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Priming metabolism with the type 5 phosphodiesterase: the role of cGMP-hydrolyzing enzymes

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Abstract

The cyclic guanosine monophosphate (cGMP) signaling system is one of the most prominent regulators of many physiopathological processes in humans and rodents. It has been strongly established as an accomplished cellular signal involved in the regulation of energy homeostasis and cell metabolism, and pharmacological enhancement of cGMP has shown beneficial effects in metabolic disorders models. cGMP intracellular levels are finely regulated by phosphodiesterases (PDEs). The main enzyme responsible for the degradation of cGMP is PDE5. Preclinical and clinical studies have shown that PDE5 inhibitors (PDE5i) have beneficial effects on improving insulin resistance and glucose metabolism representing a promising therapeutic strategy for the treatment of metabolic disorders. This review aims to describe the molecular basis underlying the use of PDE5i to prompt cell metabolism and summarize current clinical trials assessing the effects of PDE5i on glucose metabolism.

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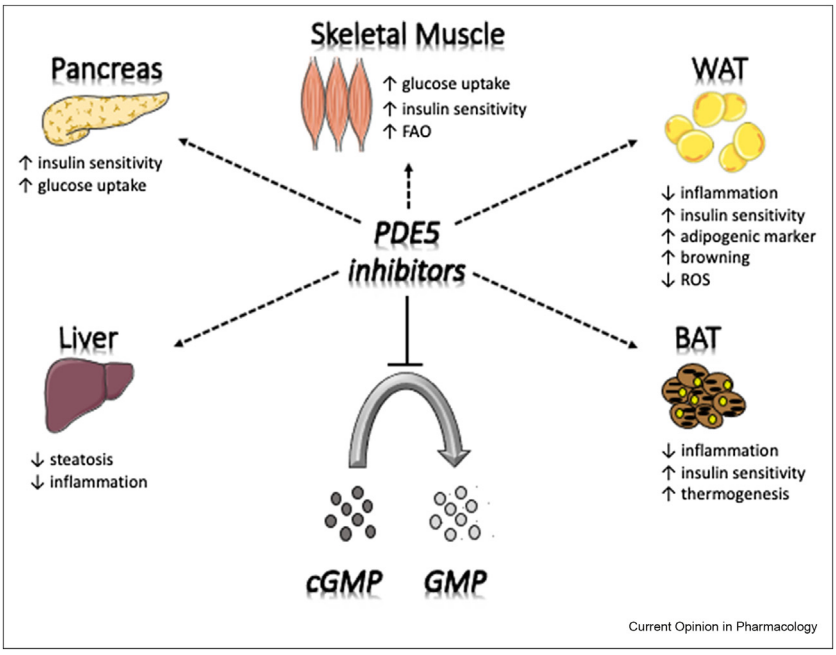
Introduction

The increasing prevalence of obesity, type 2 diabetes (T2DM) and other endocrine-metabolic disorders underscores the need to develop new therapeutic strategies [1]. Insulin resistance (IR) has been recognized as the step preceding their development, with diabetes ensuing when insulin secretory capacity fails to compensate the increased insulin body requirements [2]. Prevention strategies include weight loss and exercise activity but require patients' compliance and strict adherence; lifestyle interventions are difficult for patients to maintain and the weight loss achieved tends to be regained over time [3]. Pharmacological treatments include metformin and thiazolidinediones, and both drugs demonstrated efficacy in reducing the progression toward diabetes [4,5], but their use is not free of adverse effects [6], although metformin exhibits a safer profile [4]. In this context, one of the promising targets is the second messenger cGMP, whose levels are finely regulated by Phosphodiesterases (PDEs) and, in particular, PDE5. Many authors have investigated the efficacy of PDE5 inhibition in the regulation of glucose and lipid metabolism. PDE5 inhibitors (PDE5i) activity has long been used as an effective treatment for erectile dysfunction and pulmonary hypertension [7,8], and much evidence suggest the efficacy and safety of PDE5i in other pathological conditions, such as cardiovascular diseases [9–11] and endocrine-metabolic disorders [12–14]. Based on recent *in vitro* and *in vivo* findings, this review summarizes (i) the molecular mechanisms underlying the effects of PDE5i on glucose and lipid homeostasis (Figure 1) (ii) the data derived from clinical trials assessing possible beneficial effects on humans (Table 1).

