Letter to the Editor

Resveratrol Increases Intracellular NAD^+ Levels Through Up regulation of The NAD^+ Synthetic Enzyme Nicotinamide Mononucleotide Adenylyltransferase

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Resveratrol is a polyphenol with major health benefits[1] that is thought to operate through direct activation of the 'anti-aging' enzyme SIRT1[2]. However recent reports have challenged this 'direct-activation' hypothesis[3-4], suggesting the mechanism by which resveratrol increases SIRT1 function is still unknown. We report for the first time that resveratrol induces a dose dependent increase in activity of the NAD^+ synthetic enzyme nicotinamide mononucleotide adenylyl transferase (NMNAT1). Activation of NMNAT1 by resveratrol in cultured primary human astrocytes and neurons increased NAD^+ levels by up to 5 fold. As SIRT1 requires NAD^+ as a substrate to perform its gene silencing function[5], higher NAD^+ levels will enhance SIRT1 activity[6]. This finding suggests that resveratrol may promote SIRT1 function by enhancing NAD^+ synthesis in whole cell systems without requiring direct activation.

Resveratrol is a non-vitamin antioxidant common in the diet and particularly abundant in teas, juices, and red wines that is thought to harbour major health benefits[1]. Experiments in single and multicellular organisms and human tissue cultures suggest that resveratrol can extend the lifespan in diverse organisms by activating the NAD^+ dependent, enzyme, SIRT1[7]. The neuroprotective ability of SIRT1 induced by resveratrol has been demonstrated *in vitro*[6]. While resveratrol has been claimed to be a direct SIRT1 activator using the Fluor *de* Lys-SIRT1 peptide substrate, recent reports indicate that this finding may represent an experimental artefact[3-4]. Our study indicates resveratrol directly activates the enzyme NMNAT1 which likely influences downstream SIRT1 activity**.**

We demonstrate for the first time that resveratrol significantly increases intracellular NAD^+ levels in primary human astrocytes and neurons (Fig 1). We have shown that resveratrol can induce a dose dependent increase in NAD^+ of 3-5 fold in human astrocytes (Fig 1A) and neurons (Fig 1B) after 24-hour treatment. It has been known for some time that resveratrol can influence NAD^+ responsive enzymes, such as the energy-sensing AMP-activated kinase (AMPK)[8] and SIRT2[2] and its mammalian homologue, SIRT1[2, 9]. However to date no study has reported a link between resveratrol and changes in NAD^+ concentration. Elevated cellular levels of NAD^+, the required substrate for SIRT1, will increase its activity.

Nicotinamide mononucleotide adenylyltransferase (NMNAT) is an NAD^+ synthetic enzyme which catalyses the conversion of nicotinamide mononucleotide (NMN) to NAD^+.

NMNAT1 is the predominant human isoform of NMNAT and is located in the nucleus[6]. Increased NMNAT1 expression has been reported to protect against axonal degradation via increased NAD^+ production in mouse neurons[6]. In the same study, neurons treated with resveratrol prior to axotomy showed a decrease in axonal degeneration that was comparable to that obtained with NAD^+ [6]. It has also been previously demonstrated that increased SIRT1 deacetylase activity can protect against axonal degradation in models of AD, although the exact mechanism is still unknown[10]. As NAD^+ is an essential substrate for SIRT1, the effect of resveratrol on SIRT1 proteins may be due, at least in part, to an NMNAT mediated increase in NAD^+.

We tested the effect of resveratrol on NMNAT activity in cell homogenates of human foetal astrocytes and human recombinant-NMNAT1. We found that resveratrol (200 μM) increased NMNAT1 activity in crude astrocytic homogenates by 70% within 24 hours (Fig 1C). Using human recombinant NMNAT1, we observed that resveratrol lowered the K_m for the substrate NMN by up to 3-fold, and increased V_{max} for the recombinant NMNAT1 by up to 5 fold in a dose dependent manner (Table 1). Our results are consistent with resveratrol acting as a heterotropic allosteric modulator of NMNAT1 at an as yet unidentified site. Further work is required to characterise this novel resveratrol-NMNAT1 interaction.

Our observation that resveratrol increases NAD^+ levels in primary human brain cells by acting on NMNAT supports the view that this polyphenol has considerable therapeutic potential; particularly for the treatment of neurodegenerative diseases. As NMNAT can accelerate NAD^+ synthesis from all three substrates, quinolinic acid, nicotinic acid and nicotinamide[11], NMNAT activation by resveratrol represents an ideal natural therapeutic to replenish NAD^+ levels. Maintenance of higher cellular NAD^+ will enhance SIRT1 activity, and other NAD^+ dependent pathways impacting positively on cell viability and longevity. This work has therefore formed the basis of relevant patent applications.

