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The role of PGC-1α in mitochondrial transcription factors: A promising pathway in the treatments of mitochondrial diseases activated by *Gynostemma pentaphyllum*

Ferro M. and Channan G.

FG Scientifica and Science Department at Nutrition Formulators Inc., Miramar, Fl, 3260 Executive way, Parkway, Miramar, Fl 33025 Miramar, Fl, USA.

ABSTRACT

The peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α), being a key transcription factor in mitochondrial biogenesis, interacts as a coactivator of several mitochondrial nuclear transcription factors, such as nuclear respiratory factor (NRF) 1 and 2, estrogen-related receptor α (ERRα), as well as non-nuclear receptor myocyte-enhancing factor 2 (MEF-2). *Gynostemma pentaphyllum* (GP) is a plant used in many countries, mainly in Asia, having strong activity in PGC-1α. A wide range of GP pharmacological properties has been reported, including anti-inflammatory and antioxidant activity, lipid metabolism modulator, and neuroprotective activity. The activation of PGC-1α by exogenous factors has become a promising strategy in the treatment of various pathologies, especially when focused on mitochondria. In our review, we found numerous benefits of GP in controlling age-associated metabolic diseases, mainly in diabetes type 2, fat liver, and obesity. Some studies have also reported that, due to the strong phosphorylation of AMP protein kinase (AMPK) exerted by GP, GP-treated mice showed a significant increase in Sirtuin 1 (SIRT1) mRNA expression, a protein that acts directly on cellular aging processes. However, further studies in humans could provide more proof of its efficiency in diseases such as metabolic syndrome as well as any other pathology that involves changes in mitochondrial functions.

Keywords: *Gynostemma pentaphyllum*, PCG-1α, mitochondria, AMPK, SIRT1, metabolic syndrome.

***Correspondence to Author:**
Ferro M.
FG Scientifica and Science Department at Nutrition Formulators Inc., Miramar, Fl, 3260 Executive way, Parkway, Miramar, Fl 33025 Miramar, Fl, USA.

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Introduction

The peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) is a key transcription factor in mitochondrial biogenesis encoded by the Ppargc1a gene [1]. PGC-1α interacts as a coactivator of several nuclear transcription factors, mainly in transcription factors that act in mitochondrial biogenesis, such as nuclear respiratory factor (NRF) 1 and 2, estrogen-related receptor α (ERRα), as well as non-nuclear receptor myocyte-enhancing factor 2 (MEF-2) [2,3]. The NRF1 and 2 regulate the mitochondrial transcription factor A (Tfam) expression, another transcription factor encoded in the nucleus, essential for the replication and transcription of mitochondrial DNA [4] (Fig. 1). PGC-1α expression level is high in tissues where there is high energy demand with an abundant presence of mitochondria, such as in brown adipose tissue (BAT), heart, skeletal muscle, brain, and kidney, while the expression level is low in the liver, and very low in white adipose tissue (WAT) [5].

In muscle tissue, exercise-induced pathways involve the activation of AMP-activated protein kinase (AMPK), which has been shown to regulate phosphorylation and activation of PGC-1α [6]. The increase in exercise-induced AMP: ATP ratio activates AMPK and consequently increases transcription, and PGC-1α activity [7]. AMPK, which is activated through the aforementioned transcription factors, when phosphorylated also plays an important role in the expression of the mitochondrial protein encoded in the nucleus, mainly in the Tfam gene [8,9]. In vivo studies have shown that AMPK is activated in mouse muscles during treadmill running and in human muscles during cycle exercise in a time- and exercise-intensity-dependent manner [6,10,11].

