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Modulatory effect of resveratrol on *SIRT1, SIRT3, SIRT4, PGC1α* and *NAMPT* gene expression profiles in wild-type adult zebrafish liver

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Abstract Sirtuins (SIRTs) are NAD^+-dependent deacetylases that catalyze the hydrolysis of acetyl-lysine residues. They play an important role in many physiological and pathophysiological processes, such as the regulation of lifespan and the prevention of metabolic diseases. In this study, we analyzed the effect of resveratrol on the gene expression levels of *SIRT1*, *SIRT3*, *SIRT4*, *PGC1α*, and *NAMPT*, as well as its effect on NAD^+ and NADH levels, in the liver of non stressed or non impaired wild-type zebrafish. Semiquantative RT-PCR assays showed that resveratrol did not change the mRNA levels of *SIRT1* and *PGC1α* but decreased the expression levels of the *SIRT3*, *SIRT4*, and *NAMPT* genes. The decrease in *NAMPT* mRNA levels was accompanied by an increase in NADH levels, thereby decreasing the NAD^+/H ratio. Taken together, our results suggest that resveratrol plays a modulatory role in the transcription of the *NAMPT*, *SIRT3*, and *SIRT4* genes. Zebrafish is an interesting tool that can be used to understand the mechanisms of SIRTs and NAMPT metabolism and to help develop therapeutic compounds. However, further investigations using healthy experimental animals are required to study the modulation of the *SIRT* and *NAMPT* genes by resveratrol before it is used as a nutraceutical compound in healthy humans.

Keywords Resveratrol · Sirtuins · Pgc1 alpha · NAMPT · Liver

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Introduction

Resveratrol is a naturally occurring, biologically active substance found in many plants [1]. It has been reported to increase the lifespan of *Saccharomyces cerevisiae* [2], *Caenorhabditis elegans*, *Drosophila melanogaster* [3], and the fish *Nothobranchius furzeri* [4], but not that of rodents. The mechanism underlying this effect has been associated with the potent activation of sirtuin 1 (SIRT1) [2]. However, little is known about the interaction between resveratrol and other sirtuins (SIRTs). Since resveratrol has been shown to have potential chemo protective activity [5], several health benefits have been reported in the literature. These include the delay of onset or prevention of age-related diseases such as autoimmune diseases and inflammation [6], neurodegeneration [7], heart disease, metabolic

disorders, such as obesity and type-2 diabetes [8] and several types of cancer [9, 10].

Although several studies have been conducted to investigate the effect of resveratrol in various diseases, its mechanism of action is still not well understood. Resveratrol has been reported to act on multiple targets [1]. The majority of studies on the interaction of resveratrol with SIRTs have used stressed, impaired, or knockout models [11]; few studies have analyzed this interaction in healthy experimental animals. Nevertheless, resveratrol is being used as a nutraceutical compound and tested in healthy volunteers although it has not been found to have any correlation with the modulation of SIRTs [12, 13] or other genes. Therefore, it is of great interest to understand whether and how resveratrol modulates SIRTs in healthy or wild-type experimental animals.

SIRTs are a class of evolutionarily conserved NAD^+-dependent deacetylases found in living organisms [14]. They are essential enzymes that catalyze the hydrolysis of acetyl-lysine residues [15]. Sir2p, which is found in prokaryotes, has attracted considerable attention because of its effect on the lifespan of yeast cells [16]. In mammals, seven SIRT-related genes have been identified, namely, *SIRT1–7* [17]. The encoded enzymes have diverse cellular locations and target multiple factors and are key regulators of many physiological and pathophysiological processes that affect a broad range of metabolic cellular functions [18].

SIRT1 is the most well-studied SIRT and has been associated with several biological processes. It has been reported to modify the level of protein acetylation by either activating or suppressing them. In addition to deacetylating histones, SIRT1 also targets a number of transcription factors, including PGC1α, PPARs, FOXO, NFκB, p53, Tat, NCoR, and LXRα; this suggests that it is involved in the regulation of gluconeogenesis, mitochondrial biogenesis, resistance to oxidative stress, adipogenesis and lipolysis, glycolysis, inflammation, apoptosis, HIV transcription, cell differentiation, and angiogenesis [19].

There is little information on the other SIRTs. Recently, SIRT3 and SIRT4, which are mitochondrial proteins with deacetylase and ADP-ribosyltransferase activities, respectively [17], have also been shown to regulate cellular metabolic functions. Both proteins are found primarily in metabolically active tissues, such as the kidney, brown adipose tissue, brain, liver, and heart [20–22]. SIRT3 is a more effective deacetylase than SIRT4, since hyperacetylation is observed in SIRT3-deficient animals [23]. The most recent review proposed that these SIRTs might be the next targets for intensive studies because of their potential association with metabolic diseases [24], but their role must first be understood in a wild-type model without stress or intervention.

Different targets have been reported for SIRT3, such as acetyl-CoA synthetase [25], glutamate dehydrogenase (GDH) [23, 26], Ku70 [27], cyclophilin D [28], and AMP-activated protein kinase (AMPK) [29], through which it regulates the cellular metabolism of lipids and glucose, as well as cell survival, oxidative phosphorylation of mitochondria, and maintenance of ATP level [30]. For SIRT4, only one target has been reported—GDH—whose enzymatic activity it inhibits through ADP-ribosylation. GDH is a mitochondrial matrix enzyme that converts glutamate to α-ketoglutarate and ammonia in different tissues [21, 31]. In pancreatic islet cells, this reaction generates ATP and promotes insulin secretion [32]. One recent report also showed that SIRT4 is involved in lipid storage, where reduced levels of SIRT4 increased the mitochondrial and fatty acid metabolism in hepatocytes and liver and improved mitochondrial function in muscles [33].

