

Original Article

Protective effect of resveratrol against prostate enlargement induced by high-fat diet

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Abstract: Background: Resveratrol has shown preventing effect on prostate diseases. This research was performed to investigate the protective effect of resveratrol on prostate enlargement induced by high-fat diet (HFD). Methods: Rats were divided into four groups: normal pellet diet feeding (NPD), high-fat diet feeding (HFD), resveratrol treating (HFD+RES) and pioglitazone treating (HFD+PIO) groups. Fasting insulin and glucose level were assessed by radio-immunoassay and capillary blood glucose method respectively. Insulin resistance was evaluated by Homeostasis Model Assessment of insulin resistance index (HOMA-IR). Body weight, prostate weigh, and visceral fat weight in were measured and prostate index was calculated. The expression of PPAR γ , COX-2, NOX-2, SOD-1 and SOD-2 were revealed by using western blot analysis. Results: Insulin level in HFD group was increased than in NPD group. Resveratrol administration decreased the level of insulin and induced a decline trend in HOMA-IR. Resveratrol had similar effect with pioglitazone in decreasing prostate weight, visceral fat and PI. Furthermore, they reversed the up-regulated expression of COX-2 and NOX-2, as well as the down-regulated expression of PPAR γ , SOD-1 and SOD-2 produced by HFD-fed. Conclusion: Resveratrol has protective effects on improving prostate enlargement induced by HFD which was achieved by alleviating hyperinsulinemia, anti-inflammatory and antioxidative characteristics.

Keywords: Benign prostatic hyperplasia, resveratrol, pioglitazone, insulin resistance, PPAR γ , COX-2, NOX-2, rat

Introduction

Benign Prostate hyperplasia (BPH) is a common disease in the aging men and causes substantial adverse health effects. BPH, leading to ostensive enlargement of the prostate and in turn resulting in lower urinary tract symptoms (LUTS), has high prevalence and socio-economic burden. Therefore, it is important to determine the risk factors of BPH progression and to keep men away from them. However limited data are available concerning factors that might be protective against the development of BPH. Recently a growing amount of researches demonstrate that metabolic syndrome (MS) and/or its individual components are involved in the development and progression of BPH [1-3]. The individual components of MS, including insulin resistance [4], dyslipidemia [5], obesity [6], hypertension [7] and the syndrome itself might predispose patients to greater risks of BPH and LUTS. Available data indicate that

BPH patients have higher total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels and lower high-density lipoprotein cholesterol (HDL-C) levels compared with non-BPH patients [8]. Furthermore several reports suggested that BPH is one of components of MS [9]. With the accumulation of knowledge about MS and BPH, it has been known that insulin resistance (IR) is the cornerstone of MS and play a critical role in the growth of prostate and the progression of BPH [10, 11]. In addition to IR, inflammation and oxidative stress were reported to involve in the development and progression of BPH [12, 13]. Despite the fact that the association between prostate diseases and MS is generally accepted, the pathogenetic link still needs to be deeply elucidated.

A report has demonstrated high-fat diet led to insulin resistance, compensatory hyperinsulinemia and prostatic enlargement in Sprague-Dawley rats. Pioglitazone (PIO), a peroxisome

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proliferator-activated receptor γ (PPAR γ) agonist, normalized lipid metabolism and insulin level, improved insulin sensitivity in rats fed on high-fat diet. As a result, pioglitazone attenuated cell proliferation and enlargement of the prostate in diet-induced insulin-resistant rats [14]. However, according to previous report pioglitazone is not full safety. Its main adverse effects include: weight gain, pedal edema, bone loss and precipitation of congestive heart failure in at-risk individuals, without any increase in cardiovascular diseases/all-cause mortality [15]. Therefore, it is necessary to find alternative agents that can be used to treat this disease without obvious side-effects.

Recently several reports suggested that herb component, such as flavanol glycosides and vanillic acid, has anti-inflammatory, antioxidative and antiproliferative properties [16, 17]. Resveratrol (3,5,4'-trihydroxy-trans-stilbene, RES) is a natural plant-derived polyphenol with characteristics mentioned above [18]. Harikuma et al. reviewed that resveratrol possessed antidiabetic activity, antihyperglycemic, antioxidant and anti-inflammatory activity. More importantly, its significant antidiabetic activity equipotent tolbutamide, one kind of hypoglycemic agent, inspired us [19, 20]. Compared with pioglitazone's adverse effect, its good absorption and toleration attracted considerable attention. Mostly it was used in prostate cancer cell line or animal model. Few data about its function have been available from BPH animal model or patients.

PPAR γ , a connective molecular between systemic metabolic disease and BPH, was chosen to be a marker to explore resveratrol's protective function against prostate enlargement and insulin resistance and to verify that insulin resistance is related to BPH [14]. Cyclooxygenase-2 (COX-2) is one of the key inflammatory mediators, and was considered as a marker of inflammation to evaluate resveratrol's protective effect against prostate enlargement through anti-inflammatory property [21]. NADPH Oxidase (NOX) and superoxide dismutase (SOD)1/2 were used as markers of oxidative stress to evaluate the anti-oxidative function of resveratrol in progression of BPH [22, 23].

Thus in this study, we aimed to observe the effect of RES on improving prostate enlargement induced by high-fat diet (HFD) and dis-

cuss the mechanism involved in. We found RES had similar effect to PIO. It decreased the symptoms of BPH which might be mediated by anti-inflammatory and antioxidative characteristics.

