Supplemental information

Urolithin A improves muscle strength, exercise performance, and biomarkers of mitochondrial health in a randomized trial in middle-aged adults

Anurag Singh, Davide D'Amico, Pénélope A. Andreux, Andréane M. Fouassier, William Blanco-Bose, Mal Evans, Patrick Aebischer, Johan Auwerx, and Chris Rinsch
Supplementary Figure 1

UA supplementation improved quadriceps muscle strength (A, B) but had no impact on body composition (C, D). Related to Figure 2
Supplementary Figure 2

High dose of UA (1000mg) improves aerobic capacity at 2- and 4-months of supplementation (A, B) and exhibits a positively signal on exercise performance measures (C, D).

Related to Figure 2.
Supplementary Figure 3

Only small subset of participants had circulating levels of UA (A) at baseline and with higher circulating inflammatory biomarkers (B) C-reactive protein (CRP). Data represent mean ± s.e.m. Related to Figure 3.
Supplementary Figure 4

Principal component analysis (PCA) (A) and gene-set enrichment analysis from RNA-seq in muscle biopsies (B) show that UA supplementation increases expression of genes belonging to pathways related to mitochondria, muscle contraction and cytosolic ribosomes. Related to Figure 4.
Supplementary Figure 5.

Proteomics in muscle biopsies (A) showing UA’s impact on pathways and genes related to glycogen metabolism (B), Parkin-related biological processes (C and D), and BNIP3-mediated mitophagy (E). Related to Figure 5.
Supplementary Figure 6.

Proteomics in muscle biopsies (A) showing UA to increase pathways and genes related to mitochondria (A-D), while not impacting muscle fiber type composition (E). Related to Figure 5.
Supplementary Figure 7

Western blot (A-D) and mitochondrial to nuclear DNA analysis (E) indicate that UA increases levels of proteins linked to improved mitophagy and mitochondrial function as well as mitochondrial content. Related to Figure 5.
### Supplementary Table 1: Listing of adverse events (AE's) across groups

Total Number of Post-Emergent Adverse Events according to SOC, PT and LLT: Safety Population