Nicotinamide phosphoribosyltransferase (NAMPT), also known as pre-B-cell colony-enhancing factor (PBEF) or visfatin, is a 52 kDa protein that is most highly expressed in the liver and muscles [34]. NAMPT converts nicotinamide (NAM) to nicotinamide mononucleotide, which is converted to NAD^+ by nicotinamide adenylyltransferase in the mammalian biosynthetic pathway. NAMPT is a rate-limiting enzyme in the conversion of NAM to NAD^+ and may thus alter the NAD^+/NADH ratio, which is crucial for the activation and regulation of SIRT1 transcription [35]. Recently, NAMPT has been reported to increase the levels of SIRTs and to have cell-protective activities [36–39].

The zebrafish is a model organism widely used in various lines of biomedical research, particularly in developmental and genetic studies [40]. It is an ideal model for research because it is easy to maintain and manipulate; it is economical [41]; and its genes are highly conserved, sharing a 70–80% homology with those in humans [42]. Therefore, zebrafish may be a promising model that can be used to evaluate the modulatory effect of resveratrol on the gene expression profiles of different proteins. The aim of the present study was to analyze for the first time the potential of resveratrol to modulate the gene expression patterns of *SIRT1*, *SIRT3*, *SIRT4*, *NAMPT*, and *PGC1α* in wild-type zebrafish liver. In addition, we also sought to correlate the concentrations of NAD^+ and NADH with *NAMPT* expression.

Materials and methods

Animals

Adult wild-type zebrafish (*Danio rerio*) of both sexes were obtained from a commercial supplier (Delphis, Porto Alegre, RS, Brazil). All the fish were acclimated in a 50 l

thermostated aquarium for 2 weeks, under a 14 h/10 h light/dark controlled photoperiod. The fishes were fed twice a day with flake fish food (Alcon Basic®, Alcon, Brazil). All animal manipulations were in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals, and all procedures in the present study were approved by the Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (PUC-RS), license number 08/00082.

Resveratrol exposure

For the resveratrol treatment, we used wild-type zebrafish that were not treated with any other substance or any other intervention. The fish were exposed to solutions with two different resveratrol concentrations (5 and 50 mg/l). Resveratrol was dissolved in 100 µl of ethanol and then homogenized in 2 l of distilled water for 1 h for complete solubilization. A preliminary study was performed to verify whether the vehicle could affect the gene expression; no significant change was noted when compared to the effect observed in water control (data not shown). For resveratrol exposure, the fish were transferred to water containing the compound for 30 or 60 min, and the aquariums were completely covered to avoid degradation of the compound by ultraviolet light. The control group was kept for 1 h in distilled water. At the end of the treatment, zebrafish livers ($n = 4$) were dissected and immediately frozen in liquid nitrogen for later analysis. Each independent experiment was performed using a pool of 5 livers. SIRT3 and SIRT4 levels were analyzed after 30 min of exposure to 5 mg/l resveratrol. For NAMPT analysis, we used the same duration for all concentrations of resveratrol. To ensure that the compound reached the liver during the exposure time and the concentration of the compound added directly to the water, we determined the concentration of resveratrol by HPLC [43].

Semiquantitative RT-PCR analysis

After harvesting the livers, total RNA was isolated with TRIzol® reagent (Invitrogen) in accordance with the manufacturer's instructions. The ratio between the absorbance values at 260 and 280 nm was determined to verify RNA purity; all samples were adjusted to a final concentration of 160 ng/µl. cDNA species were synthesized with SuperScript™ First-Strand (Synthesis System for RT-PCR) from Invitrogen, according to the manufacturer's instructions. Each RNA sample (2 µg/ml) was mixed with 1 µl of 50 µM oligo (dT) and 1 µl of annealing buffer, to give a final volume of 8 µl. The samples were incubated at 65°C for 5 min in a thermal cycler; this was a 1 min step on ice when 10 µl of 2× First-Strand Reaction Mix and 2 µl of SuperScript™ III/RNaseOUT™ Enzyme Mix were added. The products were incubated for 50 min at 50°C and further incubated at 85°C for 5 min. PCR parameters, including the concentrations of $MgCl_2$ and cDNA templates and PCR cycles (25–40 cycles) were optimized (data not shown). The reactions were performed under optimal conditions that allowed product detection within the linear phase of band densitometry analyzed for each primer pair (Table 1). The β-actin primers were designed as described by Chen et al. [44] and the reactions were performed in a final volume of 20 µl. *SIRT1*, *SIRT3*, *SIRT4*, *NAMPT*, and *PGC1α* primers were designed using the Oligos 9.6 program, and the reactions were performed in a final volume of 25 µl. The gene/β-actin ratio was determined after electrophoresis on a 1.0% agarose gel stained with GelRed® and visualization under ultraviolet light by using ImageJ 1.37 for Windows. Low DNA Mass Ladder (Invitrogen) was used as a molecular marker. The gene expression patterns of *SIRT1* and *PGC1α* were determined in all treatments with resveratrol. For *SIRT3* and *SIRT4*, we determined the gene expression only in the animals exposed to 5 mg/l for 30 min. In the case of *NAMPT*, we analyzed the gene expression in the animals

