Received: 2 December 2020 | Revised: 15 January 2021 | Accepted: 1 February 2021

DOI: 10.1111/bph.15410

RESEARCH PAPER

Sodium rutin extends lifespan and health span in mice including positive impacts on liver health

Shuoshuo Li[1] | Jun Li[2] | Ruiyuan Pan[1] | Jinbo Cheng[1,3] | Qinghua Cui[4] | Jianxin Chen[5] | Zengqiang Yuan[1,2,3]

[1]The Brain Science Center, Beijing Institute of Basic Medical Sciences, Beijing, China

[2]Beijing Institute for Brain Disorders, Capital Medical University, Beijing, China

[3]Center on Translational Neuroscience, College of Life & Environmental Science, Minzu University of China, Beijing, China

[4]Department of Biomedical Informatics, Center for Noncoding RNA Medicine, School of Basic Medical Sciences, Peking University, Beijing, China

[5]School of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China

Correspondence
Jianxin Chen, PhD, Beijing University of Chinese Medicine, No. 11 Bei San Huan Dong Lu, Chaoyang District, Beijing 100029, China.
Email: cjx@bucm.edu.cn

Zengqiang Yuan, PhD, The Brain Science Center, Beijing Institute of Basic Medical Sciences, No. 27 Taiping Road, Haidian District, Beijing 100850, China.
Email: zqyuan@bmi.ac.cn; zyuan620@yahoo.com

Funding information
Beijing Natural Science Foundation, Grant/Award Number: 7161009; National Major Project of Support Program, Grant/Award Number: 2019-JCJQ-ZD-195; National Natural Science Foundation of China, Grant/Award Numbers: 81630026, 81930029

Background and Purpose: Ageing is associated with progressive metabolic dysregulation. Rutin is a metabolic regulator with a poor solubility. Using soluble sodium rutin we investigating the effect and mechanisms of rutin in ageing process.

Experimental Approach: Wild type male mice were treated with or without sodium rutin (0.2 mg·ml^{-1} in drinking water from 8-month-old until end of life. Kaplan–Meier survival curve was used for lifespan assay, ageing-related histopathology analysis and metabolic analysis were performed to determine the effects of chronic sodium rutin on the longevity. Serological test, liver tissue metabolomics and transcriptomics were used for liver function assay. SiRNA knockdown Angptl8 and autophagy flux assay in HepG2 cell lines explored the mechanism through which sodium rutin might impact the function of hepatocyte.

Key Results: Sodium rutin treatment extends the lifespan of mice by 10%. Sodium rutin supplementation alleviates ageing-related pathological changes and promotes behaviour performance in ageing mice. Sodium rutin supplementation altered the whole-body metabolism in mice, which exhibited increased energy expenditure and lower respiratory quotient. Transcriptomics analysis showed that Sodium rutin affected the expression of metabolic genes. Metabolomics analysis showed that Sodium rutin reduced liver steatosis through increased lipid β-oxidation. Sodium rutin treatment increased the autophagy level both in vivo and in vitro. The inhibition of autophagy partially abolished the sodium rutin-mediated effect on lipolysis in HepG2 cells.

Conclusion and Implications: Sodium rutin treatment extends the lifespan and health span of mice with beneficial effects on metabolism, which were achieved by enhancing the autophagy activity in hepatocytes.

KEYWORDS
ageing, autophagy, longevity, metabolism, metabolomics, sodium rutin

1 | INTRODUCTION

Ageing is a risk factor for a range of chronic diseases, including cardiovascular diseases, neurodegenerative diseases and cancer. With the deepening understanding of the ageing process, the importance of metabolic alterations, including insulin resistance, hypertension and dyslipidaemia, during ageing and in the development of ageing-related diseases, has been gradually discovered. Metabolomics studies confirmed that disordered metabolic pathways, including decreased glucose metabolism and altered lipid metabolism, were closely associated

Abbreviations: AAV, adeno-associated virus; RQ, respiratory quotient; TG, triglyceride.

with ageing pathologies and lifespan in mice (Bárcena et al., 2019; Uchitomi et al., 2019). Therefore, the maintenance of metabolic health may lead to anti-ageing and lifespan-extending effects. The liver plays a pivotal role in metabolic regulation and several studies have identified a series of alterations in ageing livers, including vacuolation, steatosis, fibrosis and increased senescence-associated β-gal expression (Schmucker, 1998, 2005). These pathological changes increased the risk of ageing-related metabolic diseases, such as diabetes and fatty liver disease. Ageing indeed increases the risk and severity of liver diseases including non-alcoholic fatty liver disease and hepatocellular carcinoma (Furman et al., 2019; Ogrodnik et al., 2017; Papatheodoridi et al., 2020; Ramirez et al., 2017). Young individuals have a better prognosis of liver diseases. Lifespan-extending strategies, such as dietary restriction and rapamycin treatment, have been reported to improve liver metabolism, indicating that liver health plays an important role in the anti-ageing process (Fontana & Partridge, 2015; Martin-Montalvo et al., 2013).

Rutin is a natural flavonoid compound and widely distributed in the plant kingdom, especially in vegetables and fruits. Rutin has attracted attention over the past decades due to its multiple functions, including anti-oxidative, anti-inflammatory, anti-tumor, anti-diabetic, neuroprotective and cardiovascular-protective effects (Alonso et al., 2014; Chua, 2013; Mahmoud, 2012). A recent study has shown that rutin has a metabolic modulator ameliorates obesity in mice though brown fat activation (Lin et al., 2017). The poor solubility of rutin in aqueous media (approximately 0.125 $g{\cdot}L^{-1}$ at room temperature) hampers the thorough investigation of its biological mechanisms and its clinical application (Samsonowicz, Kamińska, Kalinowska, & Lewandowski, 2015). In our previous study, we successfully prepared the sodium salt of rutin (hereafter called sodium rutin; Figure S1A), exhibiting improved water solubility and bioavailability (Pan et al., 2019).

In the present study, we aimed to determine the effects of chronic sodium rutin consumption on the longevity and health span of male C57BL6 mice. For this reason, we performed behaviour tests, histological analysis and metabolomics analysis. We demonstrated that sodium rutin significantly extended the lifespan and health span of mice through maintaining liver health by autophagy. The present findings render sodium rutin a promising candidate for clinical application in ageing-related diseases.

What is already known

- Rutin shows multiple functions in metabolic process regulation.

What does this study adds

- Sodium rutin treatment extended the life and health span of mice
- It acts by enhancing the autophagy activity in hepatocytes.

What is the clinical significance

- The present findings render sodium rutin a promising candidate for clinical application in ageing-related diseases.

2 | METHODS

2.1 | Animal housing

All C57BL6 mice used in this study were housed at 22°C–24°C with a 12 h dark–light cycle. Mice were provided with standard rodent chow and water ad libitum. Sodium rutin was added to the drinking water at a concentration of 0.2 $mg{\cdot}ml^{-1}$. All animal experiments were approved by the Institutional Animal Care and Use Committee at the Institute of Cognition and Brain Sciences (Beijing, China).

2.2 | Cell culture and transfection

HepG2 cells were cultured in Dulbecco's modified Eagle medium (Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), 100 $U{\cdot}ml^{-1}$ penicillin 100 $mg{\cdot}ml^{-1}$ streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. The following chemicals were added to the medium at different time points:- NaR (200 μM, 24 h), **oleic acid** (500 μM, 12 h), **chloroquine** (CQ;100 μM, 2 h) and **metformin** (2 mM, 4 h). The siRNA were transfected as previously described (Zhou et al., 2017). Briefly, siRNAs transfection were performed in complete medium with Lipofectamine 2000 (Invitrogen, Waltham, MA, USA) according to the manufacturer's protocols, cells were collected at 36–48 h after transfection for knockdown assays. si SQSTM1, (sc-29679, Santa Cruz, CA, USA). si Scramble, (sc-37007, Santa Cruz, CA, USA). si ANGPTL8-1#: AAGCCCACCAAGAATTT GAGA; si ANGPTL8-2#: TATGACAGAGCACTGGAATTC. Those two siRNAs were mixed together.

2.3 | Behaviour tests

2.3.1 | Open-field test

The locomotor activity test was conducted in a clear box (50 × 50 × 20 cm). Locomotor activity was automatically monitored with a camera above the box and analysed by ANY-maze software (ANY-maze, RRID:SCR_014289, Stoelting Co., Wood Dale, IL, USA). Each mouse was video recorded for 5 min and the total distance and average speed were analysed.

2.3.2 | Rotarod test

Motor coordination was assessed using the rotarod test. Mice were acclimatized to the rotarod platform for three consecutive days, three trials per day; each trial lasted 5 min and was performed at a constant rotarod speed of 5 rpm. On the test day, the animals were subjected to three trials on the accelerating roller (4–40 rpm in 5 min) and the time that the mice remained on the roller was recorded.

2.3.3 | Beam balance test

The balance and coordination of the animals were assessed by the beam balance test. Mice were acclimatized to a 1 × 1 × 100 cm beam for 3 days (five trials each day). On the testing day, the animals were tested on two beams of different dimensions and shapes (1 × 1 × 100 cm and Φ = 1 × 100 cm) and the time to traverse each beam was measured three times. The data represent the average traverse time.

2.3.4 | Novel object recognition test

The novel object recognition test was performed in a clear box (50 × 50 × 20 cm). Mice were accustomed to the box for 3 days. The test included two phases. Phase 1 was a 10-min familiarization period in which the animals were presented with two identical objects and phase 2 was a 5-min test in which one familiar object was replaced by a novel one. ANY-maze software was used to record and analyse data. The times spent sniffing novel and known objects were recorded.

2.3.5 | Barnes maze test

Barnes maze test was performed as previously described (Attar et al., 2013). Briefly, after 3 days of habituation in a behaviour room, mice were introduced to the Barnes maze for an adaptation session (Day 1). During the training phase (Days 2–5), mice were allowed to find the target box, which was hidden beneath one of the platform holes. Mice that failed to find the target hole were guided to it and allowed to stay there for 1 min. Each mouse was trained five times per day and data were presented as the mean value. Twenty-four hours after the last training trial, mice were tested for memory retention. The track and activity were recorded and analysed by ANY-maze software.