Role of cGMP-PKG pathway in the control of energy homeostasis

cGMP intracellular levels are finely controlled by the generating enzymes guanylyl cyclases (GCs) and the degrading enzymes, PDEs. Eleven PDE families are present in mammalian cells that, after alternative splicing, give rise to more than 100 PDE isoforms in rodents and humans [15,16]. PDE5, PDE6, and PDE9 are able to specifically hydrolyze cGMP, PDE4, PDE7, and PDE8 display high specificity for cAMP, while PDE1, PDE2, PDE3, PDE10, and PDE11 have dual specificity, modulating levels of both cAMP and cGMP [17]. cGMP regulates mitochondrial biogenesis in a broad spectrum of cells representing the main determinant for cellular metabolism [18]. The major downstream target of cGMP in adipocytes is cGMP-

Figure 1



Metabolic actions of PDE5i in the control of glucose and lipid metabolism.

dependent protein kinase (PKG), whose activity is indispensable for the proper differentiation of adipocytes [19–21]. Loss of PKG impairs the thermogenic capacity of brown adipose tissue (BAT), with reduced uncoupling protein 1 (UCP1) expression and mitochondrial content in PKGI knockout mice [19]. Moreover, brown preadipocytes isolated from PKGI knockout show defects in differentiation with reduced expression of thermogenic markers peroxisome proliferator-activated receptor gamma (PPARγ), UCP1, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) [19]. On the contrary, mice overexpressing PKG are resistant to diet-induced obesity (DIO) displaying increased insulin sensitivity, enhanced energy expenditure, increased BAT mitochondrial content, and increased expression of the thermogenic markers UCP1 and PGC-1α [22]. Importantly, activating cGMP signaling is beneficial for the adipogenic and thermogenic programs also in cultured human adipocytes [19,20,22–24]. Given that premises, targeting the cGMP-PKG axis could represent a valuable tool to control cell metabolism. cGMP-hydrolyzing PDEi efficacy is based on the ability to block the cGMP breakdown, produced by the nitric oxide (NO)-dependent activation of GC [17]. Many cGMP-hydrolyzing PDEs have been found to be expressed in adipose tissue (AT) and adipocytes in rodents and humans [25,26]. A study performed on mouse primary brown adipocytes revealed the presence of Pde1a, Pde2a, and Pde3b mRNA [27], while PDE3, PDE9 and PDE10 proteins were detected in human AT and adipocytes [28]. PDE3B represents the main regulator of lipid metabolism, adiposity, and energy status in adipocytes [29]. Based on this consideration, PDE3B deficiency resulted in cAMP/protein kinase A and 5′ adenosine monophosphate-activated protein kinase-induced increases in respiratory uncoupling and fatty acid oxidation (FAO) [30]. Moreover, Pde3b knockout mice display reduced fat mass and adipocytes size, increased insulin secretion, increased mitochondrial biogenesis and oxygen consumption [30–33]. Other cGMP-hydrolyzing PDEs are able to modulate at different levels of cell metabolism, and their absence/inhibition has beneficial effects on glucose and lipid metabolism [28,34]. Inhibition of PDE10A has been shown to increase glucose uptake and thermogenesis in BAT of different experimental models of obesity [35]. Moreover, Pde10 knockout mice are resistant to DIO and related metabolic disturbances. *In vivo* administration of PDE1 inhibitors reduced weight gain in mice fed with normal chow and high-fat diet (HFD) [36]. Table 2 summarizes the metabolic phenotype of mouse models resulting from genetic alteration of cGMP/PKG/PDEs signaling. However, the main enzyme responsible for the degradation of cGMP is PDE5 [17]. PDE5 mRNA expression was first detected in human subcutaneous adipocytes [37]. Interestingly, the highest levels of PDE5 protein were detected in preadipocytes, and these levels decreased during adipocytes maturation [21]. Several experimental findings suggest that selective PDE5 blockade increases

Table 1

Summary of RCTs studying PDE5i effects on metabolism.