Table 1 PCR primers for *SIRT1, SIRT3, SIRT4, PGC1α*, *NAMPT* and amplification conditions

Sirtuin	PCR conditions		
	Primers (5′–3′)	Tm (°C)	Cycles
SIRT 1	F: CAGCTCTGCTACAATTCATCGCGTC		
	R: AATCTCTGTAGAGTCCAGCGCGTGTG	62	30
SIRT 3	F: CATTAAATGTGGTGGAACAAGAGGCCTG		
	R: AGTTCCTCTCCTTTGTAATCCCTCCGAC	61	30
SIRT 4	F: TGTGGTGAACTGACTCCTCGTGCTGAGC		
	R: CGGAAGTTTTCTTTCACTAGCAGCGAGG	63	30
PGC1a	F: CCCCTTTGCCCTGACCTGCCTGAG		
	R: GAAGGACAGCTCTGATCACTGGCATTGG	62	35
NAMPT	F: GGAAGCAAAGGAAGTGTACCGGGAAC		
	R: TCGTGCAGCTTATATTCAAGCCCGTC	59	35

Tm melting temperature

exposed for 30 min to both concentrations of resveratrol (5 and 50 mg/l).

NAD^+ and NADH concentrations

The coenzymes were measured using the NAD^+/NADH colorimetric assay kit (BioChain Hayward, CA, USA-LGC Biotechnology) in accordance with the manufacturer's instructions. Frozen tissues (20 mg) were washed in cold phosphate-buffered saline and homogenized with NAD^+ or NADH extraction buffer. Forty microliters of the supernatant was added to 80 µl of the working reagent. The plate was mixed briefly and thoroughly, and the optical density (OD) was read at time zero (OD_0) and at 15 min (OD_{15}) after incubation at room temperature. The data were obtained using a SPECTRAmax Gemini XS microplate spectrofluorometer with the SoftMax Pro 5 software (Molecular Devices) at 565 nm. The concentrations of NAD^+ and NADH (in µM) were calculated from the difference between the experimental readings and the standard curve. The assay was performed with control and both concentrations of resveratrol (5 and 50 mg/l) after 60 min of exposure. For each group, the procedure was performed with $n = 4$, and the samples were analyzed in duplicate.

Statistical analysis

Data processing and statistical analysis were performed using Microsoft Excel and GraphPad Prism 5. The optical densitometry results were expressed as mean (SD) and statistically compared by one-way analysis of variance (one-way ANOVA) followed by Tukey's post hoc test or by unpaired *t*-test; $P \leq 0.05$ was considered significant.

Results

Resveratrol does not alter the mRNA level of the *SIRT1* gene in wild-type zebrafish liver

The fish in the resveratrol group were treated with 2 doses of the compound: a lower concentration (5 mg/l) and a concentration 10 times higher (50 mg/l), for 2 different durations of exposure: 30 and 60 min. The concentrations of resveratrol and the durations of treatment were chosen after preliminary assays. In these assays, we did not observe any difference in the transcriptional levels of *SIRT1* in the liver of zebrafish at 3 different concentrations of the compound after 2 durations of exposure: 60 min and 7 days (data not shown). In our experiments, we observed no significant changes in *SIRT1* mRNA levels after resveratrol treatment, neither at different doses nor after different durations of exposure (Fig. 1).

Resveratrol negatively regulates *SIRT3* and *SIRT4* gene expression

Resveratrol has received little attention in terms of the regulation of other SIRTs, especially in a non stressed model. Therefore, we examined the gene expression levels of two mitochondrial SIRTs—*SIRT3* and *SIRT4*—after exposure to the compound in a non impaired model. We observed a significant decrease in the *SIRT3* and *SIRT4* mRNA levels after resveratrol exposure (5 mg/l, 30 min) (for both genes: $P \leq 0.05$, *t*-test) (Fig. 2).

PGC1α mRNA levels after resveratrol treatment

SIRTs are believed to be involved in the modulation of several transcription factors, such as those related to mitochondrial biogenesis. It is well known that PGC1α is deacetylated and regulated by SIRT1 and, as recently demonstrated, by SIRT3 [45]. In order to detect any SIRT activity, we analyzed the *PGC1α* gene expression in our model after resveratrol exposure. We did not find any change in the gene expression of the transcription factor PGC1α (Fig. 3).