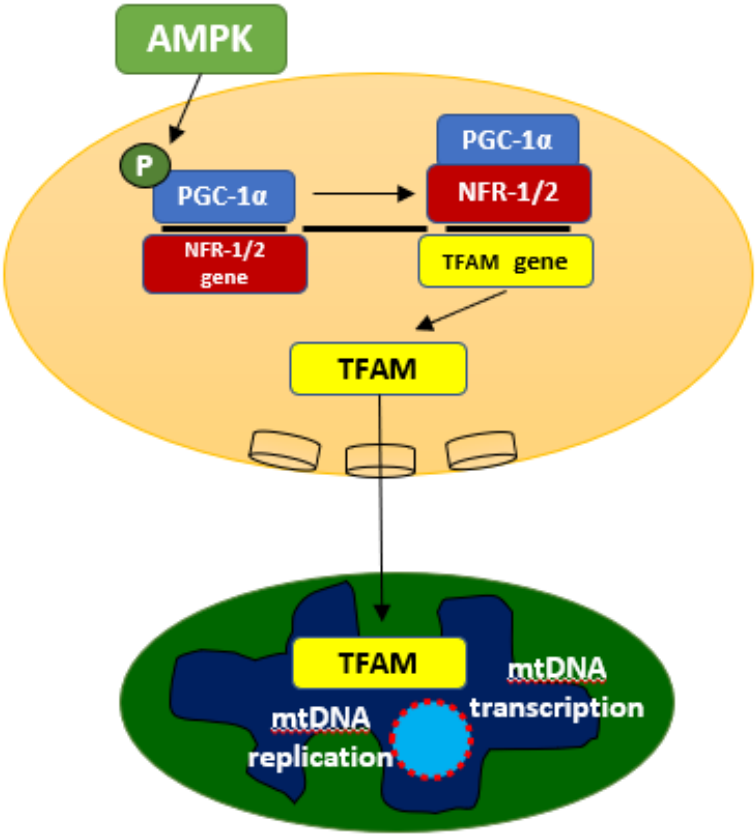

Figure 1: AMPK: AMP-activated protein kinase; **PGC-1α:** peroxisome proliferator-activated receptor-gamma coactivator 1 alpha**; NRF 1 & 2:** nuclear respiratory factor 1 & 2; **TFAM:** mitochondrial transcription factor A; **mtDNA:** mitochondrial DNA.

The biochemical cascade of mitochondrial transcription factors is not restricted exclusively by physical exercise, but also by exposure to cold and fasting [12]. In addition to physical exercise activating AMPK, the presence of calcium in calmodulin-dependent protein kinase IV (CaMKIV), in response to physical exercise, induces phosphorylation of cyclic AMP (cAMP) response element-binding (CREB) protein [13]. This protein exerts strong activity in the PGC-1α transcription, increasing its expression, and consequently positively influencing the other transcription factors responsible for mitochondrial biogenesis [14] (Fig 2). Likewise, CREB protein phosphorylation was also observed in cold and fasting exposure, but through the metabolic pathway of cAMP and consequently protein kinase A (PKA) [15, 16].

