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Efficacy of resveratrol in male urogenital tract dysfunctions: an evaluation of pre-clinical data

FB Calmasini[1]* , FH Silva[2], EC Alexandre[3] and E Antunes[3]
[1]*Department of Structural and Functional Biology, University of Campinas (UNICAMP), Campinas, SP, Brazil*
[2]*Laboratory of Multidisciplinary Research, São Francisco University (USF), Bragança Paulista, SP, Brazil*
[3]*Department of Pharmacology, Faculty of Medical Science, University of Campinas (UNICAMP), Campinas, SP, Brazil*

Abstract

Resveratrol is a polyphenol found naturally in fruits and plants. Recently, studies in humans and animal models have suggested beneficial properties of this polyphenol, such as improvements to metabolic and lipid profiles, along with antioxidant, anti-inflammatory and anti-proliferative effects. In the urogenital tract (UGT), resveratrol has also been tested clinically and experimentally as a therapeutic drug in several diseases; however, the translational efficacy of resveratrol, especially in UGT, is still a matter of debate. In the present review, we address the pre-clinical efficacy of resveratrol in UGT-related dysfunctions, focusing on lower urinary tract symptoms, non-cancerous prostatic disease (benign prostatic hyperplasia and prostatitis) and erectile dysfunction. *In vitro* studies indicate that resveratrol reduces inflammatory markers and oxidative stress, and improves endothelial function in UGT organs and cells isolated from humans and animals. Despite displaying low oral bioavailability, *in vivo* administration of resveratrol largely improves erectile dysfunction, benign prostatic hyperplasia, prostatitis and voiding impairments, as evidenced in different animal models. Resveratrol also acts as a microbiota modulator, which may explain some of its beneficial effects *in vivo*. In contrast to the large amount of pre-clinical data, there are insufficient clinical trials to establish resveratrol treatment efficacy in human UGT-related diseases. In summary, we provide an overview of the *in vivo* and *in vitro* efficacy of resveratrol in animal and human UGT dysfunctions, which may support future clinical trials.

Key words: **Prostatic diseases: Lower urinary tract: Erectile dysfunction: Polyphenol**

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Introduction

Resveratrol is a natural polyphenol compound found in several plants, such as grapes, peanuts and berries. It was first isolated in 1939, but only gained considerable attention a few decades later, when the French paradox was described[1]. Recently, the use of different *in vitro* and *in vivo* experimental approaches as well as experiments in cells isolated from animals and humans has revealed that resveratrol exerts antioxidant, anti-inflammatory and anti-proliferative effects[2,3]. Resveratrol also prevents metabolic diseases by ameliorating glucose and lipid homoeostasis and reducing fat accumulation and inflammatory biomarkers[4,5]. However, much less attention has been directed to the effects of resveratrol on urogenital tract (UGT) diseases, which manifest mainly with lower urinary tract symptoms (LUTS), prostatic disorders (benign prostatic hyperplasia (BPH) and prostatitis) and erectile dysfunction (ED). Epidemiological studies have shown a positive correlation between LUTS, prostatic dysfunction and ED[6,7]. These UGT diseases typically share common pathophysiological mechanisms, including an unbalanced oxidative state in which increased production of reactive oxygen species (ROS) is favoured and antioxidant capacity is reduced, as demonstrated in the bladder, urethra, prostate and corpus cavernosum[8–14]. By targeting these mechanisms, a number of studies have confirmed that therapy with resveratrol ameliorates UGT diseases. Therefore, in preparation for this review, we searched the literature for pre-clinical studies evaluating the efficacy of resveratrol on LUTS, BPH, ED and non-cancerous prostatic diseases in animal models and in human and animal isolated cells and, herein, provide up-to-date knowledge on the efficacy of resveratrol in UGT diseases.

Putative mechanisms of action of resveratrol

The antioxidant effect of resveratrol is clearly the most studied property of this polyphenol. Resveratrol acts as an antioxidant through at least two different pathways; that is, resveratrol increases antioxidant system efficacy and/or reduces ROS production in the targeted tissue. At the molecular level, resveratrol reduces nicotinamide adenine dinucleotide phosphate (NADPH) activity by inhibiting gp91^{phox} translocation from the cytosol to the membrane, thus impairing NADPH assembly[15].

* **Corresponding author:** Fabiano Beraldi Calmasini; email: fabiano.b.calmasini@gmail.com

Resveratrol also reduces NADPH oxidase mRNA expression and increases protein expression of superoxide dismutase[16].

The mechanism by which resveratrol modulates gene expression is still poorly understood; however, it has been proposed that sirtuin-1 (sirt-1) activation by resveratrol plays a key role in this process. Sirt-1 is an important protein linked with longevity and numerous intracellular genetic regulations. Transcription factors such as nuclear factor κB (NF-κB), tumour suppressor p53 and Forkhead box class O (FOXO) are all targets of sirt-1. Furthermore, sirt-1 is implicated in heterochromatin formation and histone hypoacetylation, thereby producing gene repression[17]. Sirt-1 seems to work in synergy with AMP-activated protein kinase (AMPK) to promote cell metabolism adaptations through activation of transcription factors and/or co-activators such as peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) and FOXO, which are also implicated in sirt-1-induced gene expression[18]. However, whether the modulation of gene expression by resveratrol in UGT also involves sirt-1 activation remains unclear. Furthermore, in UGT, pathways in addition to the sirt-1 pathway have been shown to be activated by resveratrol, suggesting a complex and non-specific mechanism of action for this polyphenol, which open fields for future studies.

Protective effects of resveratrol on male urogenital tract dysfunction

LUTS, ED, BPH and prostatitis are the most common UGT diseases. The main risk factors for these diseases include arterial hypertension, obesity, diabetes and ageing, which itself increases inflammation and oxidative status, thereby worsening the incidence and/or progression of UGT diseases[19–21]. Within this context, resveratrol has emerged as a potential candidate for the treatment of such diseases. Herein, we detailed the current data on resveratrol actions in male UGT disorders, emphasising LUTS, prostatic diseases and ED. Table 1 and Fig. 1 summarise the main effects and the proposed mechanisms of resveratrol in the abovementioned diseases.

