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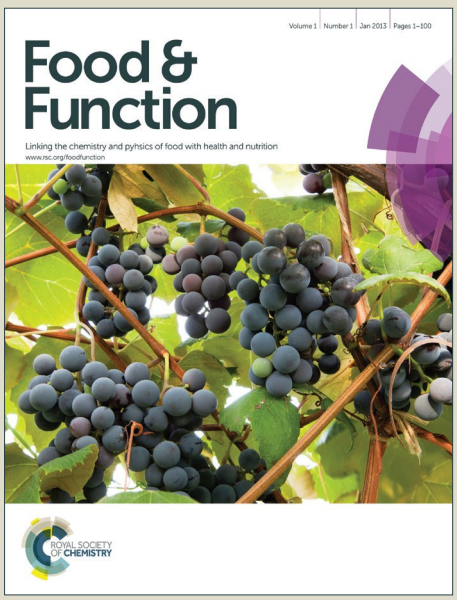

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Rutin protects against aging-related metabolic dysfunction

Tianyi Li, Sufang Chen, Tao Feng, Jie Dong, Yuanyuan Li, Hua Li*

Department of Elderly Endocrinology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450000, Henan Province, China.

* Corresponding author. Address: Department of Elderly Endocrinology, The First Affiliated Hospital of Zhengzhou University, Jianshe East Road 1, Zhengzhou, 450000, Henan Province, China. E-mail address: hua_li15@126.com.

Abstract

Aging is a complex process which is accompanied by multiple related chronic diseases. Among them, metabolic dysfunction is one of the most important aging-related disorders. In the present study, we aimed to investigate the effect of rutin on aging-related metabolic dysfunction. We found that the increase of fasting blood glucose, insulin levels, blood pressure and HOMA-IR in aged rats was significantly inhibited by rutin. In addition, rutin improved glucose and insulin tolerance in aged rats, as reflected by decreased glucose level in IPGTT and IPITT test. Rutin treatment notably increased Akt and IRS-1 phosphorylation in livers of old rats. The increase of inflammatory markers, such as IL-1β and TNFα, was prevented by the rutin administration. Moreover, in circulation and livers of old rats, rutin treatment significantly decreased the content of TG. Rutin also inhibited the increase of serum AST and ALT levels. Furthermore, rutin treatment markedly inhibited aging-related mitochondrial dysfunction, ER stress, and oxidative stress, as evidenced by increased oxygen consumption rate and activities of Na^+/K^+-ATPase and Ca_2^+-ATPase, decreased expression of ATF3 and GRP78, decreased level of MDA, increased content of GSH and enhanced activity of SOD in aged rats. We show that the administration of rutin could effectively improve aging-related metabolic dysfunction. The amelioration of inflammation, lipid accumulation, mitochondrial dysfunction, ER stress, and oxidative stress may be involved in the effect of rutin on aging-related metabolic dysfunction. These findings provide novel insights into the potential use of rutin in the intervention of aging and its related metabolic diseases.

Key words: rutin; aging; metabolic dysfunction; inflammation; lipid accumulation; oxidative stress

1. Introduction

Aging is believed to be one of the most important social problems in developed and several developing countries, because of longer life expectancy in recent decades [1]. The increase of aging is accompanied by multiple related chronic diseases. Among them, metabolic dysfunction is one of the most important aging-related disorders. Thus, the intervention measures of aging-related metabolic dysfunction have been paid large amount of attention. Hyperglycemia, insulin resistance, and disorder of lipid metabolism are hallmarks of metabolic dysfunction [2, 3].