2.4 | Histology

Histopathological analysis was performed in 24-month-old animals. After killing the animals (mice was killed by CO_2 and decaptation was carried out), organs were dissected and fixed in 4% buffered formalin. Sections (4 μm thick) of paraffin-embedded tissues from the heart, gastrocnemius muscle, liver and kidney were cut and stained with haematoxylin and eosin, periodic acid–Schiff stain and Masson trichrome stain. Sections of the liver (20 μm thick) were cut and stained with oil red O and β-gal. Slides were digitalized using a virtual slide system (Nanozoomer HT2.0, Hamamatsu, Japan). Computer-assisted analysis software (Image-Pro Plus, RRID:SCR_007369, Media Cybernetics, Rockville, MD, USA) was used to assess age-related features. Fluorescence slides were observed and photographed with a Nikon A1 confocal microscope (Nikon, Melville, NY, USA).

2.5 | Transmission electron microscopy

Liver tissues were immediately harvested from mice and cut into 1 × 1 × 1 mm blocks. After fixation, tissues were cut into 70-nm-thick sections and stained with lead citrate and uranyl acetate. Autophagosomes and lysosomes were visualized with a Hitachi transmission electron microscope (Hitachi H-7650, Japan).

2.6 | Energy intake, energy expenditure and activity measurements

The experiment was performed as previously described (Wu, Liu, et al., 2017). Briefly, mice were housed in cages with free access to food and water. Food intake and oxygen consumption were measured for two consecutive days using a LabMaster system (TSE Systems, Bad Homburg, Germany). Consumed oxygen, eliminated CO_2, food intake and energy expenditure were automatically recorded. Ambulatory activity of each mouse was measured using the optical beam technique (Opto-M3, Columbus Instruments, Columbus, OH, USA).

2.7 | Glucose tolerance test

The animals were fasted for 16 h but maintained free access to drinking water. The blood glucose level was determined with a OneTouch Ultra device (Bayer, Germany) 0, 15, 30, 60 and 120 min after the intraperitoneal injection of glucose (2 $g \cdot kg^{-1}$).

2.8 | RNA-sequencing and qPCR

Total RNA was extracted from mice liver using TRIzol Reagent (Invitrogen, 15596026). Four samples from mice of the same experimental group were mixed to obtain the sequencing sample, which was then sequenced twice. RNA sequencing was performed by Annoroad Gene Technology (Beijing, China). qPCR was performed as previously reported (Cheng et al., 2020).

2.9 | Metabolomics

After killing the animals (Dislocation of the neck), 30 mg of liver tissue was immediately harvested. The samples were stored at −80°C and then extracted as previously described (Shui et al., 2010). Metabolomics analysis was performed by LipidALL Technology (Beijing, China).

2.10 | Determination of triglyceride, cholesterol, β-hydroxybutyrate and ATP concentrations

Triglyceride and cholesterol concentrations were measured using commercial measurement kits (E1013 and E1015, Applygen, Beijing, China) according to the manufacturer's instructions. β-hydroxybutyrate concentration was measured by an ELISA kit (Yuanye Bio-Technology, Shanghai, China) and ATP concentration was determined using the Cell Viability Assay kit (G7571, Promega, USA).

2.11 | Adeno-associated virus plasmid injection for autophagy flux detection

The detection plasmid adeno-associated virus (AAV)-GFP-RFP-LC3 (GFP/RFP, green/red fluorescent protein; LC3, microtubule-associated protein light chain 3) (OBio, Shanghai, China) was used for autophagy flux detection in mice. For this purpose, mice were intravenously injected with AAV-GFP-RFP-LC3 ($1.0–1.2 \times 10^{12}$ PFU·ml^{-1}) through the tail vein. Four weeks after injection, mice were killed and the liver was harvested, fixed with 4% paraformaldehyde and embedded in optimal cutting temperature compound (OCT, 4583, Sakura Finetek, Torrance, CA, USA). The liver was then cut into 20-μm-thick sections, which were analysed and captured with a Nikon A1 confocal microscope (Nikon, Melville, NY, USA).

2.12 | Bioinformatics analysis

The Gene Set Enrichment Analysis tool (Subramanian et al., 2005) was used for the enrichment analysis of the expression data. We prepared gene sets based on Gene Ontology (GO) annotation files, which were downloaded from NCBI (Coordinators, 2018). Then, we extracted the Gene Ontology terms related to mouse and compared them against the RNA expression data. Gene Set Enrichment Analysis was performed with default parameters.

2.13 | Immunoblotting

Immunoblotting was performed as previously described (Wu, Sun, et al., 2017). Briefly, tissues or cells were lysed in lysis buffer,and protein concentration was determined by BCA Protein Assay Kit (Beyotime, China). Proteins were separated on polyacrylamide gel and transferred to nitrocellulose (GE Amersham, 10600002). The membrane was blocked in 5% milk and subsequently probed with primary antibodies overnight at 4°C and then incubated with HRP-conjugated secondary antibodies for protein detection. The antibodies used were anti-SQSTM1 (Cell Signaling Technology, 5114S, 1:1000), ant- LC3 (Cell Signaling Technology, 4599S, 1:1000), anti-ANGPTL8 (Proteintech Cat# 66641-1-Ig, RRID:AB_2882000), anti-β actin (Cwbiotech, CW0096, 1:1000), GAPDH (CWBio Cat# CW0100M, RRID:AB_2801390) HRP tagged Goat anti-rabbit, (Cwbiotech, CW0103, 1:5000), HRP tagged Goat anti-mouse, (Cwbiotech, CW0102, 1:5000). Western blotting has been conducted the experimental detail provided conforms with BJP Guidelines (Alexander et al., 2018).

2.14 | Data and analysis

Blinding and random assignment of different groups of mice in this study were in accordance of the guidelines of *British Journal of Pharmacology*. The C57BL6 mice were randomly divided to the control group and sodium rutin treatment group. Data collection and evaluation of all experiments were performed in a double-blind manner. Statistical analysis was undertaken for studies where each group size was at least $n = 5$, except for transcriptomics and metabolomics, in which $n = 4$. The group size selection for each protocol or study was based on the designation of group size that were previously published for similar experiments (Martin-Montalvo et al., 2013; Mitchell et al., 2018; Xie et al., 2018; Xu et al., 2018). The declared group size is the number of independent values,and statistical analysis was done using these independent values. In multigroup studies with parametric variables, post hoc tests were conducted only if F in ANOVA achieved statistical significance ($P < .05$) and there was no significant variance in homogeneity. No outliers were removed in these experiments. The data and statistical analysis comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology. Most data were shown with the original values. Bioinformatic, QPCR, and western blot statistic data were normalized to control group, the Y axis shown as changing fold. Two-tailed Student's t-test and ANOVA analysis were used for statistical analysis by GraphPad 6 (GraphPad Software, RRID:SCR_002798, San Diego, CA 92108). Data are expressed as mean ± SEM. Statistical significance was defined as follows $^*P < .05$.

2.15 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal

for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

2.16 | Materials

All chemicals and reagents were purchased from Sigma-Aldrich (St. louis, MO, USA) unless stated otherwise.

3 | RESULTS

3.1 | Sodium rutin treatment extends the lifespan and health span of C57BL6 mice

To investigate the long-term effect of sodium rutin treatment on male C57BL6 mice, we added sodium rutin to the drinking water at a concentration of 0.2 mg·ml^{-1}. Mice had free access to the sodium rutin/water solution from 32 weeks old to end of life. After 8 months of sodium rutin supplementation, we found that sodium rutin treatment did not affect food intake and water consumption of the mice (Figure S1B,C). Biochemical and necropsy examinations of 24-month-old mice revealed no obvious biochemical and pathological changes in the liver and kidney (Figure S1D–H). A log-rank test-based analysis revealed that the lifespan of sodium rutin-treated mice was significantly higher than that of untreated mice (Figure 1a). The mean lifespan was increased by approximately 79 days in the sodium rutin-supplemented group (Figure 1b) and the maximum lifespan was increased approximately 3 months (Figure 1c).

To determine whether sodium rutin supplementation affects ageing-associated pathology, we performed a series of experiments, including morphological, behavioural and histopathological analyses. Morphological traits were characterized by histomorphometric parameters and sodium rutin treatment markedly delayed ageing-related morphological changes, such as kyphosis, cataract and baldness in 24-month-old mice (Figure 2a,b). Then, the differences in the locomotor ability between the treatment and control groups were assayed using the open-field, rotarod and beam balance tests. The open-field test results showed that sodium rutin treatment significantly improved the locomotion ability of mice because animals in the treatment group exhibited longer total distances and higher mean speeds than those in the control group (Figure 2c,d). Compared to the control group, the sodium rutin-treated group stayed longer on the rotarod, indicating sodium rutin-induced improvements in the motor and coordination abilities (Figure 2e). In the beam balance test, sodium rutin-treated mice passed the beam faster and slipped less frequently than untreated mice, indicating better balance and motor abilities in sodium rutin-treated mice (Figure 2f,g). Previous studies showed that memory and cognitive functions significantly decreased in aged mice (Brockmole & Logie, 2013; Wallis et al., 2016). To evaluate the effect of sodium rutin on the learning and memory capabilities of mice, we performed the Barnes maze and novel object recognition tests using aged mice (24 months). Barnes maze test results indicated that sodium rutin treatment improved the long-term memory in aged mice as sodium rutin-treated mice exhibited shorter latencies to reach the target hole (Figure 2h,i). Moreover, the novel object recognition test showed that sodium rutin increased the exploration ability of mice, indicating an improvement in their cognitive function (Figure 2j,k). Overall, these findings demonstrated that sodium rutin treatment could significantly improve the health span in aged mice.