Material and methods

Animals and experimental proposal

Wistar rats (150-200 g) were purchased from the animal center of Hebei University. All rats were adaptively for one week, and then divided to four groups. (1) NPD group: The rats in NPD group, used as control group, were fed with normal pullet diet (NPD) which was paired with high fat diet (HFD) purchased from TROPHIC Animal Feed High-tech Co. LTD (Nantong, Jiangsu, China). (2) HFD group: The rats, which were used to induce the main components of metabolic syndrome (MS), were fed with HFD purchased from the same company as in control group for fourteen weeks. (3) HFD+RES group: The animals in this group were fed with HFD firstly, and then at the end of the tenth week, resveratrol (RES, 50 mg•kg⁻¹, 1/d) was given together with HFD for another 4 weeks. (4) HFD+PIO group: The protocol in this group is as same as in HFD+RES group, except for pioglitazone (PIO, 20 mg•kg⁻¹, 1/d) administration. Rats in all groups were kept under same environment and supplied with sufficient water. At that time, body weights of every rat were measured before they were sacrificed at the end of fourteenth week. Then animals were sacrificed by cervical dislocation. Visceral fat weight and prostate weight were assessed. The right ventral lobe of prostate was preserved at -80°C for the molecular studies and left ventral lobe of the prostate was used for histological evaluation using hematoxylin and eosin staining. All procedures involving animals complied with the European Community guidelines for the use and care of laboratory animals and approved by Animal Ethical Committee of Hebei Medical University, China.

Chemicals and reagents

Resveratrol was purchased from Liding Biotechnology limited (Shanghai, China) with the purity of 99.9% and was combined with β -cyclodextrin (Fengyuan Bio-technology limited, Jiangsu, China) followed the method described previously before used [24]. Pioglitazone

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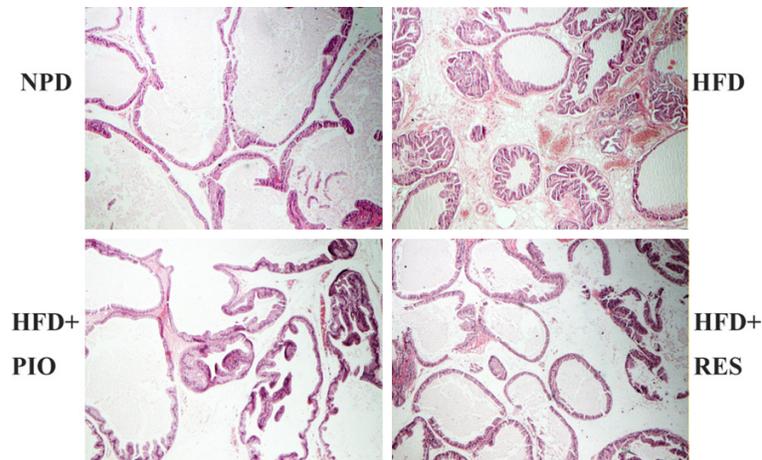


Figure 1. Impact of resveratrol on prostate issue induced by HFD. In the glandular lumens of rats fed by HFD, quantity secretion was founded, the number of epithelia cell layer, infolding of epithelial layer in the prostatic acini and mesenchyma were increased. Lots of adipose cells were observed. Resveratrol or pioglitazone similarly improved the modification found in HFD.

was purchased from Shengtianyu Bio-technology limited (Wuhan, China) with the purity of 99% and was dissolved in pure water. Protease inhibitor and phosphoric acid protease inhibitors were obtained from Roche Mannheim (Germany).

The antibody to PPAR γ (Abcam, ab209350), COX-2 (Abcam, ab52237), NOX2 (Abcam, ab-80508) and Superoxide Dismutase (SOD)1/2 (Abcam, ab13498/ab13534) were supplied by Abcam (Cambridge, MA, USA). PVDF filters and the enhanced chemiluminescence detecting reagents were obtained from Millipore Corporation (Billerica, MA, USA). PPAR γ , COX-2, NOX-2 and SOD1/2 were determined using commercial kits purchased from the bio-technology limited company (Shijiazhuang, China).

Biochemical parameters

Under intraperitoneal injection of 7% chloral hydrate, the blood samples (0.8 ml) were collected from the orbital plexus in rats. The serum was separated by centrifugation (3000 g \times 5 min) and stored at -80°C and it was used for detecting the variations of lipid metabolism parameters and the levels of insulin in biochemical laboratory of the third Hospital of Hebei Medical University. The level of fasting glucose in blood obtained from the end of rat's tail was measured by Roch glucometer. The levels of insulin and glucose and lipid metabolism parameters in fasting serum were

determined at the end of the fourteenth weekend. The Homeostasis Model Assessment of insulin resistance (HOMA-IR) was calculated by using the formula: $HOMA-IR = (\text{fasting insulin}) \times (\text{fasting glucose})/22.5$.

Anatomic parameter

At the end of drug treatment, the body weight, prostate weight and visceral fat weight were assessed. The prostate index (PI) of each rat was calculated with the ratio of prostate weight to body weight.