Rutin, also called 3, 3', 4', 5, 7-pentahydroxyflavone-3-rhamnoglucoside, is a flavonoid of the flavonol-type. It is reported that rutin could be found in many typical plants, such as buckwheat, passion flower, apple, and tea, and is an important dietary constituent of food and plant-based beverages [4, 5]. In addition, rutin is the 3-Orhamnoglucoside form of quercetin, which is the most abundant flavonol in vegetables and fruits [6]. A number of studies have shown that rutin and its metabolites and derivatives possess the capability to regulate metabolic function [7]. For instance, in 2006, Kamalakkannan et al. found that rutin exhibited antihyperglycaemic effect in streptozotocin-induced diabetic Wistar rats [8]. Stanely et al. showed that rutin exerted protective effects on lipids, lipoproteins, lipid metabolizing enzymes and glycoproteins in streptozotocin-induced diabetic rats [9]. Cai et al. showed that rutin suppressed the glucotoxicity in rat pancreatic beta cells [10]. Rutin was shown to attenuate metabolic changes, nonalcoholic steatohepatitis, and cardiovascular remodeling in high-carbohydrate, high-fat diet-fed rats [10]. In a recent study, Pyrzanowska et al. discovered that long-term rutin have benefits on behavioral and neurochemical changes in aged WAG male rats [11]. However, whether rutin is able to protect against aging-related metabolic dysfunction is still not known.

In the present study, we aimed to investigate the effect of rutin on metabolic function under aging condition and to elucidate the possible mechanisms. The results showed that the administration of rutin could effectively improve aging-related metabolic dysfunction. The amelioration of inflammation, lipid accumulation, mitochondrial dysfunction, ER stress, and oxidative stress may be involved in the effect of rutin on aging-related metabolic dysfunction.

2. Experimental section

2.1 Chemicals and reagents

Rutin was purchased from Sigma. β-actin antibody was purchased from Santa Cruz. Akt, P-Akt,

IRS-1, P-IRS-1, ATF3 and GRP78 antibodies were purchased from CST. Most the other chemicals were purchased from Sigma.

2.2 Animals and treatment

Animal care was in accordance with the guidelines for the Care and Use of Laboratory Animals and the principles presented by Zhengzhou University and approved by the Ethical Committee of Zhengzhou University (20141015). All efforts were made to reduce suffering and the number of animals used. Male young (6 months) and old (20 months) Sprague-Dawley rats were purchased from Experimental Animal Centre of Zhengzhou University.

Rats were maintained under a 12 h light/dark cycle at 23 ± 1°C and 50 ± 5% relative humidity under specific pathogen-free conditions. Animals were maintained on a standard diet that was purchased from Experimental Animal Centre of Zhengzhou University (Supplemental figure 1). Twenty-month-old rats were randomly divided into three groups (n = 8 rats per group) as follows: Old, Old + 25 mg/kg rutin (25 mg/kg/day for 6 weeks), and Old + 50 mg/kg rutin (50 mg/kg/day for 6 weeks). Six-month-old rats (8 rats) were used as age controls. In the experiments, the rutin powder was added to 2% ethylcellulose solution (5 ml) to form the suspension and then rats were given rutin by gastric gavage. Body weights were weighed and fasting blood glucose level was detected after 12-h fasting. After the experiment, animals were euthanized, and perirenal adipose tissue was dissected and weighed. Blood samples and livers were collected for further analysis.

2.3 Measurement of systolic blood pressure.

The systolic blood pressure (SBP) was measured using the tail-cuff blood pressure system (Kent Scientific, Torrington, USA).

2.4 Biochemical measurements

After the experiment, serum was isolated and liver tissues were homogenized in saline for the biochemical detection. Insulin, IL-1β, TNFα, and TG levels were measured by commercial kits purchased from CUSABIO Technology, China, according the manufacturer's instructions. HOMA-IR was then calculated as follows: HOMA-IR = fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5. ALT and AST levels were measured by commercial kits purchased from Nanjing Jiancheng, China, according the manufacturer's protocols.

2.5 IPGTT and IPITT

The intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test

(IPITT) were performed according to studies reported previously [12]. In brief, prior to each test, rats were fasted for 6 h and 4 h, respectively. Then, a baseline blood sample was taken from their tail and each rat received either i.p. glucose, 1 g/kg body weight or i.p. insulin 0.75 U/kg body weight. Tail blood samples were drawn at, 30, 60, and 120 min after the injection and were analyzed immediately for glucose content.

2.6 Evaluation of oxidative stress

For the evaluation of oxidative stress, serum was isolated and liver tissues were homogenized in saline, and malonaldehyde (MDA) and glutathione (GSH) level and superoxide dismutase (SOD) activity were determined by commercial assay kits according the manufacturer's protocols.

