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Metabolome analysis of effect of aspirin on *Drosophila* lifespan extension

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ABSTRACT

Effective approaches for drug development involve the repurposing of existing drugs which are already approved by the FDA. Aspirin has been shown to have many health benefits since its discovery as a nonsteroidal anti-inflammatory drug (NSAID) to treat pain and inflammation. Recent experiments demonstrated the longevity effects of aspirin in *Drosophila*, but its mechanism remains to be explored. In order to elucidate the effects of drug on metabolism, we carried out the metabolic analysis of aspirin-treated flies. The results identified 404 active metabolites in addition to the extended lifespan and improved healthspan in fly. There were 28 metabolites having significant changes between aspirin-treated group and the control group, out of which 22 compounds were found to have detailed information. These compounds are reported to have important functions in energy metabolism, amino sugar metabolism, and urea metabolism, indicating that aspirin might be playing positive roles in the fly's lifespan and healthspan improvement. Because of the conservation of major longevity pathways and mechanisms in different species, the health benefits of aspirin administration could be extended to other animals and humans as well.

1. Introduction

One of the most efficient strategies for development of anti-ageing drugs is to screen the already approved drugs, which have not been recognized for anti-ageing potential yet. In the past decade, many studies found that several drugs, such as rapamycin, metformin and trametinib, can extend the lifespan and reduce age-related diseases in model organisms (Powers et al., 2006, Harrison et al., 2009, Bjedov et al., 2010). One of the drugs, aspirin (acetylsalicylic acid) (Fig. 1A), a prototypic cyclooxygenase inhibitor, is the most widely used medicine for treating several medical conditions such as pain, fever and inflammation. Along with exerting the anti-inflammatory effects, it was also reported that long-term use of aspirin could improve many health aspects. Several epidemiological, preclinical, and clinical studies showed that the chronic use of aspirin could significantly reduce the risk of numerous cancers, such as colorectal, lung, and breast cancer (Thun et al., 1991, Schreinemachers and Everson, 1994, Luciani et al., 2007). It regulates the activity of a number of pro-inflammatory signaling molecules, such as TGF- β (Redondo et al., 2003) and PDGF (Rozing et al., 2009). High-dose aspirin was reported to improve glucose metabolism and reduce fatty acid levels in patients with type-2 diabetes (Hundal et al., 2002). In addition, aspirin could also inhibit the atherosclerotic plaque formation (Mehta et al., 2004), and control the development of aggregate-related proteotoxicity (Gasparini et al., 2004). Epidemiological studies showed the protective effects of aspirin during neurodegenerative diseases, including Parkinson's, Alzheimer's, and Huntington's Diseases (Esposito et al., 2007). Aspirin also exhibited extension of the lifespan in mice (Strong et al., 2008), worm (Wan et al., 2013) and fruitfly (Danilov et al., 2015).

Metabolomics is a branch of chemical biology that profiles metabolites in cells and organisms, using liquid chromatography (LC)-mass spectrometry (MS) technique, which is an important tool to study the molecules which are < 1.5 kDa. LC-MS can analyze metabolic regulation along with proteomes and transcriptomes. Recently, it was reported that, among 133 compounds identified in human blood, 101 are also found in the fission yeast, *S pombe* (Chaleckis et al., 2014), implying that many metabolites might be evolutionarily conserved. Metabolite profiling is an emerging method which aims to characterize a large number of small molecules in biological systems and identify proximal markers of biological activity (Kristal and Shurubor, 2005, Kotze et al., 2013). Recently, metabolite profiling was used to explore metabolic signatures of ageing in young and old mice (Houtkooper et al., 2010). Quantitative measurements of an array of metabolites in individuals can give insight of health and disease states as well as the effects of nutrition, drugs, and stress. Moreover, comprehensive information about

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individual variation in metabolites could impact the future of medical science (Goodacre et al., 2004, Nicholson and Lindon, 2008).

In order to explore the change in metabolome after administration of aspirin on *Drosophila* longevity, the liquid chromatography mass spectrometry (GC–MS)-based metabolite profiling was used to explore drug specific metabolite patterns in *Drosophila*. We showed that aspirin can extend the lifespan and heath span of *Drosophila*. The metabolomics analysis revealed 28 significantly different metabolites between drug-treated and control groups. These compounds have important roles in energy metabolism, sugar metabolism, and the urea cycle. In addition, some metabolites related to inflammation showed significant change. Taken together, results suggested that anti-ageing roles of aspirin in *Drosophila* were mainly involved in energy reduction, oxidation resistance and anti-inflammation.

2. Materials and methods

2.1. Fly foods

Dietary restriction medium (1xSYA) contained 100 g/l yeast (MP Biomedicals, USA), 50 g/l sucrose (Tate & Lyle, UK), 15 g/l agar (Sigma-Aldrich, UK), and 30 ml/l Nipagin (Chemlink Specialities, UK) and 3 ml/l propionic acid (Sigma-Aldrich, UK). The fully-fed medium (2xSYA) was prepared in the same way, except that it contained 200 g/l yeast. The method of food preparation is described in our previous paper (Yang et al., 2016). Different concentrations of aspirin (Sigma-Aldrich, UK) (0.2 μM; 0.5 μM; 1 μM; 5 μM; 10 μM; 50 μM; 100 μM; 400 μM; 1000 μM) were dissolved in Mili-Q water and added to SYA food respectively.