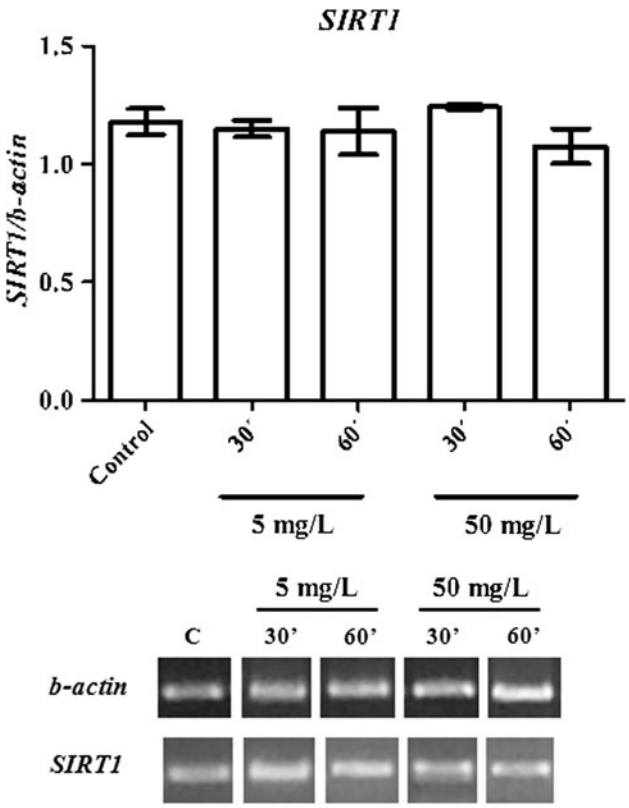

Fig. 1 *SIRT1* expression in zebrafish liver after exposure to resveratrol (5 and 50 mg/l) for 30 and 60 min. The *graph* shows the *SIRT1*/*β-actin* ratio obtained by optical densitometry analysis (mean ± S.D., $n = 4$). A representative gel of RT-PCR experiments is also shown

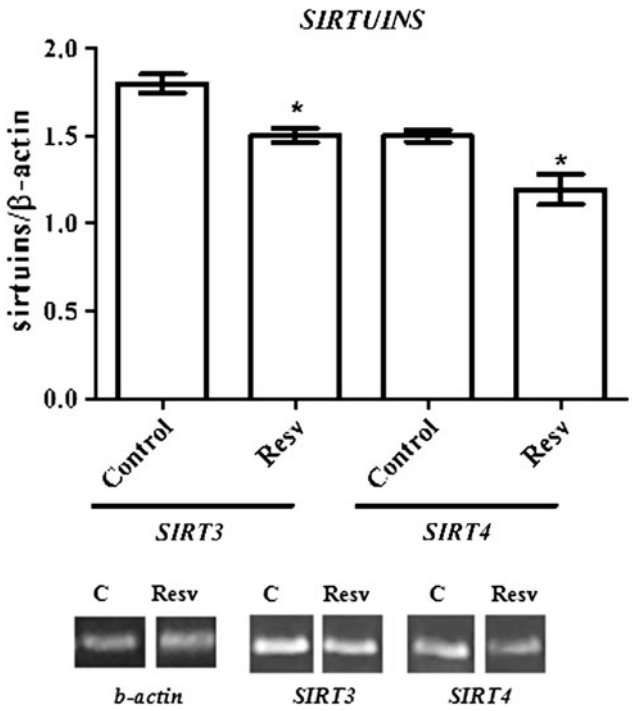

Fig. 2 *SIRT3* and *SIRT4* expression in zebrafish liver after exposure to resveratrol (5 mg/l) for 30 min. The *graph* shows the *SIRTUIN/β-actin* ratio obtained by optical densitometry analysis (mean ± S.D., $n = 4$). A representative gel of RT-PCR experiments is shown. * Significant difference compared to control group (Unpaired *t*-test, $P \leq 0.05$)

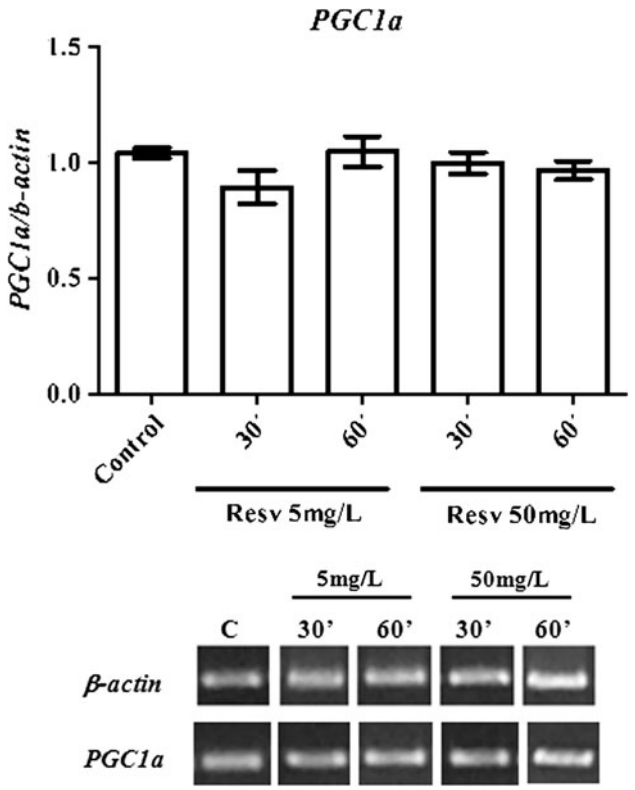

Fig. 3 *PGC1α* expression in zebrafish liver after exposure to resveratrol (5 and 50 mg/l) for 30 and 60 min. The *graph* shows the *PGC1α/β-actin* ratio obtained by optical densitometry analysis (mean ± S.D., $n = 4$). A representative gel of RT-PCR experiments is shown

Resveratrol modulates *NAMPT* expression

In yeast, the *PNC1* gene is essential for maintaining NAD^+ levels, while in mammals, maintenance of NAD^+ levels is mediated by *NAMPT*. In this study, we determined the mRNA levels of *NAMPT* and investigated whether they are correlated to the levels of NAD^+/NADH in the liver of zebrafish exposed to resveratrol. Resveratrol had a more significant effect on the gene expression of *NAMPT* than the control (Fig. 4). We observed a significant decrease in *NAMPT* mRNA levels at a higher concentration of resveratrol, which was accompanied by an increase in cellular levels of NADH and, consequently, a decrease in the NAD^+/NADH ratio (Table 2). In the group treated with 5 mg/l resveratrol, we did not observe a significant decrease in the mRNA level of NAMPT; the NADH level increased, whereas the NAD^+ level remained the same, thereby increasing the NAD^+/NADH ratio.