2.7 Western blot

After the experiment, liver tissues were isolated and were lysed with ice-cold RIPA buffer (TIANGEN). The lysates were centrifuged at 13,200 rpm for 1 hr at 4°C to produce whole-cell extracts. BCA method was used to quantify protein content. 50 μg protein was separated on a 10% SDS-polyacrylamide gel and transferred onto a polyvinylidene difluoride membrane. After blocking with 8% BSA prepared in TBS for 1 h at room temperature, membranes were incubated with primary antibodies (Cell signaling technology, 1:1000) overnight at 4°C. After washing with TBST buffer for four times, blots were then incubated with horseradish peroxidase-linked IgG secondary antibodies (Pierce, IL, USA) for 30 min at 37°C. Enhanced chemiluminescence was performed by ECL (Pierce, IL, USA). Bands were finally captured with an image analysis system (Bio-Rad) and quantified with Quantity One software (Bio-Rad).

2.8 Mitochondrial function determination

For the determination of mitochondrial function, fresh livers were harvested and homogenized in oxygen-saturated PBS, and then oxygen consumption was recorded for 10 min using a Clark Oxygen Electrode. The activities of the Na^+/K^+-ATPase and Ca_2^+-ATPase were determined, using commercial assay kits (Nanjing Jiancheng Company) following the manufacturer's protocols. Enzyme specific activity was expressed as mmol Pi released per hour per mg of protein.

2.9 Statistical analysis

Results are presented as mean ± SEM. Statistical analysis was performed by GraphPad Software. The statistical significance of differences between groups was analyzed via one-way analysis of variance followed by a Tukey's Multiple Comparison Test for multiple comparisons. P value <0.05

was considered to be statistically significant.

3. Results

3.1 Rutin improved metabolic dysfunction in aged rats.

Body weights were measured to evaluate the general effects of rutin in aged rats. In Fig. 1A, we showed that the administration of rutin significantly inhibited the increase of body weights. In Fig. 1B, the results showed that the increase of the weights of perirenal white adipose tissue in aged rats was notably reversed by rutin. In aging rats, systolic blood pressure (SBP) increased, which was suppressed by the treatment of rutin (Table 1). In Table 1, the results showed that fasting blood glucose and insulin level increased notably in old rats, compared with that of young control rats. The administration of rutin dose-dependently reduced fasting blood glucose and insulin levels in old rats (Table 1). Moreover, rutin significantly decreased HOMA-IR in aged rats (Table 1). IPGTT was conducted to evaluate the glucose tolerance in rats and the results showed that after glucose challenge, glucose level in old rats were higher than that of young control rats (Table 2). The AUC of IPGTT in old rats was significantly increased, confirming the occurrence of glucose intolerance (Table 2). In contrast, the administration of rutin decreased glucose levels in IPGTT and AUC of IPGTT in a dose-dependent manner (Table 2). Moreover, IPITT was conducted to evaluate the insulin tolerance in rats. The results showed that after insulin challenge, glucose level in old rats were higher than that of young control rats (Table 2). The AUC of IPITT in old rats was significantly increased, confirming the occurrence of insulin intolerance (Table 2). In contrast, the administration of rutin inhibited the increase of glucose levels in IPITT and AUC of IPITT in old rats in a dose-dependent manner (Table 2). These results demonstrated that rutin administration protected against aging-related metabolic dysfunction in old rats in a dose-dependent manner.

3.2 Rutin improved insulin signaling in aged rats.

To evaluate the effect of rutin on insulin signaling transduction, Akt and insulin receptor substrate 1 (IRS-1) phosphorylation in livers was detected by western blot. The results showed that in old rats, phosphorylation of Akt (Fig. 1A) and IRS-1 (Fig. 1B) was reduced compared with that of young control rats. The administration of rutin significantly inhibited the decrease of phosphorylation of Akt (Fig. 1A) and IRS-1 (Fig. 1B) in old rats. The results indicated that rutin improved insulin signaling transduction in aged rats.