2.2. Fly lifespan

All procedures were followed as per our previously published work (Fan et al., 2015, Yang et al., 2016). All experiments were conducted at 25 °C on a 12 h light:12 h dark cycle, at a constant humidity of 65%. Flies were reared at a standard larval density of ~300 flies per bottle, and all experimental adults were collected within 12 hour period after eclosion. Flies were allowed to mate for 48 h after eclosion before the experimental females were separated out under CO_2 anaesthesia. Females were then randomly allocated to the experimental food treatments and housed in plastic vials containing food at a density of 10 flies per vial, with 10 vials per condition ($n = 100$). Flies were transferred to a fresh food source 3 times per week, during which any deaths and censors were recorded. Lifetime fecundity was measured as the cumulative total for days 7, 14, 21 and 28 of the mean number of eggs laid per female fly over each 24-hour period. Eggs in each vial were counted by eye using a light microscope after 18–24 hour exposure to flies. Lifespan experiment was repeated 3 times.

2.3. Paraquat, DDT and H_2O_2 stress

Mated female flies were kept on the experimental food types for 7 days before being transferred to the stress conditions. The orally administered stressors were made up as follows: 1xSYA containing 20 mM paraquat (Sigma-Aldrich), 1.5% agar medium containing 5% H_2O_2 (Sigma-Aldrich) and 50 g/l sucrose, or plain 1.5% agar medium for the starvation experiment (Wu et al., 2016).

2.4. Heat shock

Mated female flies were kept on the experimental food types for 7 days before being transferred to the stress conditions. Experimental flies were transferred one each into dry empty 2 ml glass vials, plugged with cotton wool and placed into a water bath set at 39 °C. The time taken for each fly to fall onto its back and stop twitching (knockout) was recorded (Wu et al., 2016).

2.5. Triacylglyceride (TAG) measurement

Mated female flies were kept on the experimental food types for 7 days before collection. Measurement of TAG level was done by using the TAG detection kits (Sigma-Aldrich).

2.6. AMP:ATP ratio

EnzyLight™ ADP/ATP ratio Assay Kit (ELDT-048) was used to measure AMP/ATP ratio according to manufacturer's manual. Each sample was measured in triplicates.

2.7. Metabolomics analysis

Metabolomics service was provided by Biotree Biotech Co., Ltd., Shanghai, China.

2.7.1. Extraction of metabolites

A total of 50 mg sample was taken into 2 ml EP tubes, and extracted with 0.4 ml extraction liquid (V methanol:V chloroform = 3:1), followed by addition of 20 μl of L-2-Chlorophenylalanine (1 mg/ml stock in dH_2O) as internal standard and mixed by vortexing for 30s. The sample was homogenized in ball mill for 6 min at 45 Hz and centrifuged for 15 min at 12000 rpm at 4 °C. The supernatant (0.35 ml) was transferred into a fresh 2 ml GC–MS glass vial.

2.7.2. Metabolites derivation

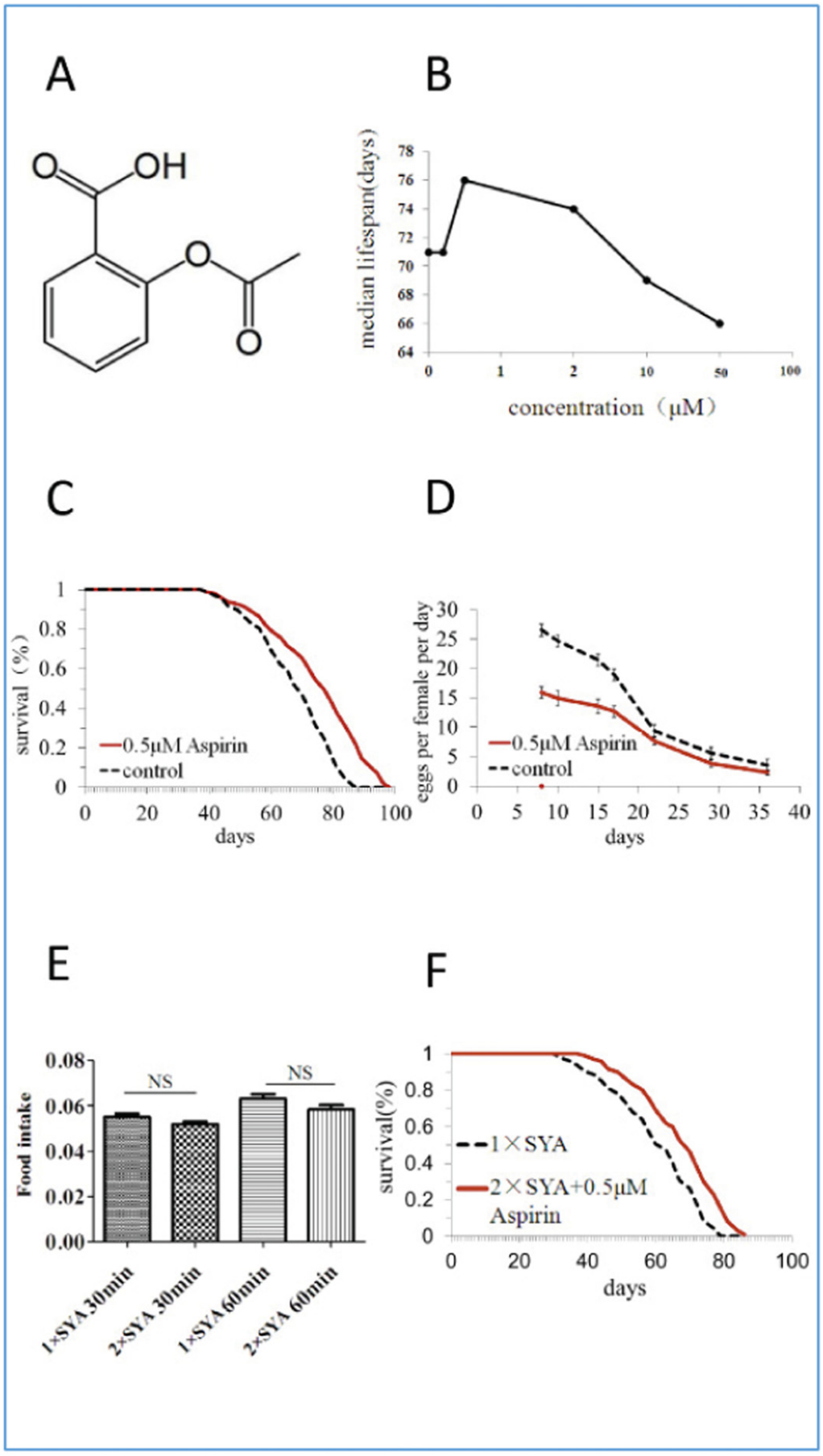
The following protocol was used to derive the metabolites. The samples were dried in a vacuum concentrator without heating and 80 μl of Methoxyamination hydrochloride (20 mg/ml in pyridine) was added in the dried extract. The samples were incubated for 30 min at 80 °C and 100 μl of the BSTFA regent was added (1% TMCS, v/v) to the sample aliquots followed by incubation at 70 °C for 2 h. Sample aliquots were mixed well for GC–MS analysis.