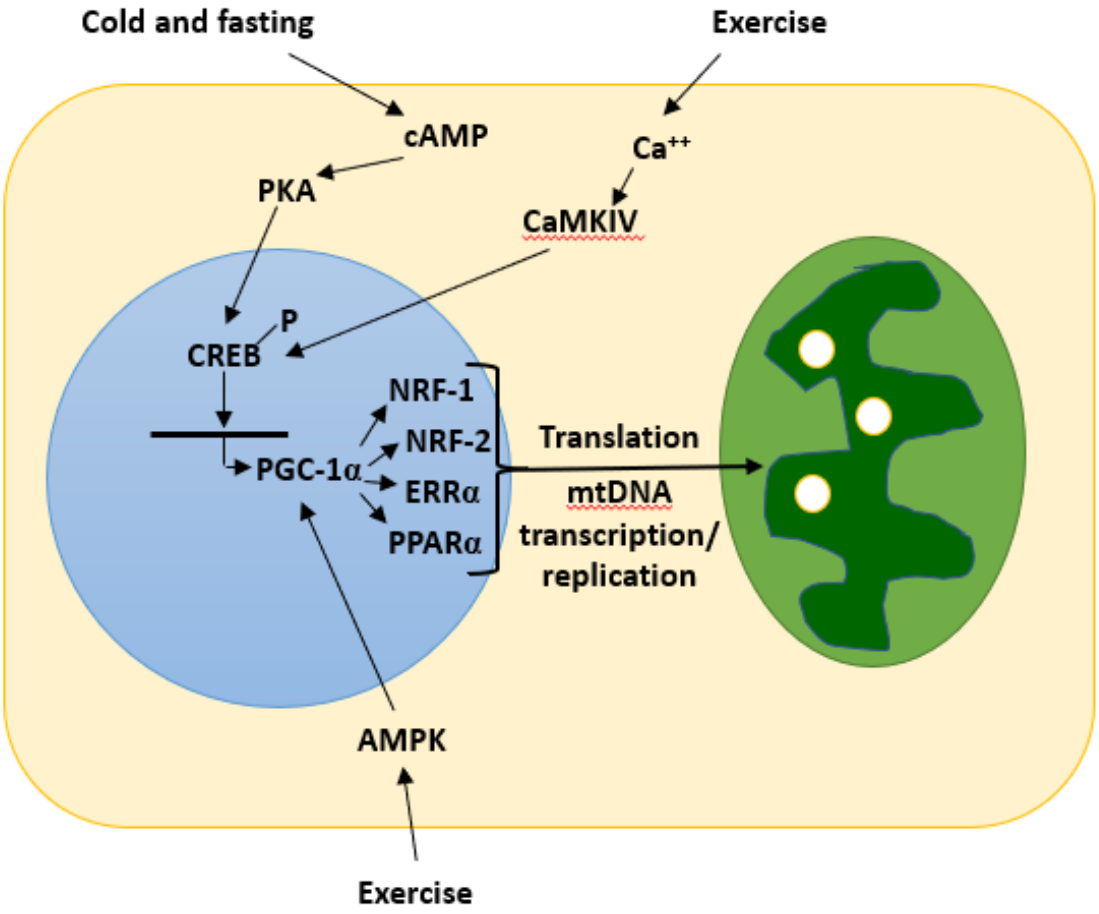

Figure 2: cAMP: cyclic AMP; **CaMKIV:** calmodulin-dependent protein kinase IV; **PKA:** protein kinase A; **CREB:** cyclic AMP (cAMP) response element-binding; **PGC-1α:** peroxisome proliferator-activated receptor-gamma coactivator 1 alpha; **NRF 1:** nuclear respiratory factor 1**; NRF 2:** nuclear respiratory factor 2; **TFAM:** mitochondrial transcription factor A; **mtDNA:** mitochondrial DNA. **ERRα**: estrogen-related receptor alpha. PPARα: peroxisome proliferator-activated receptor-alpha. **AMPK:** AMP-activated protein kinase

Gynostemma pentaphyllum

Gynostemma pentaphyllum (GP) is a plant used in many countries, mainly in Asia as

herbal medicine to treat dyslipidemia, type 2 diabetes, and inflammation [17]. Also known as Jyagulan, GP belongs to the Cucurbitaceae family, which includes cucumbers, gourds, and melons [18]. GP contains more than 230 compounds derived from this plant, including saponins, sterols, flavonoids, polysaccharides, and many others [19]. Recent studies have reported a range of pharmacological properties

of GP, including anti-inflammatory and antioxidant activity [20,21,22], modulator of lipid metabolism [23], and neuroprotective activity [24]. GP has also been used for the treatment of various diseases, including non-alcoholic liver cirrhosis [25], diabetes [26], heart diseases [27], and cancer [28, 29]. Its mechanism in apoptotic processes has drawn the scientific community's attention [19]. One of the main components of GP is gypenoside, a dammarane-like glycoside, an activator of the peroxisome proliferator-activated receptor (PPAR)-alpha [28]. So far, studies report that gypenosides isolated from GP are the main active constituents responsible for the biological activities as well as the clinical effects reported in GP therapies [30]. These dammarane-like gypenosides were isolated for the first time in 1976 [31], and since then new gypenosides have been isolated, presenting new evidence. Recent studies have reported the presence of 25% of ginsenosides in some types of gypenosides demonstrating GP to be the first plant presenting ginseng saponins outside the Araliaceae family [30]. This fact characterized it as the ginseng of the south [32].