Lower urinary tract symptoms

Normal urinary continence requires a complex interaction between the brain, nervous system and pelvic organs, namely the bladder, urethra and prostate. When any component of this system loses normal function, the micturition process can be affected and the patient may experience LUTS, characterised by bladder storage and/or filling symptoms and consisting of urinary frequency, nocturia, urgency and stream problems, thus negatively affecting the patient's quality of life[22]. Metabolic diseases such as type 2 diabetes and obesity are among the most important risk factors for LUTS, and experimental models of both of these pathological conditions have been used to test the efficacy of resveratrol as a pre-clinical approach, as illustrated in Table 1. In high-fat diet (HFD)-fed obese mice, 2-week administration of resveratrol at 100 mg/kg increased the antioxidant activity in isolated bladder, resulting in attenuation of overactive bladder, as evidenced by the reductions of non-voiding contractions, urinary frequency and detrusor smooth muscle hypercontractility[11]. Similarly, in the urethral tissue of obese mice, resveratrol increased antioxidant activity and nitric oxide (NO) production and normalised urethral smooth muscle hypercontractility, thus restoring urethral relaxation[12]. In a rat model of type 2 diabetes induced by a low dose of streptozotocin (40 mg/kg) followed by a HFD for 2 weeks, resveratrol at 10 mg/kg/d for 14 d reduced oxidative stress markers, such as malondialdehyde, 4-hydroxy-2-nonenal and 8-hydroxy-2-deoxyguanosine, and decreased serum glucose levels[23]. Considering that resveratrol treatment also reduces body weight and that it improves metabolic parameters, it is unclear whether the amelioration of obesity-induced bladder dysfunction in HFD-fed animals was the result of a direct antioxidant effect in UGT tissues or was a consequence of reduced body weight. Furthermore, the exact molecular mechanisms by which resveratrol improves bladder and urethral oxidative balance in metabolic disease models have yet to be elucidated.

In a similar fashion, resveratrol abrogated cystitis-induced bladder dysfunction by increasing antioxidant defences. In ifosfamide-induced rat cystitis, an inflammatory condition closely related to LUTS, resveratrol, given at 10 mg/kg, intraperitoneally (i.p.) for 5 d, reversed vesical epithelium degeneration and lamina propria inflammation. These effects were attributed to increased glutathione levels and reduced myeloperoxidase activity in the bladder induced by resveratrol treatment[24]. Additionally, in cyclophosphamide-induced rat cystitis, in a preventive approach, resveratrol at 20 and 40 mg/kg also reduced the oxidative status of the bladder, resulting in less epithelial degeneration and desquamation[25].

The effects of resveratrol in the bladder have also been addressed using *in vitro* assays (Table 2). For instance, resveratrol at 4 mM reduced the oxidative stress induced by hydrogen peroxidase (H_2O_2) in rabbit bladder smooth muscle and mucosa[26]. Notably, in all the *in vivo* and *in vitro* studies mentioned above, the high efficacy of resveratrol was associated with reduced systemic and/or tissue oxidative stress biomarker expression. In fact, oxidative stress has been implicated in a number of lower urinary tract disorders; however, it is important to remember that a causal relationship between increased oxidative stress and LUTS has not been shown, and hence, more specific analyses, identifying the particular reactive species involved in LUTS genesis, are needed to prove this causal relationship. Therefore, the antioxidant effects of a particular compound, such as resveratrol, can be tested against specific reactive species[27].

Finally, we cannot neglect the fact that resveratrol efficacy in treating or preventing LUTS may be related to its inhibitory effect on smooth muscle reactivity. Resveratrol at 10 and 100 μM reduced bradykinin-induced rat detrusor contraction in a concentration-dependent manner[28]. Specifically, at 100 μM, it reduced detrusor contractility by 74 %, which was associated with a reduction in cyclooxygenase activity and prostaglandin E2 generation and with the inhibition of Ca^{2+} entry through L-type Ca^{2+} channels[28]. In human and rodent bladder smooth muscle, these Ca^{2+} channels play crucial roles in agonist-induced detrusor contractility[29,30]; therefore, they may be important targets for resveratrol, but further studies are required to verify this supposition. Table 2 summarises the main effects of resveratrol in the bladder and urethra.

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Table 1. The *in vivo* effects of resveratrol in animal models of UGT dysfunctions

Tissue	Model	Animal	Dosage	Major findings	Ref.
Bladder	HFD-induced obesity	Mouse	100 mg/kg - 2 weeks (gavage)	• ↓ Detrusor hypercontractility • ↓ Cystometric impairments • ↓ Systemic and bladder oxidative stress	11
Bladder	Type 2 diabetes	Rat	10 mg/kg – 2 weeks (gavage)	• ↓ Bladder oxidative stress markers • ↓ Urothelial oedema	23
Bladder	Ifosfamide-induced cystitis	Rat	10 mg/kg – 5 d (i.p.)	• ↓ Urothelium degeneration • ↓ Lamina propria inflammation and free radical production • ↑ Plasmatic and bladder antioxidant activity	24
Bladder	Cyclophosphamide-induced cystitis	Rat	20–40 mg/kg – pre treatment (i.p.)	• ↓ Bladder inflammation and oxidative stress • ↓ Urothelial hyperplasia and degeneration	25
Bladder	Autoimmune prostatitis	Rat	10 mg/kg – 10 d (gavage)	• Improved voiding process • ↓ Detrusor fibrosis • Suppressed cell factor/c-Kit axis	50,51,58,59
Urethra	HFD-induced obesity	Mouse	100 mg/kg – 2 weeks (gavage)	• ↑ Nitric oxide levels • Restored urethral smooth muscle functioning • ↑ Urethral antioxidant activity	12
Prostate	Hormone-induced BPH	Rat	50 and 100 mg/kg of PSEER – 4 weeks (gavage)	• ↓ Prostatic size and cell proliferation	39
Prostate	Hormone-induced BPH	Rat	1 mg/kg – 4 weeks (i.p.)	• ↓ Prostatic size and cell proliferation • Restored prostatic apoptosis	38
Prostate	HFD-induced BPH	Mouse	100 mg/kg – 2 weeks (gavage)	• ↓ Prostate size and epithelial hyperplasia • ↓ Prostate smooth muscle hypercontractility • ↑ Prostatic insulin sensitivity and reduced oxidative stress	14
Prostate	Autoimmune prostatitis	Mouse and rat	10 mg/kg – 10 d (gavage)	• ↓ Prostatic fibrosis • ↓ Prostatic collagen content • ↓ Prostate inflammation • Down-regulation of c-kit–SCF axis • Restored prostatic apoptosis • Improved cell cycle arrest	47,48, 50, 101
Prostate	Hormone-induced prostatitis	Rats	10 mg/kg – 10 d (gavage)	• ↓ Prostate inflammation • ↓ Prostatic inflammatory cytokines	49
Prostate	Bacterially induced prostatitis	Mouse	100 mg/mL of PSEER – 4 weeks (gavage)	• ↓ Prostatic fibrosis • ↓ Prostate size and epithelial hyperplasia	56
CC	Hyper-cholesterolaemia-induced ED	Rabbit	8 mg/kg – 6 weeks (drinking-water)	• ↓ eNOS uncoupling • ↓ Cavernosal reactive oxygen species levels	63
CC	Hyper-cholesterolaemia-induced ED	Rabbit	4 mg/kg – 6 weeks (drinking-water)	• ↑ Endothelial CC relaxation	64
CC	Streptozotocin-induced ED	Rat	25 mg/kg – 8 weeks (gavage)	• ↑ ICP • ↑ Cavernosal nNOS/eNOS proteins expression and cGMP levels • ↓ Superoxide anion and ROS production	65
CC	Streptozotocin-induced ED	Rat	5 mg/kg – 4 weeks (gavage)	• ↑ ICP • ↑ Cavernosal smooth muscle/collagen ratio	66
CC	Streptozotocin-induced ED	Rat	5 mg/kg – 8 weeks (gavage)	• ↑ ICP • ↑ Cavernosal smooth muscle/collagen ratio • ↑ Cavernosal sirt-1 protein expression	71
CC	Radiotherapy-induced ED	Rat	10 mg/kg – 10 weeks (gavage)	• ↓ ICP • ↑ Sirt-1, eNOS and nNOS cavernosal protein expression • ↓ Cavernosal oxidative stress markers	72