We further performed histological examinations of control and sodium rutin-treated mice. Oil red O staining showed steatosis occurs in the liver from 24-month-old mice. sodium rutin treatment significantly decreased oil red O positive counts and the area percentage, which means sodium rutin supplementation markedly reduced steatosis (Figure 2l–n). The β-gal expression level is a marker of cellular senescence. We detected the higher β-gal expression level in the liver from the old control mice and sodium rutin supplementation significantly decreased β-gal expression in liver (Figure 2o-q). Furthermore, histopathology analysis showed sodium rutin supplementation reduced myocardial fibrosis in the heart, glomerulosclerosis in the kidney and atrophy in the skeleton muscle (Figure S2A–F). These results suggest that dietary supplementation with sodium rutin extends the lifespan, improves fitness and effectively alleviates a range of ageing-associated pathologies in mice.

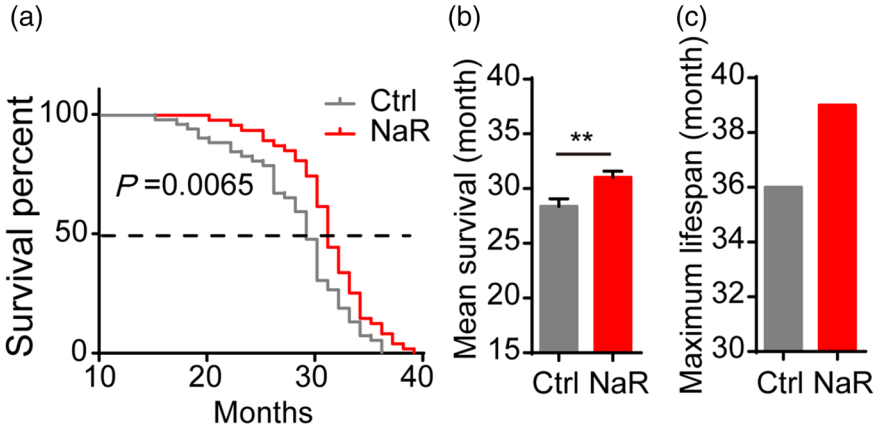

FIGURE 1 Chronic sodium rutin (NaR) treatment extends the lifespan of mice. (a) Kaplan–Meier survival curves for mice fed with normal water and NaR-supplemented water (control, n = 52; NaR, n = 47). (b) Average lifespans of control and NaR-treated mice (Student's t-test, $^*P < .05$). (c) Maximum lifespan of control and NaR-treated mice

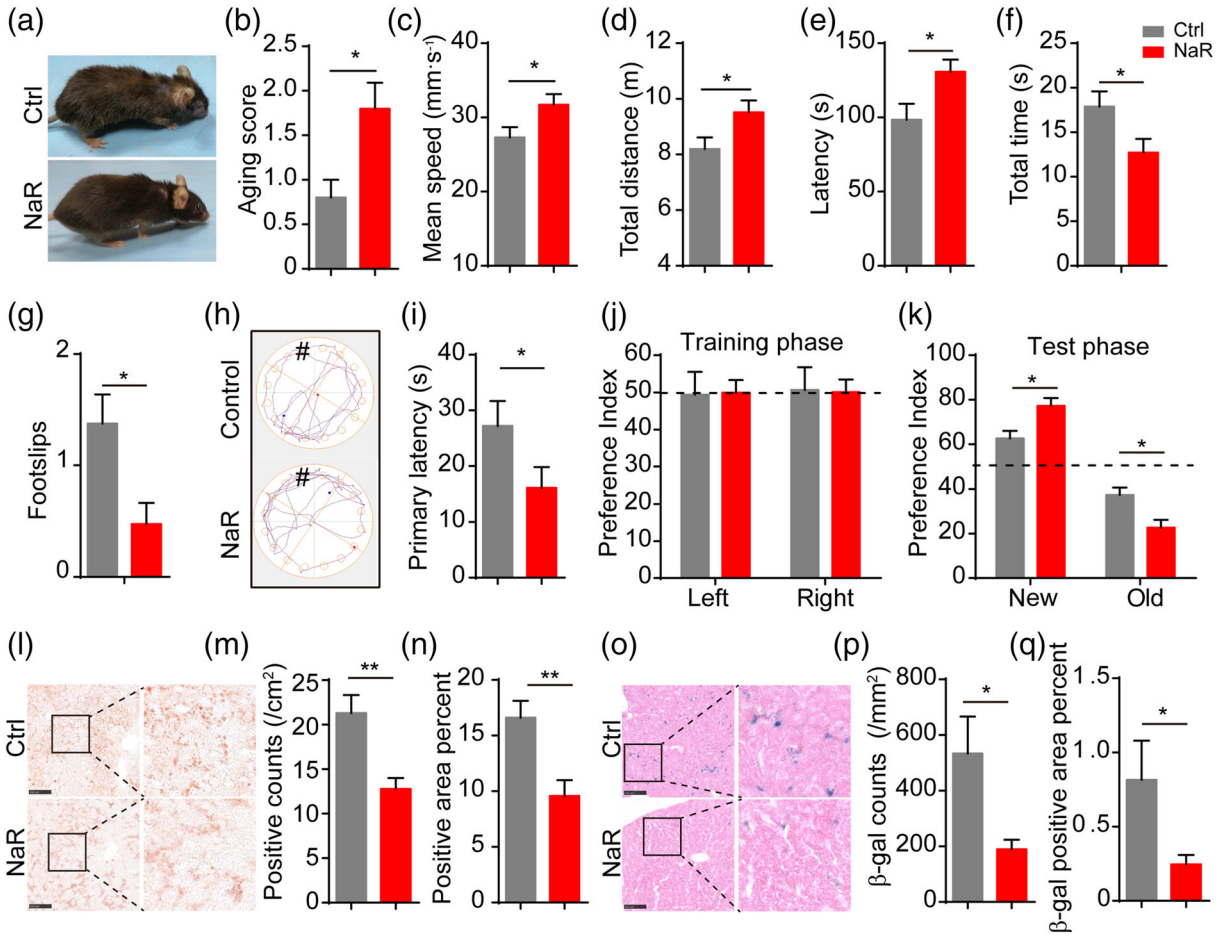

FIGURE 2 Chronic sodium rutin (NaR) treatment improves the health of ageing mice. (a) NaR treatment delays ageing-related morphological changes (kyphosis, cataract, baldness) in 24-month-old mice. (b) The score of ageing-related morphological changes (including kyphosis, cataract, baldness, each of which count 1 point, the range of ageing score is 0 to 3 points. Higher score represents more obvious ageing trait). (c) Average speeds and (d) total distances of 14-month-old mice during the open-field test. (e) Falling latencies of 16-month-old mice in the rotarod test. (f) Total traversing time and (g) number of foot slips of 28-month-old mice during the beam balance test. (h) Track plots of 19-month-old mice in the Barnes maze test; (#) represents the target hole location. (i) Primary latency times of mice to reach the target hole. Preference indices of 20-month-old mice during the (j) training and (k) test phases of the novel object recognition test. For all behaviour tests: $n \geq 8$, $^*P < .05$,. (l) Oil red O staining of liver tissue slides from 24-month-old control and NaR-treated mice; scale bar = 100 μm. (m) Statistic numbers and (n) areas of positive counts in oil red O stained liver tissue slides. (o) β-gal staining of liver tissue slides from 24-months-old control and NaR-treated mice; scale bar = 100 μm. (p) Statistic numbers and (q) areas of positive counts in β-gal stained liver tissue slides. For pathology analyses: $n = 5$ (each statistic number was derived from five slides), Student's t-test $^*P < .05$

3.2 | Sodium rutin treatment alters the whole-body metabolism

We tested whether whole-body metabolism was changed upon sodium rutin treatment. In vivo metabolism tests revealed that sodium rutin treatment significantly increased the energy expenditure in mice (Figure 3a,b). Increased energy expenditure influences the lipid metabolism in the liver (Scheja & Heeren, 2016). Also, sodium rutin-treated mice showed a lower respiratory quotient (RQ ~ 0.75) than those in the control group (RQ ~ 0.8), especially during light cycles (Figure 3c, d). A previous report showed that at an RQ value of ~0.75, fatty acids become the predominant energy source of the organism, whereas at an RQ value of ~1.0, the energy mostly comes from carbohydrates and at an RQ between 0.75 and 1.0, energy is supplied by both fats and carbohydrates (Mitchell et al., 2018). The RQ results indicated that sodium rutin-treated mice burned more fat than the control group mice. Furthermore, there was no difference in locomotive ability and food intake between both groups during the metabolic measurement period (Figure S3A–C). The present results indicated that sodium rutin treatment increased the energy expenditure in mice largely through fat burning. Consequently, the body fat percentage was significantly lower in the sodium rutin treatment group than in the control group (Figure 3e), whereas the body weights were similar between the two groups (Figure S3D). The lean mass is increased in sodium rutin-treated group (Figure 3f), which indicates that sodium rutin could increase energy expenditure (Figure 3a,b). These results suggest that sodium rutin treatment improves energy metabolism and body composition of mice.

