA24/dHPLA24
3.5
3
2.5
2
1.5
1
0.5
0
V1
V2
V3
V4
V5
V6
VISITS
sHPPA/sHPLA
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
V2
V3
V4
V5
V6
VISITS
TBWHPPA/TBWHPLA
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
V2
V3
V4
V5
V6
VISITS
uHPPA24/uHPLA24
2.5
2
1.5
1
0.5
0
V2
V3
V4
V5
V6
VISITS

Figure S8.

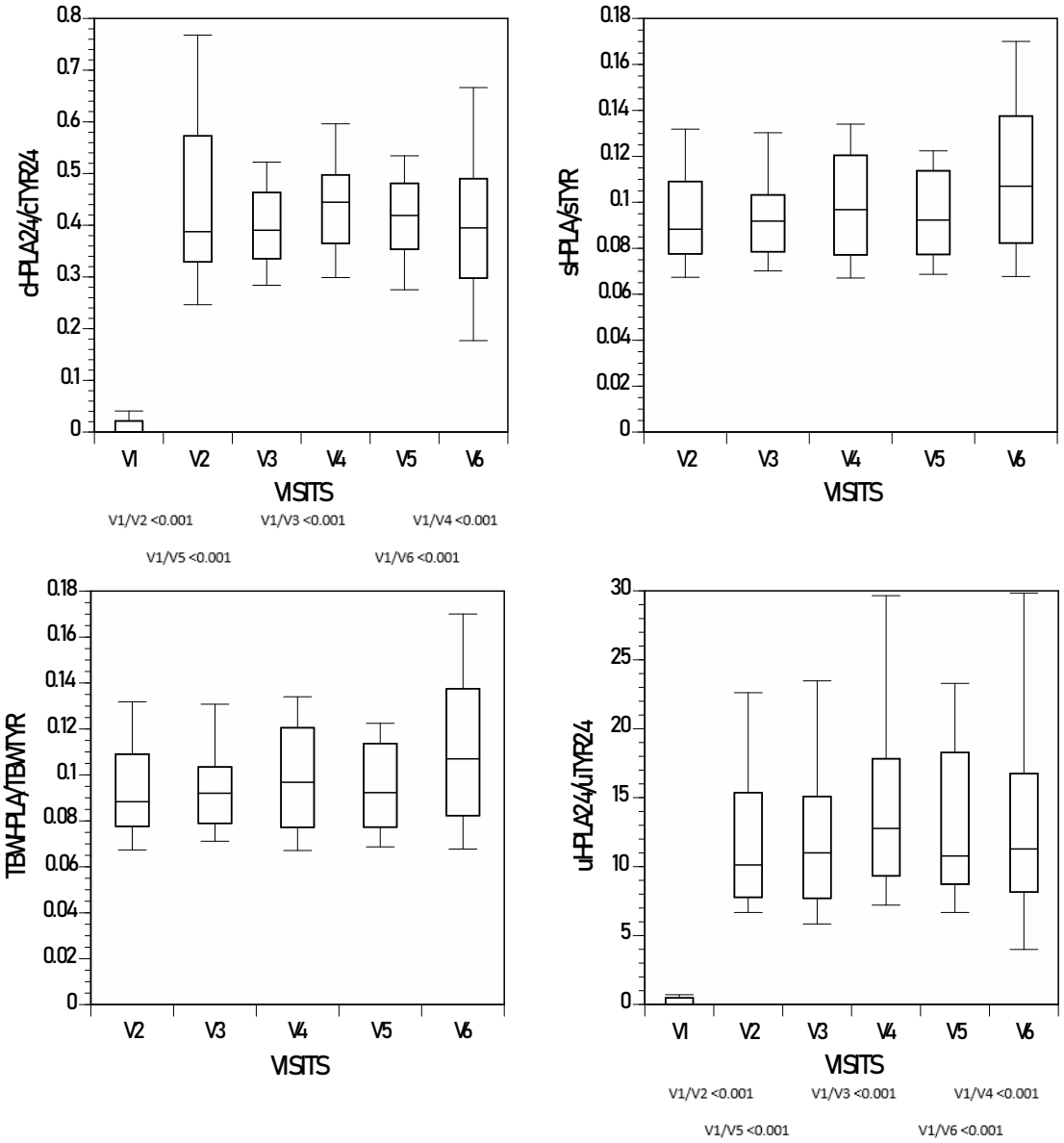

cHPLA24/cTYR24
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001
sHPLA/sTYR
VISITS
TBWHPLA/TBWTYR
VISITS
uHPLA24/uTYR24
VISITS
V1/V2 <0.001
V1/V3 <0.001
V1/V4 <0.001
V1/V5 <0.001
V1/V6 <0.001

Figure S9.