BPH, benign prostatic hyperplasia; cGMP, cyclic guanosine monophosphate; CC, corpus cavernosum; ED, erectile dysfunction; eNOS, endothelial nitric oxide synthase; HFD, high-fat diet; ICP, intra-cavernosal pressure; i.p., intraperitoneal; nNOS, neuronal nitric oxide synthase; PSEER, peanut sprout extract enriched with resveratrol; ROS, reactive oxygen species.

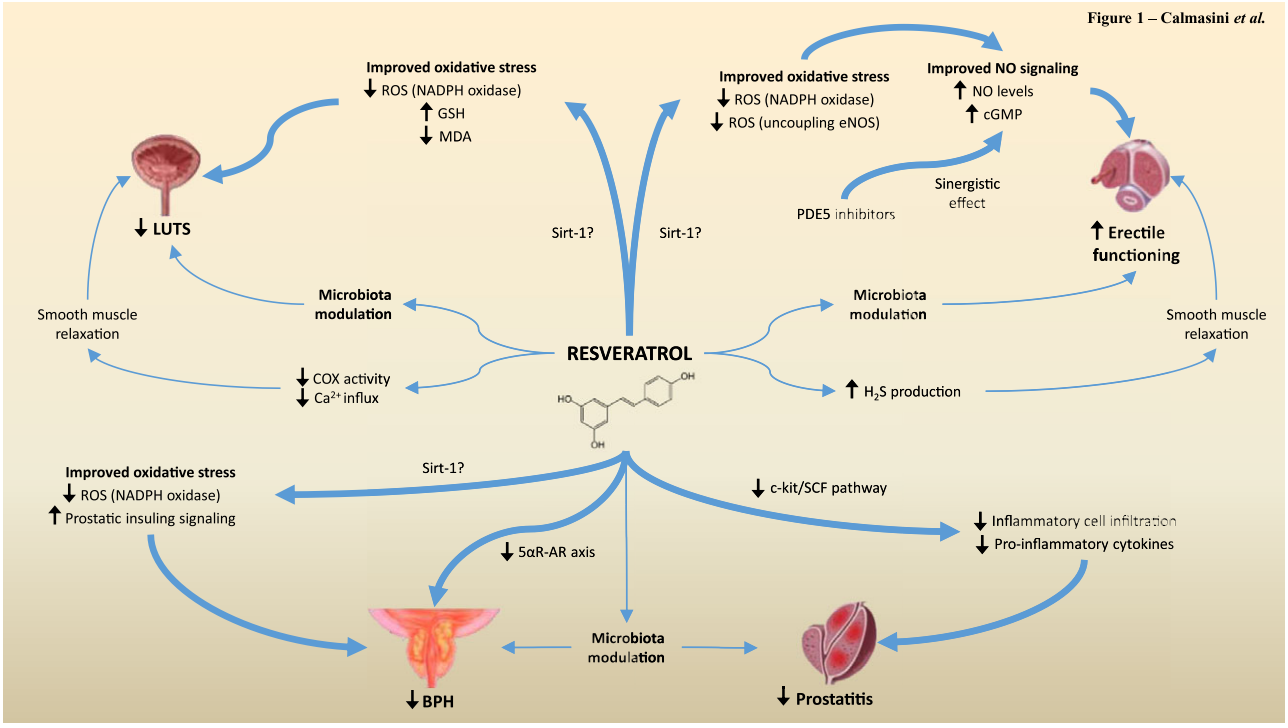

Fig. 1. Schematic representation of the suggested pathways by which resveratrol acts in the UGT and its beneficial effects. Strong evidence (represented by the thick blue arrows) indicates that resveratrol may act in the UGT by reducing oxidative stress, inflammation and cell proliferation-related pathways. A few studies (thin blue arrows) indicate that resveratrol exhibits a direct smooth muscle inhibitory effect and/or is capable of modulating the gut microbiota, resulting in improvement of UGT diseases.

Prostatic diseases

The prostate is a gland in the male genital tract that plays an important role during the fertilisation process. However, during a male's lifetime, this gland can be affected by several pathological conditions, mostly cancer, BPH and/or prostatitis. Considering that the effects of resveratrol on prostatic cancer have been reviewed in detail in the literature[31], we focus here on BPH and prostatitis.