| System Organ Class (SOC) | Preferred Term (PT) | Lower-Level Term (LLT) | Placebo n (%) | UA 500mg Dose n (%) | UA 1000 mg Dose n (%) | Between Group P-Value* |
|--------------------------|---------------------|------------------------|---------------|---------------------|-----------------------|------------------------|
| Investigations           | Liver function test abnormal Arthralgia | Abnormal liver function tests Ache wrists | 1 (2.40%) | 0 (0.00%) | 0 (0.00%) | 0.113 |
| Musculoskeletal and connective tissue disorders | Abdominal distension Hypertension | Bloating Blood pressure high Cold | 1 (2.40%) | 1 (4.20%) | 4 (11.10%) |
| Gastrointestinal disorders | Nasopharyngitis | Infections and infestations | 2 (4.80%) | 2 (8.30%) | 1 (2.80%) |
| Vascular disorders | Oral herpes Cold sores | Constipation Constipation | 0 (0.00%) | 1 (4.20%) | 0 (0.00%) |
| Infections and infestations | Muscle spasms | Cough | 1 (2.40%) | 0 (0.00%) | 1 (2.80%) |
| Musculoskeletal and connective tissue disorders | Diarrhoea | Diarrhea | 0 (0.00%) | 1 (4.20%) | 0 (0.00%) |
| Nervous system disorders | Dizziness | Dizziness | 1 (2.40%) | 0 (0.00%) | 0 (0.00%) |
| Respiratory, thoracic and mediastinal disorders | Cough | Dry cough | 1 (2.40%) | 0 (0.00%) | 1 (2.80%) |
| General disorders and administration site conditions | Fatigue | Fatigue | 2 (4.80%) | 2 (8.30%) | 0 (0.00%) |
| Infections and infestations | Influenza | Flu | 2 (4.80%) | 1 (4.20%) | 2 (5.60%) |
| Nervous system disorders | Headache | Headache | 2 (4.80%) | 4 (16.70%) | 8 (22.20%) |
| General disorders and administration site conditions | Hot flush | Hot flush | 0 (0.00%) | 1 (4.20%) | 0 (0.00%) |
| General disorders and administration site conditions | Increased appetite | Increased appetite | 2 (4.80%) | 0 (0.00%) | 0 (0.00%) |
| Musculoskeletal and connective tissue disorders | Pain in jaw | Jaw pain | 1 (2.40%) | 0 (0.00%) | 0 (0.00%) |
| Musculoskeletal and connective tissue disorders | Arthralgia | Joint pain | 1 (2.40%) | 1 (4.20%) | 0 (0.00%) |
| Musculoskeletal and connective tissue disorders | Arthralgia | Knee pain | 0 (0.00%) | 1 (4.20%) | 0 (0.00%) |
| Gastrointestinal disorders | Diarrhoea | Loose stools | 0 (0.00%) | 0 (0.00%) | 3 (8.30%) |
| Reproductive system and breast disorders | Menorrhagia | Menstrual flow excessive | 0 (0.00%) | 2 (8.30%) | 0 (0.00%) |
| Nervous system disorders | Migraine | Migraine | 0 (0.00%) | 0 (0.00%) | 1 (2.80%) |
| Musculoskeletal and connective tissue disorders | Myalgia | Muscle pain | 3 (7.10%) | 1 (4.20%) | 2 (5.60%) |
| Medical Condition | Description | Frequency | Comparison 1 | Comparison 2 | Comparison 3 |
|-------------------|-------------|-----------|--------------|--------------|--------------|
| Musculoskeletal and connective tissue disorders | Muscle strain | 0 (0.00%) | 0 (0.00%) | 1 (2.80%) |
| Gastrointestinal disorders | Nausea | 2 (4.80%) | 1 (4.20%) | 3 (8.30%) |
| Vascular disorders | Epistaxis | 1 (2.40%) | 0 (0.00%) | 0 (0.00%) |
| Nervous system disorders | Hypoaesthesia | 3 (7.10%) | 0 (0.00%) | 1 (2.80%) |
| General disorders and administration site conditions | Pain | 0 (0.00%) | 0 (0.00%) | 1 (2.80%) |
| Respiratory, thoracic and mediastinal disorders | Sneezing | 1 (2.40%) | 0 (0.00%) | 0 (0.00%) |
| Respiratory, thoracic and mediastinal disorders | Productive cough | 0 (0.00%) | 1 (4.20%) | 0 (0.00%) |
| Infections and infestations | Pneumonia | 0 (0.00%) | 0 (0.00%) | 1 (2.80%) |
| Renal and urinary disorders | Nephrolithiasis | 0 (0.00%) | 0 (0.00%) | 1 (2.80%) |
| Ear and labyrinth disorders | Tinnitus | 0 (0.00%) | 0 (0.00%) | 1 (2.80%) |
| Respiratory, thoracic and mediastinal disorders | Dyspnoea | 2 (4.80%) | 0 (0.00%) | 0 (0.00%) |
| Respiratory, thoracic and mediastinal disorders | Oropharyngeal pain | 0 (0.00%) | 0 (0.00%) | 1 (2.80%) |
| Vascular disorders | Haemoptysis | 1 (2.40%) | 0 (0.00%) | 0 (0.00%) |
| Gastrointestinal disorders | Abdominal pain upper | 1 (2.40%) | 0 (0.00%) | 0 (0.00%) |
| Gastrointestinal disorders | Faeces discoloured | 0 (0.00%) | 1 (4.20%) | 0 (0.00%) |
| Respiratory, thoracic and mediastinal disorders | Pharyngitis streptococcal | 1 (2.40%) | 0 (0.00%) | 0 (0.00%) |
| Injury, poisoning and procedural complications | Muscle rupture | 0 (0.00%) | 1 (4.20%) | 0 (0.00%) |
| Infections and infestations | Vaginal infection | 0 (0.00%) | 0 (0.00%) | 1 (2.80%) |
| Gastrointestinal disorders | Vomiting | 0 (0.00%) | 1 (4.20%) | 0 (0.00%) |
| Investigations | White blood cell count decreased | 1 (2.40%) | 0 (0.00%) | 0 (0.00%) |
Supplementary Table 2: Summary of protein information and statistical analysis from proteomics analysis