Discussion

The wild-type adult zebrafish is an interesting experimental tool that can offer several advantages in biomedical research, such as in the development of therapeutics for human diseases, since this vertebrate shares common pathophysiological pathways with humans [42]. The zebrafish is a cost-effective model that can be used to rapidly identify the lead compounds modulating specific biological processes. It can also be used as an alternative in vivo chemical screening tool for determining the bioactivity, toxicity, and off-target side effects of novel drugs, and thus for drug optimization [46]. Model may be especially suitable for studying the effects of compounds because of the simplicity of its tissue delivery system [47].

In one of our researches, the zebrafish model has been extensively used to test different substances added directly to the water. In another study, our group demonstrated that zebrafish is an interesting model for studying SIRT modulation, since the mRNAs of all SIRTs were detected in all fish tissues [48]. When the resveratrol is added in the fish environment, it is absorbed by the blood vessels of the gill and the skin; thus, the compound is rapidly diffused through systemic circulation and reaches the tissues. In this study, we confirmed this observation with resveratrol, since we detected the compound in the liver after exposure and did not detect the metabolites at the indicated time points and compound concentrations (data not shown).

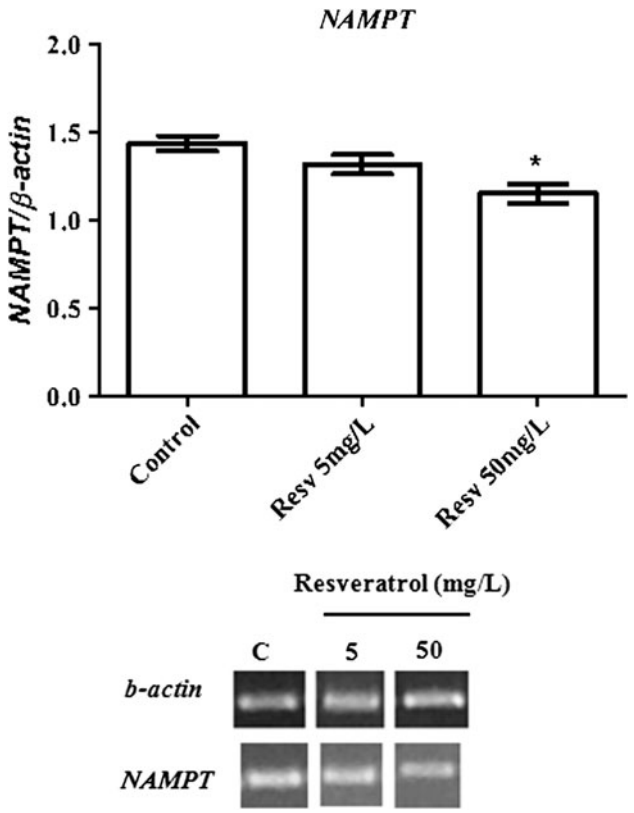

Fig. 4 *NAMPT* expression in zebrafish liver after exposure to resveratrol (5 and 50 mg/l) for 30 min. The *graph* shows the *NAMPT/β-actin* ratio obtained by optical densitometry analysis (mean ± S.D., $n = 4$). A representative gel of RT-PCR experiments is shown (one-way ANOVA, followed by Tukey's post hoc test, $P \leq 0.05$)

Table 2 Levels of NAD^+/NADH ratio, in the wild-type zebrafish liver, after resveratrol treatment

	NAD^+ (μM)	NADH (μM)	NAD^+/H
Control	2.91 ± 0.72	4.34 ± 1.90	0.67
5 mg/l	2.92 ± 0.28	6.17 ± 1.04	0.47
50 mg/l	3.13 ± 0.71	7.32 ± 2.75	0.43

The results of NAD^+ and NADH are presented as mean ± S.D. ($n = 4$)

Resveratrol has been reported in the literature as a potent activator of SIRT1 1 [2, 49, 50]. In this study, the results showed that resveratrol did not affect the *SIRT1* mRNA levels in zebrafish liver (Fig. 1). A similar result was observed in the liver of Wistar rat controls treated with resveratrol, with no change detected in *SIRT1* mRNA or protein levels [51]. By contrast, in this same study, an increase was observed in the levels of *SIRT1* transcript and protein in the rats fed a low-protein, high-carbohydrate diet plus resveratrol. An increase in the SIRT1 protein level was also detected in non stressed or non impaired rats treated with resveratrol [52]. Therefore, we think that the lack of *SIRT1* mRNA modulation by resveratrol could be due to some factors: (1) the enzyme is modulated by resveratrol in impaired or stressed models and (2) resveratrol does not modulate SIRT1 as was previously reported [53, 54], (3) and last, since the sqRT-PCR experiments do not allow a precise quantification of mRNA levels, it is not possible to completely rule out some false negative results with the methodology adopted.