Western blot analysis

The protein of prostate was separated and homogenized in lysis buffer including protease inhibitor and phosphoric acid inhibitors. The supernatant was kept at -80°C and concentration of testing protein was determined by Coomassie assay. After electrophoresed on SDS-PAGE, the samples were transferred into polyvinylidene fluoride (PVDF) membrane. Then the membranes were blocked with 5% skim milk at 37°C for 1 hour. After that the blocked membranes were incubated with primary antibodies to anti-PPAR γ , anti-COX-2, anti-NOX-2, and anti-SOD-1 and SOD-2 at 4°C overnight. The membranes were washed three times with TPBS, followed by incubation with a secondary antibody at 37°C for 1 hour. After three washes with TBST, positive bands were detected using enhanced chemiluminescence following the manufacturer's instructions. Alpha Imager gel image analysis system was used to measure integrated optical density (IOD) of every brand. The IOD ratio of PPAR γ , COX-2, NOX-2, SOD-1 and SOD-2 to GAPDH was calculated and analyzed.

Statistical analyses

Data were presented as means \pm SD. For multiple comparisons, one-way ANOVA was used to analyze obtained results. Significance of difference between two groups was assessed using Student's *t*-test. Statistically significant differences were considered at $P < 0.05$.

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Table 1. Effect of biochemical parameters in NPD, HFD, HFD+RES and HFD+PIO group at the 14th week

Groups	N	TC (mmol/L, $\bar{x} \pm SD$)	TG (mmol/L, $\bar{x} \pm SD$)	HDL (mmol/L, $\bar{x} \pm SD$)	LDL (mmol/L, $\bar{x} \pm SD$)	VLDL (mmol/L, $\bar{x} \pm SD$)	FIN (uIU/ml $\bar{x} \pm SD$)	FPG (mmol/L $\bar{x} \pm SD$)	HOMA-IR
NPD	5	1.80±0.24	0.74±0.36	0.51±0.05	0.28±0.09	0.33±0.16	23.94±2.54*	4.12±0.38	4.37±0.54*
HFD	5	2.20±0.18	0.90±0.29	0.44±0.06	0.34±0.05	0.41±0.14	35.51±7.00	4.68±0.45	7.38±1.60
HFD+RES	5	1.72±0.22*	0.38±0.22*	0.41±0.05	0.26±0.02*	0.17±0.10*	26.54±4.52*	4.82±0.31	5.68±0.98
HFD+PIO	5	2.05±0.33	0.48±0.25*	0.46±0.08	0.36±0.06	0.22±0.12*	27.41±1.78*	4.74±0.48	5.76±0.54

The FIN in HFD-fed rats is significant higher than that in NPD, RES and PIO groups. HOMA-IR in HFD group is significant higher than that in NPD group. The levels of TG and VLDL were significantly reduced at the end of the 14th in HFD+RES and HFD+PIO groups than in HFD group. The levels of TC and LDL significantly decreased in HFD+RES group than that in HFD group. No significant difference of FPG was observed among the four groups. NPD: normal pellet diet; HFD: High fat diet; RES: Resveratrol (50 mg/100 g body weight/day for 4 weeks); PIO: Pioglitazone (20 mg/100 g body weight/day for 4 weeks). TC: Total cholesterol; TG: Triglyceride; HDL: High-density lipoprotein; LDL: Low-density lipoproteins; VLDL: Very low-density lipoprotein; FIN: fasting insulin; FPG: fasting plasma glucose; HOMA-IR: Homeostasis Model Assessment of insulin resistance; BW: body weight. *P<0.05: compared with HFD group.

Table 2. Effect of anatomic parameters in NPD, HFD, HFD+RES and HFD+PIO group at the 14th week

Groups	N	BW (g $\bar{x} \pm SD$)	PW (g $\bar{x} \pm SD$)	PI (mg/g $\bar{x} \pm SD$)	VFW (g $\bar{x} \pm SD$)
NPD	5	326.20±32.27	0.41±0.07*	1.26±0.16*	6.87±1.92*
HFD	5	345.80±35.10	0.54±0.07	1.54±0.18	14.10±4.16
HFD+RES	5	349.20±19.97	0.37±0.06*	1.05±0.12*	10.31±1.49
HFD+PIO	5	374.00±40.14	0.35±0.06*	0.94±0.25*	11.69±3.65

The PW, PI and visceral fat in HFD group were significantly increased compared with in NPD group. Resveratrol or pioglitazone treatment decreased PW, PI and visceral fat weight compared with HFD fed without significant difference between HFD+RES and HFD+PIO groups. Furthermore no significant difference of BW was observed among all groups. BW: body weight; PW: prostate weight; PI: prostate index; VFW: visceral fat weight. *P<0.05 compared with HFD. The other abbreviations are the same as those in **Table 1**.

Results

Resveratrol improves hyperinsulinemia

The level of insulin in HFD group was the highest in all groups at the 14th weeks. Resveratrol or pioglitazone treatment decreased serum insulin levels without significant difference. Meanwhile there were no critical differences of the HOMA-IR among HFD group, HFD+RES group and HFD+PIO groups but there was a decline trend of HOMA-IR in both of HFD+RES group and HFD+PIO group compared with HFD group. No significant difference was observed in the fasting insulin among all groups (**Figure 1; Table 1**).

Impact of resveratrol on lipid metabolism

The level of triglyceride in serum was increased in HFD rats compared with the NPD group. Both resveratrol and pioglitazone treatment decreased the level of triglyceride compared with it in HFD group. Although no significant dif-

ference was observed, the level of triglyceride tended to elevated in HFD group than in NPD group. No difference was investigated in the level of total cholesterol among all groups (**Figure 1**).