Reference	Study design	Population	Outcomes	Drug	Results
Hill et al. Diabetes care, 2009 [26]	Randomized, crossover, double-blind.	18 subjects with metabolic syndrome	IS and β-cell function during an intravenous glucose tolerance test	Tadalafil 10 mg once daily for 3 weeks	Improved IS and β-cell function in women but not men
Jansson et al. Diabetologia, 2010 [28]	Placebo-controlled, crossover	7 post-menopausal women with T2DM versus non-diabetic matched controls	Skeletal muscle capillary recruitment (PS_{glu}) and glucose uptake measured by microdialysis	Single dose Tadalafil 20 mg	Increase in PS_{glu} and muscle glucose uptake in T2DM but not control
Murdolo et al. JCEM, 2013 [25]	Open label, active-control	8 women with post-menopausal –T2DM versus non-diabetic matched controls	Local microcirculation and regional metabolism in skeletal muscle and adipose tissue through microdialysis	Single dose Tadalafil 10 mg	Increased microvascular recruitment and glucose metabolism in skeletal muscle and adipose tissue both in T2DM and controls
Ho et al. J Am Heart Assoc, 2014 [31]	Randomized, double-blinded, placebo-controlled trial	53 adults with non-diabetic obesity–high fasting insulin levels	Difference in IR, as measured by HOMA-IR and ODI during OGTT.	Tadalafil 20 mg daily for 3 months	Improved HOMAi and ODI in severely obese patients
Ramirez et al. JCEM 2015 [21]	Randomized, double-blinded, placebo-controlled trial	21	IS and GSIS during hyperglycemic clamps	Sildenafil 75 mg per day (25 mg thrice a day) for 3 months	Enhanced IS; No effects on GSIS
Mandosi et al. Expert opin. Ther targets, 2015 [32]	Open label, active-control	28 men with T2DM	Baseline and postprandial glycemia, insulin, HbA1c, HOMAi, lipids	Sildenafil 100 mg per day (25 + 25 + 50 mg) for 3 months	Reduced postprandial glycemia, HbA1c, LDL, cholesterol and increased HDL
Fiore et al. JCEM, 2015 [8]	Randomized, double-blinded, placebo-controlled trial	59 men with type 2 diabetes mellitus	Anthropometric and metabolic parameters, VAT, EAT quantification through CMR imaging	Sildenafil 100 mg per day (25 + 25 + 50 mg) for 3 months	Reduced waist circumference and EAT
Sjogren et al. Diabet Med, 2016 [29]	Randomized, double-blinded, placebo-controlled trial	20 men and women with well-controlled T2DM	Skeletal muscle capillary recruitment (PS_{glu}) and glucose uptake measured by microdialysis	Single dose Tadalafil 20 mg before a mixed meal	Increase in PS_{glu} and muscle glucose uptake
González-Ortiz, Acta clinica Belgica, 2017 [30]	Randomized, double-blinded, placebo-controlled trial	18 male patients with obesity	Total and first phase of insulin secretion, insulin sensitivity	Tadalafil 5 mg for 28 days	No differences.

IR: insulin resistance; HOMA-IR: homeostatic model assessment for insulin resistance; ODI: oral disposition index; HOMAi: homeostatic model assessment index; OGTT: oral glucose tolerance test; VAT: visceral adipose tissue; HbA1c: glycated hemoglobin; CMR: cardiac magnetic resonance; LDL: low-density lipoprotein; HDL: high-density lipoprotein; EAT: epicardial adipose tissue; T2DM: type 2 diabetes mellitus; GSIS: glucose-stimulated insulin secretion; IS: insulin sensitivity; PS_{glu}: permeability surface area for glucose.

intracellular cGMP levels and concomitantly improves insulin resistance and peripheral glucose disposal [21]; however, the lack of Pde5 knockout mouse models hampers a deep understanding of PDE5 contribution in the regulation of glucose and lipid homeostasis.

Effect of PDE5i on adipogenesis and thermogenesis

The altered function of AT has a great impact on whole-body metabolism and represents a key driver for the development of metabolic disorders. Browning, which

Table 2

Metabolic phenotype of mouse models resulting from genetic alteration of cGMP/PKG/PDEs signaling.

