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# Increased susceptibility of mitochondria isolated from frontal cortex and hippocampus of vitamin A-treated rats to non-aggregated amyloid-$\beta$ peptides 1–40 and 1–42

de Oliveira MR, da Rocha RF, Moreira JCF. Increased susceptibility of mitochondria isolated from frontal cortex and hippocampus of vitamin A-treated rats to non-aggregated amyloid-$\beta$ peptides 1–40 and 1–42.

**Objective:** Vitamin A is a redox-active molecule and its inadvertent utilisation as a preventive therapy against ageing or neurodegeneration has become a harmful habit among humans at different ages. Mitochondrial dysfunction and redox impairment may be induced by vitamin A supplementation experimentally. Nonetheless, it is still not clear by which mechanisms vitamin A elicits such effects. Then, we performed this investigation to analyse whether mitochondria isolated from frontal cortex and hippocampus of vitamin A-treated rats are more sensitive to a challenge with amyloid-$\beta$ (A$\beta$) peptides 1–40 or 1–42.
**Methods:** Adult Wistar rats received vitamin A at 1000–9000 IU/kg/day orally for 28 days. Then, mitochondria were isolated and the challenge with A$\beta$ peptides 1–40 or 1–42 (at 0.2 or 0.1 μM, respectively) for 10 min was carried out before mitochondrial electron transfer chain enzyme activity, superoxide anion radical ($O_2^{-\bullet}$) production and 3-nitrotyrosine content quantification.
**Results:** Mitochondria obtained from vitamin A-treated rats are more sensitive to A$\beta$ peptides 1–40 or 1–42 than mitochondria isolated from the control group, as decreased mitochondrial complex enzyme activity and increased $O_2^{-\bullet}$ production and 3-nitrotyrosine content were observed in incubated mitochondria isolated from vitamin A-treated rats.
**Conclusion:** These data suggest that oral intake of vitamin A at clinical doses increases the susceptibility of mitochondria to a neurotoxic agent even at low concentrations.

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### Significant outcomes

This work shows that mitochondria isolated from the frontal cortex and hippocampus of vitamin A-treated rats are more sensitive to an *in vitro* exposition to amyloid-$\beta$ (A$\beta$) peptides 1–40 or 1–42 (at 0.2 or 0.1 μM, respectively) regarding mitochondrial complex enzyme activity, mitochondrial 3-nitrotyrosine content and superoxide anion radical ($O_2^{-\bullet}$) production. These data suggest that vitamin A supplementation at clinical doses (1000–9000 IU/kg/day) did affect both directly and indirectly the mitochondrial function, increasing the susceptibility of such organelle to a posterior deleterious stimulus.

**Limitations**

It is necessary to investigate whether other pro-oxidant agents also affect mitochondria isolated from vitamin A-treated rats.

## Introduction

Vitamin A modulates several biological events from organogenesis to maintenance of mature tissues (1). On the other hand, vitamin A-related toxicity has long been investigated, but the mechanisms by which it acts are not completely understood (2). This vitamin has been characterised as a redox agent, which is able to interfere with redox homeostasis through either an antioxidant or a pro-oxidant action. We have shown that vitamin A supplementation at pharmacological doses induces a pro-oxidant effect in some rat brain regions and impaired rat performance in different behavioural tests (3–10). Recently, increased mortality rates among users of vitamin A supplementation even at low doses were shown (11). In addition, the combination of vitamin A supplementation and smoking was shown to induce tumour growth among patients with lung cancer (12,13). Nevertheless, vitamin A utilisation at moderate to high doses (values ranging from 30 000 to 300 000 IU/day) is still being applied in the treatment of some pathologies, as for instance dermatological disturbances, leukaemia and immunodeficiency (14–17). More alarming is the fact that vitamin A supplementation at about 8500 IU/kg/day is recommended as safe during weight gain therapy for very-low-weight preterm infants (18). Vitamin A – or its derivatives, the retinoids – possesses the ability to impair the homeostasis of several mammalian brain regions and, consequently, to induce cognitive decline characterised by irritability, decreased capabilities to learning and memory, anxiety and depression, which affect directly patient's life quality and work production (17,19).

On the basis of the fact that vitamin A supplementation induces mitochondrial dysfunction *in vivo* and that mitochondrial impairment has been associated with neurodegenerative diseases, we tested here the susceptibility of mitochondria isolated from the frontal cortex and hippocampus of vitamin A-treated rats to fragment length of A$\beta$ peptides 1–40 and 1–42 at low concentrations *in vitro*, as such molecules take a pivotal role during either ageing or Alzheimer's disease progression by inducing cognitive impairment among humans (20). The aim of this work was to analyse whether mitochondria isolated from vitamin A-treated rat brain areas are more sensitive than control mitochondria when a challenge with A$\beta$ peptides was applied with respect to mitochondrial electron transfer chain (METC) enzyme activity, superoxide anion radical ($O_2^{-\bullet}$) production and nitrosative stress.