3.3 Rutin inhibited inflammation, lipid accumulation and liver injury in aged rats.

In the next step, we evaluated the effect of rutin on inflammation and lipid accumulation in aged rats. As shown in Table 3, the results showed that serum levels of IL-1β and TNFα in old rats were significantly higher than that of young rats. In rats treated by rutin, the increase of IL-1β and TNFα levels were inhibited in a dose-dependent manner (Table 3). In addition, serum and liver triglyceride (TG) levels in old rats were higher than that of young control rats (Table 3). The rutin treatment significantly decreased serum and liver TG levels in old rats, which was dose-dependent (Table 3). Moreover, the increase of serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) in old rats were significantly decreased by rutin treatment (Table 3). These results implicated that rutin ameliorated aging-related inflammation, lipid accumulation and liver injury in rats.

3.4 Rutin inhibited endoplasmic reticulum stress in aged rats.

To further explore the possible mechanism of rutin-exhibited protective effects on metabolic function in aged rats, we evaluated the effect of rutin on aging-related changes of endoplasmic reticulum (ER) function. As shown in Fig. 2, protein expression of activating transcription factor (ATF) 3 and glucose-regulated protein (GRP78) in livers of aged rats was significant higher than that of young control rats. The administration of rutin markedly reduced the protein expression of ATF3 and GRP78 in livers of age rats, which relied on the dose of rutin used (Fig. 2). These results indicated that rutin ameliorated aging-related ER stress in rats.

3.5 Rutin inhibited mitochondrial dysfunction in aged rats.

Next, we examined the effect of rutin on mitochondrial function in aged rats. As illustrated in Fig. 3A, oxygen consumption rate in livers of aged rats was significantly reduced compared with that of young control rats. In addition, in livers of old rats, The activities of the Na^+/K^+-ATPase and Ca_2^+-ATPase (Fig. 3B and C) were significantly lower than that of young control rats. Consistent with the above results, the rutin treatment could effectively inhibited the decrease of oxygen consumption rate, and Na^+/K^+-ATPase and Ca_2^+-ATPase activities in old rats (Fig. 3). These results indicated that rutin improved aging-related mitochondrial dysfunction in rats.

3.6 Rutin inhibited oxidative stress in aged rats.

Furthermore, we tested the effect of rutin on oxidative stress in aged rats. In Table 4, we showed that serum level of malondialdehyde (MDA) in old rats was significantly increased, compared with that of young control rats. The treatment of rutin suppressed the increase of MDA in those

old rats (Table 4). Moreover, glutathione (GSH) level and superoxide dismutase (SOD) activity in livers of old rats were decreased, compared with that of young control rats (Table 4). Rutin administration increased GSH level and SOD activity in aged rats in a dose-dependent manner (Table 4). The data indicated that rutin inhibited aging-related oxidative stress in rats.

4. Discussion

Rutin is a well-known flavonoid, which possess various biological capabilities. Previous studies have found that rutin is able to regulate glucose and lipid metabolism. Wang et al. [13] reported that rutin may have great therapeutic potential in the treatment of diabetic cardiomyopathy, and possibly other cardiovascular disorders, by preventing oxidative stress, inflammation and cell death. Rutin was also found to reverse or prevent metabolic changes such as abdominal fat pads and glucose tolerance, reverse or prevent changes in hepatic and cardiovascular structure and function, reverse oxidative stress and inflammation in the liver and heart, and normalize expression of liver markers in high-carbohydrate, high-fat diet-fed rats [10]. However, whether rutin could affect aging-related metabolic dysfunction is still unknown. In the current study, we evaluated the protective effect of rutin against metabolic dysfunction in aged rats and explored the possible mechanisms. We discovered that the administration of rutin in old rats significantly protected against aging-related metabolic dysfunction, as reflected by decrease of body weights, perirenal adipose tissue weights, SBP, glucose and insulin levels, and HOMA-IR, and improvement of glucose and insulin tolerance in old rats. In the further studies, we tested the effect of rutin on several pathophysiological conditions, which was associated with aging-related disorders.

Insulin resistance is one of the hallmarks of metabolic syndrome, type 2 diabetes and many other metabolic disorders [14, 15]. Moreover, insulin resistance is closely associated with aging-related metabolic pathophysiological processes [16-18]. Insulin resistance is characterized by impairment of insulin-stimulated signaling transduction. In the present study, we also examined the effect of rutin on insulin signaling transduction in aged rats. We showed that rutin treatment significantly alleviated aging-related insulin resistance, as reflected by increase of the phosphorylation of Akt and IRS-1 in livers of old rats. The data demonstrated that rutin-induced improvement of insulin sensitivity was involved in the amelioration of metabolic dysfunction in old rats.