Benign prostatic hyperplasia. BPH is a non-cancerous prostatic dysfunction characterised by abnormal epithelial and stromal cell proliferation. Prostate overgrowth and smooth muscle hypercontractility associated with BPH may lead to urethral narrowing, bladder outlet obstruction and LUTS development[32]. Considering the high epidemiological prevalence of BPH in ageing men (reaching 90 % in 90-year-old men) and the small number of commercially available drugs, the search for new molecules and pathways is crucial to improve the BPH treatment.

The ability of resveratrol to suppress cell growth has been explored extensively, especially in UGT-related proliferative diseases such as BPH. In the BPH-1 cell line, 30 μM resveratrol reduced cell proliferation by 90 %, which was attributed to an increased apoptosis rate induced by p38 MAPK activation and FOXO3a repression[33]. In human prostatic epithelial cells, lower resveratrol concentrations (2·5–10 μM) also reduced cell proliferation by inducing cellular senescence[23]. Interestingly, some of the proposed mechanisms accounting for the resveratrol-induced anti-proliferative effects are related to increased intracellular ROS production that culminates with FOXO3a repression and cellular senescence[34]. This suggests that resveratrol may act through different mechanisms depending on cell type and drug concentration and incubation time.

A recent study carried out with epithelial (BPH-1) and stromal (WPHY-1) prostatic cell lines demonstrated that resveratrol treatment at 20 μM for 72 h suppressed prostatic cell proliferation by interacting with long non-coding RNAs (lncRNAs)[35]. More specifically, in both these cell lines, resveratrol reduced the gene expression of lncRNAs DIO3 opposite strand (DIO3OS), a gene implicated in BPH pathogenesis. In addition, the authors demonstrated that DIO3OS expression is up-regulated by transforming growth factor (TGF) β1 in human prostatic cells. *In vitro* incubation with TGFβ1 (5 ng/mL for 72 h) resulted in an approximately two-fold increase in DIO3OS gene expression in BPH-1 and WPHY-1 cells, which was suppressed by resveratrol[35]. Similarly, in prostate fibroblast cells obtained from BPH patients, resveratrol (40–50 μM) reduced cell proliferation through a mechanism involving TGFβ inhibition[36].

There are several BPH animal models described in the literature, but androgen-induced BPH, in which the animals are supplemented with testosterone, associated or not with oestradiol, for 2–4 weeks, is one of the most employed models[37]. After the BPH hormonal induction period, the prostate exhibits macroscopically increased size concomitant with histological alterations evidenced by significant epithelial layer hyperplasia. Using the hormonally induced BPH model in Sprague–Dawley rats, a previous study showed that resveratrol treatment

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Table 2. The *in vitro* effects of resveratrol in human and animal UGT tissues/cells

Tissue/Cell	Source	Concentration	Major findings	Ref.
Bladder	Rabbit	4 mM	• ↓ Oxidative stress induced by hydrogen peroxide in the bladder smooth muscle and mucosa layers	26
Bladder	Rat	10 and 100 μM	• ↓ Bradykinin-induced bladder smooth muscle contractility	28
CC	Aged diabetic rats	100 μM	• ↑ Sildenafil-induced CC relaxation	67
CC	*In vitro*-induced diabetic mouse model	50 μM	• ↑ Acetylcholine-induced CC relaxation	68
CC	Mouse	Concentration-response curve (50–400 μM)	• Cavernosal smooth muscle relaxation • ↓ Cavernosal H_2S production	73
Prostate fibroblast and BPH cells	Human	40–50 μM	• ↓ TGFβ- and CXCL-12-induced cell proliferation • ↓ Fibroblast-to-myofibroblast conversion	36
BPH cells	Human	30 μM	• ↓ Cell proliferation • ↑ p38-MAPK-induced apoptosis • ↑ ROS levels	33
BPH and stromal cells	Human	20 μM	• ↓ Cell proliferation • ↓ Epithelial–mesenchymal transition	35
Prostatic epithelial cells	Human	2·5, 10 and 50 μM	• ↑ MKP5 gene expression • ↓ Cell proliferation • ↑ ROS levels • ↑ Cellular senescence	34
Prostatic epithelial cells	Human	5–50 μM	• Androgen receptor inhibition	40
Epithelial and stromal cells	Human	200–800 μg/mL of PSEER	• ↓ Cell proliferation	39
Cavernosal smooth muscle cells	Human	100 μM	• ↑ cGMP levels	66

BPH, benign prostatic hyperplasia; CC, corpus cavernosum; cGMP, cyclic guanosine monophosphate; CXCL-12, CXC motif chemokine 12; H_2S, hydrogen sulphide; PSEER, peanut sprout extract enriched with resveratrol; ROS, reactive oxygen species; TGFβ, transforming growth factor β.

(1 mg/kg. i.p. for 4 weeks) reduced both prostate size and protein expression of proliferating cell nuclear antigen, and promoted tissue apoptosis by reducing Bcl-2 family protein expression[38]. Notably, the efficacy of resveratrol in reducing BPH features was comparable to that of the 5α-reductase inhibitor finasteride, a Food and Drug Administration-approved drug used clinically to treat BPH; however, the molecular mechanisms by which resveratrol regulates protein expression in the prostate were not explored and deserve further study.

Prostatic cell proliferation in testosterone-induced rat BPH was also significantly reduced by a peanut sprout extract enriched with resveratrol (PSEER) administered at 50 and 100 mg/kg for 4 weeks by gavage[38]. PSEER-treated rats also exhibited a reduced prostate weight ratio and dihydrotestosterone levels, along with attenuation of the increase in epithelial layer thickness. Moreover, the expression of Ki-67, a marker for cell proliferation, was reduced after PSEER treatment, especially in the epithelial layer of the prostate tissue. Interestingly, PSEER treatment was as effective as finasteride in reducing prostate weight and epithelial hyperplasia, suggesting that these two compounds have similar mechanisms of action[38].