## Materials and methods

### Animals

Adult male Wistar rats (280–300 g) were obtained from our own breeding colony. They were caged in groups of five with free access to food and water and were maintained on a 12-h light-dark cycle (7:00–19:00 h), at a temperature-controlled colony room (23 ± 1 °C). These conditions were maintained constant throughout the experiments. All experimental procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 80–23, revised 1996) and the Brazilian Society for Neuroscience and Behavior recommendations for animal care. Our research protocol was approved by the Ethical Committee for animal experimentation of the Federal University of Rio Grande do Sul.

### Drugs and reagents

Arovit® (retinol palmitate, a water-soluble form of vitamin A) was purchased from Roche, Sao Paulo, SP, Brazil. Polyclonal antibody to 3-nitrotyrosine was obtained from Calbiochem, San Diego, CA, USA. All other chemicals were purchased from Sigma, St. Louis, MO, USA. Vitamin A treatment was given daily and administered during night.

### Treatment

The animals were treated once a day for 28 days. The treatments were carried out at night (i.e. when the animals are more active and take a greater amount of food) in order to ensure maximum vitamin A absorption, as this vitamin is better absorbed during or after a meal. The animals were treated once a day with vehicle (0.15 M saline; $n = 8–10$ animals), 1000 ($n = 8–10$), 2500 ($n = 8–10$), 4500 ($n = 8–10$) or 9000 IU/kg ($n = 8–10$) of retinol palmitate (vitamin A) orally via a metallic gastric tube (gavage) in a maximum volume of 0.6 ml. Adequate measures were taken to minimise pain or discomfort.



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### Mitochondrial isolation

Mitochondria from fresh rat frontal cortex and hippocampus were isolated as described elsewhere (21). Briefly, frontal cortex and hippocampus of Wistar rats were suspended in ice-cold isolation buffer A [220 mM mannitol, 70 mM sucrose, 5 mM HEPES (pH 7.4), 1 mM EGTA and 0.5 mg/ml fatty-acid free bovine serum albumin] and gently homogenised with a glass homogeniser and centrifuged at 2000 $\times g$ for 10 min at 4 °C. Approximately three quarters of the supernatant were further centrifuged at 10 000 $\times g$ for 10 min at 4 °C in a new tube. The fluffy layer of the pellet was removed by gently shaking with buffer A and the firmly packed sediment was resuspended in the same buffer without EGTA and centrifuged at 10 000 $\times g$ for 10 min at 4 °C. The mitochondrial pellet was resuspended in buffer B [210 mM mannitol, 70 mM sucrose, 10 mM HEPES-KOH (pH 7.4), 4.2 mM succinate, 0.5 mM $KH_2PO_4$ and 4 µg/ml rotenone]. This procedure, which was designed to isolate intact mitochondria rather than to recover all of that present in the frontal cortex and hippocampus, yielded about 7 mg of mitochondrial protein per gram of tissue.

### Mitochondrial challenge with A$\beta$ peptides 1–40 and 1–42

After obtaining mitochondria from rat frontal cortex and hippocampus, we incubated mitochondria with A$\beta$ peptides 1–40 (0.2 µM) or 1–42 (0.1 µM) to verify whether the organelles isolated from vitamin A-treated rats are more sensitive to a low concentration of A$\beta$ peptides, which would alter mitochondrial function only if it were previously affected through vitamin A supplementation. The challenge was carried out in buffer B at room temperature in open tubes for 10 min before the quantification of mitochondrial respiratory chain enzyme activity, superoxide anion radical ($O_2^{-\bullet}$) production and 3-nitrotyrosine content analyses.

### METC activity

*Complex I–CoQ–III activity.* It was determined by following the increase in absorbance due to the reduction of cytochrome *c* at 550 nm with 580 nm as the reference wavelength ($\epsilon = 19.1\ mM^{-1}\ cm^{-1}$). The reaction buffer contained 210 mM mannitol, 70 mM sucrose, 20 mM potassium phosphate, pH 8.0, 2.0 mM KCN, 10 µM EDTA, 50 µM cytochrome *c* and 20–45 µg supernatant protein. The reaction started by the addition of 25 µM NADH and was monitored at 30 °C for 3 min before the addition of 10 µM rotenone, after which the activity was monitored for an additional 3 min. Complex I–III activity was the rotenone-sensitive NADH:cytochrome *c* oxidoreductase activity (22).