Chronic inflammation is a basic pathophysiological process aging and the associated diseases of aging [19]. Chronic inflammation is closely associated with the pathogenesis of insulin resistance through influencing lipid accumulation, mitochondrial function, and ER stress, etc [19, 20]. In the current study, we assessed the effect of rutin on inflammation in old rats. The results showed that rutin suppressed aging-related inflammation, as evidenced by significant decrease of IL-1β and TNFα levels in aged rats, indicating that the alleviation of inflammation may be involved in rutin-exerted metabolic regulation at aging context.

Obesity and/or increased ectopic lipid accumulation in livers or muscles are major characteristics as life span increases [21]. Obesity and increased ectopic lipid accumulation contributes to chronic and systemic inflammation, insulin resistance and the metabolic consequences [19, 22, 23]. In our study, we found that the increase of circulating and liver TG content and serum levels of ALT and AST in old rats was inhibited by rutin significantly, indicating that rutin could effectively improve aging-related ectopic lipid accumulation and liver injury, which may be involved in the beneficial effect of ruin on metabolic function.

Aging is a complex process, involving the changes of various organelles. Among them, mitochondria and ER are the most important organelles that play pivotal roles in the pathogenesis of aging-related disorders. Mitochondrial dysfunction is considered to be a major risk of insulin resistance in both the adult and elderly [24, 25]. Moreover, ER stress is also a characterized alteration in aging individuals, which contributes to inflammation and insulin resistance [26, 27]. In our study, we also examined the possible effect of rutin on mitochondrial and ER function in old rats. We discovered that rutin treatment significantly inhibited mitochondrial dysfunction, as reflected by increase of oxygen consumption rate and activities of Na^+/K^+-ATPase and Ca_2^+-ATPase, and suppressed ER stress, as reflected by decreased expression of ATF3 and GRP78, in livers of aged rats. The results demonstrated that improvement of mitochondrial dysfunction and ER stress may participate in the aging-related metabolic regulation by rutin.

Oxidative stress, defined as increased generation of ROS and its oxidative products of biomacromolecules, plays a fundamental role in the process of aging and various related disorders [28-31]. Oxidative stress is the underlying mechanism and the consequence of lipid accumulation [32], inflammation [33], mitochondrial dysfunction [34] and ER stress [35], resulting insulin resistance and other metabolic outcomes. In our study, we evaluated the effect of rutin on

oxidative stress status in the elderly. We showed that rutin administration significantly inhibited aging-related oxidative stress, as evidenced by decrease of MDA level, increase of GSH content and SOD activity, indicating the involvement of reduction of oxidative stress in the beneficial effect of rutin in aged rats.

Previous studies have found other agents that could improve aging-related metabolic dysfunction [36-38]. In the present study, we have confirmed the beneficial effect of rutin for the intervention of aging-related metabolic disorders. Since rutin is an important constituent in many kinds of human food materials, it provides a potent option for the intervention of metabolic syndrome in old individuals. Most of those effects of rutin studied in the present research were dose-dependent. The effect of high dose of rutin was better than that of low dose. Further studies are required to search for the most efficient dose of rutin used for the intervention of metabolic syndrome and compare the efficiency of rutin with other metabolism-regulating agents.

In conclusion, in the present study, we found that the administration of rutin could effectively improve aging-related metabolic dysfunction (Fig. 4). The amelioration of inflammation, lipid accumulation, mitochondrial dysfunction, ER stress, and oxidative stress may be involved in the effect of rutin on aging-related metabolic dysfunction (Fig. 4). These findings provide novel insights into the potential use of rutin in the intervention of aging and its related metabolic diseases.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Figure legends

Fig. 1 Effect of rutin on insulin signaling in aged rats.

20 month rats were treated by rutin for 6 weeks. After the experiment, liver tissues were collected and Akt (A) and IRS-1 (B) phosohorylation was detected by western blot. Representative blots were shown and results were also expressed as mean ± SEM. *$p < 0.05$.

Fig. 2 Effect of rutin on ER stress in aged rats.