Effects of resveratrol on anatomic parameters

The prostate weight, visceral fat and PI in HFD group rats were increased significantly compared to NPD rats. Resveratrol treatment significantly decreased prostate weight, visceral fat and PI produced by HFD-fed. The

effect was similar with pioglitazone. There was no significant difference of body weight was observed among all groups (**Table 2**).

Histologic examination of prostate

Increasing secretion, number of epithelia cell layer and infolding of epithelial layer in the prostatic acini mesenchyma were increased and lots of adipose cells were founded in the glandular lumens of rats in HFD group than in NPD group. Resveratrol improved the histologic performance in prostate noted above reflected in varying degrees, increasing epithelial layer recede, decreasing infolding epithelial layer and reducing mesenchyma cells and the effect is similar with pioglitazone.

Effect of resveratrol treatment on the elevated expression of PPAR γ

The expression of PPAR γ was significantly decreased in HFD group than in NPD group. When treated with resveratrol or pioglitazone,

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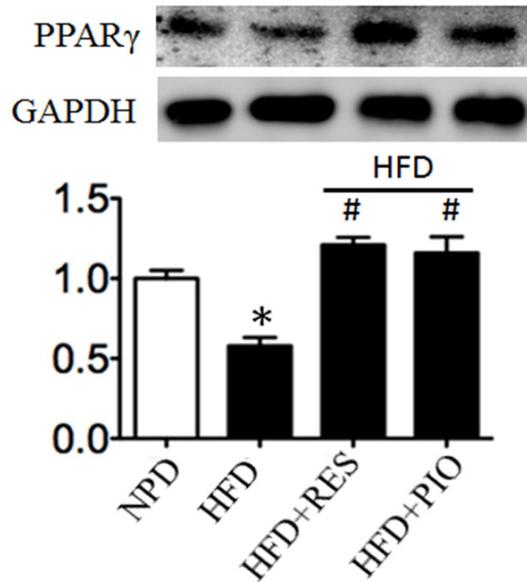


Figure 2. Impact of resveratrol on PPAR γ , the connection of systemic metabolic disease and BPH. Resveratrol and pioglitazone treatment elevated the suppressed expressions of PPAR γ in prostate gland of HFD-fed rats. PPAR γ : peroxisome proliferator-activated receptor γ .

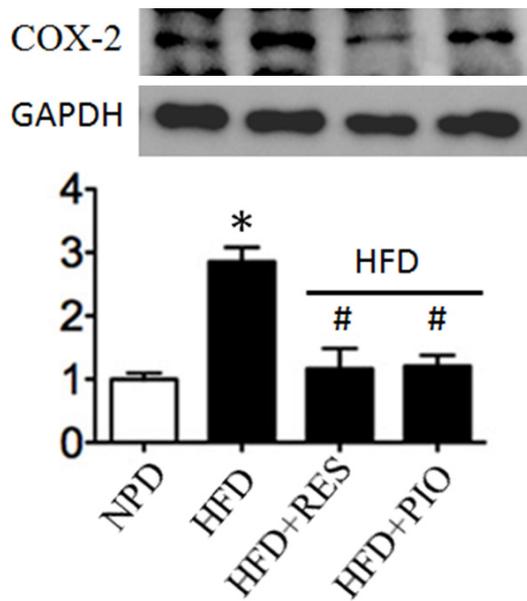


Figure 3. Impact of resveratrol on COX-2, a critical inflammatory mediator. Resveratrol and pioglitazone treatment inhibited the elevated expressions of COX-2 in prostate gland of HFD-fed rats. COX-2: Cyclooxygenase-2.

the expression of PPAR γ was both significantly promoted compared with HFD group. No significant difference of the PPAR was observed

among HFD+RES, HFD+PIO and NPD groups (**Figure 2**).

Effect of resveratrol treatment on the decreased expression of COX-2

The expression of COX-2 is obviously increased in HFD group than in NPD group. Resveratrol or pioglitazone treatment counterbalanced the change of COX-2 expression compared with HFD group. Among HFD+RES, HFD+PIO and NPD groups, there was no significant difference of the expression of COX-2 (**Figure 3**).

Effect of resveratrol treatment on the oxidative stress

The expression of NOX-2 was up-regulated in the HFD group than in NPD group. Resveratrol or pioglitazone administration down-regulated the increased protein level of NOX-2. Similarly, the expression of SOD-1 and SOD-2 were down-regulated in HFD group compared to NPD group and the above changes were reversed by resveratrol or pioglitazone given. Among HFD+RES, HFD+PIO and NPD groups, there was no significant difference in the level of NOX-2, SOD-1 and SOD-2 (**Figure 4**).

Discussion

Resveratrol is a polyphenol produced by several plants. Clinically, it has been reported that resveratrol has a wide range of beneficial effects for health, such as cardioprotective, neuroprotective, and immunomodulatory function as well as improving insulin sensitivity. The latter is very important because it is a critical risk factor of BPH. These properties, together with good absorption and tolerance, would make it an attractive agent in prostatic diseases [18]. A number of reports provided convincing evidence that insulin-resistance closely involved in BPH. Based on these opinions, we try to investigate the protective effect of resveratrol on prostate enlargement induced by HFD. As far as we know, it is the first time that resveratrol was used to intervene a BPH animal model induced by HFD.