*Complex II–CoQ–III activity.* It was measured by following the increase in absorbance due to the reduction of cytochrome *c* at 550 nm with 580 nm as the reference wavelength ($\epsilon = 21\ mM^{-1}\ cm^{-1}$). The reaction mixture consisting of 170 mM mannitol, 70 mM sucrose, 40 mM potassium phosphate, pH 7.4, 16 mM succinate was preincubated with 50–100 µg supernatant protein at 30 °C for 30 min. Subsequently, 4.0 mM sodium azide and 7.0 µM rotenone were added and the reaction started by the addition of 0.6 µg/ml cytochrome *c* and monitored for 5 min at 30 °C (23).

*Complex IV activity.* It was measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome *c* at 550 nm with 580 nm as the reference wavelength ($\epsilon = 19.15\ mM^{-1}\ cm^{-1}$). The reaction buffer contained 220 mM mannitol, 70 mM sucrose, 10 mM potassium phosphate, pH 7.0, 0.6 mM *n*-dodecyl-$\beta$-D-maltoside, 2–4 µg supernatant protein and the reaction was started with the addition of 0.7 µg reduced cytochrome *c*. The activity of complex IV was measured at 25 °C for 10 min (24).

### Indirect ELISA to 3-nitrotyrosine

To realise indirect enzyme-linked immunosorbent assay (ELISA), rat brain regions were rapidly homogenised ($T < 1$ min) in lysis buffer containing 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton-X-100, 10% glycerol, 2 mM sodium orthovanadate and Complete™ protease inhibitor cocktail (Roche). Indirect ELISA was performed to analyse changes in the content of nitrotyrosine by utilising a polyclonal antibody to nitrotyrosine (Calbiochem) diluted 1:2000 in phosphate-buffered saline (PBS), pH 7.4, with 5% albumin. Briefly, microtitre plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in PBS with 5% albumin. Plates were then washed four times with wash buffer (PBS with 0.05% Tween-20), and the specific antibodies were added to the plates for 2 h at room temperature. After washing (four times), a second incubation with peroxidase-conjugated anti-rabbit antibody (diluted 1:1000) for 1 h at room temperature was carried out. After addition of substrates (hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine, 1:1 – v:v), the samples were read at 450 nm in a plate spectrophotometer. Results are expressed as changes in percentage among the groups and the experiments were performed in triplicate.



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### Superoxide anion radical ($O_2^{-\bullet}$) production

Briefly, to obtain submitochondrial particles (SMPs), frontal cortex and hippocampus were dissected and homogenised in 230 mM mannitol, 70 mM sucrose, 10 mM Tris-HCl and 1 mM EDTA (pH 7.4). The samples were centrifuged for 10 min at 600 $\times g$ (4 °C). The supernatants were then centrifuged (8000 $\times g$ for 10 min at 4 °C) two times to isolate mitochondria. Then, freezing and thawing (three times) the mitochondrial solution gave rise to superoxide dismutase-free SMP. The SMP solution was also washed (twice) with 140 mM KCl, 20 mM Tris-HCl (pH 7.4) to ensure mitochondrial superoxide dismutase (SOD) release from mitochondria (centrifugation to wash at 5400 $\times g$ for 10 min at 4 °C). To quantify $O_2^{-\bullet}$ production, SMP was incubated in reaction medium consisting of 230 mM mannitol, 70 mM sucrose, 10 mM HEPES-KOH (pH 7.4), 4.2 mM succinate, 0.5 mM $KH_2PO_4$, 0.1 µM catalase and 1 mM epinephrine, and the increase in the absorbance (auto-oxidation of adrenaline to adrenochrome) was read at 480 nm in a spectrophotometer at 32 °C, as previously described (3,25). The experiments were performed in triplicate.

### Statistical analyses

Results are expressed as means ± SEM; *p*values were considered significant when $p < 0.05$. Differences in experimental groups were determined by one-way analysis of variance (ANOVA) followed by the *post hoc* Duncan's test whenever necessary.

## Results

### METC enzyme activity

As depicted in Fig. 1a, vitamin A supplementation at 4500 or 9000 IU/kg/day induced an increase in complex I–III enzyme activity in mitochondria incubated in the absence of A$\beta$ peptides ($p < 0.05$). However, this increase was abolished in the presence of A$\beta$ peptides 1–40 or 1–42, which decreased complex I–III enzyme activity ($p < 0.05$). Complex I–III enzyme activity of mitochondria isolated from saline-treated rats was not affected by the challenge with A$\beta$ peptides.

Similarly, increased complex I–III enzyme activity was observed in mitochondria isolated from hippocampus of the rats that were administered vitamin A supplementation at 4500 or 9000 IU/kg/day ($p < 0.05$; Fig. 1b). A$\beta$ peptides challenge induced a decrease in complex I–III enzyme activity of mitochondria isolated from vitamin A-treated rats at any dose ($p < 0.05$; Fig. 1b).