Accession number, Gene ID and Official Protein name of proteins indicated in the paper, together with change over baseline expression values (AVG Log Ratio), p. value and FDR.

| Accession ID | Gene ID | Protein Name | Group         | AVG Log Ratio | Absolute AVG Log Ratio | Ratio | Pvalue | FDR  |
|--------------|---------|--------------|---------------|---------------|------------------------|-------|--------|------|
| P35573       | AGL     | GDE_HUMAN    | Placebo       | -0.06         | 0.06                   | 0.96  | 0.00   | 0.03 |
| P35573       | AGL     | GDE_HUMAN    | UA 1000 mg    | 0.04          | 0.04                   | 1.03  | 0.00   | 0.08 |
| P35573       | AGL     | GDE_HUMAN    | UA 1000 mg    | 0.05          | 0.05                   | 1.04  | 0.00   | 0.00 |
| P11217       | PYGM    | PYGM_HUMAN   | Placebo       | -0.15         | 0.15                   | 0.90  | 0.00   | 0.01 |
| P11217       | PYGM    | PYGM_HUMAN   | UA 1000 mg    | 0.06          | 0.06                   | 1.04  | 0.01   | 0.18 |
| P11217       | PYGM    | PYGM_HUMAN   | UA 1000 mg    | 0.08          | 0.08                   | 1.05  | 0.01   | 0.13 |
| P62253       | UBE2G1  | UBE2G1_HUMAN | Placebo       | 0.54          | 0.54                   | 1.45  | 0.44   | 0.67 |
| P62253       | UBE2G1  | UBE2G1_HUMAN | UA 500 mg     | 0.62          | 0.62                   | 1.54  | 0.02   | 0.24 |
| P62253       | UBE2G1  | UBE2G1_HUMAN | UA 1000 mg    | -0.38         | 0.38                   | 0.77  | 0.23   | 0.57 |
| P61088       | UBE2N   | UBE2N_HUMAN  | Placebo       | -0.02         | 0.02                   | 0.99  | 0.64   | 0.73 |
| P61088       | UBE2N   | UBE2N_HUMAN  | UA 500 mg     | 0.17          | 0.17                   | 1.12  | 0.01   | 0.17 |
| P61088       | UBE2N   | UBE2N_HUMAN  | UA 1000 mg    | 0.10          | 0.10                   | 1.07  | 0.03   | 0.30 |
| Q712K3       | UBE2R2  | UBE2R2_HUMAN | Placebo       | 0.08          | 0.08                   | 1.06  | 0.30   | 0.61 |
| Q712K3       | UBE2R2  | UBE2R2_HUMAN | UA 500 mg     | 0.22          | 0.22                   | 1.16  | 0.02   | 0.25 |
| Q712K3       | UBE2R2  | UBE2R2_HUMAN | UA 1000 mg    | 0.16          | 0.16                   | 1.11  | 0.89   | 0.79 |
| P49721       | PSMB2   | PSB2_HUMAN   | Placebo       | -0.06         | 0.06                   | 0.96  | 0.23   | 0.57 |
| P49721       | PSMB2   | PSB2_HUMAN   | UA 500 mg     | 0.08          | 0.08                   | 1.05  | 0.01   | 0.19 |
| P49721       | PSMB2   | PSB2_HUMAN   | UA 1000 mg    | -0.01         | 0.01                   | 0.99  | 0.68   | 0.75 |