In fact, resveratrol has anti-androgenic effects. The 5α-reductase-androgen receptor axis was suppressed by PSEER treatment, explaining in part the prevention of prostate enlargement in testosterone-induced BPH in rats[38]. The anti-androgenic mechanism of resveratrol seems to involve at least two pathways: (i) the inhibition of 3-hydroxysteroid dehydrogenase, an enzyme that catalyses essential steps in the formation of all classes of active steroid hormones, and (ii) allosteric androgen receptor agonist, inhibiting *in vitro* androgen receptor activation by dihydrotestosterone in a concentration-dependent manner[39]. Intriguingly, this inhibitory effect was more pronounced in rats (IC_{50} 3·87 ± 0·06 μM) than in humans (IC_{50} 8·48 ± 0·04 μM), which may help explain the low efficacy of resveratrol in some translational studies[40]. This negative modulation of the androgen pathway by resveratrol may be of particular relevance to BPH conditions because the reduction in dihydrotestosterone levels is a standard approach to BPH treatment.

With respect to the male reproductive system, clinical studies indicate that obese patients are more likely to develop BPH[41]. Although the mechanism of obesity-induced prostatic dysfunction is not completely understood, high levels of oxidative stress are implicated in this process. Accordingly, obese animals have been used to better understand BPH physiopathology and to test new drugs, especially antioxidant drugs. Mice fed a HFD for 12 weeks exhibited prostate overgrowth and prostatic epithelial hyperplasia associated with increased ROS levels. A 2-week treatment with resveratrol (100 mg/kg, once daily by oral gavage) reversed the HFD-induced oxidative stress in the prostate, which was accompanied by reduced prostatic overgrowth and epithelial hyperplasia. Resveratrol also reduced prostatic smooth muscle hypercontractility in obese mice[14]. Similarly, resveratrol exerts its protective effect in UGT cells in part by reducing ROS production, thereby improving the oxidative balance, as demonstrated in prostate epithelial cells[42].

Obesity-associated ROS production has also been implicated in insulin resistance and hyper-insulinaemia. In HFD obese rats, hyper-insulinaemia was associated with BPH[43]. Similarly, the prostate of HFD-fed obese mice presented defective insulin action and increased levels of ROS, both of which were improved by resveratrol[14]. The exact link between preserved

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insulin signalling in the prostate and BPH pathophysiology in resveratrol-treated animals is still unclear. In vascular and bladder tissues, insulin stimulates NO synthesis through IRS-1/PI3K-Akt/eNOS pathway activation[30,44]. Therefore, it is possible that impaired obesity-induced insulin signalling in the prostate results in low levels of NO, favouring the contractile machinery and cell proliferation of the prostate.

In addition to ameliorating UGT diseases, resveratrol exerts some effects under healthy conditions. In normal epithelial and stromal cells, 24-h *in vitro* incubation with PSEER reduced the cell count and viability in a concentration-dependent manner. An initial effect of PSEER was observed at 200 μg/mL in both cell types, reaching 50 % inhibition at 800 μg/mL[45]. PSEER suppressed cell proliferation by arresting the cell cycle in the G1 phase. The molecular mechanisms proposed for this inhibitory effect involve decreased expression of the proteins cyclin D1 and CDK4 (which are related to G1 cell cycle progression) and increased p21WAF1 protein expression (a negative cell cycle regulator). In addition, the expression of fibroblast growth factor (FGF), 5α-reductase and AR was reduced after incubation with PSEER, suggesting a mechanism independent of oxidative stress[45].

Prostatitis. Prostatic inflammation, also referred to as prostatitis, has recently been implicated in BPH pathogenesis and may contribute to LUTS[46]. Importantly, prostatitis may affect men of all ages, and it is estimated that one-half of all men will face this condition during life.

The approved pharmacological treatments for prostatitis involve anti-microbial therapy and anti-inflammatory drugs. Therefore, drugs such as resveratrol, which exhibits an anti-inflammatory profile, have been tested experimentally to treat this condition. In rats, a 10-d treatment with resveratrol (10 mg/kg per gavage) reduced autoimmune prostatitis-induced mast cell infiltration in the prostate[47]. Using the same animal model of prostatitis, a previous study demonstrated reduced prostatic leucocyte infiltration after resveratrol treatment (10 mg/kg daily per gavage)[48]. Similarly, in a rat model of 17-β-oestradiol-induced prostatitis, a 10-d treatment with resveratrol (10 mg/kg daily per gavage) reduced the infiltration of inflammatory cells in the prostate[49]. The abovementioned studies indicate that resveratrol promotes beneficial effects in experimental models of prostatitis by suppressing inflammatory cell infiltration in the prostate, even in a short-term treatment of 10 d. The attenuation of prostatitis by resveratrol may account for histological improvements, as evidenced by reduced epithelial layer height and fibrosis, as well as by epithelial and stromal hyperplasia[48,49].

One of the proposed mechanisms to explain the reduction of inflammatory cell infiltration in the prostatic tissue by resveratrol treatment is the suppression of the prostatic c-kit-stem cell factor (SCF) axis, an important pathway related to inflammation and carcinogenesis[50]. The suppression of the c-kit/SCF axis by resveratrol is accompanied by reductions in prostatic inflammation and leucocyte infiltration[51]. In addition, resveratrol decreases pro-inflammatory cytokine levels in the prostate, such as interleukin (IL)-6, IL-8, tumour necrosis factor (TNF)α and TGFβ, which may be associated with the reduced presence of inflammatory cells[49]. Among these cytokines, TGFβ plays a pivotal role in prostatitis-induced fibrosis. This cytokine has been associated with the conversion of fibroblasts to myofibroblasts, which contribute to prostate remodelling and collagen deposition[52]. The exact mechanism by which resveratrol inhibits TGFβ activity has not been completely elucidated; however, evidence has implicated at least two different pathways: upstream inhibition of microRNA (especially miR-17) and downstream inhibition of Smad complex proteins[53,54]. Furthermore, in hepatocytes, the positive relationship between the SCF axis and TGFβ has been demonstrated to act as a positive feedback loop involving STAT3 and Smad2 activation[55]. Therefore, it is possible that this same positive loop plays a role in prostatic inflammation and that resveratrol may exert its therapeutic effect by inhibiting this loop[48].

Resveratrol-enriched extracts have also been tested in prostatitis assays. PSEER (containing 14·85 μg/mL resveratrol) suppressed prostate enlargement and stromal fibrosis induced by intraurethral injection of *Escherichia coli* in mice. After mice were treated with 200 μL of PSEER for 4 weeks, the number of colony-forming units in the prostate was reduced from $2{\cdot}4 \times 10^5$ to $0{\cdot}6 \times 10^5$, which may explain the structural amelioration seen in PSEER-treated mice[56].