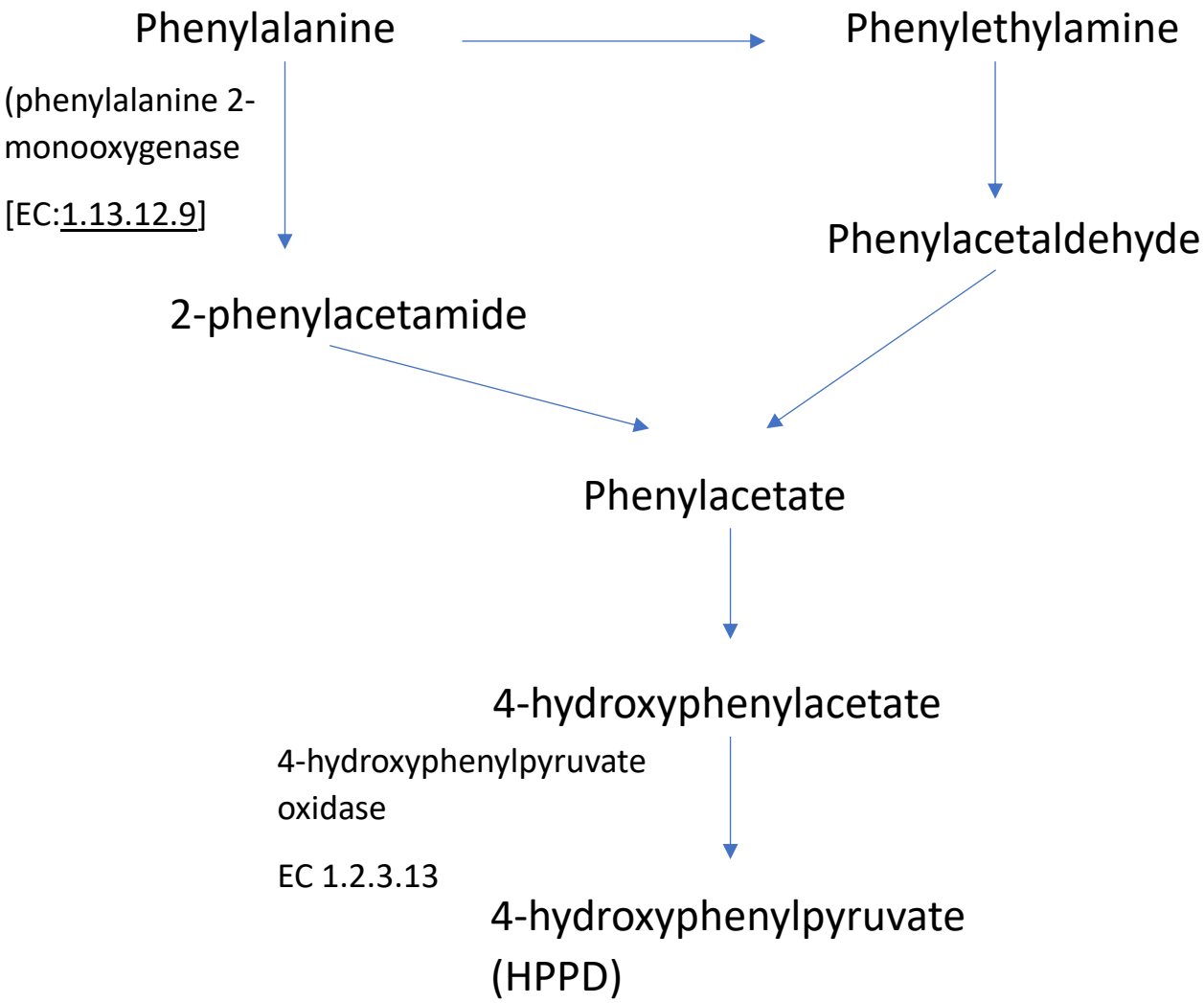

Phenylalanine
Phenylethylamine
(phenylalanine 2-monooxygenase
[EC:1.13.12.9]
Phenylacetaldehyde
2-phenylacetamide
Phenylacetate
4-hydroxyphenylacetate
4-hydroxyphenylpyruvate oxidase
EC 1.2.3.13
4-hydroxyphenylpyruvate (HPPD)

Figure S10.

