

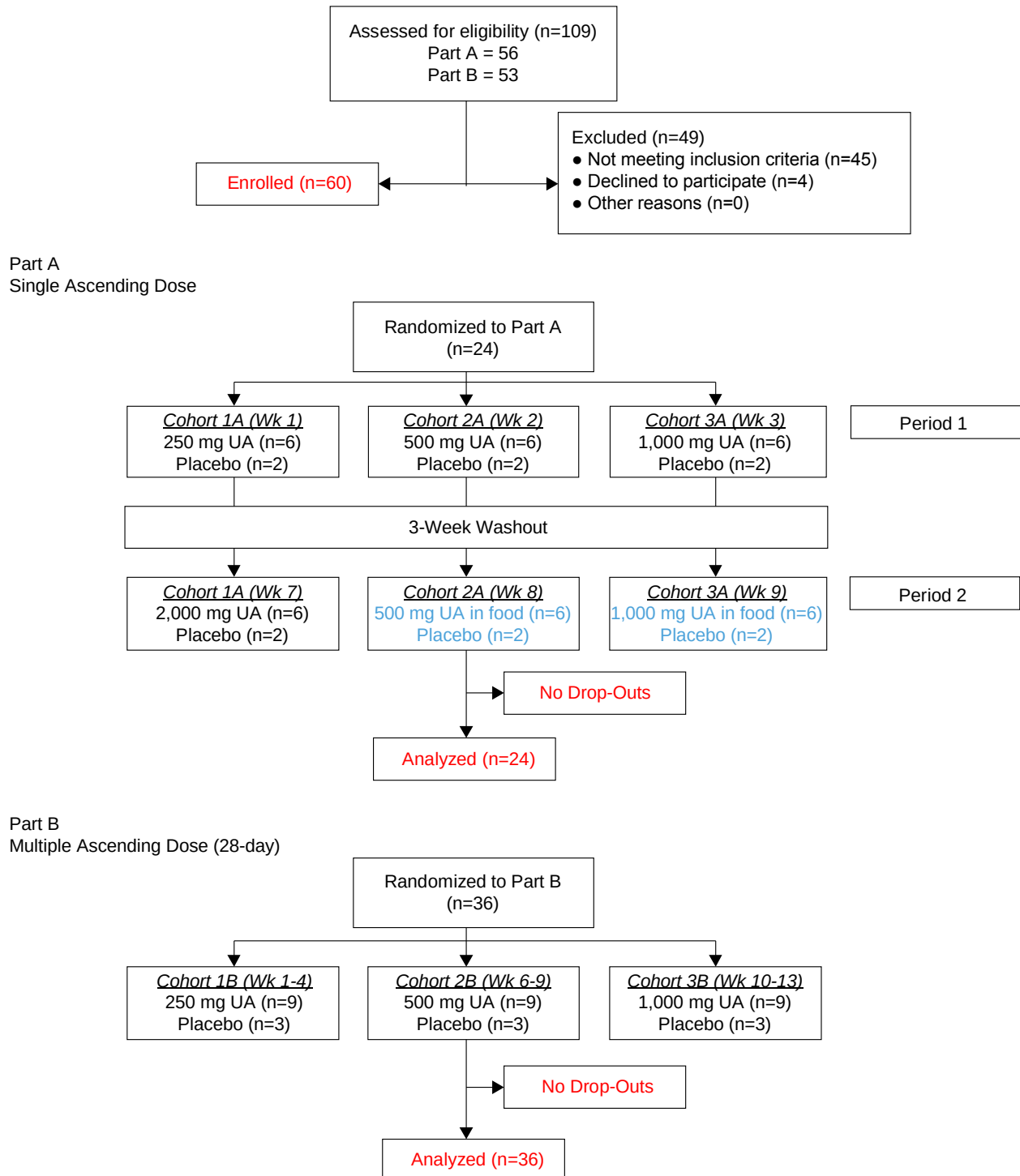
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The mitophagy activator urolithin A is safe and induces a molecular signature of improved mitochondrial and cellular health in humans