According to Fig. 1c, complex II–III enzyme activity did not change in the mitochondria obtained from the frontal cortex of vitamin A-treated rats and incubated in the absence of A$\beta$ peptides. On the contrary, it was observed that A$\beta$ peptides challenge decreased complex II–III enzyme activity only in the mitochondria obtained from vitamin A-treated rats (Fig. 1c; $p < 0.05$). A similar effect was observed in mitochondria isolated from the hippocampus of the rats that received vitamin A supplementation at any dose tested (Fig. 1d; $p < 0.05$).

Complex IV enzyme activity of the mitochondria incubated in the absence of A$\beta$ peptides did not change in the rats that were treated with vitamin A supplementation (Fig. 1e). However, it was observed that there was decreased complex IV enzyme activity in mitochondria isolated from the frontal cortex of vitamin A-treated rats when A$\beta$ peptides were added to the incubation media ($p < 0.05$; Fig. 1e). Decreased complex IV enzyme activity was observed in the mitochondria isolated from the hippocampus of the rats that were administered vitamin A supplementation at 4500 or 9000 IU/kg/day and whose mitochondria were incubated in the absence of A$\beta$ peptides ($p < 0.05$; Fig. 1f). When A$\beta$ peptides were present in the incubation media, it was observed that there was decreased complex IV enzyme activity in the mitochondria obtained from the hippocampus of the rats that were treated with vitamin A supplementation at any dose tested ($p < 0.05$; Fig. 1f).

### Superoxide anion radical ($O_2^{-\bullet}$) production

As shown in Fig. 2a, mitochondria isolated from the frontal cortex of the rats that were administered vitamin A supplementation at 2500, 4500 or 9000 IU/kg/day produced more $O_2^{-\bullet}$ than control mitochondria even in the absence of A$\beta$ peptides ($p < 0.05$). However, an increment in $O_2^{-\bullet}$ production in the presence of A$\beta$ peptides was observed ($p < 0.05$; Fig. 2a). Mitochondria obtained from the hippocampus of the rats that received vitamin A supplementation at 2500, 4500 or 9000 IU/kg/day produced significantly more $O_2^{-\bullet}$ than mitochondria isolated from control rats in the absence of A$\beta$ peptides ($p < 0.05$; Fig. 2b). The incubation with A$\beta$ peptides increased $O_2^{-\bullet}$ production in hippocampal mitochondria obtained from vitamin A-treated rats ($p < 0.05$; Fig. 2b).

### Mitochondrial membrane 3-nitrotyrosine content

Mitochondrial membranes obtained from the frontal cortex of vitamin A-treated rats show increased 3-nitrotyrosine content even in the absence of A$\beta$ peptides in the incubation media ($p < 0.05$; Fig. 2c).



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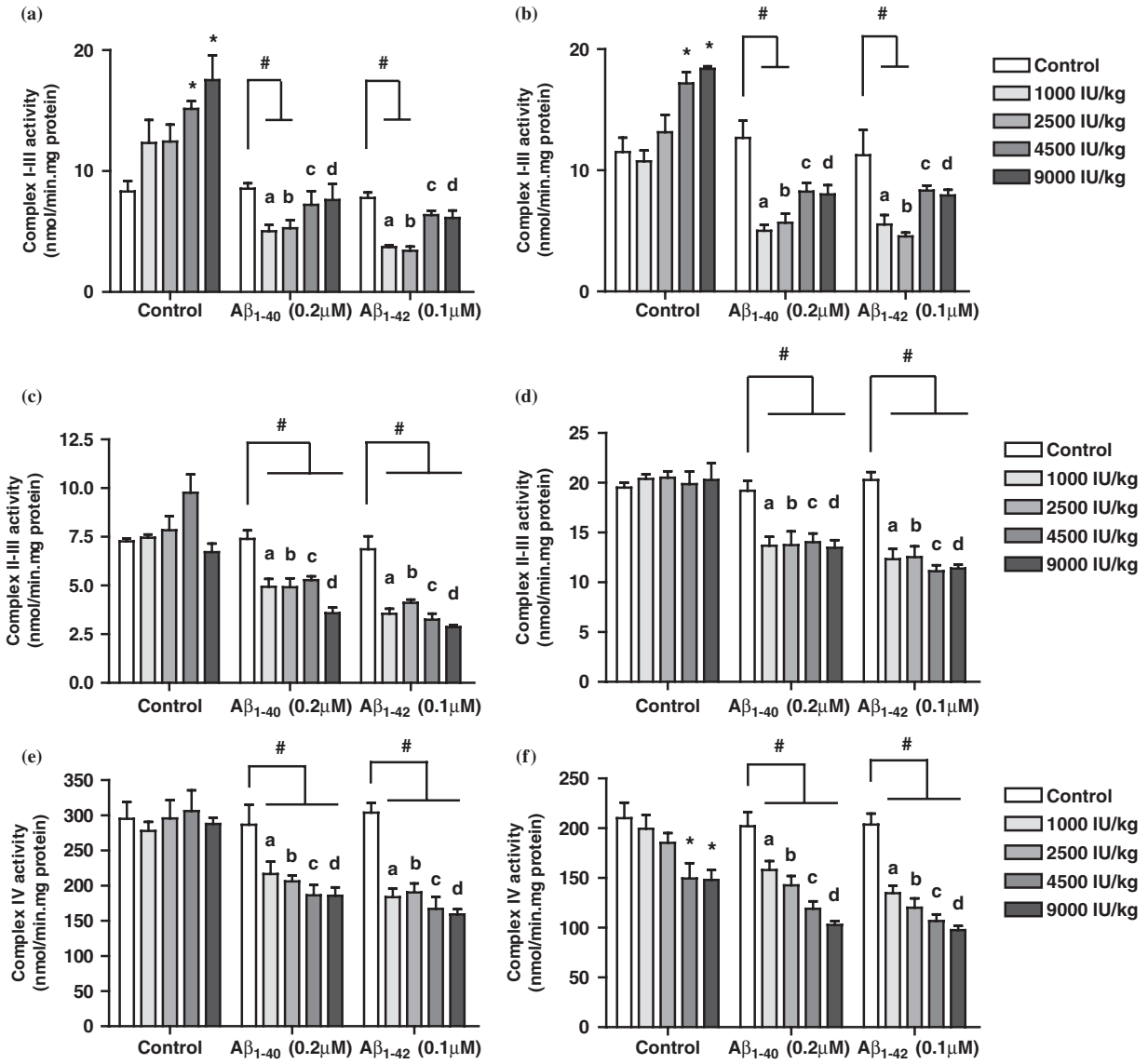