| P62333       | PSMC6   | PRS10_HUMAN  | Placebo       | -0.06         | 0.06                   | 0.96  | 0.29   | 0.60 |
| P62333       | PSMC6   | PRS10_HUMAN  | UA 500 mg     | 0.13          | 0.13                   | 1.09  | 0.01   | 0.12 |
| P62333       | PSMC6   | PRS10_HUMAN  | UA 1000 mg    | -0.09         | 0.09                   | 0.94  | 0.95   | 0.81 |
| Q06323       | PSME1   | PSME1_HUMAN  | Placebo       | -0.17         | 0.17                   | 0.89  | 0.03   | 0.30 |
| Q06323       | PSME1   | PSME1_HUMAN  | UA 500 mg     | 0.24          | 0.24                   | 1.18  | 0.00   | 0.08 |
| Q06323       | PSME1   | PSME1_HUMAN  | UA 1000 mg    | 0.01          | 0.01                   | 1.01  | 0.39   | 0.65 |
| Q12983       | BNIp3   | BNIp3_HUMAN  | Placebo       | 0.27          | 0.27                   | 1.21  | 0.97   | 0.81 |
| Q12983       | BNIp3   | BNIp3_HUMAN  | UA 500 mg     | -0.18         | 0.18                   | 0.88  | 0.31   | 0.61 |
| Q12983       | BNIp3   | BNIp3_HUMAN  | UA 1000 mg    | -0.46         | 0.46                   | 0.73  | 0.20   | 0.55 |
| P25705       | ATP5F1A | ATPA_HUMAN   | Placebo       | -0.01         | 0.01                   | 0.99  | 0.60   | 0.72 |
| P25705       | ATP5F1A | ATPA_HUMAN   | UA 500 mg     | -0.04         | 0.04                   | 0.97  | 0.24   | 0.57 |
| P25705       | ATP5F1A | ATPA_HUMAN   | UA 1000 mg    | 0.11          | 0.11                   | 1.08  | 0.00   | 0.00 |
| O75251       | NDUF7   | NDUS7_HUMAN  | Placebo       | -0.11         | 0.11                   | 0.93  | 1.00   | 0.81 |
| O75251       | NDUF7   | NDUS7_HUMAN  | UA 500 mg     | 0.02          | 0.02                   | 1.01  | 0.66   | 0.74 |
| O75251       | NDUF7   | NDUS7_HUMAN  | UA 1000 mg    | 0.24          | 0.24                   | 1.18  | 0.01   | 0.11 |
| P31930       | UQCRC1  | QCR1_HUMAN   | Placebo       | -0.02         | 0.02                   | 0.98  | 0.05   | 0.36 |
| P31930       | UQCRC1  | QCR1_HUMAN   | UA 500 mg     | -0.02         | 0.02                   | 0.98  | 0.02   | 0.25 |
| P31930 | UQRC1 | QCR1_HUMAN | UA 1000 mg | 0.10 | 0.10 | 1.07 | 0.60 | 0.72 |
| Q99798 | ACO2  | ACON_HUMAN | Placebo    | 0.09 | 0.09 | 1.07 | 0.17 | 0.52 |
| Q99798 | ACO2  | ACON_HUMAN | UA 500 mg  | 0.14 | 0.14 | 1.10 | 0.13 | 0.48 |
| Q99798 | ACO2  | ACON_HUMAN | UA 1000 mg | 0.19 | 0.19 | 1.14 | 0.00 | 0.02 |
| O75390 | CS    | CISY_HUMAN | Placebo    | -0.04 | 0.04 | 0.97 | 0.17 | 0.52 |
| O75390 | CS    | CISY_HUMAN | UA 500 mg  | 0.21 | 0.21 | 1.15 | 0.62 | 0.73 |