2.7.3. Test

GC/TOFMS analysis was performed using an Agilent 7890gas chromatograph system coupled with a Pegasus HT time-of-flight mass spectrometer. The system utilized a DB-5MS capillary column coated with 5% diphenyl cross-linked with 95% dimethylpolysiloxane (30 m × 250 μm inner diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). A 1 μl aliquot of the analyte was injected in splitless mode. Helium was used as the carrier gas, the front inlet purge flow was 3 ml min^{-1}, and the gas flow rate through the column was 1 ml min^{-1}. The initial temperature was kept at 50 °C for 1 min, then raised to 290 °C at a rate of 10 °C min^{-1}, then kept for 5 min at 290 °C. The injection, transfer line, and ion source temperatures were 280, 270, and 220 °C, respectively. The energy was −70 eV in electron impact mode. The mass spectrometry data were acquired in full-scan mode with the m/z range of 50–500 at a rate of 20 spectra per second after a solvent delay of 366 s.

2.7.4. Data processing

Chroma TOF4.3X software of LECO Corporation and LECO-Fiehn Rtx5 database were used for raw peaks extraction, the data baselines filtering and calibration of the baseline, peak alignment, deconvolution analysis, peak identification and integration of the peak area (Kind et al., 2009). The RI (retention time index) method was used in the peak identification, and the RI tolerance was 5000.


A
O
OH
O
O
B
median lifespan(days)
78
76
74
72
70
68
66
64
0
1
2
10
50
100
concentration（μM）
C
survival（%）
1
0.8
0.6
0.4
0.2
0
0.5μM Aspirin
control
0
20
40
60
80
100
days
D
eggs per female per day
30
25
20
15
10
5
0
0.5μM Aspirin
control
0
5
10
15
20
25
30
35
40
days
E
Food intake
0.08
0.06
0.04
0.02
0.00
NS
NS
1×SYA 30min
2×SYA 30min
1×SYA 60min
2×SYA 60min
F
survival(%)
1
0.8
0.6
0.4
0.2
0
1×SYA
2×SYA+0.5μM
Aspirin
0
20
40
60
80
100
days

3. Results

3.1. Aspirin extends Drosophila lifespan

We first investigated the effects of aspirin on *Drosophila* lifespan when administered at different concentrations. Higher concentrations of drug (100 μM; 400 μM; 1000 μM) on dietary restriction (DR) food (1xSYA) caused short median lifespan (Supplementary Fig. S1). The lower concentrations of aspirin (0.2 μM; 0.5 μM; 1 μM; 5 μM; 10 μM; 50 μM) on DR food were further tested, and dose-response analysis showed that fruit fly raised at 0.5 μM of aspirin displayed the longest median lifespan extension by up to 12.5% (Fig. 1B). We chose 0.5 μM as the best dose on 1xSYA food for the subsequent experiments. Aspirin at a concentration of 0.5 μM led to a significant lifespan extension as shown in Fig. 1C. Also aspirin reduced female fecundity (Fig. 1D), which was consistent with previous report (Danilov et al., 2015).

To evaluate if the lifespan benefits of aspirin were indirectly caused by the drug being aversive and repellant and causing the flies to self-impose dietary restriction (DR), we assessed the effect of drug on food ingestion. During 30 min and 60 min feeding assay, aspirin showed no effect on food consumption (Fig. 1E). We also tested whether aspirin can extend *Drosophila* lifespan in a fully fed medium(2xSYA)and the result indicated that aspirin can extend fly lifespan under fully-fed condition as well (Fig. 1F).

3.2. Aspirin improved Drosophila healthspan

To understand the causal mechanisms of increased lifespan with the drug, we then assessed whether aspirin could improve fly health conditions during the ageing. When administered with a toxic dose of H_2O_2 and paraquat, the aspirin-treated flies displayed a significant increase in the ability to resist oxidative stresses (Fig. 2A–B). Also upon testing the response of flies to heat shock stress, we found that control flies were significantly less resistant than aspirin treated flies (Fig. 2C). Furthermore, we found that aspirin-treated flies showed greater resistance to starvation than control flies (Fig. 2D), suggesting a possible mechanistic relationship between longevity and ability to resist environmental stresses.