*Fig. 1.* Vitamin A supplementation effects on METC enzyme activity in a challenge with A$\beta$ peptides 1–40 or 1–42. (a) Frontal cortex complex I–III enzyme activity; (b) hippocampal complex I–III enzyme activity; (c) frontal cortex complex II–III enzyme activity; (d) hippocampal complex II–III enzyme activity; (e) frontal cortex complex IV enzyme activity and (f) hippocampal complex IV enzyme activity. Data are mean ± SEM ($n = 8$–10 per group). $^{*}p < 0.05$ (vitamin A vs. control group), $^{\#}p < 0.05$ (vitamin A vs. control group in the presence of A$\beta$ peptides 1–40 or 1–42), $^{a,b,c,d}p < 0.05$ (vitamin A dose in the presence of A$\beta$ peptides 1–40 or 1–42 vs. the same vitamin A dose in the absence of A$\beta$ peptides 1–40 or 1–42; one-way ANOVA followed by the *post hoc* Duncan's test).

Interestingly, although increased 3-nitrotyrosine content in mitochondrial membranes of frontal cortex was observed, it was not changed by the challenge with A$\beta$ peptide 1–40 (Fig. 2c). On the other hand, the challenge with A$\beta$ peptide 1–42 did increase 3-nitrotyrosine content in membranes of mitochondria that were obtained from the frontal cortex of vitamin A-treated rats ($p < 0.05$; Fig. 2c).

We observed increased 3-nitrotyrosine content in mitochondrial membranes obtained from the hippocampus of vitamin A-treated rats in the absence of A$\beta$ peptides challenge ($p < 0.05$; Fig. 2d). However, the challenge with either A$\beta$ peptides 1–40 or 1–42 did increase 3-nitrotyrosine content in hippocampal mitochondrial membranes obtained from vitamin A-treated rats ($p < 0.05$; Fig. 2d).

## Discussion

Neurodegenerative pathologies affect dramatically life quality of patients suffering from such diseases. It has been shown that there was decreased life expectancy and production at work. Moreover, the governmental costs for treatment of