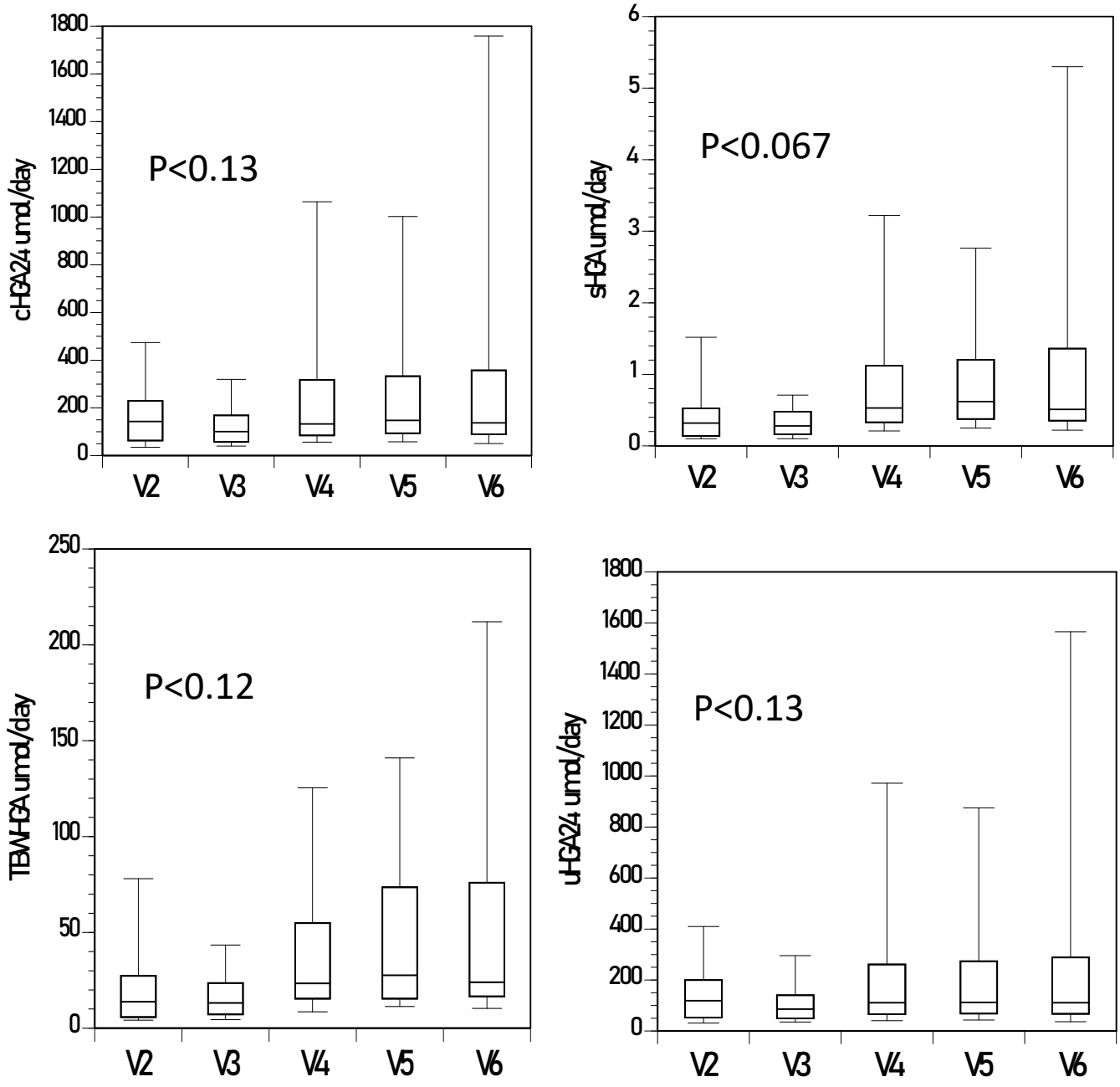

cHGA24 umol/day
P<0.13
V2
V3
V4
V5
V6
sHGA umol/day
P<0.067
TBWHGA umol/day
P<0.12
uHGA24 umol/day
P<0.13

Figure S11.