20 month rats were treated by rutin for 6 weeks. After the experiment, liver tissues were collected and ATF3 and GRP78 expression was detected by western blot. Representative blots were shown and results were also expressed as mean ± SEM. *$p < 0.05$.

Fig. 3 Effect of rutin on mitochondrial function in aged rats.

20 month rats were treated by rutin for 6 weeks. After the experiment, liver tissues were collected and oxygen consumption in fresh livers was detected using a Clark Oxygen Electrode. The activities of the Na^+/K^+-ATPase and Ca_2^+-ATPase were determined, using commercial assay kits. $p < 0.05$.

Fig. 4 General effect of rutin on aging-related metabolic dysfunction.

Table 1 Effect of rutin on body weight, adipose tissue weight, systolic blood pressure (SBP), fasting glucose and insulin levels and HOMA-IR.

Group	Body weight (g)	Weight of perirenal adipose tissue (g)	SBP(mmHg)	Fasting blood glucose level (mg/dl)	Fasting insulin level (ng/dl)	HOMA-IR
Young	554 ± 37	4.6 ± 1.2	111.4 ± 6.8	67.6 ± 5.6	1.7 ± 0.2	0.4 ± 0.1
Old	662 ± 30 *	17.7 ± 3.2 *	171.8 ± 10.2 *	114.1 ± 11.4 *	4.0 ± 0.3 *	11.4 ± 2.4 *
25 mg/kg Rutin	634 ± 36 #	14.8 ± 2.4 #	145.8 ± 23.2 #	104.2 ± 9.1 #	3.5 ± 0.4 #	8.2 ± 2.2 #
50 mg/kg Rutin	602 ± 18#	9.9 ± 3.3 #	133.8 ± 13.9 #	86.1 ± 7.3 #	3.1 ± 0.3 #	5.0 ± 2.5 #

*p < 0.05, compared with Young. #p < 0.05, compared with Old.

Table 2 Effect of rutin on glucose and insulin tolerance.

Group	**IPGTT (mg/dl)**				
	0 min	30 min	60 min	120 min	**AUC**
Young	62.9 ± 4.8	115.7 ± 6.9	95.7 ± 6.9	76.5 ± 4.1	184 ± 17
Old	110.1 ± 4.6 *	165.2 ± 5.9 *	148.3 ± 6.1 *	136.4 ± 7.9 *	289 ± 19 *
25 mg/kg Rutin	97.5 ± 4.5 #	159.1 ± 3.6	131.7 ± 4.5 #	111.9 ± 5.9 #	268 ± 12 #
50 mg/kg Rutin	82.5 ± 4.5 #	135.1 ± 5.6 #	117.7 ± 4.5 #	95.9 ± 5.9 #	233 ± 21 #
	IPITT (mg/dl)				
	0 min	30 min	60 min	120 min	**AUC**
Young	75.1 ± 5.5	48.4 ± 4.5	42.2 ± 4.2	43.8 ± 5.5	115 ± 9
Old	124.7 ± 5.6 *	92.5 ± 6.2 *	77.7 ± 6.3 *	88.9 ± 5.2 *	191 ± 7 *
25 mg/kg Rutin	118.1 ± 5.8	78.3 ± 4.8 #	66.7 ± 6.6 #	73.5 ± 4.9 #	173 ± 9 #
50 mg/kg Rutin	89.1 ± 5.6 #	59.3 ± 5.0 #	52.7 ± 6.2 #	58.5 ± 6.0 #	147 ± 6 #

IPGTT: intraperitoneal glucose tolerance test; IPITT: intraperitoneal insulin tolerance test. *$p < 0.05$, compared with Young. #$p < 0.05$, compared with Old.

Table 3 Effect of rutin on inflammation and lipid accumulation.