Due to the anatomical proximity among the prostate, bladder and urethra, prostatitis has been shown to negatively impact lower urinary tract functioning[57]. In rats, the induction of autoimmune prostatitis led to voiding dysfunction, as evidenced by increases in bladder capacity, voiding pressure and residual volume. Oral treatment with resveratrol at 10 mg/kg for 10 d attenuated the impaired voiding process by mechanisms involving the suppression of the c-kit/SCF axis in the bladder[58]. Resveratrol also reduced TGFβ-induced bladder fibrosis secondary to prostatitis by down-regulating TGFβ, Wnt and β-catenin protein expression. Histological analysis revealed that prostatitis led to bladder smooth muscle disarrangement and increased α-SMA protein expression, a marker for myofibroblasts. Resveratrol restored the control levels of all the protein expressed in the bladder, lowering the fibrotic area[59]. However, considering that resveratrol also promoted changes in the prostate by reducing prostatic inflammation, it is difficult to ascertain whether the improvements by resveratrol on prostatitis-induced bladder impairments reflect a local vesical action or just a consequence of the ameliorated prostatitis condition.

The efficacy of resveratrol in prostatitis-induced LUTS in rats was also assessed in the absence and presence of solifenacin, a competitive and selective muscarinic receptor (M_3) antagonist, clinically approved by the Food and Drug Administration and other regulatory organisations worldwide to treat LUTS. The combination of resveratrol plus solifenacin produced a synergistic effect, further reducing all the abnormal cystometric parameters in comparison with resveratrol alone. Corroborating the literature, prostatitis led to overexpression of bladder c-kit and SCF proteins, which were reduced to the same extent by treatment with resveratrol alone or in combination with solifenacin, indicating different targets for both drugs[58].

Erectile dysfunction

Erectile dysfunction is characterised by the incapacity of achieving or maintaining an erection sufficient for intercourse.

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The activation of the NO-soluble guanylyl cyclase-cyclic GMP (NO–sGC–cGMP) axis is crucial to induce corpus cavernosum smooth muscle relaxation and penile erection[60]. Reduced NO bioavailability in the corpus cavernosum due to abnormal NO–cGMP signalling has been implicated in ED pathophysiology[61]. Epidemiological data show that ED is closely related to obesity, diabetes and dyslipidaemia, supporting a causal link between these conditions and ED[62]. As reported in the literature, the high efficacy of resveratrol in ameliorating ED has been largely confirmed by using different experimental approaches. Similar to lower urinary tract smooth muscle, resveratrol treatment increases cavernosal relaxation and improves ED mainly by reducing oxidative stress, thus ameliorating the antioxidant activity of the cavernosal tissue (as detailed below). This improved oxidative status by resveratrol treatment in the corpus cavernosum also involves enhanced activities of endothelial (eNOS) and neuronal nitric oxide synthase (nNOS) enzymes.

Hyper-cholesterolaemic model of erectile dysfunction and resveratrol. Using a hyper-cholesterolaemia-induced ED model in rabbits, a previous study showed that both preventive and therapeutic oral treatments with resveratrol (8 mg/kg/d for 6 weeks) ameliorated impaired cavernosal endothelium-dependent relaxation. This beneficial effect was associated with a resveratrol-induced reduction in NADPH oxidase activity in the corpus cavernosum of hyper-cholesterolaemic animals. The improved oxidative status induced by resveratrol also ameliorated the eNOS/NO pathway, reducing the levels of uncoupling eNOS[63]. Similarly, a lower resveratrol dose (4 mg/kg/d for 6 weeks, oral) improved endothelium-dependent corpus cavernosum relaxation in hyper-cholesterolaemic rabbits[64]. Interestingly, the hyper-cholesterolaemic diet did not alter the endothelium-independent cavernosal relaxation induced by sodium nitroprusside, corroborating the idea that resveratrol acts as an endothelial protector in cavernosal tissue[64].

Diabetic models of erectile dysfunction. In streptozotocin-induced diabetes, the oral administration of resveratrol at 25 mg/kg/d for 8 weeks ameliorated ED by increasing both the cavernosal cGMP levels and the nNOS/eNOS expression in diabetic rats[65]. Moreover, resveratrol reduced ROS production in the corpus cavernosum from the diabetic group, reinforcing an important antioxidant mechanism triggered by this polyphenol. Interestingly, the combination of resveratrol (25 mg/kg/d) plus the phosphodiesterase-5 (PDE5) inhibitor sildenafil (5 mg/kg for 8 weeks) resulted in a synergistic effect by further improving ED in diabetic rats, which was also attributed to the antioxidant properties and up-regulation of the NO–cGMP pathway[65]. The same synergism between resveratrol and another PDE5 inhibitor (vardenafil) was observed in humans[66]. These findings indicate that this synergistic effect may be associated with the activation of the NO–cGMP pathway itself, not a non-specific molecule-related effect. This synergism was also reported using corpus cavernosum *in vitro*. In aged diabetic rats, *in vitro* incubation of corpus cavernosum with resveratrol at 100 μM for 45 min enhanced sildenafil-induced relaxation[67]. A lower concentration (50 μM) of resveratrol also improved acetylcholine-induced corpus cavernosum relaxation in diabetic mice[68].

One of the proposed mechanisms by which resveratrol up-regulates the NO–cGMP pathway activity in the corpus cavernosum is the activation of sirt-1, an important NOS modulator in several vascular and nonvascular tissues[69]. Sirt-1 is expressed in the human and rodent corpus cavernosum[70,71], and its expression is reduced in the cavernosal tissue of diabetic rats, which may be implicated in ED[71]. In addition, oral intake of resveratrol at doses of 5 and 10 mg/kg/d for 8 weeks is capable of up-regulating sirt-1 protein expression in ED models of diabetes and radiotherapy[71,72].