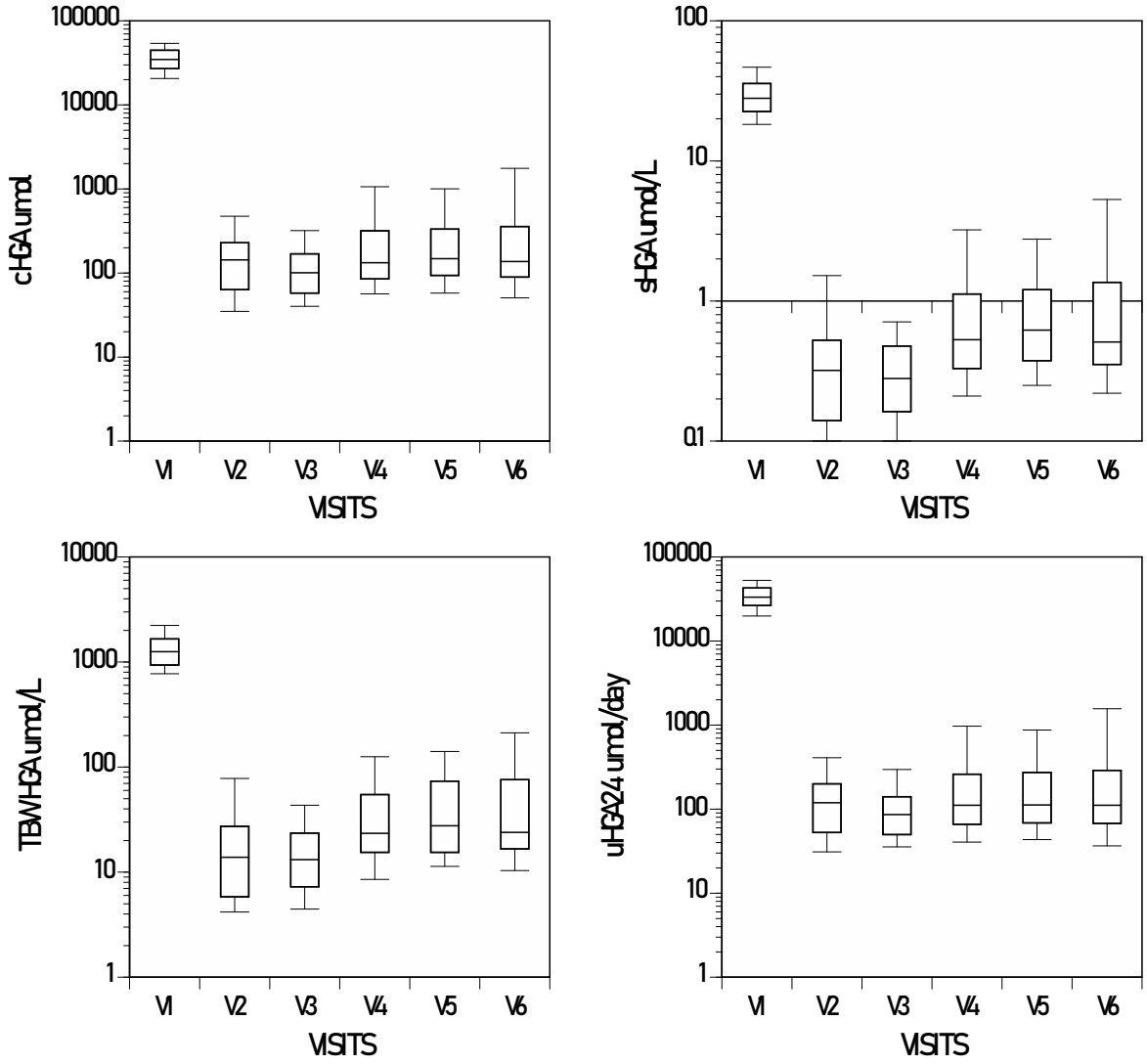
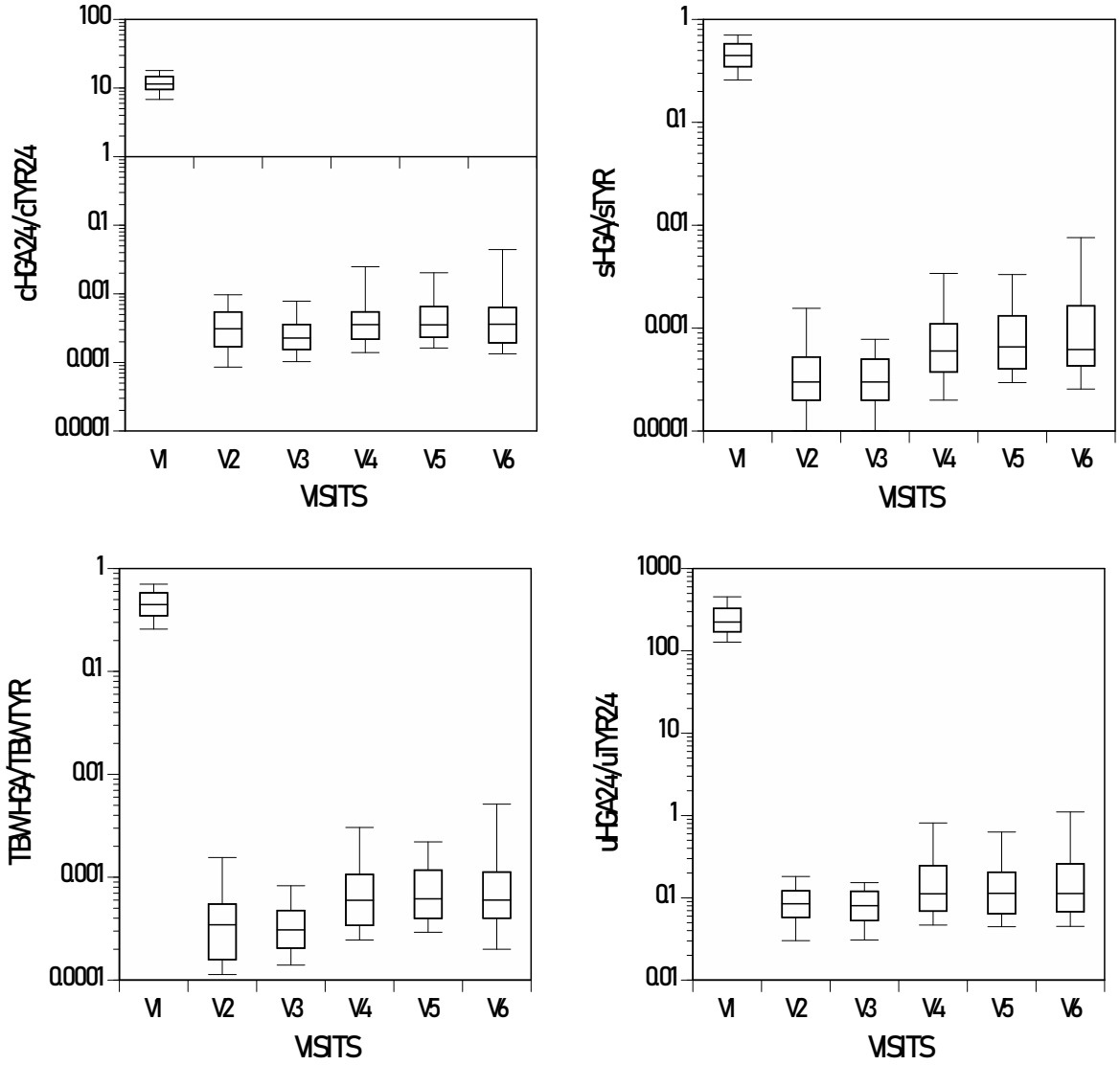

cHGAumol
sHGAumol/L
TBWHGAumol/L
uHGA24umol/day
VISITS
V1
V2
V3
V4
V5
V6

Figure S12.


cHGA24/cTYR24
sHGA/sTYR
TBMHGA/TBMTYR
uHGA24/uTYR24
100
10
1
0.1
0.01
0.001
0.0001
1000
0.01
V1
V2
V3
V4
V5
V6
VISITS