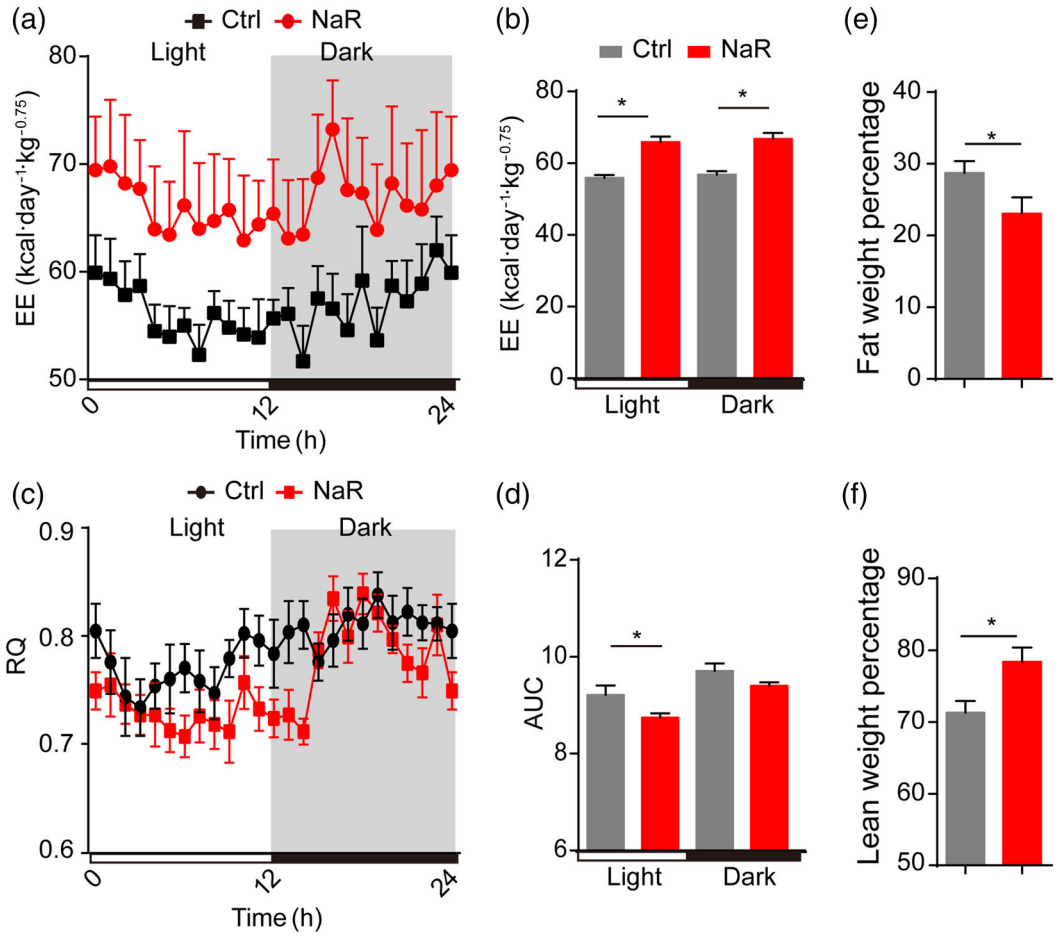

FIGURE 3 Sodium rutin (NaR) treatment alters whole-body metabolism. (a) Energy expenditure of the control and NaR treatment groups during light and dark periods. (b) Average energy expenditure of the control and NaR treatment groups during light and dark periods. (c,d) Respiratory quotients of the control and NaR treatment groups during light and dark periods. (e) Fat and (f) lean weight percentages of the control and NaR treatment group mice measured by echo MRI. Metabolic analyses were conducted using 19-month-old mice. $n \geq 8$, $^*P < .05$

3.3 | Sodium rutin treatment significantly alters the expression of metabolic genes in the liver

The liver is essential for the maintenance of the lipid and glucose homeostasis and plays an important role in energy balance. To better understand the response of the liver to long-term sodium rutin treatment, we performed RNA-sequencing analysis (Table S1). Total RNA was extracted from the liver of 24-month-old mice. We identified 94 differentially expressed genes with the more stringent methodology analysis (adjusted $P < .05$, or more than twofold changes) (Figure 4a). Gene enrichment analysis of the differentially expressed genes showed that sodium rutin treatment significantly increased the expression of genes related to the lipid droplet metabolism, which was downregulated in the control group (Figure 4b). These results are consistent with the obtained histopathological parameters. Furthermore, genes involved in the respiratory chain were also upregulated in the sodium rutin treatment group (Figure 4c). These data indicate that sodium rutin treatment could improve liver energy metabolism. In addition, we found that the expression levels of genes related to the p53 (Lyn) and reactive oxygen species pathways were markedly downregulated in the sodium rutin treatment group; these pathways are closely related to ageing processes (Figure S4A,B). Gene ontology analysis showed that sodium rutin treatment led to the downregulation of genes that are mainly enriched in the inflammatory response, whereas genes mainly enriched in metabolic response were upregulated (Figure 4d). Our data strongly suggest that sodium rutin treatment altered the metabolic gene expression pattern in the liver, improving the global energy expenditure.

3.4 | sodium rutin treatment alters liver metabolic profiling

Using qPCR, we confirmed that the expression of genes involved in the lipid metabolism was altered, including *Far1* (fatty acyl-CoA reductase 1, synthesis of monoesters and ether lipids), *Steap4* (STEAP family member 4, FFAs metabolism), *Il1rn* (interleukin 1 receptor antagonist, cholesterol metabolism), *Isyna1* (inositol-3-phosphate

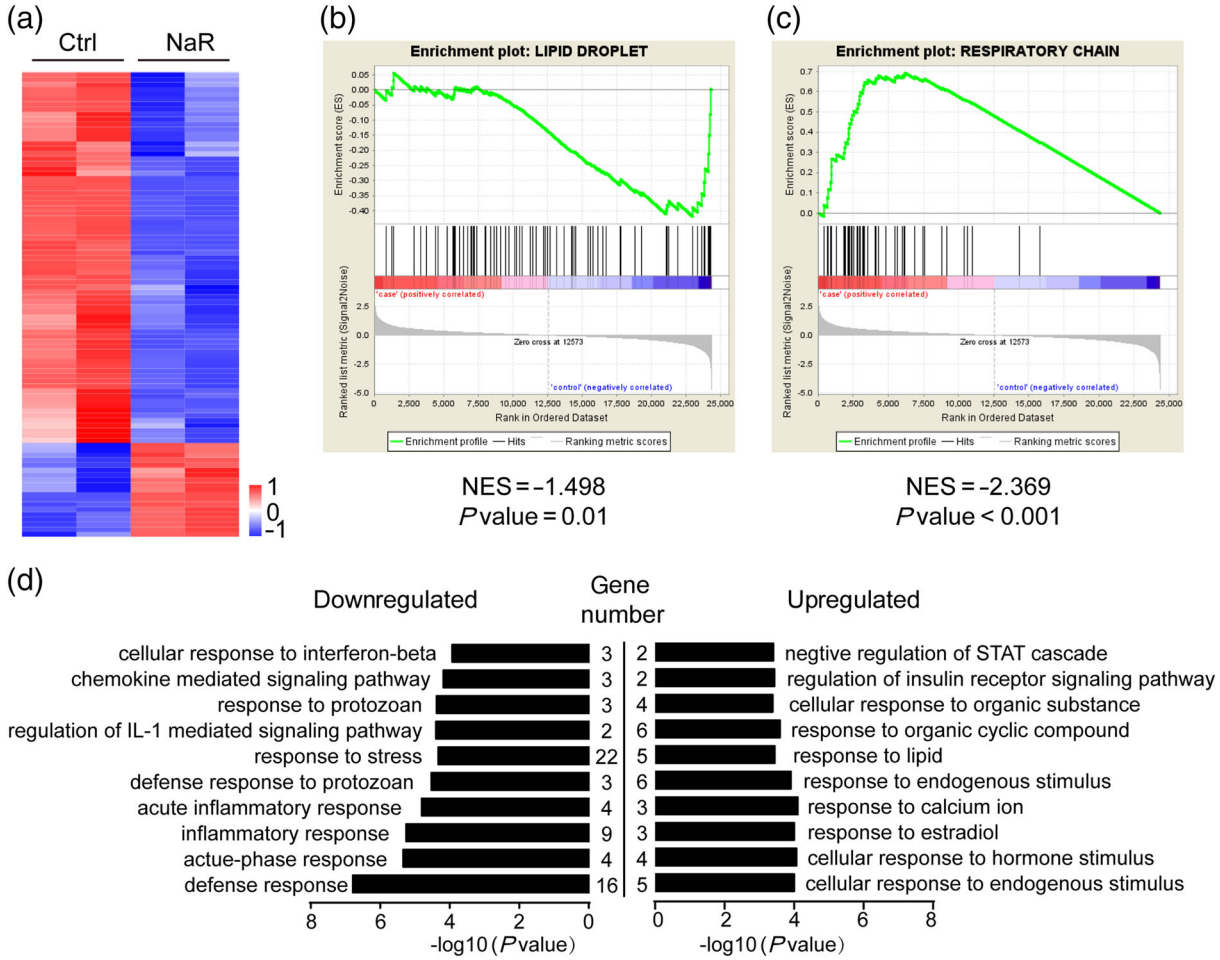

FIGURE 4 Sodium rutin (NaR) treatment upregulates lipid metabolic genes. (a) Heat map showing significantly changed genes after NaR treatment. Gene Set Enrichment Analysis (GSEA) results of significantly changed genes enriched in the (b) droplet pathway and (c) respiratory chain. (d) Gene ontology analysis of significantly changed genes

synthase 1, phosphoinositide metabolic), *Lcn2* (lipocalin 2, lipid droplet formation), ***Enho*** (energy homeostasis associated, lipogenesis) and *Angptl8* (angiopoietin-like 8, lipogenesis) (Figure 5a). To further explore the effect of sodium rutin on liver metabolism, we performed liver metabolomics and found that sodium rutin influenced the abundance of metabolites (Figure 5b and Table S2, S3). The levels of the metabolites from the tricarboxylic acid cycle and lipid metabolism were altered the most upon sodium rutin treatment. As depicted of the metabolic pathway based on liver metabolites analysis in Figure 5c, the sodium rutin treatment group exhibited lower triglyceride, diglyceride and cholesterol levels than the control group (Figure 5d,e), which are the major components of the lipid droplet metabolism in hepatocytes. In contrast, the levels of phospholipids related to membrane fluidity were increased in the sodium rutin treatment group, including phosphatidylethanolamines, phosphatidylcholines, phosphatidylglycerols and phosphatidylserines. Concomitantly, intermediates of the tricarboxylic acid cycle were upregulated, such as isocitric acid, succinic acid and malic acid, suggesting a higher level of lipolysis. sodium rutin treatment also increased the amount of β-hydroxybutyrate (Figure 5f), suggesting that sodium rutin promotes lipolysis by enhancing fat oxidation. Since fatty acid oxidation leads to enhanced tricarboxylic acid cycle activity and ATP production, we determined the ATP production in the liver. Our results showed no significant difference in ATP production between the two groups (Figure S5A). As fatty acids and glucose play a key role in energy balance, we analysed the glycolysis level and found that sodium rutin treatment slowed down glycolysis by changing the expression level of glycolysis-related genes (Figure S5B,C). These results indicated that sodium rutin-induced effects rely on fat oxidation as a predominant energy source.