Group	Serum IL-1β (ng/l)	Serum TNFα (ng/l)	Serum TG (mg/dl)	Liver TG (mmol/g pro.)	Serum ALT (U/L)	Serum AST (U/L)
Young	9.8 ± 1.5	11.0 ± 2.6	142 ± 19	76.2 ± 8.7	33.5 ± 12.1	157.2 ± 21.2
Old	20.4 ± 3.2 *	23.2 ± 5.7 *	236 ± 24 *	104.6 ± 10.3 *	142.6 ± 42.1 *	261.7 ± 77.3 *
25 mg/kg Rutin	18.2 ± 1.3 #	19.2 ± 3.3 #	214 ± 11 #	98.2 ± 6.1 #	108.3 ± 33.5 #	216.7 ± 56.5 #
50 mg/kg Rutin	15.6 ± 2.1#	16.6 ± 1.7 #	184 ± 15 #	85.4 ± 7.2 #	78.7 ± 14.8 #	186.5 ± 48.0 #

TNFα, tumor necrosis factor; TG, triglyceride; ALT, alanine transaminase; AST, aspartate transaminase. *$p < 0.05$, compared with Young. #$p < 0.05$, compared with Old.

Table 4 Effect of rutin on oxidative stress.

Group	Serum			Liver		
	MDA (μmol/ml)	GSH (μmol/ml)	SOD (U/ml)	MDA (μmol/g pro.)	GSH (μmol/g pro.)	SOD (U/g pro.)
Young	0.72 ± 0.19	0.042 ± 0.008	142 ± 19	28.6 ± 4.2	0.098 ± 0.015	1.51 ± 0.23
Old	1.52 ± 0.22 *	0.019 ± 0.005 *	236 ± 24 *	17.6 ± 2.1 *	0.027 ± 0.008 *	0.71 ± 0.25 *
25 mg/kg Rutin	1.34 ± 0.15 #	0.031 ± 0.005 #	214 ± 11 #	22.6 ± 1.8 #	0.036 ± 0.012 #	1.12 ± 0.09 #
50 mg/kg Rutin	1.18 ± 0.16 #	0.038 ± 0.008 #	184 ± 15 #	25.8 ± 2.9 #	0.054 ± 0.011 #	1.33 ± 0.19 #

MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase. *$p < 0.05$, compared with Young. #$p < 0.05$, compared with Old.

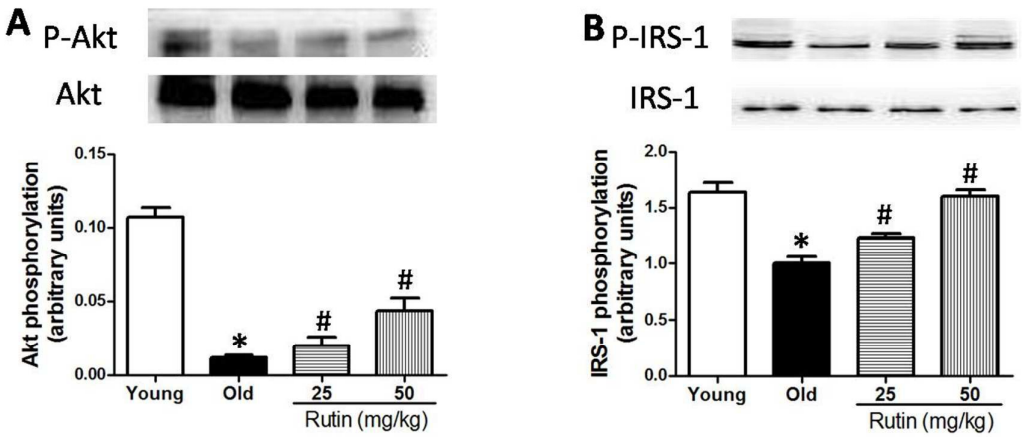

Fig. 1 Effect of rutin on insulin signaling in aged rats.

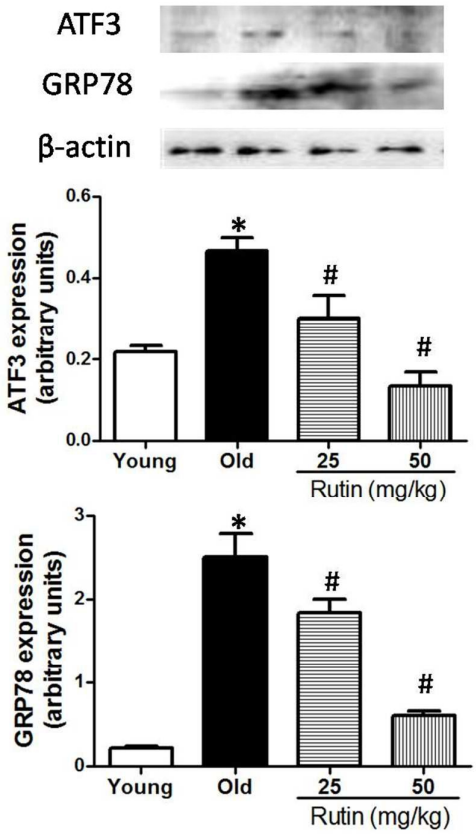

Fig. 2 Effect of rutin on ER stress in aged rats.