The regulation of SIRT1 by resveratrol may be more complex than first thought. It is not also well defined whether SIRT1 is regulated at the transcriptional, translational, or post-translational level. Previous studies have reported an increase in the enzyme level at different steps above mentioned, but most of these studies used an impaired or stressed model. Other important factors to be considered are the influence of tissue type on modulation, differences in the tissue distribution of resveratrol, the manner of compound administration, and food interference [49–51, 55, 56].

We sought to determine the effect of resveratrol on other SIRTs. To this end, we investigated the *SIRT3* and *SIRT4* genes, since both these mitochondrial enzymes are detected in metabolically active tissues, such as the liver, and are sensitive to nutrient signals.

Whether SIRT3 is modulated by resveratrol remains unclear. In rat cardiac ischemic tissue, an increase in SIRT3 protein levels was reported after 14 days of resveratrol exposure [37]. However, resveratrol inhibits the ethanol-induced increase in the *SIRT3* mRNA levels in mouse liver [57]. However, in adipocytes, resveratrol up-regulated the activity of mitochondrial genes, including *SIRT3* [58]. In a non stressed or non impaired model, we observed a decrease in *SIRT3* gene expression with resveratrol treatment. In the literature, *SIRT3* regulation by resveratrol has been demonstrated in healthy experimental models; however, no studies have been performed in humans, although resveratrol is already being used as a nutraceutical compound. Furthermore, no previously reported study has demonstrated the interaction between resveratrol and *SIRT3* gene expression, as was done in the experimental model used in this study.

Most of the studies on SIRT3 and its effects on cell metabolism have employed an *SIRT3*-knockout model or have been performed under calorie restriction, with the latter intervention being known to up-regulate the SIRT3 protein [20, 22, 30, 59]. $SIRT3^{-/-}$ knockout mice appear to be phenotypically normal under baseline conditions; however, these animals showed a decrease in fatty acid oxidation, hypoglycemia, and inefficient thermogenesis under calorie restriction [59]. SIRT3 deficiency also leads to a decrease in the ATP levels in the liver, kidney, and heart cells in vivo [30, 59].

Among the SIRTs analyzed in this study, SIRT4 is still poorly understood in terms of the mechanism of its regulation. The results of this study showed a decrease in *SIRT4* gene expression in response to resveratrol (Fig. 3). SIRT4 modulation, similar to SIRT3 modulation, has been more frequently reported after fasting, and little is known about its response to resveratrol exposure. In mice, under calorie restriction, an increase in GDH activity was observed after the activation of SIRT4 was inhibited, without affecting the protein levels in the pancreas and liver [21, 60]. This increase has been associated with the stimulation of insulin secretion by amino acids. In the hepatocytes and liver of *SIRT4*-knockout mice, increases in the fatty acid oxidative capacity and mitochondrial biogenesis have been demonstrated [33]. One study showed increased levels of SIRT4 protein after treatment with resveratrol in the heart of rats after ischemia. However, the effect of this activation was not fully explained [37]. As with *SIRT3*, until now there has been no report on the interaction between resveratrol and *SIRT4* gene expression in a healthy experimental model, as was done in this study.

A known target of SIRT1 and, more recently, of SIRT3 is the transcription factor PGC1α. PGC1α is an important regulator of mitochondrial biogenesis and gluconeogenesis. The lack of *PGC1α* mRNA modulation observed in this study is consistent with the SIRT modulation and also suggests that resveratrol does not activate SIRT1. A similar result was observed in mice after resveratrol exposure [55]. The study reported that under calorie restriction, *PGC1α* gene expression did not increase in the liver of $SIRT1^{-/-}$ knockout mice unlike in their wild-type counterparts [56].

One important mechanism of SIRT activation is the biosynthesis of NAD^+ by NAMPT [36, 38]. Since SIRTs are deacetylases, their mechanism is dependent on the availability of this cofactor. In 2 other studies involving chronic exposure to resveratrol, an increase in NAMPT protein expression was observed in cardiac tissues of rats, which was accompanied by an increase in the levels of the proteins SIRT1, SIRT3, and SIRT4 [37]. In MAGI cells, resveratrol did not change either the *NAMPT* mRNA or the NAD^+ level, but reversed the decrease caused by HIV Tat [61]. In our study, we detected decreases in *NAMPT* gene expression (Fig. 4) and in the levels of the cofactors NAD^+ and NADH (Table 2). Furthermore, there was a correlation between *NAMPT* gene expression and the levels of the cofactors, which was expressed as the NAD^+/NADH ratio, especially at a higher dose of resveratrol. This is reflected in the changes in protein levels or, more specifically, in activity modulation [62], mediated by resveratrol. Although at a lower concentration of resveratrol, the NAD^+/NADH ratio did not change significantly, we did observe a tendency of a decreasing ratio.

As is the case with SIRTs, modulation of *NAMPT* gene expression will be better understood in wild-type animals, but most of the literature presents results obtained from impaired experimental models. High levels of NAMPT have been detected in pathological conditions such as obesity, atherosclerosis, and metabolic syndrome [63–65]; however, decreased levels NAMPT have been observed in human adults with nonalcoholic fatty liver disease [66] and cirrhosis [67].