3.5 | Sodium rutin treatment maintains liver fitness by promoting hepatocyte autophagy

Previous reports showed that autophagy contributes to the maintenance of intracellular amino acid levels, lipophagy-mediated lipolysis and oxidative metabolism in hepatocytes (Ezaki et al., 2011; Sinha et al., 2014). Since the metabolomics results showed that sodium rutin treatment increased the levels of several amino acids and fatty acid

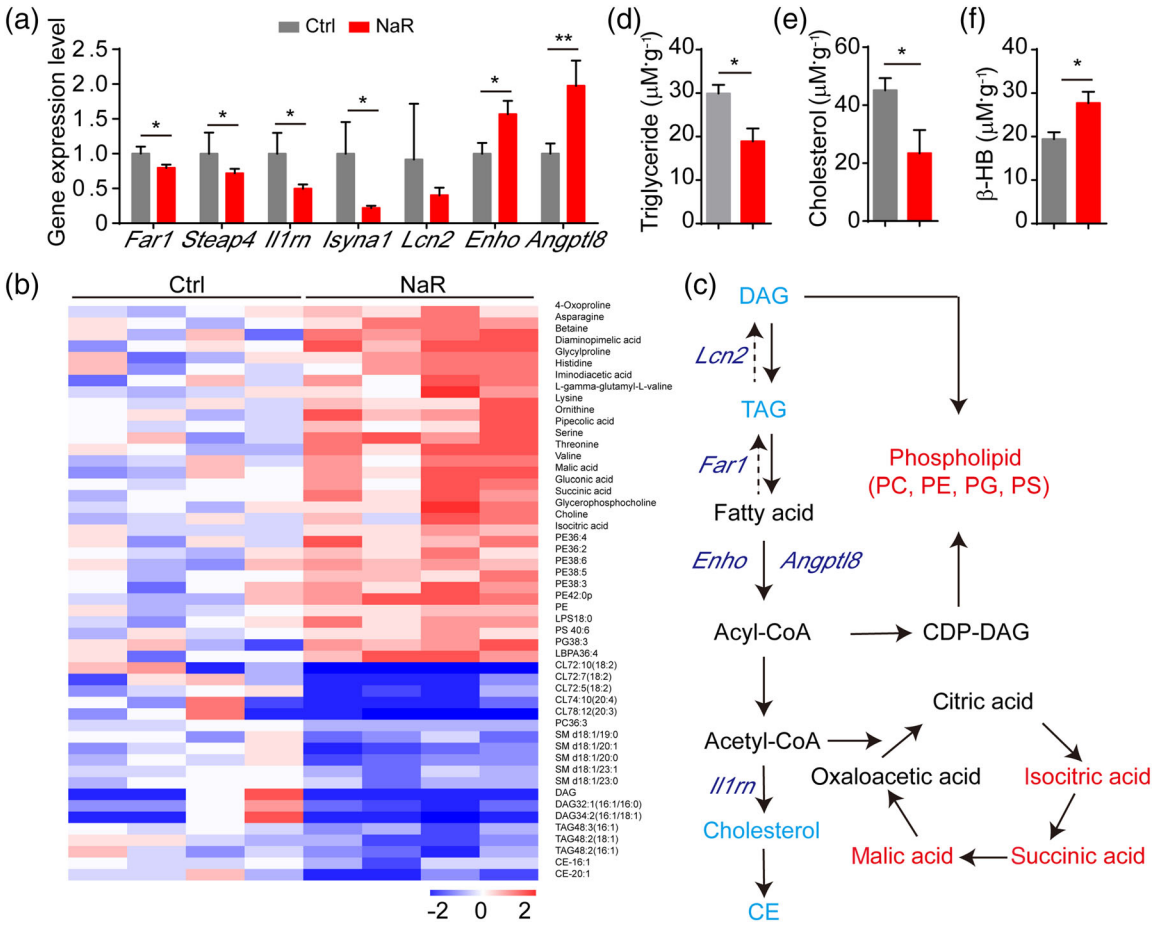

FIGURE 5 Sodium rutin (NaR) treatment alters liver metabolic profiles. (a) Expression levels of genes associated with the lipid metabolism in the liver assessed by qPCR. (b) Heat map showing changed metabolites derived from metabolomics. (c) Diagram depicting altered lipid metabolite concentrations in the liver in response to NaR treatment (red, upregulated; blue, downregulated). (d) Triglyceride, (e) cholesterol, and (f) β-hydroxybutyrate (β-HB) concentrations in the liver. Mice were 24 months old. $n = 4$, $^*P < .05$

β-oxidation (Figure 5F), we proposed that sodium rutin increases hepatocytic autophagy. Transcriptomics showed that sodium rutin treatment upregulated the gene expression of angiopoietin-like protein 8 (*Angptl8*) (Figure 5a). *Angptl8* reportedly enhances the p62-dependent autophagic degradation pathway (Zhang et al., 2017). To test this hypothesis, we employed three different methods to assess the autophagic degradation activity in murine hepatocytes. First, we examined the protein levels of p62 and LC3 in the liver tissue of 24-month-old mice. The expression of the p62 protein increases when autophagy is inhibited and decreases when it is induced (Bjørkøy et al., 2009). Immunoblotting showed that the expression levels of p62 and LC3 II/I were decreased and increased, respectively, in the liver tissue from sodium rutin-treated mice compared with the corresponding values in the control group, indicating that sodium rutin treatment increased the autophagic flux in the murine liver (Figure 6a, b). Second, electron microscopy of the liver tissue showed that sodium rutin increased autophagic induction in the liver, as evidenced by the enhanced formation of autophagosomes (Figure 6c,d). We also observed increased lipophagy in the sodium rutin treatment group (Figure S6A), suggesting that sodium rutin administration may reduce steatosis through lipophagy (Sinha et al., 2014). Finally, we injected 24-month-old mice with the AAV-RFP-GFP-LC3 expression plasmid to explore the effect of sodium rutin treatment on the autophagic flux in vivo (Kimura, Noda, & Yoshimori, 2007). In the present experimental setup, GFP-tagged LC3 only labelled autophagosomes, while RFP labelled both autophagosomes and autolysosomes; therefore, autophagosomes appeared as yellow dots and autolysosomes as red dots. We observed that sodium rutin increased the fraction of autolysosomes (red foci), indicating that sodium rutin treatment promotes autophagy flux (Figure 6e–g). Since lysosomes are the site of degradation of obsolete macromolecules, such as lipids, during autophagy, we examined whether sodium rutin-promoted lipid droplet clearance depends on autophagy. Chloroquine was used to block the fusion of autophagosomes with lysosomes. sodium rutin treatment enhanced autophagy flux in HepG2 cells, reflected by the increased LC3 II/I and decreased p62 accumulation, which could be blocked by chloroquine (Figures 6h,i and S6B). Electron microscopy of the HepG2 cells showed that sodium rutin increased autophagy induction, as evidenced by the enhanced formation of autophagosomes (Figure S6B). Overall, those results showed sodium rutin treatment