Gynostemma pentaphyllum induces AMPK phosphorylation

AMPK is an important intracellular modulator. This protein regulates the energy balance of all cells in response to energy supply and demand as well as a signal for other proteins that act as gene transcription factors [33]. Several metabolic diseases are related to AMPK activity including obesity, type 2 diabetes, and dyslipidemia. Being able to activate fat oxidation and glucose uptake, AMPK simultaneously inhibits fat and cholesterol synthesis, exerting numerous benefits on cells [34,35]. When activated, AMPK phosphorylates downstream targets that inhibit anabolic pathways, particularly those which consume ATP, such as fatty acid and cholesterol synthesis [36]. Simultaneously, the catabolic pathways producing ATP like glycolysis, fatty acid oxidation, and the glucose uptake itself are also stimulated, favoring the control of numerous diseases related to the metabolic syndrome [37]. In vitro studies revealed that phosphorylation of AMPK, as well as acetyl-CoA carboxylase (ACC), was increased in the presence of damulin A and B when these saponins were purified from GP leaves. Consequently, an increase in beta-oxidation in response to AMPK and ACC phosphorylation was also observed [38]. However, one of the biochemical pathways for AMPK stimulated by GP is in activating PGC-1α, a master regulator of mitochondrial biogenesis processes [6,38].

Gynostemma pentaphyllum activates PGC-1α

The role of PGC-1α in controlling mitochondrial biogenesis is already well reported in scientific studies. PGC-1α increases mitochondrial biogenesis and cellular respiration rates, improving substrate absorption and utilization for energy production [39]. PGC-1α directly coactivates not only nuclear transcription factors but also non-nuclear transcription factors such as MEF-2 [40] and the forkhead O-box (FOXO) [41]. Among nuclear transcription factors, PGC-1α coactivates PPARs [42,43], the thyroid hormone receptor (TR) on the uncoupling protein (UCP-1) promoter [44], glucocorticoid receptors (GRs) [42], estrogen receptors (ERs) [42] and others. With its ability to simultaneously coactivate these transcription factors, PGC-1α can coordinate and modulate a transcriptional program on energy metabolism [45]. Therefore, the activation of PGC-1α by exogenous factors has become a promising strategy in the treatment of various pathologies, especially when focusing on mitochondria [46]. Thus, GP administration has shown expressive results in both animals and humans in obesity type 2 diabetes, and cancer control [19,26,47]. AMPK and sirtuin 1 (SIRT1) have also been reported to directly affect PGC-1α activity through phosphorylation and deacetylation processes, respectively [39]. Although the physiological aspects and their molecular consequences on these proteins are still not well defined, recent studies with transgenic models in vivo suggest that AMPK, SIRT1, and PGC-1α can act as an

orchestrated group to regulate metabolism [17,46,48,49]. In the studies by Kim et al, GP and gypenoside L promoted a significant increase in mRNA expression levels of the PGC-1α gene (Ppargc1a), genes that regulate lactate metabolism (Esrra and Mct1), adipocyte browning gene fibronectin type III containing domain 5 (Fndc5), the glycogen synthase gene (GYS), and the lipid metabolism gene carnitine palmitoyltransferase 1b (Cpt1b). In addition, GP and gypenoside induced phosphorylation of AMPK, p38, and SIRT1 [50]. This study also demonstrated that the treatment with GP and gypenoside significantly stimulated the expression of genes associated with the antioxidant stress response, such as Ucp2 and 3, nuclear factor-erythroid factor 2-related factor 2 (Nrf2), and superoxide dismutase 2 (Sod2) [50].