Flies with aspirin treatment displayed significantly higher levels of triacylglyceride (TAG) than control flies (Fig. 2E), demonstrating that lifespan extension is associated with increased TAG levels. On the other hand, we examined the expression levels of the ADP/ATP ratio, and found that the ADP/ATP ratio was significantly increased in aspirin-treated group compared to control group (Fig. 2F), indicating the possibility that the lower levels of energy promoted the phosphorylation of AMPK by LKB1, and subsequently stimulates Foxo and inhibits TOR signaling to induce downstream effects (Inoki et al., 2012).

3.3. Effect of aspirin on Drosophila metabolome

To further characterize the aspirin-treated flies at metabolomics level, the gas chromatography mass spectrometry (GC–MS)-based metabolite profiling was used. We collected 6 samples in triplicates from aspirin-treated and control groups at the 7 day of age. By OPLS-DA analysis, after eliminating the batch differences within the group, the aspirin-treated group and the control group can be well separated (Fig. 3A), suggesting significant differences in metabolites between two groups. Two control samples displayed identical profiles that's why only five spots can be seen on PCA score plot instead of six. The potential differences in metabolism can be seen in the loading plot (Fig. 3B).

We identified 404 metabolites from 685 valid peaks from chromatograms in aspirin treated fly group. Twenty-eight metabolites were significantly altered between drug-treated and control groups, of which 22 compounds can be traced back with names (Table 1, Supplementary Table S2). The variable importance in the projection (VIP) statistic of the first principal component of OPLS-DA model (threshold > 1), together with the p-value (threshold < 0.05), were used for selecting significant variables responsible for group separation. Six kinds of compounds were significantly down-regulated in aspirin-treated flies, which were succinic acid, proline, aspartic acid, norvaline, tartronic acid and norleucine. These were related to reduction of the energy usage and to bring down the inflammation. The remaining 22 compounds were significantly up-regulated and involved in purine metabolism, urea cycle, sugar and alcohol metabolic pathway.

The six up-regulated unknown compounds could not be identified with names due to unavailability in our metabolites library. To cluster those unknown metabolites, we performed the hierarchical clustering analysis across the metabolites and samples by using Spearman distance metric (Qizheng et al., 2002) and average linkage (Eisen et al., 1998) methods. The clustering analysis allowed grouping of certain groups of chemicals showing biochemical relevance with a co-regulatory expression pattern in accordance with the different genotypes. The results showed that unknown No.6 compound was clustered with octadecanol and unknown No.2 was classified with gluconic lactone1, which indicated that they may be involved in amino sugar metabolism. The unknown No.5, 1 and 4 were classified with sorbitol. The unknown No.3 compound was placed between cellobiose and methyl palmitoleate (Fig. 4). The functions of these unknown metabolites are presumed to be similar to their neighbors.

To further analyze the metabolic pathway of these 28 compounds identified in our study, we drew a metabolome view map (Fig. 5) based on the mataboanalyst website (Aittokallio and Schwikowski, 2006), which revealed that the enriched pathways ($p < 0.05$) were of alanine, aspartate and glutamate metabolism, arginine and proline metabolism, glycorophospolipid metabolism, purine metabolism, fructose and mannose metabolism and galactose metabolism. The metabolites were mainly involved in amino acid metabolism and amino sugar metabolism.

Based on the results of common key different metabolic pathways from aspirin-treated and control groups, we manually integrated key differential metabolic pathways (Fig. 6). Three key metabolic pathways were involved in amino sugar metabolism, citrate cycle and urea cycle with 11 significantly different metabolites from our findings.

4. Discussion

We demonstrated that aspirin can increase the *Drosophila* longevity and enhance the fly healthspan. We also identified

Fig. 1. Effect of aspirin on *Drosophila* lifespan. (A) Chemical structure of aspirin. (B) The effect of different concentrations of aspirin on *Drosophila* median lifespan. (C) 0.5 μM of aspirin treatment extends the lifespan of w^{Dah} females, compared to control flies on 1XSYA food ($p < 0.05$, *t*-test). (D) The influence of 0.5 μM aspirin on daily average egg production in *Drosophila* on 1XSYA food ($p < 0.05$, *t*-test). (E) Aspirin has no effect on the food consumption between control and drug-treated group ($p > 0.05$, *t*-test). (F) 0.5 μM of aspirin extends *Drosophila* lifespan under fully-fed condition, 2XSYA food ($p < 0.05$, *t*-test).