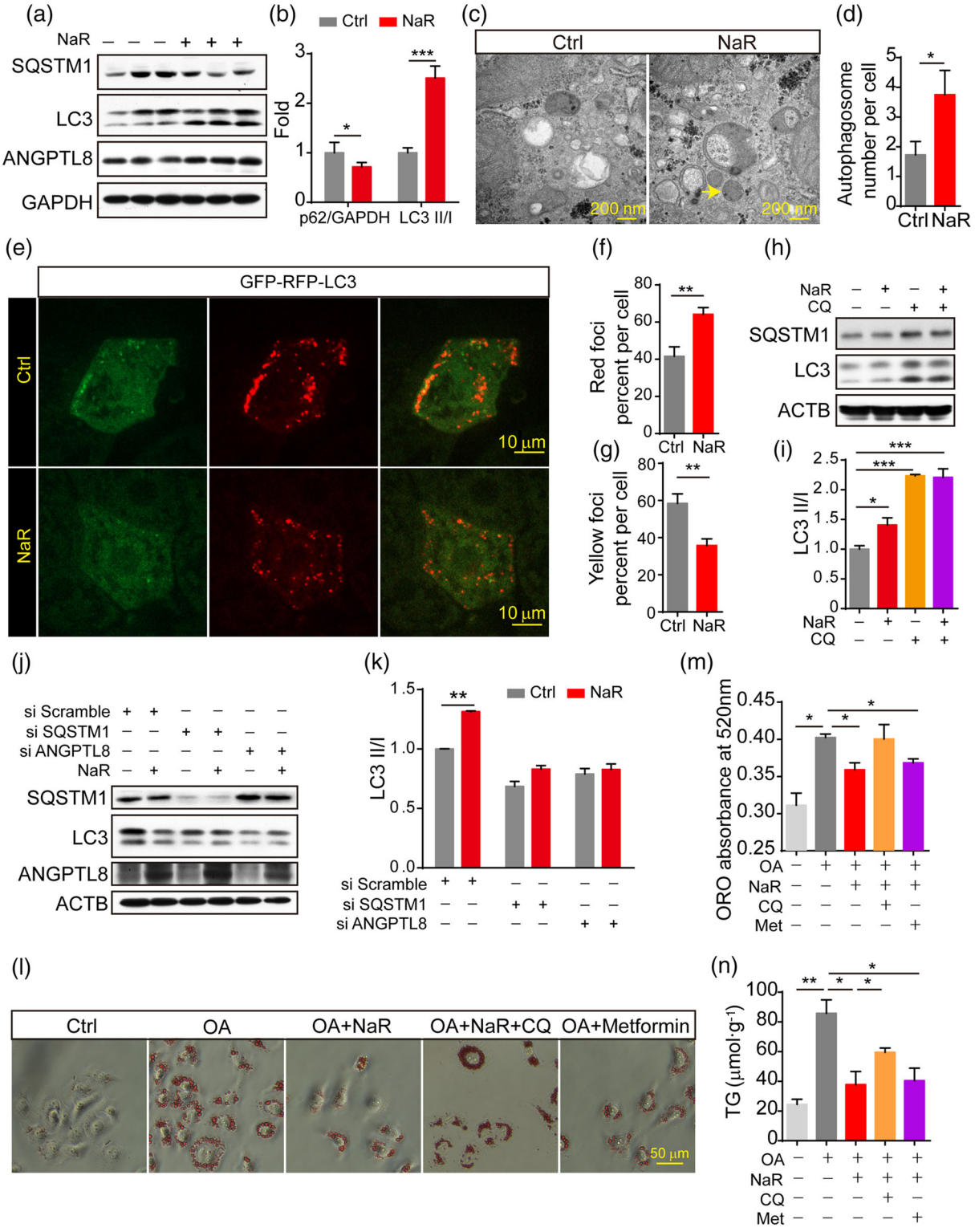

FIGURE 6 Sodium rutin (NaR) treatment improves autophagy activity in hepatocytes. (a,b) Western blot analysis of the expression levels of p62 and LC3 in the liver from 24-month-old mice. (c) Transmission electron microscopy shows autophagosome (yellow arrow) in liver (scale bar = 200 nm). (d) Statistic results of autophagosome in control and NaR treatment group per cell basis (n = 6). (e–g) Representative and quantificative image of early autophagosomes (yellow foci) and autolysosomes (red foci) in murine liver with AAV-RFP-GFP-LC3 infection (n = 6, scale bar = 10 μm). (h–k) Western blot results and quantification of p62 and LC3 protein levels in HepG2 cells with different treatment (quantification data were observed from three dependent experiment, and the data normalized to the control, one-way ANOVA analysis was performed in (i) and two-way ANOVA analysis was performed in (k), (*P < .05,). (l) Oil red O staining of HepG2 cells after each treatment with metformin as a positive control (scale bar = 50 μm). (m,n) Oil red O absorbance at 520 nm and TG concentration of HepG2 cells with each treatment (NaR: 200 μM, 24 h; oleic acid: 500 μM, 12 h; chloroquine (CQ): 100 μM, 2 h; metformin: 2 mM, 4 h; Student's t-test, *P < .05,)

enhanced autophagy level in HepG2 cells. In order to illustrate the sodium rutin-induced autophagy enhancement was achieved by ANGPTL8, we performed *Angptl8* knockdown by siRNA and found that *Angptl8* knockdown, similar to chloroquine, alleviated the sodium rutin's effect on autophagic flux (Figures 6j,k and S6D,E). It has been reported that metformin attenuated ageing process though activating autophagic flux (Kulkarni et al., 2020). Here, we use metformin as a positive control. The oil red O staining and lipid quantification experiments showed that sodium rutin or metformin treatment significantly reduced steatosis in HepG2 cells, which could be blocked by chloroquine in HepG2 cells (Figure 6l–n). Therefore, we concluded that sodium rutin treatment maintains liver fitness by promoting autophagy activity in hepatocytes.

4 | DISCUSSION

Ageing is one of the most challenging issues in the fields of population and health research nowadays. Here, we demonstrated that chronic sodium rutin treatment significantly extended the lifespan in wild-type C57BL6 mice by 9.3% and, more importantly, increased the health span by significantly delaying ageing-related morphological changes. Those results were consistent with the previous report that rutin promote longevity in *Drosophila melanogaster* (Chattopadhyay et al., 2017). Our study for the first time demonstrated this effect in mammals. Furthermore, whole-body metabolic investigations, metabolomics and transcriptomics revealed that sodium rutin treatment promoted liver fitness by modulating lipid metabolism. We also demonstrated that the hepatoprotective effect of sodium rutin was achieved by enhancing hepatocytic autophagy. Thus, our study strongly implicates that sodium rutin is a promising drug candidate for targeting ageing.

Ageing is a process of progressive impairment of homeostasis at the genomic, cellular, tissue and whole-organism levels, increasing the risk of diseases and death (Timchenko, 2009). At the cellular level, multiple biological alterations accumulate over time, including genomic stability, epigenetic alterations, telomere attrition, loss of proteostasis, dysregulation of nutrient sensing, altered intracellular communication, mitochondrial dysfunction, stem cell exhaustion and cellular senescence. The crosstalk between the ageing-related dysfunction and metabolism dysregulation had been reported in previous studies (López-Otín, et al., 2016). At the whole-organism level, the ageing phenotypes usually comprise lower voluntary running, lower energy expenditure, glucose intolerance and reduced fatty acid oxidation (Azzu & Valencak, 2017). In our study, all the above ageing-related phenotypes were significantly reversed by sodium rutin treatment. The liver is the central organ for metabolic homeostasis and liver fitness reportedly plays an important role in the anti-ageing effects of nutritional interventions, such as caloric and protein restrictions (Jové et al., 2014; Vermeij et al., 2016). In accordance, we found that sodium rutin markedly promoted liver metabolic fitness and alleviated ageing pathological features in aged mice.

Low grade inflammation is a hallmark of ageing. In this study, our transcriptomic analysis showed that sodium rutin treatment has an anti-inflammatory effect in liver. According to Gene Ontology analysis, the genes involved in inflammation pathway were significantly downregulated in sodium rutin treatment group (Figure 4d). Actually, our previous work showed that sodium rutin treatment exhibits neuroprotective property by suppressing microglia-derived proinflammatory cytokine production and enhancing microglia phagocytosis (Pan et al., 2019). Here, we also observed that sodium rutin treatment reduced the number of senescent cells in vivo (Figure 2o–q). Together, our data indicated sodium rutin treatment could alleviate senescence-associated secretory phenotype. Thus, we argue sodium rutin confers a senolytic property.

Autophagic dysfunction during ageing leads to the aggregation of damaged organelles or obsolete cytoplasmic proteins. It has been reported that autophagy defects contribute to liver pathologies; thus, enhancing autophagy activity could maintain the energy balance and eliminate damaged cellular components or structures, rendering autophagy a therapeutic target for the treatment of liver disorders (Schneider & Cuervo, 2014). In addition, enhanced autophagy activity increases the lifespan of model organisms (Eisenberg et al., 2016; Fernández et al., 2018). In the present study, sodium rutin treatment significantly promoted autophagy activity in hepatocytes. Autophagy-mediated lipid droplet degradation by β-oxidation reduces steatosis and the associated breakdown of macromolecules provides cellular fuel and promotes cellular fitness. Thus, autophagy promotes liver fitness by maintaining energy homeostasis and reducing cellular senescence. Our results showed that sodium rutin-mediated steatosis reduction was at least partly dependent on autophagy; however, the crosstalk between the lipid metabolism and autophagy is complex. Lipid metabolism products, such as phosphatidylethanolamines and phosphatidylserines, can also modulate autophagy; the mechanism of lipid metabolic regulating autophagy needs further study.

The search for chemicals affecting longevity has attracted much attention. For example, **resveratrol**, a well-studied natural compound, extended the lifespan in various species (Baur et al., 2006; Pallauf, Rimbach, Rupp, Chin, & Wolf, 2016); however, its clinical application is limited due to its low bioavailability, pharmacokinetics and tissue distribution (Hubbard & Sinclair, 2014), despite being nontoxic to animals (Baur & Sinclair, 2006). Metformin, a clinical drug for the treatment of type 2 diabetes, has recently been reported to extend the lifespan in lower animals and mammals (Chen et al., 2017; Martin-Montalvo et al., 2013). Metformin is used in a variety of clinical applications (Thomas & Gregg, 2017) and represents a promising drug for the treatment of ageing and ageing-related diseases. Rutin is a natural compound found in fruits and vegetables and the average daily intake is approximately 1.5 mg (Kimira, Arai, Shimoi, & Watanabe, 1998). In the present study, rutin was prepared as a sodium salt to improve its water solubility and bioavailability. The chosen doses were made basing on the clinic dosage of troxerutin (0.2–1 g/70 kg·day^{-1}), which is a trihydroxyethyl form of rutin. The dosage in mice is 0.52–2.6 mg·day^{-1} according to the ratio of body surface area in human versus mice

(387.9:1). Here, the chosen dose is 0.6–0.8 mg·day^{-1} in mice. Long-term administration of rutin showed no side effects on the liver and kidney in aged mice (Figure S1). Therefore, we suggest that sodium rutin could be used in anti-ageing therapy and for the treatment of ageing-associated diseases.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Natural Science Foundation of China (81630026 and 81930029 to Z Yuan), National Major Project of Support Program (2019-JCJQ-ZD-195 to Z Yuan) and Beijing Natural Science Foundation (7161009 to Z Yuan).