Genetic alteration	Phenotype/Biological effects	References
PKGI$^{-/-}$	Reduced BAT mass Impairment of browning Reduced mitochondrial biogenesis Reduced adiponectin levels	*Haas B.* et al. *2009* [19]; *Mitschke, M.M.* et al., *2013* [20];
cGK-Tg	Reduced body weight Increased mitochondrial content Increased fat oxidation Improved insulin sensitivity Decreased glucose levels Increased oxygen consumption	*Miyashita K.* et al. *2009* [22].
Pde3b$^{-/-}$	Increased basal glucose production Defects in TG storage Increased FA biosynthesis Decreased adipocytes size Enhanced catecholamine-stimulated lipolysis Enhanced insulin-stimulated lipogenesis Increased browning	*Berger K.* et al. *2009* [31]; *Choi Y.H.* et al. *2006* [32]; *Guirguis E.* et al. *2013* [33].
Pde4b$^{-/-}$	Reduced fat pad weights Reduced adipocyte size Decreased serum leptin levels Reduced HFD-induced inflammation	*Zhang R.*et al. *2009* [34].
Pde9a$^{-/-}$	Reduced body weight Reduced fat mass	*Omar B.*et al. *2011* [28].
Pde10a$^{-/-}$	DIO resistance Improved insulin sensitivity	*Nawrocki A.R.* et al. *2014* [35].

BAT: brown adipose tissue; TG: triglycerides; FA: fatty acids; HFD: high-fat diet; DIO: diet-induced obesity.

consists of the induction of thermogenically active adipocytes in white fat depots [38], leads to protection against metabolic derangements in a mouse model of metabolic syndrome [39]. It has been established that browning improves whole-body homeostasis and insulin sensitivity in humans and mouse models [40,41]. Therefore, increasing BAT activity emerged as an attractive target for endocrine-related disorders. Many pieces of evidence suggest an involvement of the cGMP-PKG pathway in adipogenesis in the 3T3-L1 cell line [20] and the use of sildenafil in these cells has been shown to promote adipogenesis through the activation of the cGMP-PKG pathway [20]. In particular, PDE5 blockade during 3T3-L1 preadipocyte differentiation increased intracellular lipid droplets, as well as the expression of adipocyte-specific genes PPARγ, Fas, and Adiponectin [21]. Sildenafil treatment enhances the expression of adipogenic markers, such as PPARγ and Fabp4 also in murine primary white adipocytes [20,42]. Furthermore, *in vitro* treatment of murine primary white adipocytes with sildenafil induces browning and increases expression of the thermogenic markers UCP1 and PGC-1α [20], while acute exposure of primary human white adipocytes to PDE5i stimulates aromatase expression positively affecting metabolism [43].

Effect of PDE5i on lipolysis

Lipolysis is responsible for the hydrolysis of triacylglycerol stored in AT and is catalyzed by hormone-sensitive lipase (HSL) and adipose tissue triglyceride lipase, yielding free fatty acid (FFA) and glycerol [44]. Activation of HSL is dependent on increased levels of cAMP and subsequent activation of protein kinase A [45]. However, an intrinsic lipolytic pathway is coupled to PKG signaling because PKG is able to phosphorylate HSL, stimulate mitochondrial biogenesis and improve insulin signaling to counteract DIO [46]. PDEs inhibition has been known to enhance adipocyte lipolysis, and in particular, PDE3i can induce high levels of lipolysis in human adipocytes *in vitro* [26]. Moreover, the use of a PDE1i increases HSL phosphorylation suggesting the induction of lipolytic pathway [36], while PDE4 can limit the rate of basal lipolysis in rat adipocytes [47]. Given the ability of PDE3 and PDE4 to influence lipolysis the inhibition of both PDEs is required for efficient stimulation of lipolysis in murine and rat adipocytes [26]. PDE5i in human subcutaneous adipocytes [37] and visceral adipocytes [21] does not impair lipolysis rate probably for the slight magnitude of the increase in cGMP levels in these cells.

Effect of PDE5i on energy expenditure and fat oxidation

Following lipolysis, the free fatty acid is released into the blood and transported to the working muscle for oxidation [48]. Several compounds are known to influence energy expenditure and fat oxidation [49]. PDE5i-induced cGMP enhancement results in increased expression and activity of PPARα, a master controller of mitochondrial FAO [50]. Subtherapeutic doses of PDE5i combined with Leucine result in activation of FAO, marked improvement in insulin sensitivity and reversal of hepatic steatosis and inflammation in DIO mice [51]. Moreover, PDE5i treatment of 3T3-L1 cells significantly increases basal oxygen consumption rate, maximal OxPhos capacity together with increased FAO rate [52]. In men, testosterone plays a pivotal role in restraining FA storage in femoral adipose tissue via suppression of lipoprotein lipase and acyl-coenzyme A synthetase activities, and testosterone deficiency has been demonstrated to alter FA storage [53].