Finally, it is important to highlight that resveratrol may also induce cavernosal relaxation by mechanisms in addition to increasing NO production. For instance, resveratrol directly relaxed the mouse corpus cavernosum with potency at an application of approximately 1 mM through mechanisms involving increased hydrogen sulphide (H_2S) production[73], suggesting that H_2S mediated cavernosal relaxation at high concentrations of resveratrol. Collectively, these literature data indicate that resveratrol has a protective effect on erectile function in several pathological conditions and that this polyphenol may be a valuable alternative treatment applied in association with PDE5 inhibitors or in conditions where PDE5 inhibitors do not work effectively as monotherapy.

Pharmacokinetics of resveratrol

Resveratrol comprises three hydroxyl groups and two phenolic rings that confer high lipophilic properties. This molecular characteristic of resveratrol confers a high oral absorption rate (approximately 70 % in humans) but rather low bioavailability[74]. This apparent paradoxical effect may be explained by at least two complementary pathways: (i) resveratrol is extensively metabolised in the liver before reaching circulation and (ii) this polyphenol may enter the enterohepatic cycle, which may retard and/or decrease its oral absorption[75]. After a single oral dose of resveratrol of 25 mg, low plasmatic levels were detected in humans (less than 10 ng/mL or approximately 40 nM); however, when considering resveratrol in addition to its metabolites, the overall concentration was found to be 500 ng/mL or 2 μM[76]. The major conjugated metabolites produced during resveratrol metabolism are sulphate and glucuronide, which are suggested to contribute to the beneficial effects of resveratrol treatment in experimental and clinical studies.

In all the aforementioned studies involving animal models, the resveratrol dosage used in the *in vivo* protocols ranged between 1 and 100 mg/kg/d (Table 1). It was reported that oral administration of 2 mg/kg resveratrol to rats achieves peak plasma concentrations of 2·6 μM at 10 min after intake[77]. Using 50 mg/kg orally in rats, the plasma concentration of resveratrol was as high as 10 μM[78]. However, when we consider the high concentrations of resveratrol used in the *in vitro* assays (between 10 and 100 μM) and its reduced oral bioavailability, only a few studies suggested that the *in vivo* actions could be

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explained by a direct effect of resveratrol on the targeted tissue (Table 2).

Despite its low bioavailability and tissue distribution, resveratrol exhibits an array of beneficial effects in humans and animals. It has been suggested that resveratrol can act directly in the liver during its first pass, which would not be dependent on its bioavailability[79]. Moreover, resveratrol may produce some of its beneficial effects by acting directly on the gut cells. For example, intra-duodenal infusion of resveratrol in HFD-fed mice enhanced insulin sensitivity and lowered hepatic glucose production through a mechanism involving the sirt-1 receptor and AMPK protein activation in the gut[80].

Resveratrol as a microbiota modulator

The microbiota of the gut is the largest in the human body in terms of diversity and number of microbes and is composed mainly of five different phyla, namely Bacteroidetes, Firmicutes, Verrucomicrobia, Actinobacteria and Proteobacteria[81]. The ratio between the phyla generally varies between individuals according to the lifestyle and other environmental factors that impact the host organism. Gut microbiota dysbiosis is associated with a number of diseases, exerting a pivotal role in the health–disease balance. Metabolic diseases, such as type 2 diabetes and obesity, have been closely related to low microbiota diversity and altered microbiota composition. For instance, a reduced number of Bacteroidetes is found in the gut of animal models of metabolic syndrome, while the levels of Firmicutes species are increased. The altered ratio, in addition to the low microbiota diversity, increases calorie harvesting, which may contribute to adiposity. Some species such as Firmicutes, are capable of metabolising polysaccharides that are excreted in a balanced gut microbiota[82].

Recently, growing evidence has supported a role for resveratrol as a microbiota modulator[83]. Resveratrol is capable of promoting gut microbiota changes by inhibiting *Enterococcus faecalis* growth, as well as promoting *Lactobacillus* and *Bifidobacterium* populations, both of which are involved in gut permeability and integrity[84]. Therefore, it is possible that resveratrol may exert its beneficial effects in part by improving microbiota diversity, especially under impaired metabolic conditions. In fact, mice fed a HFD to induce obesity and treated with resveratrol at 200 mg/kg/d for 12 weeks exhibited an increased number of *Lactobacillus* and *Bifidobacterium* (both belonging to the Actinobacteria phylum) and a decreased number of *Enterococcus faecalis* in the gut. Each of these bacterial genera is negatively and positively associated with body weight gain, respectively. Furthermore, the ratio between Bacteroidetes and Firmicutes bacteria, which negatively correlates with body weight, was increased after resveratrol treatment[84].

In addition to the changes in body weight, resveratrol also improves glucose homoeostasis by a mechanism involving gut microbiota modulation. Obese mice fed a diet containing resveratrol exhibited reduced fasting glucose and glucose tolerance, an effect partially lost when the obese mice were treated with antibiotics to deplete the microbiota[85]. Experiments involving faecal microbiota transplantation have also been conducted to further explore the role of resveratrol-induced microbiota modulation and its relationship with glucose homoeostasis. The faecal microbiota obtained from control mice fed a resveratrol-supplemented diet was transplanted to HFD-fed obese mice[86]. These authors found that faecal microbiota transplantation improved glucose homoeostasis in the obese group, as evidenced by the reduced area under the curve (AUC) in the glucose tolerance test. Interestingly, the sterile faecal content from control mice fed resveratrol also improved blood glucose levels in the obese group, suggesting that post-biotics also contribute to resveratrol-induced metabolic improvements[87].

Concerning UGT specifically, the resveratrol efficacy in ameliorating UGT diseases through gut microbiota modulation has been poorly addressed; however, a few studies have shown an important link between UGT and gut microbiota. Under physiological conditions, the gut microbiota is important for maintaining bladder structure and functioning. The mRNA expression of ninety-seven genes was found to be up- or down-regulated in the bladder of germ-free mice, which may be involved in the reduced bladder size and weight in these animals[87]. The abundance of *Faecalibacterium* along with the reduced number of *Bifidobacterium* was recently reported in patients with overactive bladder syndrome[88]. Similarly, a negative correlation between *Lachnospiraceae*, *Blautia* and LUTS in adult men was found[89]. The cross-talk between gut microbiota and UGT dysfunctions was also reported in inflammation-induced rat dysbiosis. The gut inflammation induced by colonic instillation of 2,4,6-trinitrobenzenesulphonic acid was associated with impaired detrusor contractility with no accompanying inflammatory signs[90]. Additionally, patients diagnosed with irritable bowel syndrome are 2·12- and 2·38-fold more likely to develop organic and psychogenic ED, respectively[91].