AUTHOR CONTRIBUTIONS

S.L. designed and performed all the experiments and wrote the manuscript. J.L. helped with animal experiments. R.P. helped with preparing sodium rutin. J.C. helped with analysing the data and autophagy-related experiment. Q.C. helped with analysing bioinformatics data. J.C. and Z.Y. supervised this project.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design & Analysis and Animal Experimentation and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

ORCID

Jianxin Chen https://orcid.org/0000-0003-4067-5638

REFERENCES

Alexander, S. P. H., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, C., Buneman, O. P., Cidlowski, J. A., Christopoulos, A., Davenport, A. P., Fabbro, D., Spedding, M., Striessnig, J., Davies, J. A., & CGTP Collaborators. (2019). The Concise Guide to PHARMACOLOGY 2019/20: Introduction and other protein targets. *British Journal of Pharmacology*, *176*(Suppl 1), S1–S20. https://doi.org/10.1111/bph.14747

Alexander, S. P. H., Roberts, R. E., Broughton, B. R. S., Sobey, C. G., George, C. H., Stanford, S. C., Cirino, G., Docherty, J. R., Giembycz, M. A., Hoyer, D., Insel, P. A., Izzo, A. A., Ji, Y., MacEwan, D. J., Mangum, J., Wonnacott, S., & Ahluwalia, A. (2018). Goals and practicalities of immunoblotting and immunohistochemistry: A guide for submission to the British Journal of Pharmacology. *British Journal of Pharmacology*, *175*(3), 407–411. https://doi.org/10.1111/bph.14112

Alonso, C., Rubio, L., Touriño, S., Martí, M., Barba, C., Fernández-Campos, F., Coderch, L., & Parra, J. L. (2014). Antioxidative effects and percutaneous absorption of five polyphenols. *Free Radical Biology & Medicine*, *75*, 149–155. https://doi.org/10.1016/j.freeradbiomed.2014.07.014

Attar, A., Liu, T., Chan, W. T., Hayes, J., Nejad, M., Lei, K., & Bitan, G. (2013). A shortened Barnes maze protocol reveals memory deficits at 4-months of age in the triple-transgenic mouse model of Alzheimer's disease. *PLoS One*, *8*(11), e80355. https://doi.org/10.1371/journal.pone.0080355

Azzu, V., & Valencak, T. G. (2017). Energy metabolism and ageing in the mouse: A mini-review. *Gerontology*, *63*(4), 327–336. https://doi.org/10.1159/000454924

Bárcena, C., Valdés-Mas, R., Mayoral, P., Garabaya, C., Durand, S., Rodríguez, F., Fernández-García, M. T., Salazar, N., Nogacka, A. M., Garatachea, N., Bossut, N., Aprahamian, F., Lucia, A., Kroemer, G., Freije, J. M. P., Quirós, P. M., & López-Otín, C. (2019). Healthspan and lifespan extension by fecal microbiota transplantation into progeroid mice. *Nature Medicine*, *25*(8), 1234–1242. https://doi.org/10.1038/s41591-019-0504-5

Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K., Pistell, P. J., Poosala, S., Becker, K. G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K. W., Spencer, R. G., … Sinclair, D. A. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*, *444*(7117), 337–342. https://doi.org/10.1038/nature05354

Baur, J. A., & Sinclair, D. A. (2006). Therapeutic potential of resveratrol: The in vivo evidence. *Nature Reviews. Drug Discovery*, *5*(6), 493–506. https://doi.org/10.1038/nrd2060

Bjørkøy, G., Lamark, T., Pankiv, S., Øvervatn, A., Brech, A., & Johansen, T. (2009). Monitoring autophagic degradation of p62/SQSTM1. *Methods in Enzymology*, *452*, 181–197. https://doi.org/10.1016/S0076-6879(08)03612-4

Brockmole, J. R., & Logie, R. H. (2013). Age-related change in visual working memory: A study of 55,753 participants aged 8–75. *Frontiers in Psychology*, *4*, 12–16. https://doi.org/10.3389/fpsyg.2013.00012

Chattopadhyay, D., Chitnis, A., Talekar, A., Mulay, P., Makkar, M., James, J., & Thirumurugan, K. (2017). Hormetic efficacy of rutin to promote longevity in *Drosophila melanogaster*. *Biogerontology*, *18*(3), 397–411. https://doi.org/10.1007/s10522-017-9700-1

Chen, J., Ou, Y., Li, Y., Hu, S., Shao, L.-W., & Liu, Y. (2017). Metformin extends lifespan through lysosomal pathway. *eLife*, *6*, e31268. https://doi.org/10.7554/eLife.31268

Cheng, J., Liao, Y., Dong, Y., Hu, H., Yang, N., Kong, X., Li, S., Li, X., Guo, J., Qin, L., Yu, J., Ma, C., Li, J., Li, M., Tang, B., & Yuan, Z. (2020). Microglial autophagy defect causes Parkinson disease-like symptoms by accelerating inflammasome activation in mice. *Autophagy*, *16*, 2193–2205. https://doi.org/10.1080/15548627.2020.1719723

Chua, L. S. (2013). A review on plant-based rutin extraction methods and its pharmacological activities. *Journal of Ethnopharmacology*, *150*(3), 805–817. https://doi.org/10.1016/j.jep.2013.10.036

Coordinators, N. R. (2018). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, *46*(D1), D8–D13. https://doi.org/10.1093/nar/gkx1095

Eisenberg, T., Abdellatif, M., Schroeder, S., Primessnig, U., Stekovic, S., Pendl, T., Harger, A., Schipke, J., Zimmermann, A., Schmidt, A., Tong, M., Ruckenstuhl, C., Dammbrueck, C., Gross, A. S., Herbst, V., Magnes, C., Trausinger, G., Narath, S., Meinitzer, A., … Madeo, F. (2016). Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nature Medicine*, *22*(12), 1428–1438. https://doi.org/10.1038/nm.4222

Ezaki, J., Matsumoto, N., Takeda-Ezaki, M., Komatsu, M., Takahashi, K., Hiraoka, Y., Taka, H., Fujimura, T., Takehana, K., Yoshida, M., Iwata, J., Tanida, I., Furuya, N., Zheng, D. M., Tada, N., Tanaka, K., Kominami, E.,

& Ueno, T. (2011). Liver autophagy contributes to the maintenance of blood glucose and amino acid levels. *Autophagy*, *7*(7), 727–736. https://doi.org/10.4161/auto.7.7.15371

Fernández, Á. F., Sebti, S., Wei, Y., Zou, Z., Shi, M., McMillan, K. L., He, C., Ting, T., Liu, Y., Chiang, W. C., Marciano, D. K., Schiattarella, G. G., Bhagat, G., Moe, O. W., Hu, M. C., & Levine, B. (2018). Disruption of the beclin 1–BCL2 autophagy regulatory complex promotes longevity in mice. *Nature*, *558*(7708), 136–140. https://doi.org/10.1038/s41586-018-0162-7

Fontana, L., & Partridge, L. (2015). Promoting health and longevity through diet: From model organisms to humans. *Cell*, *161*(1), 106–118. https://doi.org/10.1016/j.cell.2015.02.020

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. *Nature Medicine*, *25*(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0

Harding, S. D., Sharman, J. L., Faccenda, E., Southan, C., Pawson, A. J., Ireland, S., Gray, A. J. G., Bruce, L., Alexander, S. P. H., Anderton, S., Bryant, C., Davenport, A. P., Doerig, C., Fabbro, D., Levi-Schaffer, F., Spedding, M., Davies, J. A., & NC-IUPHAR. (2018). The IUPHAR/BPS guide to PHARMACOLOGY in 2018: Updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Research*, *46*(D1), D1091–D1106. https://doi.org/10.1093/nar/gkx1121

Hubbard, B. P., & Sinclair, D. A. (2014). Small molecule SIRT1 activators for the treatment of aging and age-related diseases. *Trends in Pharmacological Sciences*, *35*(3), 146–154. https://doi.org/10.1016/j.tips.2013.12.004

Jové, M., Naudí, A., Ramírez-Núñez, O., Portero-Otín, M., Selman, C., Withers, D. J., & Pamplona, R. (2014). Caloric restriction reveals a metabolomic and lipidomic signature in liver of male mice. *Aging Cell*, *13*(5), 828–837. https://doi.org/10.1111/acel.12241

Kimira, M., Arai, Y., Shimoi, K., & Watanabe, S. (1998). Japanese intake of flavonoids and isoflavonoids from foods. *Journal of Epidemiology*, *8*(3), 168–175. https://doi.org/10.2188/jea.8.168

Kimura, S., Noda, T., & Yoshimori, T. (2007). Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent-tagged LC3. *Autophagy*, *3*(5), 452–460. https://doi.org/10.4161/auto.4451

Kulkarni, A. S., Gubbi, S., & Barzilai, N. (2020). Benefits of metformin in attenuating the hallmarks of aging. *Cell Metabolism*, *32*(1), 15–30. https://doi.org/10.1016/j.cmet.2020.04.001

Lin, J., Hu, T., Zhang, H., Zhang, C., Zhou, H., Ye, R., Qi, X., Zhai, B., Huang, W., Liu, S., Xie, W., Liu, Q., Liu, X., Cui, C., Li, D., Zhan, J., Cheng, J., Yuan, Z., & Jin, W. (2017). Rutin ameliorates obesity through brown fat activation. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *31*(1), 333–345. https://doi.org/10.1096/fj.201600459RR

López-Otín, C., Galluzzi, L., Freije, J. M. P., Madeo, F., & Kroemer, G. (2016). Metabolic control of longevity. *Cell*, *166*(4), 802–821. https://doi.org/10.1016/j.cell.2016.07.031

Mahmoud, A. M. (2012). Influence of rutin on biochemical alterations in hyperammonemia in rats. *Experimental and Toxicologic Pathology*, *64*(7–8), 783–789. https://doi.org/10.1016/j.etp.2011.01.016

Martin-Montalvo, A., Mercken, E. M., Mitchell, S. J., Palacios, H. H., Mote, P. L., Scheibye-Knudsen, M., Gomes, A. P., Ward, T. M., Minor, R. K., Blouin, M. J., Schwab, M., Pollak, M., Zhang, Y., Yu, Y., Becker, K. G., Bohr, V. A., Ingram, D. K., Sinclair, D. A., Wolf, N. S., … de Cabo, R. (2013). Metformin improves healthspan and lifespan in mice. *Nature Communications*, *4*, 2192–2212. https://doi.org/10.1038/ncomms3192

