Supplementary material

Legend to tables and figures

Supplementary tables

Table S1. Demographic and other data in SONIA 2.

Table S2. Further metabolic data in the control group in SONIA 2 (n = 69)

Table S3. Linear regression relationships of measured data for sTYR, uUREA_{24} and sNIT (linear regression coefficient shown as R and its statistical significance shown by p value).

Table S4. Linear regression relationships of derived data for sTYR, uUREA_{24} and sNIT (linear regression coefficient shown as R and its statistical significance shown by p value).

Table S5. Dietary approach to sTYR in the United Kingdom National Alkaptonuria Centre.

Supplementary figures

Figure S1. The tyrosine 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 nitisinone. (HPPR – 4-hydroxyphenylpyruvate reductase)

Figure S2. Study design of SONIA 2 indicating the visits when assessments including 24-h urine and blood samples were taken.

Figure S3. Changes in sHGA, uHGA_{24}, TBW_{HGA}, and cHGA_{24} across the sTYR groups of <701, 701-900, 901-1100 and >1100 µmol/L (p values indicated for within-group comparison where statistical significance was achieved).

Figure S4. Changes in sTYR, uTYR_{24}, TBW_{TYR}, and cTYR_{24} across the sTYR groups of <701, 701-900, 901-1100 and >1100 µmol/L (p values indicated for within-group comparison where statistical significance was achieved).

Figure S5. Changes in sHPPA, uHPPA_{24}, TBW_{HPPA}, and cHPPA_{24} across the sTYR groups of <701, 701-900, 901-1100 and >1100 µmol/L (p values indicated for within-group comparison where statistical significance was achieved).

Figure S6. Changes in sHPLA, uHPLA_{24}, TBW_{HPLA}, and cHPLA_{24} across the sTYR groups of <701, 701-900, 901-1100 and >1100 µmol/L (p values indicated for within-group comparison where statistical significance was achieved).

Figure S7. Changes in sHGA/sTYR, uHGA_{24}/uTYR_{24}, TBW_{HGA}/TBW_{TYR}, and cHGA_{24}/cTYR_{24} across the sTYR groups of <701, 701-900, 901-1100 and >1100 µmol/L (p values indicated for within-group comparison where statistical significance was achieved).

Figure S8. Changes in sTYR/sPHE, uTYR_{24}/uPHE_{24}, TBW_{TYR}/TBW_{PHE}, and cTYR_{24}/cPHE_{24} across the sTYR groups of <701, 701-900, 901-1100 and >1100 µmol/L (p values indicated for within-group comparison where statistical significance was achieved).
Figure S9. Changes in uUREA$_{24}$, and uUREA$_{24}$/kg across the sTYR groups of <701, 701-900, 901-1100 and >1100 µmol/L (p values indicated for within-group comparison where statistical significance was achieved).

Figure S10. Regression graphs showing relationships between sTYR and uUREA$_{24}$ and sNIT

Figure S11. Regression graphs showing relationships between sTYR and sPHE, sHPPA and sHPLA
Table S1

|              | Control group | Nitisinone group |
|--------------|---------------|-----------------|
| Numbers of patients | 69 | 69 |
| Male | 40 | 45 |
| Female | 29 | 24 |
| Age years | 47·7 (10·2) | 47·4 (11·9) |
| Weight kg | 74·1 (15·6) | 74·8 (14·8) |
| Body Mass Index kg/M² | 26·4 (4·6) | 27·3 (4·2) |
| uHGA₂₄ (µmol/day) | 35394 (13868) | 35019 (13124) |
| sHGA (µmol/L) | 28·3 (8·7) | 31·7 (11·2) |
| sTYR (µmol/L) | 64·5 (15·5) | 66·3 (15·1) |

Table S2

|              | HGA (µmol) | TYR (µmol/day) | PHE (µmol) | HPPA (µmol) | HPLA (µmol) |
|--------------|------------|---------------|------------|-------------|-------------|
| TBW          | 1450 (726) | 2942 (1214)   | 2701 (887) | <LLoQ       | <LLoQ       |
| 24-h Urine   | 32151 (12842) | 165 (134)   | 85 (63)    | 79 (217)    | 72 (98)     |
| TBW+URINE    | 33590 (13169) | 3113 (1296) | 2778 (910) | 93 (339)    | 76 (118)    |