Gynostemma pentaphyllum in diabetes

There are many antidiabetic drugs currently available for the management of type 2

diabetes. However, because these drugs exhibit several limitations, such as side effects and high secondary failure rates [51], in addition to the very high treatment costs, diabetic patients and health professionals are considering alternative ways to use herbal medicines with antidiabetic properties. Asian countries have demonstrated that herbal medicines have played an important role in the control of type 2 diabetes [52]. Scientific evidence has already proved that GP tea has shown antidiabetic effects in patients with type 2 diabetes, both as a single treatment and as a complementary therapy to sulfonylureas with good safety data [53]. In a study by Huyen et al, 16 patients diagnosed with type 2 diabetes were evaluated using GP tea. In this study, the inclusion criteria were (1) newly diagnosed patients with type 2 diabetes accordingly, (2) age 40 to 70 years old, (3) antidiabetic drug naive, (4) mean (of two) fasting blood glucose (FPG) measurements from 7 to 11 mmol/L, and (5) glycosylated hemoglobin from 7 to 9%. Exclusion criteria were (1) previous pharmacological treatment for diabetes, (2) chronic complications related to type 2 diabetes, (3) smokers, and (4) high titers of GAD and IA-2 antibodies [53]. The results of this study demonstrated that GP tea exerted a significant antidiabetic effect by increasing insulin sensitivity. The authors reported that GP tea was primarily responsible for lowering glucose levels, as all patients received similar diets and exercise therapies. The main antidiabetic role of GP tea was also proven by reducing fasting plasma glucose (FPG), and glucose responses to insulin during somatostatin-insulin-glucose infusion test (SIGIT) following the GP treatment with reverse effect after switching to placebo treatment, while plasma insulin during SIGIT did not change following the treatment with GP tea [53].

Some other studies have shown both the antidiabetic effect of GP and its protective effect on kidney health [54]. A substance called phanoside, a saponin of the dammarane type, was isolated from the ethanol extract and tested in insulin release stimulation in pancreatic islets isolated from rats [55]. Phanoside-induced insulin release was via the K-ATP channel and L-type $Ca2^{+}$ channel [56,57]. Studies demonstrated that phanoside dose-dependent stimulated insulin release at both low and high glucose levels, suggesting its insulin-secreting effect was not glucose-dependent, which could lead to severe hypoglycemia, similar to the sulfonylurea antidiabetic drug [55,57]. On the other hand, gylongiposide I, which was identified while screening for the active components of GP, was shown to be different, exhibiting unique abilities to stimulate insulin release at high glucose levels, but limited effects at low glucose concentrations [58,59]. Many studies suggested that GP can be a new antidiabetic drug used to reduce postprandial blood glucose, mainly to activate AMPK [38,47,58,59]. Compared to traditional oral hypoglycemic drugs, which do not have protective effects on the kidneys, GP has shown itself to be the complete opposite, demonstrating protective effects on the kidneys while remarkably reducing blood glucose [53,60]. These studies also demonstrated that GP significantly improved congestion and the epithelial system

of renal tubules in diabetic mice.

Despite all these GP benefits, the main glucose control mechanism lies in the activation of AMPK. This protein when activated proves to be a key regulator of both glucose and lipid metabolism [61]. AMPK, in addition to increased glucose uptake and glycogen synthesis, stimulates the translocation of glucose transporter 4 (GluT4) to the cell membrane, as well as up-regulating the expression of GluT4 [62]. Therefore, AMPK activators such as GP were considered promising candidates for type 2 diabetes mellitus.