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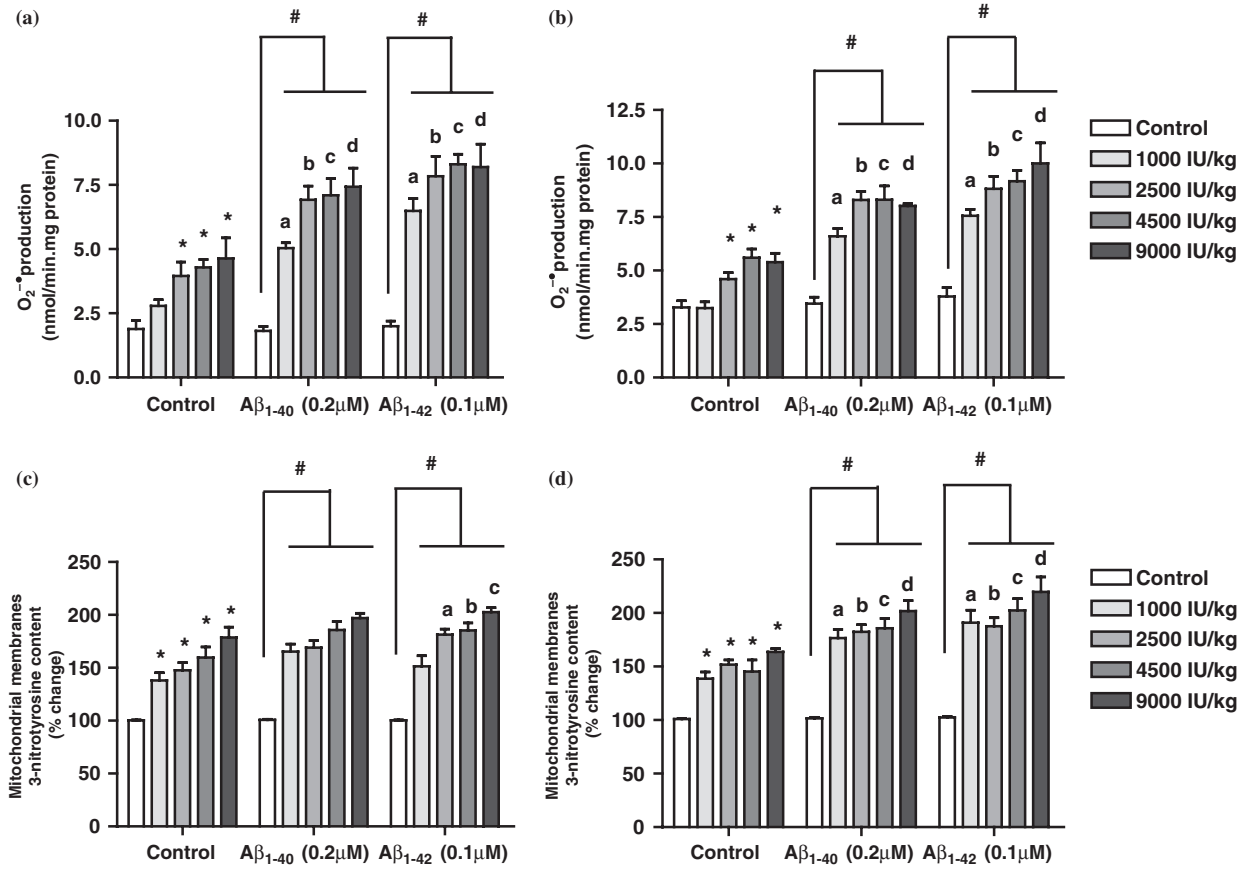


*Fig. 2.* Vitamin A supplementation effects on superoxide anion radical ($O_2^{-\bullet}$) production in (a) frontal cortex and (b) hippocampal mitochondrial incubations with or without A$\beta$ peptides 1–40 or 1–42. The effects of vitamin A supplementation on 3-nitrotyrosine content in mitochondrial membranes during incubations with or without A$\beta$ peptides 1–40 or 1–42 are shown in (c) and (d). $^{*}p < 0.05$ (vitamin A vs. control group), $^{\#}p < 0.05$ (vitamin A vs. control group in the presence of A$\beta$ peptides 1–40 or 1–42), $^{a,b,c,d}p < 0.05$ (vitamin A dose in the presence of A$\beta$ peptides 1–40 or 1–42 vs. the same vitamin A dose in the absence of A$\beta$ peptides 1–40 or 1–42; one-way ANOVA followed by the *post hoc* Duncan's test).

neuropathological conditions increase annually in both developed and developing countries (26–28). Vitamin A has been shown to induce neurotoxicity and also impaired mammalian behaviour [as reviewed in (16,17)]. Decreased metabolic rates were observed in the cerebral cortex of patients under retinoid treatment against acne (29). Hallucinations, confusion, anxiety, depression and suicide ideation may also be listed among the effects associated with vitamin A supplementation use (30). Nonetheless, it still remains on debate by which mechanisms such vitamin elicits neurotoxicity.

We have shown that mitochondria are a target of vitamin A-related toxicity *in vitro* and *in vivo*. Retinol at concentrations similar to that observed physiologically induced mitochondrial swelling and facilitated cytochrome *c* release from the organelle *in vitro* (21). Recently, we showed increased levels of oxidative stress markers in SMPs isolated from some brain regions of rats that were subjected to long-term vitamin A supplementation. This effect was accompanied by increased rates of $O_2^{-\bullet}$ production (3,6–10). In addition, vitamin A supplementation is able to impair METC enzyme activity *in vivo*, which may give rise to free radical production (3–10).