Mitchell, S. J., Bernier, M., Aon, M. A., Cortassa, S., Kim, E. Y., Fang, E. F., Palacios, H. H., Ali, A., Navas-Enamorado, I., di Francesco, A., Kaiser, T. A., Waltz, T. B., Zhang, N., Ellis, J. L., Elliott, P. J., Frederick, D. W., Bohr, V. A., Schmidt, M. S., Brenner, C., … de Cabo, R. (2018). Nicotinamide improves aspects of healthspan, but not lifespan, in mice. *Cell Metabolism*, *27*(3), 667–676.e4. https://doi.org/10.1016/j.cmet.2018.02.001

Ogrodnik, M., Miwa, S., Tchkonia, T., Tiniakos, D., Wilson, C. L., Lahat, A., Day, C. P., Burt, A., Palmer, A., Anstee, Q. M., Grellscheid, S. N., Hoeijmakers, J. H. J., Barnhoorn, S., Mann, D. A., Bird, T. G., Vermeij, W. P., Kirkland, J. L., Passos, J. F., von Zglinicki, T., & Jurk, D. (2017). Cellular senescence drives age-dependent hepatic steatosis. *Nature Communications*, *8*, 15691–15702. https://doi.org/10.1038/ncomms15691

Pallauf, K., Rimbach, G., Rupp, P. M., Chin, D., & Wolf, I. M. A. (2016). Resveratrol and lifespan in model organisms. *Current Medicinal Chemistry*, *23*(41), 4639–4680. https://doi.org/10.2174/0929867323666161024151233

Pan, R. Y., Ma, J., Kong, X. X., Wang, X. F., Li, S. S., Qi, X. L., Yan, Y. H., Cheng, J., Liu, Q., Jin, W., Tan, C. H., & Yuan, Z. (2019). Sodium rutin ameliorates Alzheimer's disease-like pathology by enhancing microglial amyloid-beta clearance. *Science Advances*, *5*(2), eaau6328. https://doi.org/10.1126/sciadv.aau6328

Papatheodoridi, A.-M., Chrysavgis, L., Koutsilieris, M., & Chatzigeorgiou, A. (2020). The role of senescence in the development of nonalcoholic fatty liver disease and progression to nonalcoholic steatohepatitis. *Hepatology*, *71*(1), 363–374. https://doi.org/10.1002/hep.30834

Ramirez, T., Li, Y.-M., Yin, S., Xu, M.-J., Feng, D., Zhou, Z., Zang, M., Mukhopadhyay, P., Varga, Z. V., Pacher, P., Gao, B., & Wang, H. (2017). Aging aggravates alcoholic liver injury and fibrosis in mice by downregulating sirtuin 1 expression. *Journal of Hepatology*, *66*(3), 601–609. https://doi.org/10.1016/j.jhep.2016.11.004

Samsonowicz, M., Kamińska, I., Kalinowska, M., & Lewandowski, W. (2015). Alkali metal salts of rutin—Synthesis, spectroscopic (FT-IR, FT-Raman, UV-VIS), antioxidant and antimicrobial studies. *Spectrochimica Acta. Part a, Molecular and Biomolecular Spectroscopy*, *151*, 926–938. https://doi.org/10.1016/j.saa.2015.07.027

Scheja, L., & Heeren, J. (2016). Metabolic interplay between white, beige, brown adipocytes and the liver. *Journal of Hepatology*, *64*(5), 1176–1186. https://doi.org/10.1016/j.jhep.2016.01.025

Schmucker, D. L. (1998). Aging and the liver: An update. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *53*(5), B315–B320.

Schmucker, D. L. (2005). Age-related changes in liver structure and function: Implications for disease ? *Experimental Gerontology*, *40*(8–9), 650–659. https://doi.org/10.1016/j.exger.2005.06.009

Schneider, J. L., & Cuervo, A. M. (2014). Liver autophagy: Much more than just taking out the trash. *Nature Reviews Gastroenterology & Hepatology*, *11*(3), 187–200. https://doi.org/10.1038/nrgastro.2013.211

Shui, G., Guan, X. L., Low, C. P., Chua, G. H., Goh, J. S. Y., Yang, H., & Wenk, M. R. (2010). Toward one step analysis of cellular lipidomes using liquid chromatography coupled with mass spectrometry: Application to *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* lipidomics. *Molecular BioSystems*, *6*(6), 1008–1017. https://doi.org/10.1039/b913353d

Sinha, R. A., Farah, B. L., Singh, B. K., Siddique, M. M., Li, Y., Wu, Y., Ilkayeva, O. R., Gooding, J., Ching, J., Zhou, J., Martinez, L., Xie, S., Bay, B. H., Summers, S. A., Newgard, C. B., & Yen, P. M. (2014). Caffeine stimulates hepatic lipid metabolism by the autophagy-lysosomal pathway in mice. *Hepatology*, *59*(4), 1366–1380. https://doi.org/10.1002/hep.26667

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., Lander, E. S., & Mesirov, J. P. (2005). Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of*

America, *102*(43), 15545–15550. https://doi.org/10.1073/pnas.0506580102

Thomas, I., & Gregg, B. (2017). Metformin; a review of its history and future: From lilac to longevity. *Pediatric Diabetes*, *18*(1), 10–16. https://doi.org/10.1111/pedi.12473

Timchenko, N. A. (2009). Aging and liver regeneration. *Trends in Endocrinology and Metabolism*, *20*(4), 171–176. https://doi.org/10.1016/j.tem.2009.01.005

Uchitomi, R., Hatazawa, Y., Senoo, N., Yoshioka, K., Fujita, M., Shimizu, T., Miura, S., Ono, Y., & Kamei, Y. (2019). Metabolomic analysis of skeletal muscle in aged mice. *Scientific Reports*, *9*(1), 10425–10435. https://doi.org/10.1038/s41598-019-46929-8

Vermeij, W. P., Dollé, M. E. T., Reiling, E., Jaarsma, D., Payan-Gomez, C., Bombardieri, C. R., Wu, H., Roks, A. J. M., Botter, S. M., van der Eerden, B. C., Youssef, S. A., Kuiper, R. V., Nagarajah, B., van Oostrom, C. T., Brandt, R. M. C., Barnhoorn, S., Imholz, S., Pennings, J. L. A., de Bruin, A., … Hoeijmakers, J. H. J. (2016). Restricted diet delays accelerated ageing and genomic stress in DNA-repair-deficient mice. *Nature*, *537*(7620), 427–431. https://doi.org/10.1038/nature19329

Wallis, L. J., Virányi, Z., Müller, C. A., Serisier, S., Huber, L., & Range, F. (2016). Aging effects on discrimination learning, logical reasoning and memory in pet dogs. *Age (Dordrecht, Netherlands)*, *38*(1), 6–24. https://doi.org/10.1007/s11357-015-9866-x

Wu, R., Liu, X. M., Sun, J. G., Chen, H., Ma, J., Dong, M., Peng, S., Wang, J. Q., Ding, J. Q., Li, D. H., Speakman, J. R., Ning, G., Jin, W., & Yuan, Z. (2017). DJ-1 maintains energy and glucose homeostasis by regulating the function of brown adipose tissue. *Cell Discovery*, *3*, 16054–16071. https://doi.org/10.1038/celldisc.2016.54

Wu, R., Sun, J.-G., Wang, J.-Q., Li, B., Liu, Q., Ning, G., Jin, W., & Yuan, Z. (2017). c-Abl inhibition mitigates diet-induced obesity through improving insulin sensitivity of subcutaneous fat in mice. *Diabetologia*, *60*(5), 900–910. https://doi.org/10.1007/s00125-016-4202-2

Xie, K., Ryan, D. P., Pearson, B. L., Henzel, K. S., Neff, F., Vidal, R. O., Hennion, M., Lehmann, I., Schleif, M., Schröder, S., Adler, T., Rathkolb, B., Rozman, J., Schütz, A. L., Prehn, C., Mickael, M. E., Weiergräber, M., Adamski, J., Busch, D. H., … Ehninger, D. (2018). Epigenetic alterations in longevity regulators, reduced life span, and exacerbated aging-related pathology in old father offspring mice. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(10), E2348–E2357. https://doi.org/10.1073/pnas.1707337115

Xu, M., Pirtskhalava, T., Farr, J. N., Weigand, B. M., Palmer, A. K., Weivoda, M. M., Inman, C. L., Ogrodnik, M. B., Hachfeld, C. M., Fraser, D. G., Onken, J. L., Johnson, K. O., Verzosa, G. C., Langhi, L. G. P., Weigl, M., Giorgadze, N., LeBrasseur, N. K., Miller, J. D., Jurk, D., … Kirkland, J. L. (2018). Senolytics improve physical function and increase lifespan in old age. *Nature Medicine*, *24*(8), 1246–1256. https://doi.org/10.1038/s41591-018-0092-9

Zhang, Y., Guo, X., Yan, W., Chen, Y., Ke, M., Cheng, C., Zhu, X., Xue, W., Zhou, Q., Zheng, L., Wang, S., Wu, B., Liu, X., Ma, L., Huang, L., & Huang, K. (2017). ANGPTL8 negatively regulates NF-κB activation by facilitating selective autophagic degradation of IKKγ. *Nature Communications*, *8*(1), 2164–2176. https://doi.org/10.1038/s41467-017-02355-w

Zhou, L., Zhang, Q., Zhang, P., Sun, L., Peng, C., Yuan, Z., & Cheng, J. (2017). c-Abl-mediated Drp1 phosphorylation promotes oxidative stress-induced mitochondrial fragmentation and neuronal cell death. *Cell Death Disease*, *8*(10), e3117. https://doi.org/10.1038/cddis.2017.524

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Li S, Li J, Pan R, et al. Sodium rutin extends lifespan and health span in mice including positive impacts on liver health. *Br J Pharmacol*. 2021;1–14. https://doi.org/10.1111/bph.15410