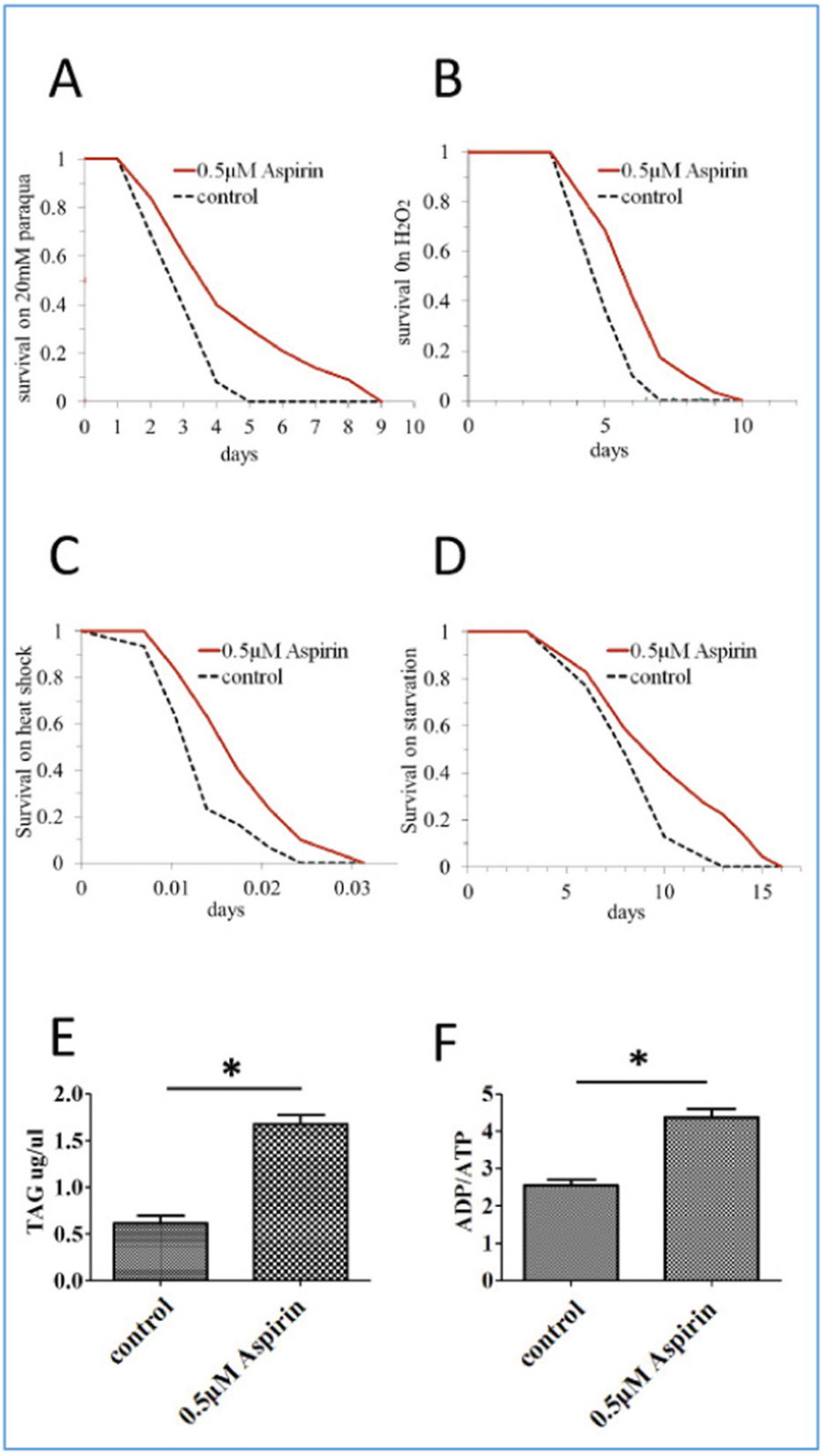


Fig. 2. The effects of aspirin on *Drosophila* against oxidative stress. (A) Aspirin-treated flies showed an increased resistance to 20 mM paraquat toxicity compared to control flies ($p < 0.05$; $n = 150$ flies per condition). (B) Aspirin-treated flies showed an increased resistance to 5% hydrogen peroxide toxicity compared to control flies ($p < 0.05$; $n = 150$ flies per condition). (C) Aspirin-treated flies were significantly more resistant to a 39 °C heat stress compared to control flies ($p < 0.005$; $n = 40$ flies per condition). (D) Aspirin significantly increased the tolerance to starvation in treated flies. ($p < 0.001$; $n = 100$ flies per condition). (E) Aspirin increased the triacylglyceride (TAG) levels in drug-treated flies ($p < 0.05$; $n = 6$; *t*-test). (F) Aspirin treatment increased the ratio of ADP/ATP ($p < 0.05$; $n = 6$; *t*-test).

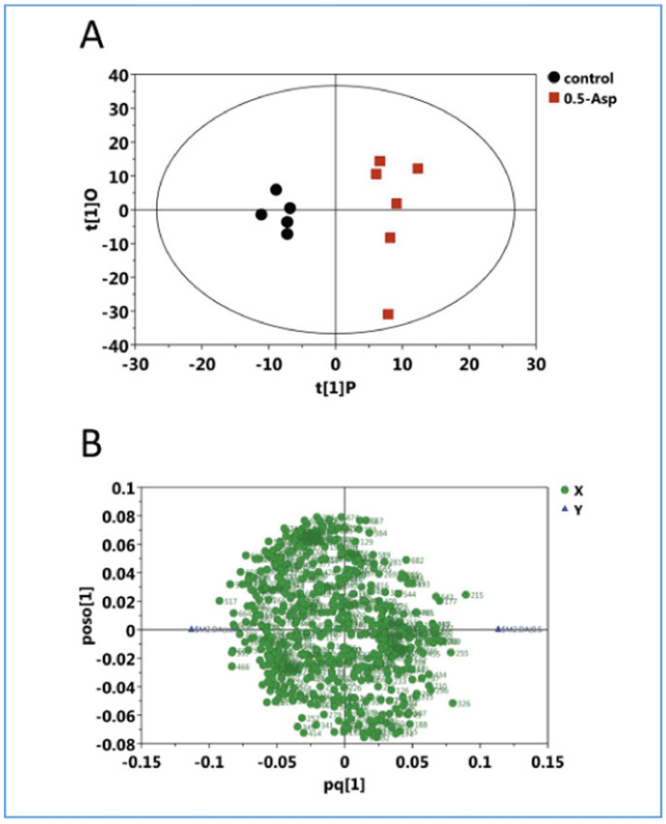


Fig. 3. PCA score plot derived from the GC–MS analysis of *Drosophila*. (A) Score plot of OPLS-DA model obtained from control and aspirin-treated flies. (B) Loading plot of OPLS-DA model obtained from control and aspirin-treated flies. t[1]P: the first principal component scores. t[1]O: the second principal component scores. poso[1]: difference within group. pq[1]: difference between groups.