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FIGURE LEGENDS

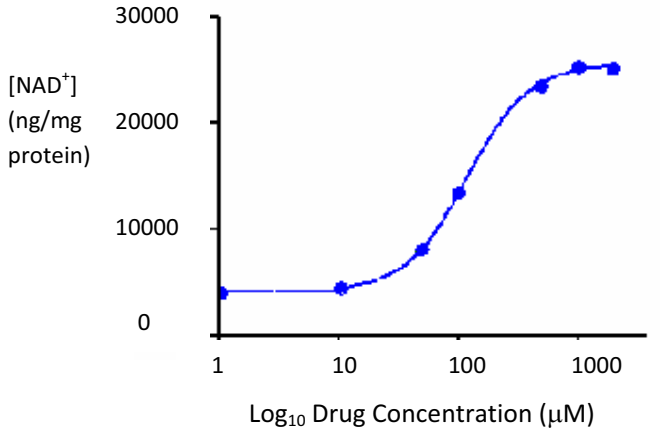
Figure 1. Resveratrol increases intracellular NAD^+ levels in (a) human foetal astrocytes and (b) human foetal neurons, after 24-hour treatment. (c) Effect of resveratrol (200 μM) on NMNAT-1 activity in astrocytic nuclear homogenates. P<0.05 compared to control, (n=4 at each drug concentration)

Table 1. Resveratrol increases human recombinant NMNAT1 activity

(A separate kinetic experiment was carried out for each substrate and resveratrol concentration)

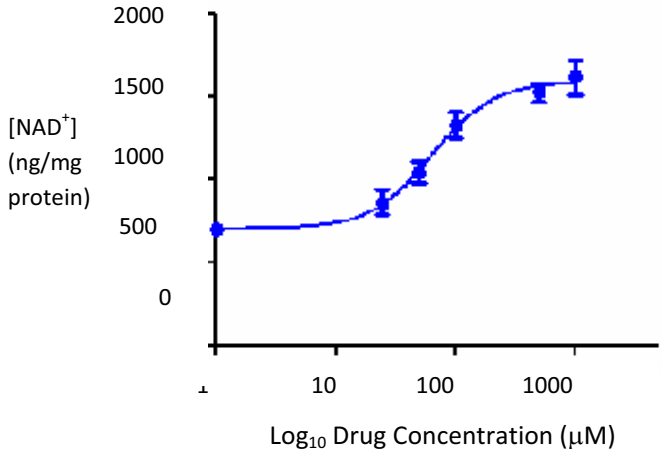
Figure 1 (Grant)

(a)



	Resveratrol
LOGIC50	2.073
Hill slope	1.705
IC50	118.4

(b)



	Resveratrol
LOGIC50	2.073
Hill slope	1.321
IC50	100.7

(c)

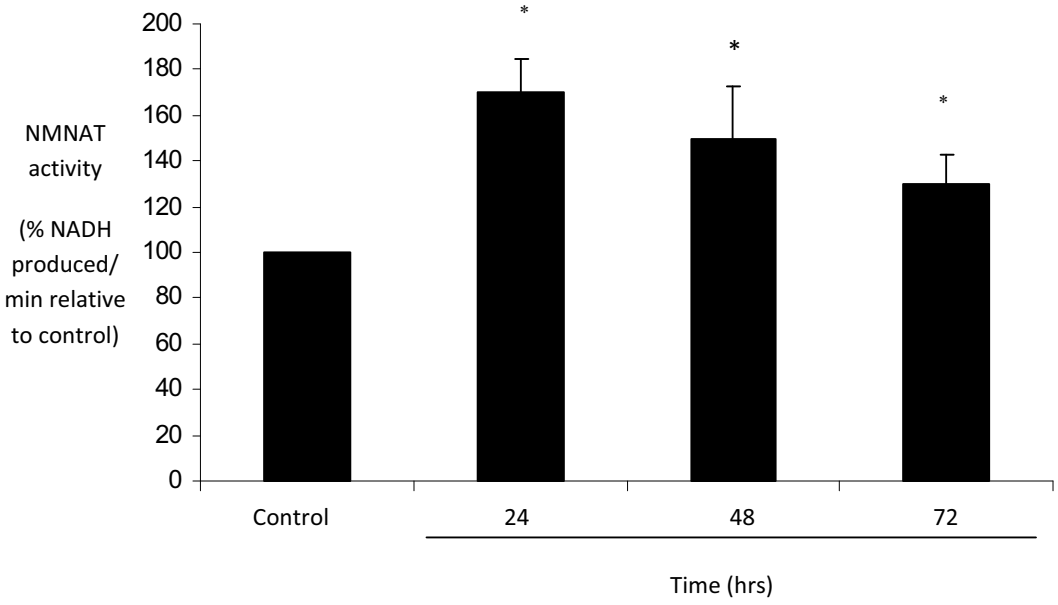


Table 1 (Grant)

Treatment	Km	Vmax (nM/min)
No Resveratrol	115	83
Resveratrol (50μM)	83	130
Resveratrol (100μM)	62	430
Resveratrol (200μM)	43	506