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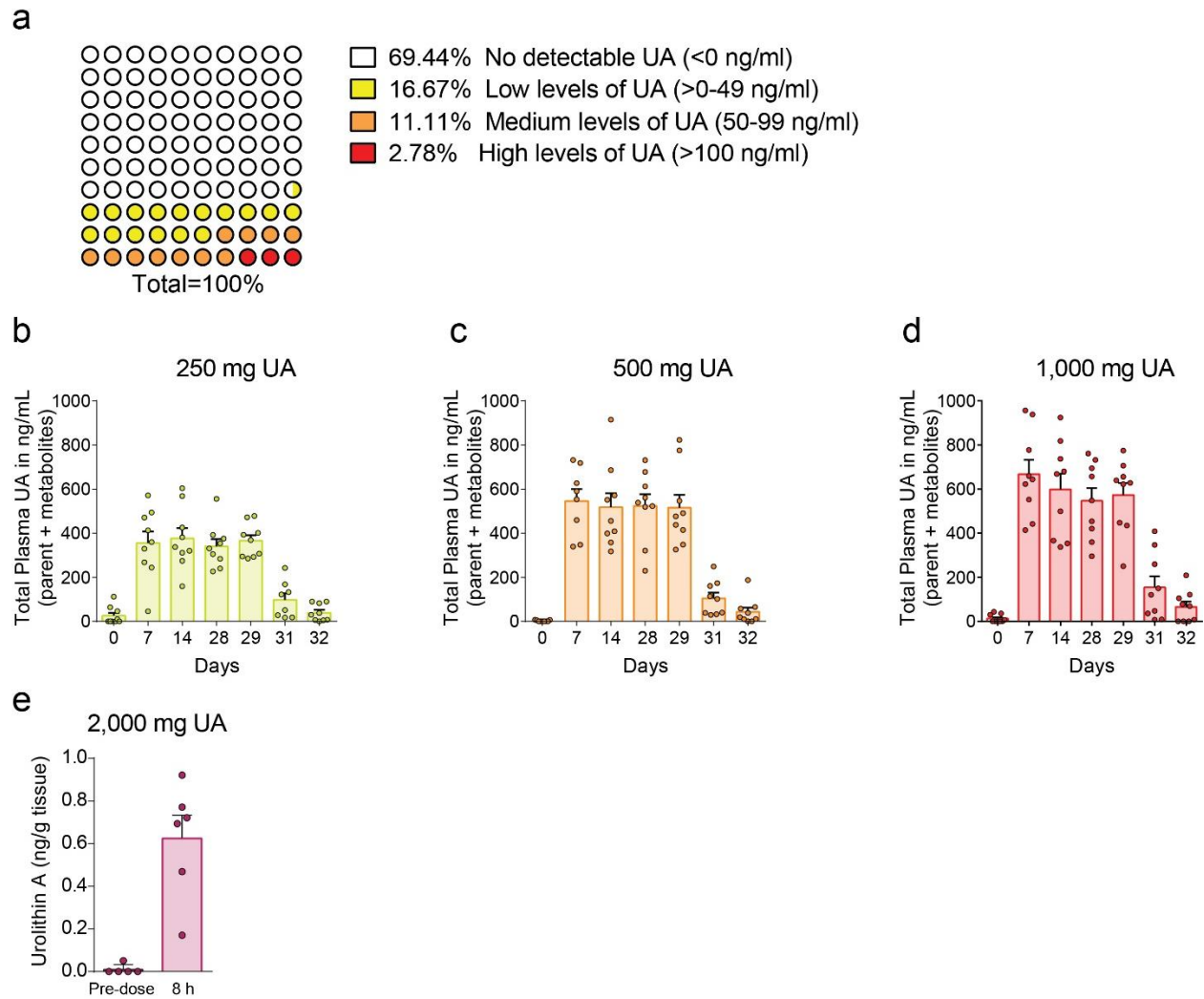
Supplementary Figure 1



Schematic CONSORT diagram of Parts A (single UA dosing) and B (28-day UA dosing) representing subjects recruitment and randomization. 60 elderly subjects were recruited, n=24 into Part A; and n=36

into Part B. Part A was a two-period design with a 3-week washout in between the two periods. In Period 1, three cohorts of eight subjects were randomized to receive 250 mg softgels containing either placebo or UA at 250, 500 or 1,000 mg. Cohort 1A continued to Period 2 in which UA or placebo at 2,000 mg was administered using softgels. Additionally, cohorts 2A and 3A were orally administered UA at doses of 500 mg and 1,000 mg and placebo in food to investigate the impact of food on the safety and bioavailability of UA. In Part B new subjects were recruited and were randomized into three cohorts of twelve subjects to receive 250 mg softgels containing either UA or placebo doses at 250 mg, 500 mg and 1,000 mg once daily for 4 weeks. No major protocol deviations were reported, there were no drop-outs and all included subjects finished the study and were in the final analysis for both the parts of the study. Timelines are indicated next to cohorts numbers between brackets (*Wk*, Week).

Supplementary Figure 2



(a) Distribution of subjects based on their pre-dosing plasma levels of UA before the first administration of UA during the multiple ascending dose study (Part B). Data represent the relative frequency in percentage. Numbers indicated between brackets correspond to total plasma UA levels (UA + UA-sulfate + UA-glucuronide) in ng/ml (n=36). **(b-d)** Total plasma UA levels (UA + UA-sulfate + UA-glucuronide) at days 0, 7, 14, 28, 29, 31 and 32 during the 28 d multiple dosing at **(b)** 250 mg of UA (n=9 biologically independent samples); **(c)** 500 mg of UA (n=9 biologically independent samples); **(d)** 1,000 mg of UA (n=9 biologically independent samples). **(e)** UA was detectable in skeletal muscle tissue eight hours after a single oral dosing of 2,000 mg UA, primarily in its parent state (n=6 biologically independent samples).