As to the MS, there is not a standard model that can mimic its entire components so far. Hammarsten J et al. proposed that given the limitations of the classic definitions of MS, it is sensible to use non-composite definitions of

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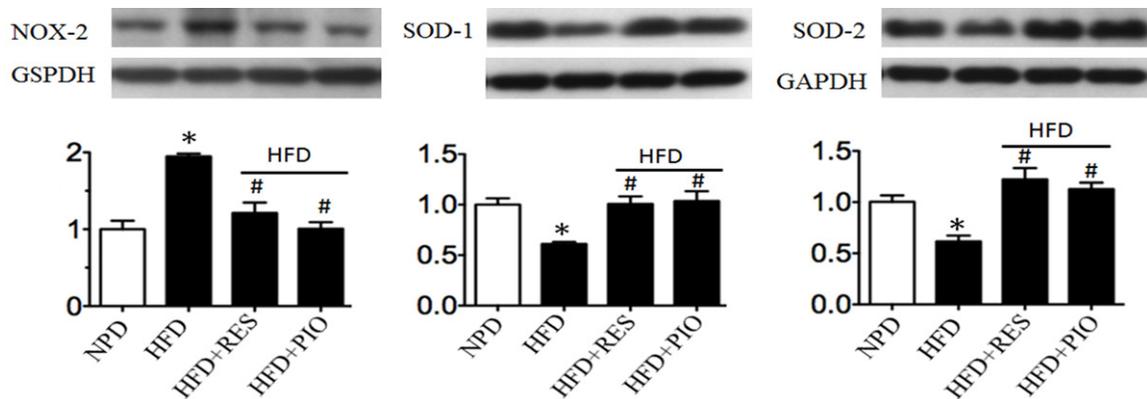


Figure 4. Impact of resveratrol on NOX, SOD, two opposite aspect of oxidative stress. Resveratrol and pioglitazone treatment inhibited the elevated expressions of NOX-2 in prostate gland of HFD-fed rats and elevated the suppressed expression of SOD-1/2. SOD: Superoxide Dismutase; NOX-2: NADPH Oxidase-2.

the disorder in urological research. In their research, the risk factor linking established aspects of the MS with urologic disorders were analyzed instead of that established definitions [25]. It is widely recognized that abdominal obesity and insulin resistance are potential MS pathogenic factors [26, 27], so the two key components of MS were chosen as impact factor of the development and progression of BPH.

Vikramet et al. reported that cell proliferation and contractility of the prostate gland increased in hyperinsulinaemic rats and proposed mechanistic links between insulin resistance and BPH [28]. In their following research, pioglitazone was used to treat prostate enlargement and insulin-resistance. Enhanced cell proliferation and prostate enlargement in insulin-resistant rats induced by HFD were attenuated [14]. Combined with others research finding insulin resistance was showed to be a critical role in BPH development and growth [11, 29, 30].

A study investigated the function of obesity on prostate volume in men who had no obvious obesity related metabolic disorder. Totally, 146 men older than 40-year without overt obesity-related disorder, such as diabetes mellitus, impaired fasting glucose, dyslipidemia or hypertension were included. Prostate volume was critically larger in the central obesity group than that in the normal waist group. Therefore a conclusion that central obesity is a key risk factor for prostatic hyperplasia was derived [31].

In the present study, the two critical components of MS, insulin resistance and visceral obesity, were mimicked as the impact factor of

BPH finally. As anticipated, visceral adipose and plasma insulin level of rats in HFD-fed group significantly increased compared to that in NPD-fed group, epithelia cell proliferation was observed in HFD-fed group. After resveratrol and pioglitazone administrations, insulin level, triglyceride and visceral adipose of rat decreased. Though there is no significant difference of HOMA-IR among RES, PIO and HFD-fed groups at the end of this experiment, the decrease trend of HOMA-IR has been gotten. Meanwhile prostate epithelia cell proliferation was improved. Prolonging the admission of RES and PIO or elevating agent's dose maybe result in a positive change of HOMA-IR.

PPAR γ is a nuclear receptor. According to literature published it is the connection between systemic metabolic disease and BPH/LUTS because it has impaction on both inflammation and insulin resistance which were speculated to be correlated with BPH [32]. As early as 2009, Escobar et al. reported that PPAR γ was a possible link between diet and augmented prostatic growth [33]. The mechanisms of BPH induced by insulin resistance and improvement of prostate enlargement by pioglitazone through up-regulating PPAR γ were discussed. Finally it was proposed that upregulation of PPAR γ may be potential treatment strategy for BPH. But we all know that full PPAR γ agonist, such as pioglitazone, has varying degrees side-effects, negative effects on body weight, fracture risk, fluid retention, heart failure and LDL cholesterol [15, 34]. Resveratrol, a natural agonist of PPAR γ agonist, emerged into our vision in the context we had mentioned above because of its safety and well tolerance.

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In our present research, the level of PPAR γ was down-regulated in HFD feeding group compared to NPD group. Administration of resveratrol or pioglitazone restored the level of PPAR γ to nearly normal in addition to improvement of hyperinsulinaemia, triglyceride, visceral adipose and prostate weight. No significantly difference between HFD+RES and HFD+PIO groups was found. It means that resveratrol up-regulated PPAR γ expression and in turn improved insulin resistance and prostate enlargement functionally similarly as pioglitazone.