Gynostemma pentaphyllum in obesity

As mentioned above, AMPK is an important metabolic activator. Many studies report its

efficiency in regulating the energy balance of the whole body, especially in metabolic diseases such as obesity, since its activation increases fat oxidation and glucose uptake [34,35]. According to in vivo studies, GP is a safe substance that does not cause side effects, even when administered for a long period [17]. These studies revealed that there were no reports of toxicity or mortality observed after long-term GP administration with doses up to 750 mg/kg in rats. The standardized aqueous extract of GP also showed no toxicity in rats [35]. Confirming GP to be a safe alternative as a chemotherapeutic agent. A large quantity of evidence has shown that GP also has a potent anti-obesity effect and can be used continuously for long periods without risk of adverse effects [17,35]. Thus, comparative studies between GP and other ingredients such as some slimming drugs have been the target of some researchers.

A comparative study with GP extract (300 mg/kg) in mice with high contents of gypenoside, gypenoside LI, and ginsenoside Rg3 exhibited a more efficient anti-obesity effect than the group that used Orlistat (30 mg/kg) [30]. This study also demonstrated that when GP was administered in high-fat diets, body weight, fat mass, white adipose tissue, and adipocyte hypertrophy were all suppressed. Likewise, the GP group exhibited lower serum levels of triglycerides, total cholesterol, and low-density lipoprotein-cholesterol when compared to the group receiving only a high-fat diet [30]. Several lipid-lowering results were directly associated with AMPK activation, and consequently with increased SIRT1 expression [17]. In studies by Gauhar et al., the GP (300 mg/kg) used, also known as actiponin, containing significant amounts of damulin A and damulin B both prevented and improved obesity in ob/ob mice, stimulating fatty acid oxidation by activating AMPK in these organs [63]. Similarly, in the same study actonine (200 mg/kg) reduced body weight and total plasma cholesterol levels without any effect on food intake [63].

Another clinical study in humans on GP role in obese individuals can be found in the work by Park et al. In this study, 80 participants were divided into two groups, GP (actiponin) 450 mg and placebo. This was a 12-week, randomized, double-blind, placebo-controlled study, followed by a 3-week screening period. The results of this study concerning anthropometric parameters showed significant changes. In the GP (actiponin) group, there was a decrease in body weight ($P = 0.021$), BMI ($P = 0.029$), body fat mass ($P < 0.0001$), and body fat percentage ($P < 0.0001$). When analyzing the changes in anthropometric parameters before and after the 12-week intervention, a decrease in waist circumference was also found (GP actiponin group: -2.49 ± 0.35 cm, placebo group: -1.33 ± 0.37, $P = 0.029$) [33]. Many other studies suggest that the role of GP in fat oxidative metabolism in preventing obesity is due to its increase in caloric energy expenditure, as well as in the activity of both brown adipose tissue and the darkening of white adipose tissue through increased AMPK activity [33,64,65].

Gynostemma pentaphyllum in aging

The aging process is a complex mechanism with more than 300 theories presented by

the scientific community [65]. Currently, one of the most accepted theories is the reactive oxygen species (ROS) related to oxidative stress arising from both mitochondrial cellular respiration and

produced by the cell membrane via NADH oxidase [67]. Among the productions of ROS is the hydroxyl radical (OH*), superoxide anions ($O2^-$), and hydrogen peroxide (H_2O_2) [68]. When cells lose the antioxidant capacity to control ROS levels, these radicals react in a harmful way in several cellular structures, including the plasma membrane, nuclear and mitochondrial membrane, nucleic acids, and proteins [69]. DNA damage in the presence of high levels of ROS causes a metabolic response activating p38MAPK for upregulating p16 [70]. This process triggers cellular senescence, leading to the development of aging-related diseases. Studies indicate that DNA damage by ROS is directly associated with the activation of cellular senescence induced by telomeres through the p53 pathway [71].