Each time point was collected on a fasted state in the morning before the daily intake of UA. All data represent mean $\pm$ SEM.

Supplementary Table 1. Dose Escalation Scheme employed in Part A and B of the UA study

Part A: Single Ascending Dose

Week	Cohort/ Group	Period	UA or Placebo Dose (mg)
1	1A	Period 1	250
2	2A	Period 1	500
3	3A	Period 1	1,000
7	1A	Period 2	2,000
8	2A	Period 2	500 (in food product yoghurt)
9	3A	Period 2	1,000 (in food product yoghurt)

Part B: Multiple Ascending Dose

Cohort/ Group	Dose (mg)
1B (Week 1-4)	250
2B (Week 6-9)	500
3B (Week 10-13)	1,000

Supplementary Table 2. Subject demographics and baseline characteristics for the different groups enrolled into Part A of the clinical study

Characteristics	Part A: Single Dosing*			
	Placebo (n=6)	250 mg / 2,000 mg (n=6)	500 mg (n=6)	1,000 mg (n=6)
Age	70.8 ± 7.4	68.0 ± 4.6	68.3 ± 5.0	67.5 ± 4.7
Gender:				
- Male	3 (50.0%)	3 (50.0%)	3 (50.0%)	3 (50.0%)
- Female	3 (50.0%)	3 (50.0%)	3 (50.0%)	3 (50.0%)
BMI (kg/m ²)	24.63 ± 1.50	23.58 ± 2.86	23.42 ± 1.78	26.75 ± 3.46
Physical Activity (METs)	262.75 ± 207.35	330.75 ± 157.65	203.50 ± 98.26	214.50 ± 181.65
Ethnic origin: Caucasian	6 (100.0%)	6 (100.0%)	6 (100.0%)	6 (100.0%)

BMI: Body mass index; METs: metabolic equivalent.

*: Values reported are Mean ± SD

Supplementary Table 3. Subject demographics and baseline characteristics for the different groups enrolled into Part B of the clinical study

Characteristics	Part B: 4-week dosing*			
	Placebo (n=9)	250 mg (n=9)	500 mg (n=9)	1,000 mg (n=9)
Age	68.2 ± 5.3	67.2 ± 5.9	65.4 ± 3.7	64.8 ± 4.2
Gender:				
- Male	3 (33.3%)	3 (33.3%)	3 (33.3%)	3 (33.3%)
- Female	6 (66.7%)	6 (66.7%)	6 (66.7%)	6 (66.7%)
BMI (kg/m ²)	26.39 ± 2.90	24.06 ± 3.33	24.71 ± 3.36	24.91 ± 2.51
Physical Activity (METs)	194.67 ± 381.06	257.83 ± 227.05	205.33 ± 212.45	63.11 ± 166.02
Ethnic origin: Caucasian	9 (100.0%)	9 (100.0%)	9 (100.0%)	9 (100.0%)

BMI: Body mass index; METs: metabolic equivalent.

*: Values reported are Mean ± SD

Supplementary Table 4. Safety: Adverse Events by classification during the Part A of the clinical study

Treatment group	MedDRA term	Serious	Intensity	Relationship with treatment	Time between last intake and occurrence; duration	Outcome
Placebo	Myalgia	No	Moderate	Unrelated	Approximately 8 d after, lasted for 25 d	Recovered/resolved
	Bronchitis	No	Moderate	Unrelated	Before 2 nd period dosing, lasted 6 d	Recovered/resolved
250 / 2,000 mg	Myalgia	No	Moderate	Unrelated	Before 2 nd period dosing, due to biopsy procedure, lasted 7 h	Recovered/resolved
500 mg	Orthostatic hypotension	No	Mild	Unrelated	Approximately 1 d after, resolved immediately	Recovered/resolved
1,000 mg	Myalgia	No	Mild	Unlikely	Approximately 1 d after, lasted 15 h	Recovered/resolved
	Headache	No	Moderate	Unlikely	Approximately 1 d after, lasted 13 h	Recovered/resolved

Adverse events (AEs) were found to be mainly linked to the study procedure of muscle biopsy, consistent with studies employing this procedure. There were no serious AEs reported during the conduct of the study. In the single ascending dose Part A, there were six non-product related AEs and no reports of any abnormal clinical and laboratory findings up to 2,000 mg UA, which was the single highest dose tested. All AEs were considered either unrelated or unlikely to be related to UA intake. All events resolved during the course of the study.