Clinically, some of cross sectional studies demonstrated the association between the presence of inflammation and increased prostate volume [12]. Evidence showed that the risk of BPH and acute urinary retention increased in men with inflammation of prostate [13]. A research used a mice model with PPAR γ deleted in luminal epithelial cells showed that prostate hyperplasia was associated with inflammation in mouse prostates [35]. Interleukins (IL) directly regulated prostate growth of mouse [36]. COX-2, as a critical inflammatory mediators induced by pro-inflammatory cytokines, was showed to contribute to the growth of prostate in a study [21]. A research showed that COX-2 was significantly increased in HFD prostate of rabbit [37]. Recently another investigation using some activated extractive of Chinese traditional herbs to treat testosterone-induced BPH. Increased level of COX-2 was found in testosterone-induced model group. The level of COX-2 decreased after herb treatment [38]. So we checked COX-2 to access the inflammatory condition in prostate of rat fed by HFD and explore the mechanism of resveratrol impact on prostate enlargement.

There is a critical relationship between PPAR γ and COX-2 expression. An analysis using human BPH samples showed that COX-2 increased with elevated inflammation [39]. It has been reported that PPAR γ regulate transcription of COX-2 directly [40]. Clinically, combination of a 5 α -reductase and COX-2 inhibition increased the apoptosis in BPH [41]. Recently a clinical study showed that COX-2 inhibitor, celecoxib, with alpha-adrenoceptor blocker could elevate therapeutic effectiveness of LUTS in men with BPH [42]. In our investigation, COX-2 expression was up-regulated by HFD. Meantime PPAR γ expression was suppressed by HFD feeding. This finding consists with previous report. It is

confirmed that HFD induce inflammation in prostate and result in prostate hyperplasia finally. Our data support this hypothesis that inflammation links to the development and progression of BPH. Resveratrol, and pioglitazone, reduced inflammatory reaction by suppressing COX-2 expression and attenuated BPH induced by HFD accompany the increase of PPAR γ . Based on finding mentioned above, we inferred that resveratrol maybe decrease COX-2 level through increasing expression of PPAR γ .

Recent years, several studies have shown that Oxidative stress (OxS) play a key role in the development and progression of BPH [43-45]. OxS refers to a broken balance between the production and detoxification of ROS. NOX 2 are crucial for superoxide generation [46]. A research has revealed that several male genital disorder, including BPH, were correlated with OxS-media pathways closely [47]. One review showed that HFD is positively concerned with an increased risk of BPH. Oxidative stress and inflammation in the prostate gland were induced by HFD, and influenced transform prostate from a normal to a diseased state. Our data demonstrated that NOX level was increased, while as SOD level was decreased in model group by HFD feeding. It is consist with the published research. However they were revived by admission of resveratrol and pioglitazone. In other word, resveratrol decreased the production of reactive oxygen species. On the other hand it increased the elimination of oxygen free radical. The result confirmed that resveratrol can attenuate prostate enlargement through its anti-oxidant bioactivity.

Conclusion

In summary, resveratrol has protective effect similar to pioglitazone on improving prostate enlargement induced by HFD. It can attenuate prostate cell proliferation and improve metabolic imbalance through upregulating PPAR γ expression. In addition, it has anti-inflammatory and antioxidative effects on alleviating hyperinsulinemia pharmacologically. These results proposed that resveratrol deserves deeply investigation as a potential candidate for the treatment of BPH accompanying with metabolic disease because of its multi-bioactivity and safety.

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Disclosure of conflict of interest

None.

Authors' contribution

All authors have read and approved of the final version of the manuscript. Shijie Yang: Protocol/project development, experimental analysis, data collection and analysis, manuscript writing/editing. Yixian Liu: Data collection or management, data analysis, manuscript writing/editing. Yuhai Qiao and Guozhu Wu: Protocol/project development, data analysis, manuscript writing/editing. Wenqing Cai: Protocol/project development, data management, manuscript editing.

Abbreviations

NPD, normal pellet diet; HFD, High fat diet; RES, Resveratrol (50 mg/100 g body weight/day for 4 weeks); PIO, Pioglitazone; TC, Total cholesterol; TG, Triglyceride; HDL, High-density lipoprotein; LDL, Low-density lipoproteins; VLDL, Very low-density lipoprotein; FIN, fasting insulin; FPG, fasting plasma glucose; HOMA-IR, Homeostasis Model Assessment of insulin resistance; BW, body weight; PW, prostate weight; PI, prostate index; VFW, visceral fat weight; COX-2, Cyclooxygenase-2; SOD, Superoxide Dismutase; NOX-2, NADPH Oxidase-2; PPAR γ , peroxisome proliferator-activated receptor γ .