Table 1
Differential metabolites in aspirin-treated flies.

Metabolites	VIP	p-Value
Succinic acid	2.32775	0.002811794
Proline	1.88705	0.023129304
Aspartic acid 1	2.06961	0.030297015
Norvaline	1.81997	0.048982918
Tartronic acid	2.08374	0.006720147
Norleucine 1	1.70061	0.0341111850
Octadecanol	1.89105	0.031314226
Sophorose 2	2.0094	0.034491354
Cellobiose 1	1.20806	0.008989200
Xanthine	2.16172	0.010641781
Palatinitol 2	1.78273	0.047752741
Mannose 1	1.05945	0.032539421
Saccharic acid	1.83647	0.028447702
Uric acid	1.37638	0.0369757020
Galactinol 1	1.71146	0.040328494
Methyl Palmitoleate	1.58619	0.048002695
2-Deoxy-D-galactose 2	2.16424	0.016664058
Prunin degr. Prod. 1	1.95172	0.002274337
Sorbitol	2.08374	0.0067201470
Gluconic lactone 1	2.402	0.0033779560
Methyl *trans*-cinnamate	1.86371	0.032485605
Galactose	2.12966	0.021661519
Unknown 1	1.89368	0.037887952
Unknown 2	1.60691	0.037049647
Unknown 3	1.98826	0.008434099
Unknown 4	2.12936	0.025075452
Unknown 5	1.72146	0.019653486
Unknown 6	2.13441	0.004962248

Metabolites in blue fonts show down-regulation whereas in red fonts indicate up-regulation. VIP value means the differences caused by the difference between the two groups of weights. p-Value, $p < 0.05$.

six compounds which were significantly down-regulated and twenty two compounds which were up-regulated significantly in aspirin-treated female flies. These compounds had important functions with energy metabolism, urea metabolism, and inflammation, indicating multiple roles of aspirin in antioxidant, anti-inflammatory.

Aspirin improved stress tolerance to heat, starvation, paraquat and H_2O_2, suggesting an association of healthspan and lifespan. It is a well-known fact that long-lived animals often have an associated increase in the ability to resist environmental stresses and this is presumed to reflect a general increase in their health (Wullschleger et al., 2006). Genetic mechanism underlying lifespan extension by aspirin were driven by AMPK and DAF-16 in worm (Wan et al., 2013) and also direct activation of AMPK by aspirin had some beneficial effects in mouse and human (Stevenson et al., 2012). More recently, the involvement of Pkh2-ypk1-lem3-tat2 pathway is reported in *Drosophila* lifespan extension (Danilov et al., 2015). However, metabolite profiles of aspirin-treated flies have not been generated yet, with an emphasis on its lifespan extension effects. Although the results have not shown the direct involvement of the relevant metabolites in above pathways at metabolic level, yet the current outcomes may open a new window for mechanical analysis of anti-ageing drugs.

Our results in metabolomics analysis showed that 28 metabolites have been significantly altered between drug-treated and control groups. Regarding down-regulated metabolites, the succinic acid participated in the Krebs cycle to protect the body against the hyperbaric oxygen, shock and convulsions. Decreased metabolism of succinic acid upon aspirin treatment had the same effect as significantly lowered level of the succinic acid under the condition of caloric restriction (Laye et al., 2015). In addition, aspirin also increased the ratio of ADP/ATP. Together, these findings indicated that aspirin reduced the energy levels in *Drosophila*. Proline has been proposed to be a potential endogenous excitotoxin (Henzi et al., 1992), therefore, the lower level of proline after addition of drug could benefit the neural cells. Aspirin also reduced the metabolism of aspartic acid and increased mannose level at the same time. There is a significant association between aspartic acid and inflammation (Kizawa et al., 2005). Mannose can combine with macrophage surface receptor, inhibit macrophage inflammation (Iwata et al., 2004) and provide resistance to bacterial infections (King et al., 2000). It also can increase and promote the wound healing ability, and exert anti-inflammatory effects (Kossi et al., 1999). Aspirin reduced the metabolism of aspartic acid and increased the metabolism of mannose, showing a positive effect on inflammation.