Supplementary Table 5. Safety: Adverse Events by classification during the Part B of the clinical study

Treatment group	MedDRA term	Seriousness	Intensity	Relationship with Treatment	Time between last intake and occurrence; Duration	Outcome
Placebo	Contusion	No	Moderate	Unrelated	Approximately 9 d after; lasted 23 d	Recovered/resolved
	Headache	No	Moderate	Unrelated	Approximately 23 d after, lasted 30 min	Recovered/resolved
	Back pain	No	Moderate	Unrelated	Approximately 1 d after, lasted 15 h	Recovered/resolved
	Headache	No	Moderate	Unrelated	Approximately 3 d after, lasted 1 hour	Recovered/resolved
	Arthralgia	No	Moderate	Unrelated	Approximately 7 d, lasted for 7 d	Recovered/resolved
	Headache	No	Mild	Unlikely	Less than 1 d, lasted for 4 d	Recovered/resolved
	Limb discomfort	No	Moderate	Unlikely	Approximately 1 d after, lasted 3 d	Recovered/resolved
250 mg	Headache	No	Moderate	Unrelated	Approximately 20 d after, lasted 1 hour	Recovered/resolved
	Headache	No	Moderate	Unrelated	Approximately 27 d after, lasted 15 h	Recovered/resolved
	Post procedural hematoma	No	Moderate	Unrelated	Less than 1 d after, lasted 15 d	Recovered/resolved
		No	Mild	Unrelated	Approximately 7 d after, lasted 24 d	Recovered/resolved
	Diarrhea	No	Moderate	Unlikely	Approximately 27 d after, lasted 9 h	Recovered/resolved
	Procedural pain	No	Mild	Unrelated	Approximately 27 d after, lasted 2 d	Recovered/resolved
	Contusion	No	Moderate	Unrelated	Approximately 29 d after, lasted 14 d	Recovered/resolved
	Post procedural hematoma	No	Moderate	Unrelated	Approximately 6 d after, lasted 21 d	Recovered/resolved
	Procedural pain	No	Mild	Unrelated	Approximately 27 d after, lasted 7 d	Recovered/resolved
	Post procedural hematoma	No	Mild	Unrelated	Less than 1 d, lasted 31 d	Recovered/resolved
		No	Mild	Unrelated	Less than 1 d, lasted 31 d	Recovered/resolved
	Procedural pain	No	Mild	Unrelated	Less than 1 d, lasted 31 d	Recovered/resolved
		No	Mild	Unrelated	Approximately 27 d after, lasted 1 d	Recovered/resolved
	Post procedural hematoma	No	Mild	Unrelated	Approximately 6 d after, lasted for 17 d	Recovered/resolved
	Syncope	No	Moderate	Unrelated	Approximately 27 d after, lasted 2 min	Recovered/resolved
	Back pain	No	Moderate	Unrelated	Approximately 3 d after, lasted for 6 d	Recovered/resolved
500 mg						

Treatment group	MedDRA term	Seriousness	Intensity	Relationship with Treatment	Time between last intake and occurrence; Duration	Outcome
	Pain in extremity	No	Moderate	Unrelated	Approximately 17 d after, lasted 30 d	Recovered/resolved
	Back pain	No	Mild	Unlikely	Approximately 18 d after, lasted 3 d	Recovered/resolved
	Headache	No	Moderate	Unrelated	Approximately 1 d after, lasted 1 d	Recovered/resolved
		No	Moderate	Unrelated	Approximately 2 d after, lasted 30 min	Recovered/resolved
		No	Moderate	Unrelated	Approximately 4 d after, lasted 30 min	Recovered/resolved
	Thermal burn	No	Moderate	Unrelated	Approximately 24 d after, lasted 9 d	Recovered/resolved
1,000 mg	Post procedural hematoma	No	Mild	Unrelated	Approximately 1 d after, lasted 25 d	Recovered/resolved
	Myalgia	No	Mild	Possible	Approximately 2 d after, lasted 6 d	Recovered/resolved
	Post procedural hematoma	No	Mild	Unrelated	Approximately 2 d after, lasted 5 d	Recovered/resolved

Thirty-one non-serious AEs were recorded in the multiple ascending dosing Part B of the study, none of which were found related to the study product intake. There were no serious AEs reported during the conduct of the study. Seven AEs were recorded in the placebo group, nine in the 250 mg group, twelve in the 500 mg group and three in the 1,000 mg group. One event in the 1,000 mg dosing was considered possibly linked to UA intake (muscle pain in the calf muscle), however, creatine kinase levels were normal and the casualty could not be linked as the event started on the third day of product intake and resolved by itself in the next few days. All the other events were determined to be either unrelated or unlikely to be related to the intervention. All AEs resolved during the course of the study.