LLoQ – lower limit of quantification
Table S3. Regression analyses of sTYR, uUREA24 and sNIT against other measured data

|                  | sTYR vs other data | uUREA24 vs other data | sNIT vs other data |
|------------------|--------------------|-----------------------|-------------------|
|                  | R                  | R                     | R                 |
| Age years        | 0.056              | -0.18***              | 0.34****          |
| Weight kg        | -0.085             | 0.27****              | -0.42****        |
| uUREA24 mmol/day | 0.12*              | uUREA24 mmol/day      | 0.29****          |
| sUREA mmol/kg    | 0.12*              | uUREA mmol/kg         |                  |
| sHGA umol/L      | 0.024              | sHGA umol/L           | -0.37****        |
| sTYR umol/L      | sHGA umol/L        | 0.15*                 | sHGA umol/L      |
| sTYR umol/L      | sPHE umol/L        | 0.16*                 | sHGA umol/L      |
| sHGA umol/L      | 0.31*****          | sPHE umol/L           | 0.13*            |
| sHPPA umol/L     | 0.28*****          | sHPPA umol/L          | 0.003            |
| sHPPA umol/L     | 0.59*****          | sHPPA umol/L          | 0.18**           |
| sNIT umol/L      | 0.21*****          | sHPPA umol/L          | 0.07             |
| sNIT umol/L      | sNIT umol/L        | -0.29*****            | sHPPA umol/L     |
| uHGA24 umol/day  | -0.06              | uHGA24 umol/day       | -0.4****         |
| uTYR24 umol/day  | 0.09               | uTYR24 umol/day       | -0.23****        |
| uPHE24 umol/day  | -0.05              | uPHE24 umol/day       | -0.16**          |
| uHPPA24 umol/day | 0.1                | uHPPA24 umol/day      | -0.18**          |
| uHPLA24 umol/day | 0.23*****          | uHPLA24 umol/day      | -0.07            |

Degree of statistical significance of R is indicated by p:< *0.05; **<0.01; ***<0.001; ****<0.0001
Table S4. Regression analyses of sTYR, uUREA24 and sNIT against other derived data

|                  | sTYR vs other data | uUREA24 vs other data | sNIT vs other data |
|------------------|--------------------|-----------------------|--------------------|
|                  | R                  | R                     | R                  |
| TBW HGA umol     | 0.02               | 0.16**                | -0.39****          |
| TBW TYR umol     | 0.71****           | 0.28****              | -0.13*             |
| TBW PHE umol     | 0.14*              | 0.26****              | -0.16**            |
| TBW HPPA umol    | 0.22****           | 0.14*                 | -0.01              |
| TBW HPLA umol    | 0.47****           | 0.23****              | 0.2***             |
| cHGA24 umol/day  | -0.05              | 0.21***               | -0.42****          |
| cTYR24 umol/day  | 0.2****            | 0.31****              | -0.14**            |
| cPHE24 umol/day  | 0.16**             | 0.28****              | -0.18**            |
| cHPPA24 umol/day | 0.12*              | 0.79****              | -0.18**            |
| cHPLA24 umol/day | 0.31****           | 0.72****              | -0.01              |

Degree of statistical significance of R is indicated by p<: *0.05; **<0.01; ***<0.001; ****<0.0001
Table S5

| sTYR (µmol/L) | Action |
|---------------|--------|
| <500          | Acceptable, no further action |
| 501 – 700     | Institute 0.9g/kg body weight protein in diet |
| 701 – 900     | Institute 0.8g/kg body weight protein in diet |
| >900          | Institute 0.8g/kg body weight protein in diet, plus tyrosine/phenylalanine-free amino-acid supplements |
| Keratopathy   | Stop nitisinone and restart after 2 months, intensify dietary protein restriction |
Figure S1

Phenylalanine

Phenylalanine hydroxylase

Tyrosine

Tyrosine aminotransferase

4-Hydroxyphenylpyruvic acid

4-Hydroxyphenylpyruvic acid dioxygenase

Nitisinone

Homogentisic acid

Homogentisate 1,2-dioxygenase

Alkaptonuria

Maleylacetoacetic acid

Maleylacetoacetic acid isomerase

Fumarylacetacetic acid

Fumarylacetacetic acid hydrolase

Acetoacetic acid + Fumaric acid

4-Hydroxyphenyllactic acid

HPPR
Figure S2

Control (no treatment)

S + R

Nitisinone

F  T

Visits  1  2  3  4  5  6  7
Months  0  3  6  12  18  24  30  36  42  48  49

S + R = Screening, baseline and Randomisation Visit
F    = Final treatment Visit
T    = Telephone follow-up Visit
18   = Interim safety questionnaires
Figure S3
Figure S3

Box plots showing distribution of TBWA (umol) and chBA24 (umol/day) across different concentration ranges of umol/L or umol/day.