We identified 22 up-regulated metabolites with possible functions in ageing. In elderly individuals, antioxidants metabolic levels are significantly lower than younger people (Chaleckis et al., 2016). We found

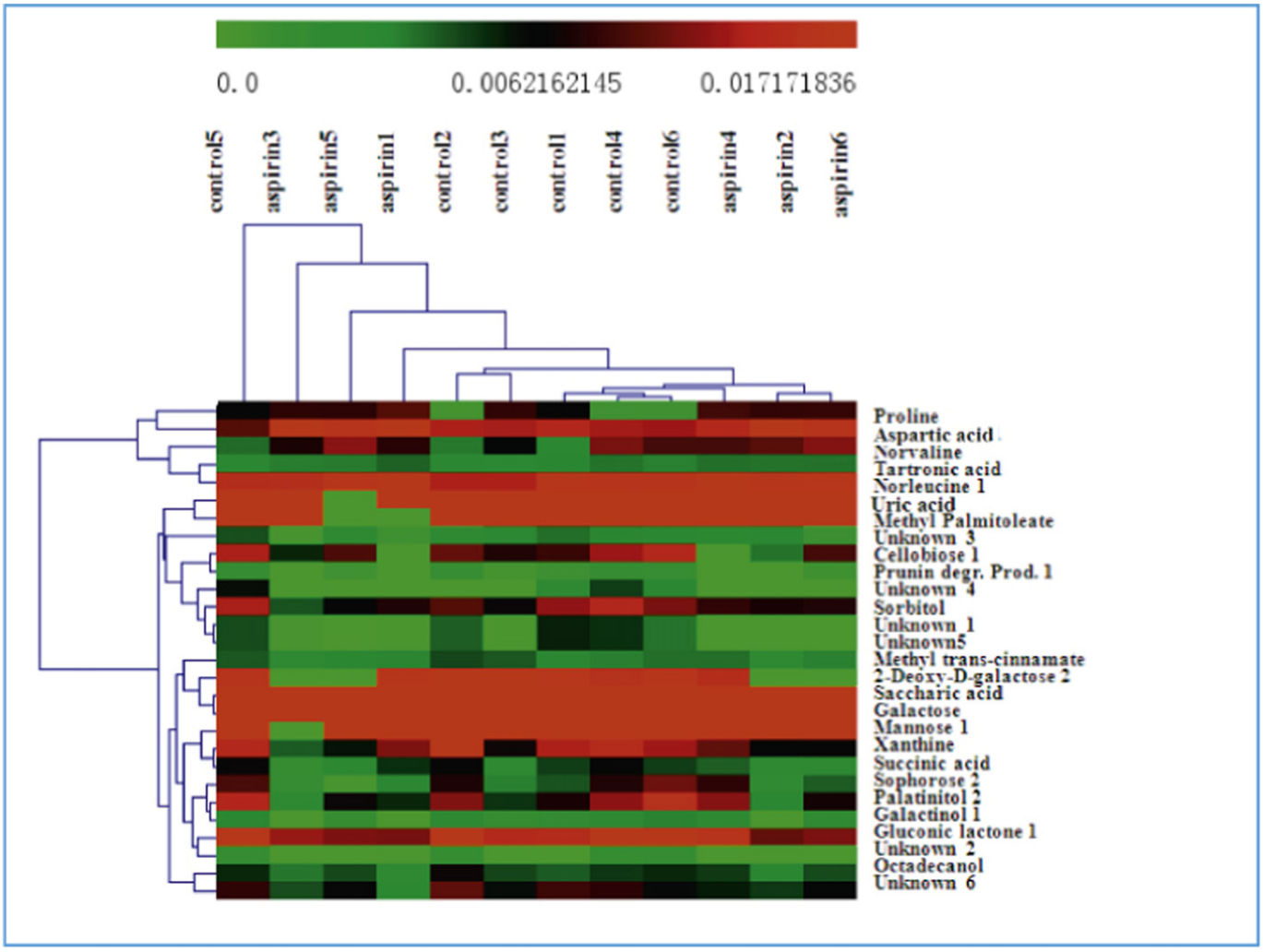


Fig. 4. The heat map of hierarchical clustering analysis. Clustering analysis was performed across the metabolites and samples by using Spearman rank correlation and average linkage methods. Each column and each row represent a fly sample and an individual metabolite, respectively.

that antioxidants, octadecanol and sophorose (Loewenberg and Chapman, 1977, Kachhy et al., 1972) metabolic levels were increased after adding aspirin, and this might play a great role against oxidation during ageing.

A gradual decline in the urea metabolism ability has been documented, during ageing (Chaleckis et al., 2016) and we found that two metabolites uric acid and xanthine involved in the urea cycle (George et al., 2011) were up-regulated. Uric acid is the final product of urea cycle and xanthine can be converted to uric acid by the action of the xanthine oxidase enzyme. So aspirin could promote the urea cycle and consequently improve the lifespan and heath span in *Drosophila*.

Previous reports showed that long-term intake of palatinito can reduce the hyperglycemia disease mortality in humans and treat diabetes (Warshaw and Powers, 1999, Livesey, 2003). Palatinito can decrease hemoglobin concentration, a deterministic sign of death in diabetes patients (Stratton et al., 2000). We found that palatinito metabolites were up-regulated, indicating that aspirin may exert its benefits through regulating the diabetes state.

Our results showed that metabolites relevant to sugar metabolism, such as Cellobiose, Galactose, Sorbitol, Mannose, 2-Deoxy-D-galactose, and Gluconic lactone, were up-regulated after the administration of aspirin. It implied that these kinds of sugar metabolites would be beneficial to animal health since it has been reported that glucose enrichment plays diverse protective roles against proteotoxicity (Tauffenberger et al., 2012).

Taken together, our metabolomic analysis suggested that aspirin can reduce the energy level, promote the urea cycle, increase the oxidation resistance and stimulate amino sugar metabolism, and consequently led to lifespan and healthspan extension in *Drosophila*. Results also suggested that administration of the up-regulated metabolites could be an effective approach to anti-ageing.

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Conflict of interest

The authors declare no competing financial interests.