Since SIRT3 and SIRT4 are important metabolic enzymes related to glucose and insulin metabolism, more attention should be paid to these proteins both in healthy and diseased models. Given that no change was observed in the *SIRT1* gene and that the levels of *SIRT3* mRNA decreased, it is plausible that there was no modulation of *PGC1α* mRNA. We also found that resveratrol influences *NAMPT* mRNA levels and the NAD^+/NADH ratio, and more studies are required to understand its mechanism and physiological effects in the wild-type model. An important finding was that resveratrol modulates gene expression in non impaired or non stressed experimental models and in vivo. We have shown that the zebrafish can be a useful tool for understanding the mechanisms of SIRT and NAMPT metabolism and for developing therapeutic compounds. However, further investigations using healthy experimental animals are required to study the modulation of the *SIRT* and *NAMPT* genes by resveratrol before it is used as a nutraceutical compound in healthy humans.

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References

1. Harikumar KB, Aggarwal BB (2008) Resveratrol: a multitargeted agent for age-associated chronic diseases. Cell Cycle 7(8): 1020–1035
2. Howitz KT et al (2003) Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 425(6954): 191–196
3. Wood JG et al (2004) Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature 430(7000):686–689
4. Valenzano DR et al (2006) Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. Curr Biol 16(3):296–300
5. Jang M et al (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275(5297): 218–220
6. Yar AS et al (2010) The effects of resveratrol on cyclooxygenase-1 and cyclooxygenase-2 mRNA and protein levels in diabetic rat kidneys. Mol Biol Rep 37(5):2323–2331
7. Marques FZ, Markus MA, Morris BJ (2009) Resveratrol: cellular actions of a potent natural chemical that confers a diversity of health benefits. Int J Biochem Cell Biol 41(11):2125–2128

8. Szkudelska K, Szkudelski T (2010) Resveratrol, obesity and diabetes. Eur J Pharmacol 635:1–8
9. Patel KR et al (2010) Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. Cancer Res 70(19): 7392–7399
10. Hsieh TC, Wu JM (2010) Resveratrol: biological and pharmaceutical properties as anticancer molecule. Biofactors 36: 360–369
11. Baur JA (2010) Biochemical effects of SIRT1 activators. Biochim Biophys Acta 1804(8):1626–1634
12. Nunes T et al (2009) Pharmacokinetics of trans-resveratrol following repeated administration in healthy elderly and young subjects. J Clin Pharmacol 49(12):1477–1482
13. Almeida L et al (2009) Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers. Mol Nutr Food Res 53(Suppl 1):S7–S15
14. Sauve AA et al (2006) The biochemistry of sirtuins. Annu Rev Biochem 75:435–465
15. Hernick M, Fierke CA (2005) Zinc hydrolases: the mechanisms of zinc-dependent deacetylases. Arch Biochem Biophys 433(1): 71–84
16. Lin SJ, Defossez PA, Guarente L (2000) Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. Science 289(5487):2126–2128
17. Michan S, Sinclair D (2007) Sirtuins in mammals: insights into their biological function. Biochem J 404(1):1–13
18. Haigis MC, Guarente LP (2006) Mammalian sirtuins—emerging roles in physiology, aging, and calorie restriction. Genes Dev 20(21):2913–2921
19. Feige JN, Auwerx J (2008) Transcriptional targets of sirtuins in the coordination of mammalian physiology. Curr Opin Cell Biol 20(3):303–309
20. Shi T et al (2005) SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. J Biol Chem 280(14):13560–13567
21. Haigis MC et al (2006) SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. Cell 126(5):941–954
22. Palacios OM et al (2009) Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1alpha in skeletal muscle. Aging 1(9):771–783
23. Lombard DB et al (2007) Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. Mol Cell Biol 27(24):8807–8814
24. Imai S, Guarente L (2010) Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases. Trends Pharmacol Sci 31(5):212–220
25. Hallows WC, Lee S, Denu JM (2006) Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. Proc Natl Acad Sci USA 103(27):10230–10235
26. Schlicker C et al (2008) Substrates and regulation mechanisms for the human mitochondrial sirtuins Sirt3 and Sirt5. J Mol Biol 382(3):790–801
27. Sundaresan NR et al (2008) SIRT3 is a stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death by deacetylation of Ku70. Mol Cell Biol 28(20): 6384–6401
28. Shulga N, Wilson-Smith R, Pastorino JG (2010) Sirtuin-3 deacetylation of cyclophilin D induces dissociation of hexokinase II from the mitochondria. J Cell Sci 123(Pt 6):894–902
29. Shi T, Fan GQ, Xiao SD (2010) SIRT3 reduces lipid accumulation via AMPK activation in human hepatic cells. J Dig Dis 11(1): 55–62
30. Ahn BH et al (2008) A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. Proc Natl Acad Sci USA 105(38):14447–14452
31. Ahuja N et al (2007) Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. J Biol Chem 282(46): 33583–33592
32. Stanley CA (2004) Hyperinsulinism/hyperammonemia syndrome: insights into the regulatory role of glutamate dehydrogenase in ammonia metabolism. Mol Genet Metab 81(Suppl 1): S45–S51
33. Nasrin N et al (2010) *SIRT4* regulates fatty acid oxidation and mitochondrial gene expression in liver and muscle cells. J Biol Chem 285:31995–32002
34. Samal B et al (1994) Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. Mol Cell Biol 14(2):1431–1437
35. Revollo JR et al (2007) Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. Cell Metab 6(5):363–375
36. Yang H et al (2007) Nutrient-sensitive mitochondrial NAD+ levels dictate cell survival. Cell 130(6):1095–1107
37. Mukherjee S et al (2009) Expression of the longevity proteins by both red and white wines and their cardioprotective components, resveratrol, tyrosol, and hydroxytyrosol. Free Radic Biol Med 46(5):573–578
38. Hayashida S et al (2010) Fasting promotes the expression of SIRT1, an NAD+ -dependent protein deacetylase, via activation of PPARalpha in mice. Mol Cell Biochem 339(1–2):285–292
39. Imai S, Kiess W (2009) Therapeutic potential of SIRT1 and NAMPT-mediated NAD biosynthesis in type 2 diabetes. Front Biosci 14:2983–2995
40. Zon LI, Peterson RT (2005) In vivo drug discovery in the zebrafish. Nat Rev Drug Discov 4(1):35–44
41. Pichler FB et al (2003) Chemical discovery and global gene expression analysis in zebrafish. Nat Biotechnol 21(8):879–883
42. Barbazuk WB et al (2000) The syntenic relationship of the zebrafish and human genomes. Genome Res 10(9):1351–1358
43. Souto AA et al (2001) Determination of trans-resveratrol concentrations in Brazilian Red Wines by HPLC. J Food Compos Anal 14:441–445
44. Chen WY et al (2004) Expression of metallothionein gene during embryonic and early larval development in zebrafish. Aquat Toxicol 69(3):215–227
45. Kong X et al (2010) Sirtuin 3, a new target of PGC-1alpha, plays an important role in the suppression of ROS and mitochondrial biogenesis. PLoS One 5(7):e11707
46. Bowman TV, Zon LI (2010) Swimming into the future of drug discovery: in vivo chemical screens in zebrafish. ACS Chem Biol 5(2):159–161
47. Rosemberg DB et al (2010) Taurine prevents enhancement of acetylcholinesterase activity induced by acute ethanol exposure and decreases the level of markers of oxidative stress in zebrafish brain. Neuroscience 171(3):683–692
48. Pereira TC et al (2011) Zebrafish as a model organism to evaluate drugs potentially able to modulate sirtuin expression. Zebrafish 8(1):9–16
49. Baur JA et al (2006) Resveratrol improves health and survival of mice on a high-calorie diet. Nature 444(7117):337–342
50. Lagouge M et al (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127(6):1109–1122
51. Franco JG et al (2010) Resveratrol (RSV) reduces lipid peroxidation and increases sirtuin1 (SIRT1) expression in adult animals programmed by neonatal protein restriction. J Endocrinol 207(3): 319–328
52. Barger JL et al (2008) Short-term consumption of a resveratrol-containing nutraceutical mixture mimics gene expression of long-term caloric restriction in mouse heart. Exp Gerontol 43(9): 859–866