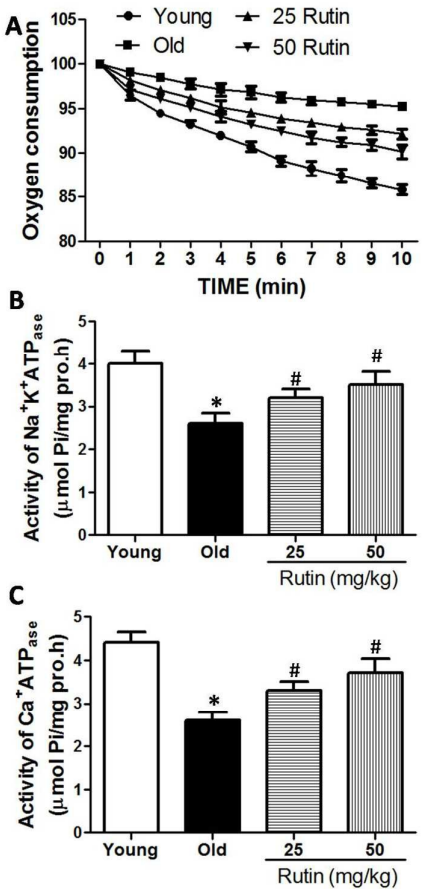

Fig. 3 Effect of rutin on ER stress in aged rats.

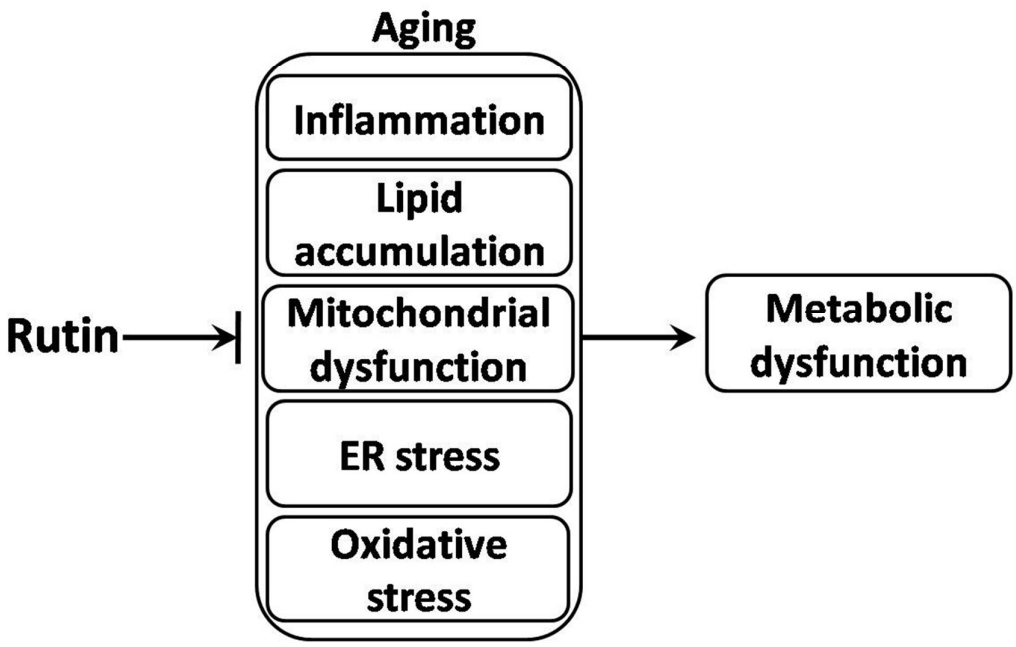

Fig. 4 General effect of rutin on aging-related metabolic dysfunction.