The mechanisms by which gut microbiota modulates UGT under physiological and pathological conditions may involve neuronal modulation (afferent and efferent nerves), neurotransmitter release, and control of food intake[92]. All these effects induced by the gut microbiota may contribute to better metabolic, hormonal and neurogenic functioning of the host. Therefore, it is likely that at least some of the beneficial effects of resveratrol in UGT dysfunction may be due to gut microbiota modulation, but surprisingly, no study has explored this possibility until now.

Future directions

To the best of our knowledge, there is just one clinical trial evaluating the efficacy of resveratrol in BPH patients. Long-term oral treatment with resveratrol (1000 mg, 4 months) in middle-aged men with metabolic syndrome indeed reduced the plasma levels of the androgen precursors androstenedione, dehydroepiandrosterone and dehydroepiandrosterone sulphate but had no significant effect on prostate size or the levels of prostate-specific antigen, testosterone and dihydrotestosterone[93]. On the other hand, resveratrol given orally for 2 months to patients with prostatic fibrosis (but with normal prostate volume) secondary to chronic prostatic inflammation resulted in reduced leucocyte

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infiltration as well as lowered Chronic Prostatic Symptom Index (by 3·5 points) and International Prostate Symptom Score (by 7 points), indicating better UGT functioning[94].

One critical issue about resveratrol that may explain the reduced number of clinical trials involving this polyphenol in the UGT is its low bioavailability, which reflects its low distribution to tissues. In an attempt to overcome this problem, several strategies have been proposed, such as the synthesis of resveratrol analogues, drug association and the development of pharmaceutical techniques. Acetylation or methylation of the three hydroxyl groups in the resveratrol molecule is a commonly used strategy reported to increase the plasma concentration by 74·5 % after intragastric administration to rats. Increases in half-life (234 %) and volume of distribution (176 %) along with reduced total body clearance (17·8 %) were also observed with these modified compounds[95].

With regard to the association of resveratrol with other drugs, a double-blind placebo-controlled and randomised multicentre study was performed to evaluate the co-administration of resveratrol and the cyclooxygenase inhibitor meloxicam in patients with knee osteoarthritis[96]. The authors reported that resveratrol 500 mg plus meloxicam 15 mg, both orally administered once per day, improved osteoarthritis-associated symptoms, such as knee physical function and pain, compared with meloxicam offered as a monotherapy. This combination was also considered safe based on clinical and biochemical analysis[96].

Different pharmaceutical formulations of resveratrol have also been developed to overcome its low bioavailability. In human volunteers, an oral formulation containing resveratrol solubilised in a lipid solution exhibited a twelve-fold increase in maximum plasma concentration and an eight-fold increase in AUC compared with dry powder of resveratrol capsules. Moreover, in HFD-fed mice, this same formulation reduced liver, colon and hypothalamus inflammation compared with dry powder of resveratrol[97]. Curiously, high-fat meals reduced AUC and the maximal plasma concentration of resveratrol in humans. Therefore, despite the high resveratrol lipophilicity, large amounts of fat reduced its bioavailability; hence, resveratrol is recommended to be taken with normal or low-fat meals[98].

Nanoparticles loaded with resveratrol have also been tested. Oral administration to rats of a nanoparticle composed of resveratrol/hydroxylpropylmethylcellulose/poloxamer 407 (1:4:1) led to a ten-fold increase in maximum plasma concentration and a three-fold increase in AUC of resveratrol compared with resveratrol[99]. A lipid-based nanocarrier system composed of a dioctadecyldimethylammonium bromide: monoolein liposomal system (1:2) was also shown to improve the stability and cellular penetration of resveratrol[100]. However, despite these innovative formulations of resveratrol, no study has tested these compounds in the healthy or diseased UGT.

Considering the low number of clinical studies addressing the effects of resveratrol on UGT dysfunctions, the results obtained from basic science should be interpreted from a human perspective, which may help to better understand the underlying pathophysiology of UGT diseases. For instance, resveratrol-induced c-kit/SCF pathway inhibition has been shown to play a role in prostate and bladder impairments in animal models[101]. This pathway has already been demonstrated in the human prostate and is activated in the prostate of BPH patients[102,103]. Therefore, drugs targeting the c-kit/SCF pathway, such as resveratrol, may be an interesting approach for future clinical trials involving UGT diseases. Similarly, the activation of sirt-1 in animal models is another proposed mechanism by which resveratrol exerts its beneficial effects in pre-clinical studies involving UGT diseases, such as ED[71,72]. Selective sirt-1 agonists have been developed and successfully tested in animal models of metabolic diseases; however, clinical trials have failed to demonstrate the efficacy of these molecules in ameliorating the glucose homoeostasis in type 2 diabetic patients[104]. However, the testing of selective sirt-1 agonists in human UGT has yet to be demonstrated. Nonetheless, this highlights the complex task in translating results from animals to humans but encourages further clinical trials of resveratrol or its modified molecules in UGT diseases. In this context, the results of analyses on the pre-clinical data from animals or isolated cells, treated resveratrol or sirt-1 agonists not only help to understand the molecular basis underlying resveratrol effects, but also guide additional clinical trials of UGT disease treatments.

Conclusion

Resveratrol is a polyphenol compound largely explored in recent decades as a therapeutic drug in a number of human and animal diseases. Its mechanisms of action are not specific (not exactly determined) but may involve receptor activation, antioxidant activity and microbiota modulation. Despite all the beneficial effects shown with *in vitro* assays and animal models, the efficacy of resveratrol in humans is still a matter of debate. In UGT diseases specifically, resveratrol ameliorates LUTS, BPH, prostatitis and ED in animal models, but there are still few studies in human tissues and insufficient clinical trials to establish resveratrol efficacy. Therefore, future studies designed to investigate the resveratrol actions on human UGT dysfunctions are required.

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Conflict of interest

There are no conflicts of interest.

Authorship

Participated in manuscript design: C.F.B., A.E.
Drafted the manuscript: C.F.B., S.F.H., A.E.C.
Edited and revised the manuscript: C.F.B., A.E.

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