| O75390 | CS    | CISY_HUMAN | UA 1000 mg | 0.33 | 0.33 | 1.25 | 0.11 | 0.46 |
| P36957 | DLST  | ODO2_HUMAN | Placebo    | -0.03 | 0.03 | 0.98 | 0.10 | 0.45 |
| P36957 | DLST  | ODO2_HUMAN | UA 500 mg  | 0.01 | 0.01 | 1.01 | 0.62 | 0.73 |
| P36957 | DLST  | ODO2_HUMAN | UA 1000 mg | 0.13 | 0.13 | 1.09 | 0.02 | 0.20 |
| P48735 | IDH2  | IDHP_HUMAN | Placebo    | -0.07 | 0.07 | 0.95 | 0.89 | 0.80 |
| P48735 | IDH2  | IDHP_HUMAN | UA 500 mg  | 0.02 | 0.02 | 1.01 | 0.41 | 0.66 |
| Q15388 | TOMM20| TOM20_HUMAN| Placebo    | -0.02 | 0.02 | 0.99 | 0.36 | 0.63 |
| Q15388 | TOMM20| TOM20_HUMAN| UA 500 mg  | -0.21 | 0.21 | 0.87 | 0.84 | 0.78 |
| Q15388 | TOMM20| TOM20_HUMAN| UA 1000 mg | 0.80 | 0.80 | 1.74 | 0.04 | 0.30 |
| P21796 | VDAC1 | VDAC1_HUMAN| Placebo    | -0.01 | 0.01 | 0.99 | 0.47 | 0.68 |
| P21796 | VDAC1 | VDAC1_HUMAN| UA 500 mg  | -0.09 | 0.09 | 0.94 | 0.20 | 0.55 |
| P21796 | VDAC1 | VDAC1_HUMAN| UA 1000 mg | 0.05 | 0.05 | 1.03 | 0.20 | 0.55 |
| O00429 | DNM1L | DNM1L_HUMAN| Placebo    | -0.18 | 0.18 | 0.89 | 0.05 | 0.36 |
| O00429 | DNM1L | DNM1L_HUMAN| UA 500 mg  | -0.11 | 0.11 | 0.93 | 0.06 | 0.38 |
| O00429 | DNM1L | DNM1L_HUMAN| UA 1000 mg | 0.06 | 0.06 | 1.04 | 0.92 | 0.80 |
| Q8IWA4 | MFN1  | MFN1_HUMAN | Placebo    | 0.08 | 0.08 | 1.05 | 0.68 | 0.74 |
| Q8IWA4 | MFN1  | MFN1_HUMAN | UA 500 mg  | -0.19 | 0.19 | 0.88 | 0.72 | 0.76 |
| Q8IWA4 | MFN1  | MFN1_HUMAN | UA 1000 mg | 0.26 | 0.26 | 1.20 | 0.89 | 0.80 |
| O95140 | MFN2  | MFN2_HUMAN | Placebo    | 0.03 | 0.03 | 1.02 | 0.50 | 0.69 |
| O95140 | MFN2  | MFN2_HUMAN | UA 500 mg  | -0.06 | 0.06 | 0.96 | 0.48 | 0.68 |
| O95140 | MFN2  | MFN2_HUMAN | UA 1000 mg | 0.14 | 0.14 | 1.10 | 0.94 | 0.80 |
| O60313 | OPA1  | OPA1_HUMAN | Placebo    | 0.11 | 0.11 | 1.08 | 0.89 | 0.79 |
| O60313 | OPA1  | OPA1_HUMAN | UA 500 mg  | 0.06 | 0.06 | 1.04 | 0.06 | 0.38 |
| O60313 | OPA1  | OPA1_HUMAN | UA 1000 mg | 0.21 | 0.21 | 1.16 | 0.79 | 0.77 |
| P12882 | MYH1  | MYH1_HUMAN | Placebo    | -0.05 | 0.05 | 0.97 | 0.01 | 0.15 |
| P12882 | MYH1  | MYH1_HUMAN | UA 500 mg  | -0.02 | 0.02 | 0.99 | 0.00 | 0.00 |
| P12882 | MYH1  | MYH1_HUMAN | UA 1000 mg | 0.02 | 0.02 | 1.02 | 0.00 | 0.00 |
| Q9UKX2 | MYH2  | MYH2_HUMAN | Placebo    | -0.12 | 0.12 | 0.92 | 0.10 | 0.45 |
| Q9UKX2 | MYH2  | MYH2_HUMAN | UA 500 mg  | 0.05 | 0.05 | 1.04 | 0.00 | 0.00 |
| Q9UKX2 | MYH2  | MYH2_HUMAN | UA 1000 mg | -0.01 | 0.01 | 0.99 | 0.00 | 0.04 |
| P12883 | MYH7  | MYH7_HUMAN | Placebo    | -0.22 | 0.22 | 0.86 | 0.05 | 0.36 |
| P12883 | MYH7  | MYH7_HUMAN | UA 500 mg  | -0.27 | 0.27 | 0.83 | 0.00 | 0.00 |
| P12883 | MYH7  | MYH7_HUMAN | UA 1000 mg | -0.03 | 0.03 | 0.98 | 0.02 | 0.22 |