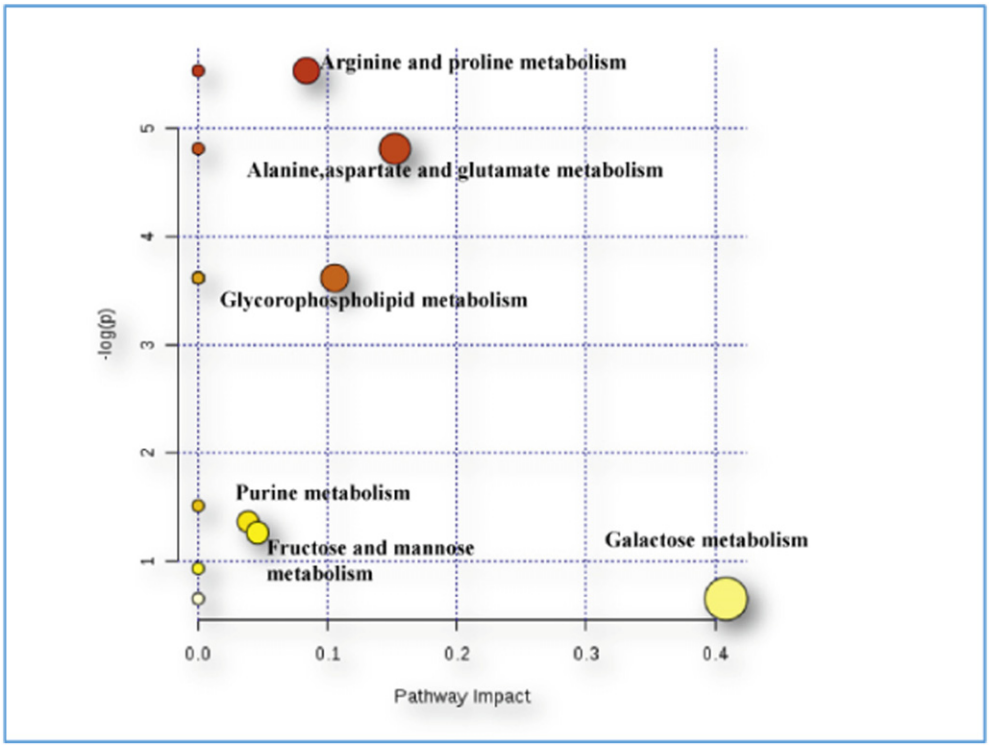

Fig. 5. Metabolome view map of the common metabolites identified in control and aspirin-treated group. The x-axis represents the pathway impact, and y-axis represents the pathway enrichment. Larger sizes and darker colors represent higher pathway enrichment and higher pathway impact values respectively.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.exger.2017.04.010.

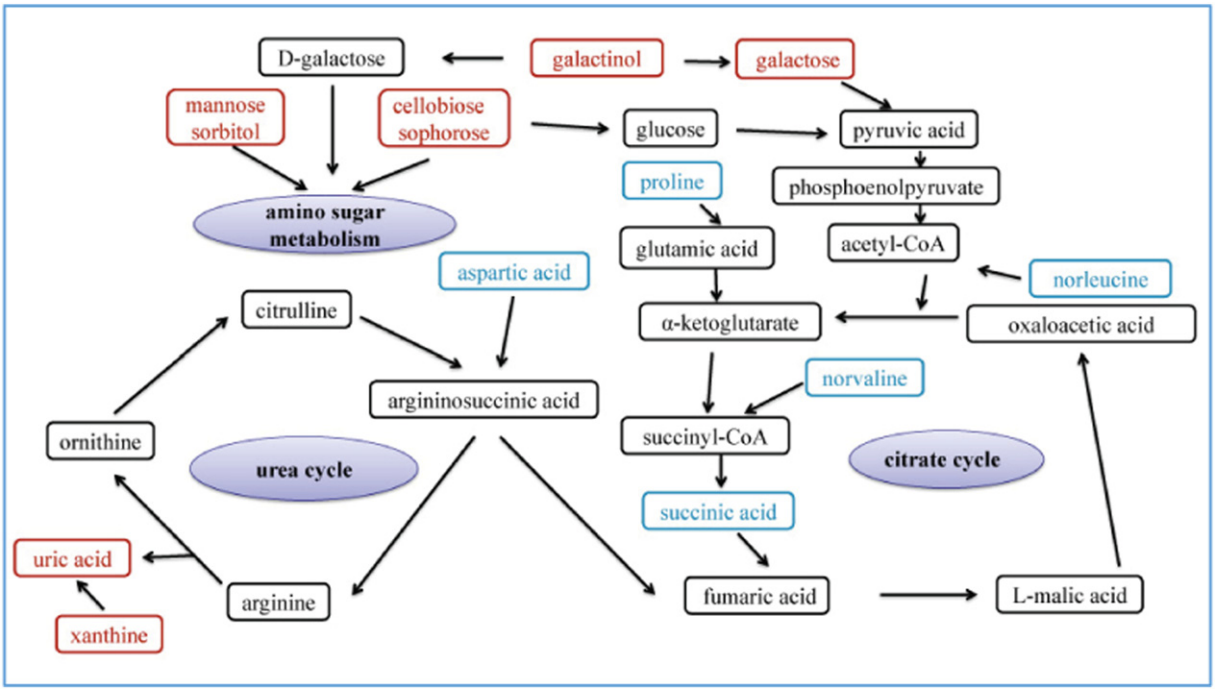

Fig. 6. The key metabolic pathways from aspirin-treated and control samples. The map illustrates significantly different metabolites in the two groups and the key metabolic pathways including amino sugar metabolism, citrate cycle and urea cycle. Blue = down-regulated metabolites, red = up-regulated metabolites.

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