53. Beher D et al (2009) Resveratrol is not a direct activator of SIRT1 enzyme activity. Chem Biol Drug Des 74(6):619–624
54. Pacholec M et al (2010) SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. J Biol Chem 285(11):8340–8351
55. Barger JL et al (2008) A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. PLoS One 3(6):e2264
56. Chen D et al (2008) Tissue-specific regulation of SIRT1 by calorie restriction. Genes Dev 22(13):1753–1757
57. Oliva J et al (2008) Sirt1 is involved in energy metabolism: the role of chronic ethanol feeding and resveratrol. Exp Mol Pathol 85(3):155–159
58. Rayalam S et al (2008) Resveratrol induces apoptosis and inhibits adipogenesis in 3T3–L1 adipocytes. Phytother Res 22(10): 1367–1371
59. Hirschey MD et al (2010) SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature 464(7285):121–125
60. Chen YR et al (2010) Calorie restriction on insulin resistance and expression of SIRT1 and SIRT4 in rats. Biochem Cell Biol 88(4):715–722
61. Zhang HS et al (2010) Nicotinamide phosphoribosyltransferase/sirtuin 1 pathway is involved in human immunodeficiency virus type 1 Tat-mediated long terminal repeat transactivation. J Cell Biochem 110(6):1464–1470
62. Kelly G (2010) A review of the sirtuin system, its clinical implications, and the potential role of dietary activators like resveratrol: part 1. Altern Med Rev 15(3):245–263
63. Dahl TB et al (2007) Increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis: possible role in inflammation and plaque destabilization. Circulation 115(8):972–980
64. Rasouli N, Kern PA (2008) Adipocytokines and the metabolic complications of obesity. J Clin Endocrinol Metab 93(11 Suppl 1):S64–S73
65. Sommer G et al (2008) Visfatin/PBEF/Nampt: structure, regulation and potential function of a novel adipokine. Clin Sci 115(1): 13–23
66. Dahl TB et al (2010) Intracellular nicotinamide phosphoribosyltransferase protects against hepatocyte apoptosis and is down-regulated in nonalcoholic fatty liver disease. J Clin Endocrinol Metab 95(6):3039–3047
67. de Boer JF et al (2009) Plasma levels of PBEF/Nampt/visfatin are decreased in patients with liver cirrhosis. Am J Physiol Gastrointest Liver Physiol 296(2):G196–G201