In this work, we show, for the first time, that mitochondria isolated from the frontal cortex and hippocampus of vitamin A-treated rats are more sensitive to a challenge with A$\beta$ peptides at low doses regarding METC enzyme activity, $O_2^{-\bullet}$ production and nitrosative stress. A$\beta$ peptides take a central role in Alzheimer's disease and their increased levels have been mentioned as a cause of this pathology (31). A$\beta$ peptides interact with mitochondria *in vivo* physiologically, but such interaction becomes harmful depending on other circumstances, as for instance the redox and bioenergetics environments (32–34). Moreover, A$\beta$ intramitochondrial accumulation occurs before extracellular A$\beta$ deposition during Alzheimer's disease (35). It was recently shown that mitochondrial bioenergetic impairment preceded Alzheimer's disease pathology in an animal



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model of this pathology, and it was positively correlated with increased mitochondrial A$\beta$ levels (36). Then, based on the data obtained in our work, we suggest that vitamin A supplementation may induce a predisposition to mitochondrial deficit elicited by the presence of A$\beta$ peptides, favouring a loss of energetic homeostasis.

It is noteworthy that A$\beta$ peptides decreased cortical and hippocampal METC enzyme activities of the rats that were administered vitamin A at any dose tested. In some cases, vitamin A supplementation alone induced an increase in METC enzyme activities, which was decreased by the combination of previous vitamin A supplementation and A$\beta$ peptides. On the other hand, when vitamin A supplementation was not effective in altering METC activity, it rendered mitochondria to be more sensitive to A$\beta$ peptides challenge (Fig. 1).

The mechanism by which A$\beta$ peptides interfere with the function of mitochondria (complex I–III, complex II–III and complex IV enzyme activity) isolated from vitamin A-treated rats still remains to be clarified, but by decreasing mitochondrial complex enzyme activity, A$\beta$ peptides may increase $O_2^{-\bullet}$ production due to electron leakage and consequent $O_2$ partial reduction at complex IV, as previously reviewed (20). Then, based on the data presented here, we propose that vitamin A supplementation *in vivo* facilitates mitochondrial dysfunction in future events that may interfere with mitochondrial dynamics.

The data presented here may be useful, at least in part, to show that the use of vitamin A supplements as an antioxidant during the treatment (or prevention) of neurodegenerative diseases should be revised. The faddism around utilisation of vitamin supplements in an attempt to prevent ageing progress, for example, may otherwise trigger negative, and perhaps irreversible, consequences.

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## References

1. Lane MA, Bailey SJ. Role of retinoids signaling in the adult brain. Prog Neurobiol 2005;**75**:275–293.
2. Snodgrass SR. Vitamin neurotoxicity. Mol Neurobiol 1992;**6**:41–73.
3. De Oliveira MR, Moreira JCF. Acute and chronic vitamin A supplementation at therapeutic doses induces oxidative stress to submitochondrial particles isolated from cerebral cortex and cerebellum of adult rats. Toxicol Lett 2007;**173**: 145–150.
4. De Oliveira MR, Silvestrin RB, Mello e Souza T, Moreira JCF. Oxidative stress in the hippocampus, anxiety-like behavior and decreased locomotory and exploratory activity of adult rats: effects of sub acute vitamin A supplementation at therapeutic doses. Neurotoxicology 2007;**28**: 1191–1199.
5. De Oliveira MR, Pasquali MAB, Silvestrin RB, Mello e Souza T, Moreira JCF. Vitamin A supplementation induces a prooxidative state in the *striatum* and impairs locomotory and exploratory activity of adult rats. Brain Res 2007;**1169**:112–119.
6. De Oliveira MR, Moreira JCF. Impaired redox state and respiratory chain enzyme activities in the cerebellum of vitamin A-treated rats. Toxicology 2008;**253**:125–130.
7. De Oliveira MR, Silvestrin RB, Mello E, Souza T, Moreira JCF. Therapeutic vitamin A doses increase the levels of markers of oxidative insult in *substantia nigra* and decrease locomotory and exploratory activity in rats after acute and chronic supplementation. Neurochem Res 2008; **33**:378–383.
8. De Oliveira MR, Oliveira MWS, Behr GA, Hoff MLM, Da Rocha RF, Moreira JCF. Evaluation of the effects of vitamin A supplementation on adult rat substantia nigra and striatum redox and bioenergetics states: mitochondrial impairment, increased 3-nitrotyrosine and $\alpha$-synuclein, but decreased D2 receptor contents. Progr Neuropsychopharmacol Biol Psychiatry 2009;**33**:353–362.
9. De Oliveira MR, Oliveira MWS, Da Rocha RF, Moreira JCF. Vitamin A supplementation at pharmacological doses induces nitrosative stress on the hypothalamus of adult Wistar rats. Chem Biol Interact 2009;**180**:407–413.
10. De Oliveira MR, Oliveira MWS, Behr GA, Moreira JCF. Vitamin A supplementation at clinical doses induces a dysfunction in the redox and bioenergetics states, but did not change neither caspases activities nor TNF-alpha levels in the frontal cortex of adult Wistar rats. J Psychiatr Res 2009;**43**:754–762.
11. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systemic review and meta-analysis. J Am Med Assoc 2007;**297**: 842–857.
12. Omenn GS, Goodman G, Thornquist M et al. The beta-carotene and retinol efficacy trail (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. Cancer Res 1994; **54**:2038s–2043s.
13. Omenn GS, Goodman GE, Thornquist MD et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 1996;**334**: 1150–1155.
14. Tsunati H, Iwasaki H, Kawai Y, Tanaka T, Ueda T, Uchida M, Nakamura T. Reduction of leukemia cell growth in a patient with acute promyelocytic leukemia treated by retinol palmitate. Leukemia Res 1990;**14**: 595–600.
15. Tsunati H UEDAT, Uchid AM, Nakamura T. Pharmacological studies of retinol palmitate and its clinical effect in patients with acute non-lymphocytic leukemia. Leukemia Res 1991;**15**:463–471.
16. Allen LH, Haskell M. Estimating the potential for vitamin A toxicity in women and young children. J Nutr 2002; **132**:2907S–2919S.