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References

- [1] Ozden C, Ozdal OL, Uryancioglu G, Koyuncu H, Gokkaya S, Memis A. The correlation between metabolic syndrome and prostatic growth in patients with benign prostatic hyperplasia. *Eur Urol* 2007; 51: 199-206.
- [2] Parsons JK. Modifiable risk factors for benign prostatic hyperplasia and lower urinary tract symptoms: new approaches to old problems. *J Urol* 2007; 178: 395-401.
- [3] De Nunzio C, Aronson W, Freedland SJ, Giovannucci E, Parsons JK. The correlation between metabolic syndrome and prostatic diseases. *Eur Urol* 2012; 61: 560-570.
- [4] Gacci M. Editorial comment on Zhang et al.: impact of metabolic syndrome on benign prostatic hyperplasia in elderly Chinese men. *Urol Int* 2014; 93: 214-219.
- [5] Gacci M, Vignozzi L, Sebastianelli A, Salvi M, Giannessi C, De Nunzio C, Tubaro A, Corona G, Rastrelli G, Santi R, Nesi G, Serni S, Carini M, Maggi M. Metabolic syndrome and lower urinary tract symptoms: the role of inflammation. *Prostate Cancer Prostatic Dis* 2013; 16: 101-106.
- [6] Kristal AR, Arnold KB, Schenk JM, Neuhauser ML, Weiss N, Goodman P, Antvelink CM, Penson DF, Thompson IM. Race/ethnicity, obesity, health related behaviors and the risk of symptomatic benign prostatic hyperplasia: results from the prostate cancer prevention trial. *J Urol* 2007; 177: 1395-1400.
- [7] Rohrmann S, Smit E, Giovannucci E, Platz E. Association between markers of the metabolic syndrome and lower urinary tract symptoms in the Third National Health and Nutrition Examination Survey (NHANES III). *Int J Obes (Lond)* 2005; 29: 310-316.
- [8] Parsons JK, Bergstrom J, Barrett-Connor E. Lipids, lipoproteins and the risk of benign prostatic hyperplasia in community-dwelling men. *BJU Int* 2008; 101: 313-318.
- [9] Hammarsten J, Hogstedt B. Clinical, haemodynamic, anthropometric, metabolic and insulin profile of men with high-stage and high-grade clinical prostate cancer. *Blood Press* 2004; 13: 47-55.
- [10] Vikram A, Jena G, Ramarao P. Insulin-resistance and benign prostatic hyperplasia: the connection. *Eur J Pharmacol* 2010; 641: 75-81.
- [11] Vikram A, Jena GB, Ramarao P. Increased cell proliferation and contractility of prostate in insulin resistant rats: linking hyperinsulinemia with benign prostate hyperplasia. *Prostate* 2010; 70: 79-89.
- [12] Shankar E, Bhaskaran N, MacLennan GT, Liu G, Daneshgari F, Gupta S. Inflammatory signaling involved in high-fat diet induced prostate diseases. *J Urol Res* 2015; 2.
- [13] McVary KT. A review of combination therapy in patients with benign prostatic hyperplasia. *Clin Ther* 2007; 29: 387-398.
- [14] Vikram A, Jena G, Ramarao P. Pioglitazone attenuates prostatic enlargement in diet-induced