Table 1 - In vivo clinical studies with GP in different pathologies

Authors	Clinical evaluation	Year	in vivo	Main Target
Qifa et al.	Protective effect of GP against I/R-induced renal injury	2016	Mice	Creatinine and blood urea nitrogen levels
Hesse et al.	Phytoprotective effects of GP gypenosides on the gastrointestinal tract and kidney of indomethacin-treated rats.	2007	Rats	Cecal hemoglobin and plasma haptoglobin
Gauhar et al.	The beneficial effect of GP on improving obesity	2012	Mice	Body weight, liver weight and blood cholesterol levels
Park et al.	Antiobesity effect of GP extract (actiponin)	2014	Human	Abdominal fat area,body weight, body fat mass, percentage BF, and BMI
Lee et al.	Anti-obesity effect of GP extract enriched in gypenoside L, gypenoside LI and ginsenoside Rg3	2019	Mice	Triglyceride, total cholesterol, PPARγ AMPK, C/EBPα, SIRT1, FAS, AP2 and CPT1
Zhao et al.	The protective effects of GP against I/R-induced hepatic injury in mice	2014	Mice	Bax, cytochrome-c, caspase-3-8, and Bcl-2
He et al.	The protective effects of gypenosides from GP on fatty liver disease	2012	Rats	Triglyceride, cholesterol, LDL-C, HDL-C, MDA, ALT, AST, SOD and PPARα
Gao et al.	The role of GP saponins as the main compound responsible for anti-diabetes	2016	Rats	HbA1c level, glucose,Glutathione peroxidase, Nrf2, and SOD
Huyen et al.	The effect of GP tea on insulin sensitivity in drug-naive type 2 diabetic patients	2013	Human	Glucose, insulin level, and HbA1c level
Rao et al.	The effect of GP extract (ActivAMP®) with respect to improving body composition in overweight males and females.	2022	Human	Dual-energy X-rays to assess body composition, anthropometric measures, Triglyceride, ALT, AST, Gamma-GT, Cholesterol, Glucose, insulin, TNF-α, IL-8 and IL-10

Sirtuins, a family of 7 proteins (SIRT 1 - 7) also play an essential role in controlling cellular senescence and prolonging the lifespan of the organism [72]. Its action mechanisms are linked to the regulation of several cellular processes. The age-related delay in telomere wear is one of

the main processes mediated by sirtuins, mainly by SIRT 1 [73]. However, there are several other cellular signaling mechanisms modulated by sirtuins, including insulin/IGF-1, AMPK, and FOXO [74]. With its main activity being deacetylation, studies have revealed that sirtuins have been associated with DNA repair, control of inflammation as well as delaying aging. SIRT1 specifically is considered the most conserved NAD^+-dependent protein deacetylase in mammals [75]. As SIRT1 is activated via AMPK phosphorylation [76], once phosphorylated, AMPK activates SIRT1, which in turn acts on DNA deacetylation processes [77]. Due to the strong phosphorylation on AMPK exerted by GP, studies in mice reported that GP showed a significant increase in SIRT1 mRNA expression [17]. Other studies have shown that some saponins type dammarane and cucurbitacin, are present in GP and demonstrate agonist activity towards the SIRT1 protein [78]. The results of GP administration on these specific metabolism proteins indicate that this plant has a promising potential in alternative treatments for age-related diseases.

Conclusion

This association between AMPK, SIRT1 and PCG-1alpha seems to be positively influenced by GP supplementation. This herb can have numerous benefits in controlling age-associated metabolic diseases. However, further studies in humans could provide further evidence of its efficiency in diseases such as metabolic syndrome as well as any other pathology that involves changes in mitochondrial functions. Numerous studies in animals and humans have already demonstrated the action of GP on mitochondrial biogenesis and its control in diabetes, obesity, and hepatic steatosis. The effects of GP on the three proteins AMPK, SIRT1, and PGC-1alpha are already well documented in the scientific literature. However, despite the promising results so far, new human studies about the active principle of GP, gypenosides, as well as its pharmacokinetics and pharmacodynamics still lack more data to finally place GP as an innovative option for the treatment of aging diseases.

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