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17. Myhre AM, Carlsen MH, Bohn SK, Wold HL, Laake P, Blomhoff R. Water-miscible emulsified, and solid forms of retinol supplements are more toxic than oil-based preparations. Am J Clin Nutr 2003;**78**:1152–1159.
18. Mactier H, Weaver LT. Vitamin A and preterm infants: what we know, what we don't know, and what we need to know. Arch Dis Child Fetal Neonatal Ed 2005;**90**:103–108.
19. Jick SS, Kremers HM, Vasilakis-Scaramozza C. Isotretinoin use and risk of depression, psychotic symptoms, suicide, and attempted suicide. Arch Dermatol 2000;**136**: 1231–1236.
20. Halliweel B. Oxidative stress and neurodegeneration: where are we now? J Neurochem 2006;**97**:1634–1658.
21. Klamt F, De Oliveira MR, Moreira JCF. Retinol induces permeability transition and cytochrome c release from rat liver mitochondria. Biochim Biophys Acta 2005;**1726**: 14–20.
22. Shapira AH, Mann VM, Cooper JM et al. Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency in Parkinson's disease. J Neurochem 1990;**55**: 2142–2145.
23. Fisher JC, Ruitenbeek W, Berden JÁ et al. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. Clin Chim Acta 1985;**153**:23–36.
24. Rustin P, Chretien D, Bourgeron T, Gérard B, Rötig A, Saudubray JM, Munnich A. Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta 1994;**228**:35–51.
25. Poderoso JJ, Carreras MC, Lisdero C, Riobo N, Schopfer F, Boveris A. Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles. Arch Biochem Biophys 1996;**328**:85–92.
26. Ferri CP, Prince M, Brayne C et al. Global prevalence of dementia: a Delphi consensus study. Lancet 2005;**366**: 2112–2117.
27. Kalaria RN, Maestre GE, Arizaga R et al. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. Lancet Neurol 2008;**7**:812–826.
28. Hawton K, van heeringen K. Suicide. Lancet 2009;**373**: 1372–1381.
29. Bremner JD, Fani N, Ashraf A et al. Functional brain imaging alterations in acne patients treated with isotretinoin. Am J Psychiatr 2005;**162**:983–991.
30. O'Reilly K, Bailey SJ, Lane MA. Retinoid-mediated regulation of mood: possible cellular mechanisms. Exp Biol Med 2008;**233**:251–258.
31. Berg D, Youdim MBH, Riederer P. Redox imbalance. Cell Tissue Res 2004;**318**:201–213.
32. Manczak M, Anekonda TS, Henson E, Park BS, Quinn P, Reddy H. Mitochondria are a direct site of A$\beta$ accumulation in Alzheimer's disease neurons: implications for frre radical generation and oxidative damage in disease progression. Hum Mol Genet 2006;**15**:1437–1449.
33. Chen X, Yan SD. Mitochondrial A$\beta$: a potential cause of metabolic dysfunction in Alzheimer's disease. IUBMB Life 2006;**58**:686–694.
34. Pavlov PF, Petersen CH, Glaser E, Ankarcrona M. Mitochondrial accumulation of APP and A$\beta$: significance for Alzheimer's disease pathogenesis. J Cell Mol Med 2009;**13**:4137–4145.
35. Du H, Guo L, Fang F et al. Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. Nat Med 2008;**14**:1097–1105.
36. Yao J, Irwin RW, Zhao L, Nilsen J, Hamilton RT, Brinton RD. Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A 2009;**106**: 14670–14675.



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