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- insulin-resistant rats by altering lipid distribution and hyperinsulinaemia. *Br J Pharmacol* 2010; 161: 1708-1721.
- [15] Shah P, Mudaliar S. Pioglitazone: side effect and safety profile. *Expert Opin Drug Saf* 2010; 9: 347-354.
- [16] Calixto-Campos C, Carvalho TT, Hohmann MS, Pinho-Ribeiro FA, Fattori V, Manchope MF, Zarpelon AC, Baracat MM, Georgetti SR, Casagrande R, Verri WA Jr. Vanillic acid inhibits inflammatory pain by inhibiting neutrophil recruitment, oxidative stress, cytokine production, and NFkappaB activation in mice. *J Nat Prod* 2015; 78: 1799-1808.
- [17] Chung KS, Cheon SY, An HJ. Effects of resveratrol on benign prostatic hyperplasia by the regulation of inflammatory and apoptotic proteins. *J Nat Prod* 2015; 78: 689-694.
- [18] Jasinski M, Jasinska L, Ogrodowczyk M. Resveratrol in prostate diseases-a short review. *Cent European J Urol* 2013; 66: 144-149.
- [19] Harikumar KB, Aggarwal BB. Resveratrol: a multitargeted agent for age-associated chronic diseases. *Cell Cycle* 2008; 7: 1020-1035.
- [20] Orhan N, Aslan M, Orhan DD, Ergun F, Yeşilada E. In-vivo assessment of antidiabetic and antioxidant activities of grapevine leaves (*Vitis vinifera*) in diabetic rats. *J Ethnopharmacol* 2006; 108: 280-286.
- [21] Sciarra A, Mariotti G, Salciccia S, Autran Gomez A, Monti S, Toscano V, Di Silverio F. Prostate growth and inflammation. *J Steroid Biochem Mol Biol* 2008; 108: 254-260.
- [22] Touati S, Montezano AC, Meziri F, Riva C, Touyz RM, Laurant P. Exercise training protects against atherosclerotic risk factors through vascular NADPH oxidase, extracellular signal-regulated kinase 1/2 and stress-activated protein kinase/c-Jun N-terminal kinase downregulation in obese rats. *Clin Exp Pharmacol Physiol* 2015; 42: 179-185.
- [23] Pace G, Di Massimo C, De Amicis D, Corbacelli C, Di Renzo L, Vicentini C, Miano L, Tozzi Ciancarelli MG. Oxidative stress in benign prostatic hyperplasia and prostate cancer. *Urol Int* 2010; 85: 328-333.
- [24] Tian Shi-F, LC-J, Wang F, Bu WW, Wu SW. Effect of resveratrol encapsulated by hydroxypropyl- β -cyclodextrin on the expression of liver lipid metabolism related genes of mice. *Food Science and Technology* 2011; 36: 45-49.
- [25] Hammarsten J, Pecker R. Urological aspects of the metabolic syndrome. *Nat Rev Urol* 2011; 8: 483-494.
- [26] Reaven GM. Role of insulin resistance in human disease. *Diabetes* 1988; 37: 1595-1607.
- [27] Zhang C, Rexrode KM, Dam RM, Li TY, Hu FB. Abdominal obesity and the risk of all-cause, cardiovascular, and cancer mortality. *Circulation* 2008; 117: 1658-67.
- [28] Vikram A, Tripathi DN, Ramarao P, Jena GB. Intervention of D-glucose ameliorates the toxicity of streptozotocin in accessory sex organs of rat. *Toxicol Appl Pharmacol* 2008; 226: 84-93.
- [29] Zheng JX, Xiao YC, Hu YR, Hao M, Kuang HY. Exendin-4 shows no effects on the prostatic index in high-fat-diet-fed rat with benign prostatic hyperplasia by improving insulin resistance. *Andrologia* 2015; 47: 236-242.
- [30] Qu X, Huang Z, Meng X, Zhang X, Dong L, Zhao X. Prostate volume correlates with diabetes in elderly benign prostatic hyperplasia patients. *Int Urol Nephrol* 2014; 46: 499-504.
- [31] Lee SY, Min HG, Choi SH, Kim YJ, Oh SW, Kim YJ, Park YS, Kim SS. Central obesity as a risk factor for prostatic hyperplasia. *Obesity* 2006; 14: 172-179.
- [32] Jiang M, Strand DW, Franco OE, Clark PE, Hayward SW. PPARgamma: a molecular link between systemic metabolic disease and benign prostate hyperplasia. *Differentiation* 2011; 82: 220-236.
- [33] Escobar EL, Gomes-Marcondes MC, Carvalho HF. Dietary fatty acid quality affects AR and PPAR γ levels and prostate growth. *Prostate* 2009; 69: 548-558.
- [34] Lindberg M, Astrup A. The role of glitazones in management of type 2 diabetes. A dream or a nightmare? *Obes Rev* 2007; 8: 381-384.
- [35] Jiang M, Fernandez S, Jerome WG, He Y, Yu X, Cai H, Boone B, Yi Y, Magnuson MA, Roy-Burman P, Matusik RJ, Shappell SB, Hayward SW. Disruption of PPARgamma signaling results in mouse prostatic intraepithelial neoplasia involving active autophagy. *Cell Death Differ* 2010; 17: 469-481.
- [36] Jerde TJ, Bushman W. IL-1 induces IGF-dependent epithelial proliferation in organ development and reactive hyperplasia. *Sci Signal* 2009; 2: ra49.
- [37] Vignozzi L, Morelli A, Sarchielli E, Comeglio P, Filippi S, Cellai I, Maneschi E, Serni S, Gacci M, Carini M, Piccinni MP, Saad F, Adorini L, Vannelli GB, Maggi M. Testosterone protects from metabolic syndrome-associated prostate inflammation: an experimental study in rabbit. *J Endocrinol* 2012; 212: 71-84.
- [38] Yang X, Yuan L, Xiong C, Yin C, Ruan J. Abacopteris penangiana exerts testosterone-induced benign prostatic hyperplasia protective effect through regulating inflammatory responses, reducing oxidative stress and anti-proliferative. *J Ethnopharmacol* 2014; 157: 105-113.
- [39] Wang W, Bergh A, Damber JE. Chronic inflammation in benign prostate hyperplasia is associated with focal upregulation of cyclooxygenase-2, Bcl-2, and cell proliferation in the

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- glandular epithelium. *Prostate* 2004; 61: 60-72.
- [40] Subbaramaiah K, Lin DT, Hart JC, Dannenberg AJ. Mechanisms of signal transduction-peroxisome proliferator-activated receptor α ligands suppress the transcriptional activation of cyclooxygenase-2. Evidence for involvement of activator protein-1. *J Biol Chem* 2001; 276: 12440-12448.
- [41] Sciarra A, Mariotti G, Salciccia S, Gomez AA, Monti S, Toscano V, Di Silverio F. Prostate growth and inflammation. *J Steroid Biochem Mol Biol* 2008; 108: 254-260.
- [42] Jhang JF, Jiang YH, Kuo HC. Adding Cyclooxygenase-2 inhibitor to alpha blocker for patients with benign prostate hyperplasia and elevated serum prostate specific antigen could not improve prostate biopsy detection rate but improve lower urinary tract symptoms. *Int J Clin Pract* 2013; 67: 1327-1333.
- [43] Aryal M, Pandeya A, Gautam N, Baral N, Lam-sal M, Majhi S, Chandra L, Pandit R, Das BK. Oxidative stress in benign prostate hyperplasia. *Nepal Med Coll J* 2007; 9: 222-224.
- [44] Szewczyk-Golec K, Tyloch J, Czuczejko J. Antioxidant defense system in prostate adenocarcinoma and benign prostate hyperplasia of elderly patients. *Neoplasma* 2015; 62: 119-123.
- [45] Vital P, Castro P, Ittmann M. Oxidative stress promotes benign prostatic hyperplasia. *Prostate* 2016; 76: 58-67.
- [46] Seta F, Rahmani M, Turner PV, Funk CD. Pulmonary oxidative stress is increased in cyclooxygenase-2 knockdown mice with mild pulmonary hypertension induced by monocrotaline. *PLoS One* 2011; 6: e23439.
- [47] Kullisaar T, Türk S, Punab M, Mändar R. Oxidative stress-cause or consequence of male genital tract disorders? *Prostate* 2012; 72: 977-983.