- TBWA (umol): Ranges from less than 701 umol/L to greater than 1100 umol/L.
- chBA24 (umol/day): Ranges from less than 701 umol/day to greater than 1100 umol/day.

Each box plot represents the median, interquartile range, and outliers for each concentration range.
Figure S4

Comparison of urinary tyrosine (μmol/L) across different concentration ranges:

- <701 μmol/L vs. 701-900 μmol/L: p<0.0001
- <701 μmol/L vs. >1100 μmol/L: p<0.0001
- 701-900 μmol/L vs. >1100 μmol/L: p<0.0001

Comparison of urinary tyrosine excretion (μmol/day) across different concentration ranges:

- <701 umol/L vs. 701-900 umol/L vs. 901-1100 umol/L vs. >1100 umol/L: p<0.0001
Figure S5

![Box plot showing distribution of HPPA levels in different concentration ranges. The top plot shows HPPA concentrations in the range of <701 umol/L, 701-900 umol/L, 901-1100 umol/L, and >1100 umol/L. The bottom plot shows uHPPA24 concentrations in the same ranges.]

- <701 umol/L vs >1100 umol/L: p<0.001
- 701-900 umol/L vs >1100 umol/L: p<0.001
Figure S5

[Box plots showing data distribution for TBM/ARA and d-PHE4 umol/day across different umol/L ranges, with statistical significance noted for comparisons between groups.]

- **TBMA/ARA umol/L**:
  - <701 umol/L vs >1100 umol/L: p<0.001
  - 701-900 umol/L vs >1100 umol/L: p<0.001

- **d-PHE4 umol/day**:
  - <701 umol/day vs >1100 umol/day
  - 701-900 umol/day vs >1100 umol/day
  - 901-1100 umol/day vs >1100 umol/day
  - >1100 umol/day vs >1100 umol/day
Figure S6

![Box plots showing sHLAumol/L and uHLAumol/d results](image)

- <701 vs 701-900 p<0.05
- <701 vs >1100 p<0.001
- 701-900 vs >1100 p<0.001
- 901-1100 vs >1100 p<0.001
Figure S6

- <701 vs 901-1100 p<0.001
- <701 vs >1100  p<0.001
- 701-900 vs 901-1100 p<0.05
- 701-900 vs >1100  p<0.001
- 901-1100 vs >1100 p<0.001
Figure S7
Figure S7

![Box plots for different umol/L ranges](image)

- **TBHBA/TVR**
  - P < 0.023

- **cH3C2/H2R24**
  - No significant difference noted
Figure S8

<701 vs 701-800 \ p<0.0001  
<701 vs >1100 \ p<0.0001  
701-900 vs >1100 \ p<0.0001  
901-1100 vs >1100 \ p<0.0001

<701 vs 701-900 \ p<0.001  
<701 vs 901-1100 \ p<0.0001  
701-900 vs 901-1100 \ p<0.05  
701-900 vs >1100 \ p<0.001
Figure S8

<701 vs 701-800  p<0.0001  
<701 vs >1100   p<0.0001  
701-900 vs >1100  p<0.0001  
901-1100 vs >1100  p<0.0001

<701 vs 701-800  p<0.0001  
<701 vs >1100   p<0.0001  
701-900 vs >1100  p<0.0001  
901-1100 vs >1100  p<0.0001
Figure S9
Figure S10

**sTYR vs uUREA24**

$R = 0.12$, $p < 0.05$

**sNIT vs sTYR**

$R = 0.21$, $p < 0.0001$
Figure S11

**sTYR vs sPHE**

R 0.31, p<0.0001

**sTYR vs sHPPA**

R 0.28, p<0.0001

**sTYR vs sHPLA**

R 0.59, p<0.0001