Effect of PDE5i on insulin sensitivity and secretion

The involvement of PDE5 in insulin signaling has been claimed from the observation that chronic treatment with PDE5i in a mouse model of diet-induced IR increases not only cGMP levels but also insulin sensitivity and muscle glucose uptake counteracting the detrimental effects of HFD on endothelial function [54]. Moreover, a study performed on rabbits demonstrated that long- and short-term treatment with tadalafil was able to reduce triglycerides accumulation in visceral adipose tissue in an experimental model of diet-induced metabolic syndrome. Tadalafil is able to counteract HFD-related alterations by restoring insulin sensitivity, increasing expression of thermogenic markers, reducing ROS, and prompting preadipocytes differentiation toward a metabolically healthy phenotype [55]. Moreover, short-term treatment with PDE5i udenafil has been demonstrated to reduce body weight, visceral fat mass ad appetite in high-fat-fed mice mostly because of a reduction of leptin plasma levels [56]. cGMP pathway appears to regulate glucose metabolism through the upstream activation of NO also in pancreatic β-cells [57]. Preclinical and clinical studies have shown that PDE5i have beneficial effects not only on improving β-cell function but also on increasing insulin sensitivity of other peripheral tissues (such as skeletal muscle cells and adipocytes) thus improving IR [58–61]. Physiological concentrations of NO enhance hepatic insulin response through the canonical sGC/PKG pathway [62] while endothelial NO/cGMP/VASP signaling attenuates hepatic IR induced by high-fat feeding [63]. In human skeletal muscle cells, tadalafil has been demonstrated to regulate lipid homeostasis via IRS-1 [59], and acute administration of PDE5 inhibitor zaprinast was able to enhance insulin-mediated microvascular perfusion [64].

Clinical trials

The first proof-of-concept study testing the hypothesis that PDE5i could improve glucose homeostasis was a randomized, crossover, double-blind trial performed in individuals with metabolic syndrome [65]. 18 patients were treated for 3 weeks with 10 mg tadalafil once daily. Insulin sensitivity and β-cell function were measured during an intravenous glucose tolerance test. The results showed that daily treatment with tadalafil improved β-cell function, insulin sensitivity and secretion in women but not men. Interestingly, the PDE5i-mediated improvement in β-cell function was measured in subjects exhibiting baseline fasting hyperglycemia as opposite to euglycemic ones suggesting that PDE5i are more effective during metabolic impairment. The study did not address possible underlined mechanisms and the sex difference in response to PDE5i was explained by the higher blood glucose in enrolled women, or rather in the different sensitivity to cGMP degradation that can be linked to genetic variants in female subjects [66].

Sex differences in response to PDE5i treatment are recognized [67]. A possible explanation for the sex difference in the response to PDE5i treatment could rely on the gonadal status of the enrolled subjects. It is now recognized that intra-myocyte effects of sildenafil are estrogen-dependent in females, [68] and on the other hand, testosterone is crucial for PDE5 expression, NO generation, and response to PDE5i in men [69]. Unfortunately, neither the age nor the gonadal status was described by the authors.

Later, a possible explanation for PDE5i peripheral effect on insulin sensitivity was proposed [70–73]. By using muscle microdialysis, Jansson et al. [70] performed a placebo-controlled, crossover trial to assess the effects of a single dose of 20 mg of tadalafil or matching placebo on muscle capillary recruitment and forearm glucose uptake in 7 post-menopausal women with T2DM on the fasting state. They showed an acute positive effect on capillary recruitment and glucose uptake [70]. The same results were then confirmed in an randomized controlled trial (RCT) enrolling a mixed population of 20 women and men with well-controlled T2DM treated with a single dose of 20 mg of tadalafil before a mixed meal test. However, in this study, no differences were found for circulating concentrations of baseline or postprandial glucose, insulin, triglycerides or free fatty acids between groups [71]. The authors speculated that the expansion of the endothelial surface area, through the recruitment of additional microvasculature, could play an important role in modulating the muscle sensitivity to both insulin- and contraction-dependent glucose disposal.

Conflicting results came from a subsequent study performed by the same group on 8 post-menopausal

women with T2DM compared to non-diabetic matched controls treated with a single dose of 10 mg of tadalafil [74]. The aim of the study was to explore the acute effects of tadalafil on local microcirculation and regional metabolism in skeletal muscle and AT through intramuscular and subcutaneous microdialysis. The authors demonstrated that acute tadalafil administration increases muscle capillary recruitment, non-oxidative glucose metabolism and glucose conversion to lactate in skeletal muscle and AT irrespective of IR. However, no differences in permeability surface area for glucose (PS_{glu}) and/or regional glucose uptake were found. The authors discussed the conflicting results arguing with the total dose (10 vs 20 mg) and duration (acute vs chronic) of tadalafil administration, which was lower than previous results.

A similar lack of metabolic effects was seen in a clinical trial performed in 18 obese men on which 5 mg of tadalafil for 28 days had no effects on insulin sensitivity or total/first phase of insulin secretion [75]. Whether the lack of the effects could be due to the length of treatment or the total daily dose of PDE5i must be confirmed in dedicated trials.

Another human evidence of the beneficial effects of PDE5i on AT comes from a clinical trial on 59 men with T2DM treated with 100 mg of sildenafil or a matching placebo for 12 weeks [12]. Sildenafil treatment reduced waist circumference and epicardial adipose tissue without affecting BMI. The authors also demonstrated the modulation of miR-22-3p and SIRT1 pathways associated with the beneficial effects of PDE5i on AT remodeling.

The same group also gave the only evidence of metabolic improvement in terms of glucose metabolism in 28 men with T2DM treated with sildenafil 100 mg daily or matching placebo for 3 months. In this cohort, sildenafil reduced postprandial glycemia, HbA1c, low-density lipoprotein cholesterol and increased high-density lipoprotein cholesterol.

Later, Ho et al. [57] performed a clinical trial to examine the effects of 3 months of high dose tadalafil treatment on IR and insulin secretion in 53 adults with non-diabetic obesity with elevated fasting insulin levels [57]. Participants were randomized to receive either oral tadalafil 20 mg daily or matching placebo for 3 months. Oral glucose tolerance tests were performed to examine the effect of tadalafil on IR. The results showed that in individuals with severe obesity, tadalafil improved HOMAi and oral disposition index (a measure of β-cell compensation for IR) without sex by treatment interactions.

However, the study used estimates of β-cell function and IR derived from glucose tolerance test rather than the gold standard, a more precise and physiologic estimate of insulin sensitivity, the hyperinsulinemic-euglycemic clamp.

Convincing results came from a clinical trial measuring glucose-stimulated insulin secretion and estimating insulin sensitivity through hyperglycemic clamps. The authors tested the hypothesis that 3-months treatment with 75 mg of sildenafil increases insulin secretion and improves tissue insulin sensitivity in overweight patients with prediabetes [58]. The results confirmed sildenafil improved insulin sensitivity index, but no effects were found on acute or late phase glucose-stimulated insulin secretion. A trend toward higher disposal index was found in the sildenafil arm.

Finally, in a very recent trial on 43 non-obese men with erectile dysfunction, 2-months treatment with 5 mg of tadalafil improved body composition, by increased abdominal lean mass, and improved endothelial function [76]. The effects were directly related with serum insulin and inversely related to estrogen levels. Interestingly, all the beneficial effects of tadalafil were lost after 2 months withdrawal.

Further large, well-designed, prospective trials are needed to draw definitive conclusion regarding the contribute of sex and/or different degree of metabolic impairment (insulin resistance, obesity and diabetes mellitus) on the beneficial effects of PDE5i treatment.

Conclusions

NO-cGMP-PKG signaling pathway plays a pivotal role in the regulation of glucose and lipid metabolism. Although molecular mechanisms are still not completely understood, given the wide expression of PDE5 in metabolic active tissues and the safety of PDE5i, these data suggest that PDE5i may have favorable metabolic effects by improving insulin sensitivity and glucose metabolism before the development of clinical diabetes. This opens a new therapeutic strategy in the prevention of metabolic diseases.

Conflict of interest statement

Nothing declared.

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