



# Persistence of the benefit of an antioxidant therapy in children and teenagers with Down syndrome



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## ABSTRACT

This study examined the effect of an antioxidant intervention in biomarkers of inflammation and oxidative stress (OS) in the blood of Down syndrome (DS) children and teenagers during four different stages. A control group was composed by healthy children ( $n = 18$ ), assessed once, and a Down group composed by DS patients ( $n = 21$ ) assessed at the basal period ( $t_0$ ), as well as after 6 months of antioxidant supplementation ( $t_1$ ), after 12 months (after interruption of the antioxidant intervention for 6 months) ( $t_2$ ), and again after further 6 months of antioxidant supplementation ( $t_3$ ). Biomarkers of inflammation (myeloperoxidase activity – MPO and levels of IL-1 $\beta$  and TNF- $\alpha$ ) and OS (thiobarbituric acid reactive substances – TBARS, protein carbonyls – PC), reduced glutathione (GSH), uric acid (UA) and vitamin E levels, as well as antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and gamma-glutamyltransferase (GGT) activities, were measured after each period. After the antioxidant supplementation, the activities of SOD, CAT, GPx, GR, GGT and MPO were downregulated, while TBARS contents were strongly decreased, the contents of GSH and vitamin E were significantly increased, and no changes in G6PD and GST activity as well as in UA and PC levels were detected. After the interruption of the antioxidant therapy for 6 months, DS patients showed elevated GPx and GGT activities and also elevated UA and TBARS levels. No changes in SOD, CAT, GR, GST, G6PD and MPO activities as well as in GSH, vitamin E, PC, TNF- $\alpha$  and IL-1 $\beta$  levels were detected. The results showed that the antioxidant intervention persistently attenuated the systemic oxidative damage in DS patients even after a relatively long period of cessation of the antioxidant intervention.

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## 1. Introduction

Down syndrome (DS) or Trisomy 21 is the most common chromosome abnormality in humans and oxidative stress (OS) is part of the fundamental biology of DS (Pagano & Castello, 2012; Parisotto et al., 2014; Pueschel, 1990). This increased OS

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involves one of the key enzymes associated with the detoxification of reactive oxygen species (ROS), Cu–Zn superoxide dismutase (SOD-1), which is located on chromosome 21. There is consequently a gene dosage effect in DS leading to ~50% greater levels of SOD-1 and elevated endogenous production of hydrogen peroxide ( $H_2O_2$ ), and potentially of hydroxyl radical ( $\cdot OH$ ) (Garlet et al., 2013; Lott, 2012), which is the most reactive and deleterious ROS inducing damage in proteins, lipids and DNA (Halliwell & Gutteridge, 2007).

We previously demonstrated that DS individuals presented depleted levels of GSH (Parisotto et al., 2014). The induction of GGT and G6PD activity found in the present study is probably counteracting the GSH depletion. The complex redox system of GSH involves GPx, GR, GST, as well as GGT. The redox cycling of oxidized glutathione is catalyzed by GR, whereas the supply of NADPH, the major reducing agent for this redox cycling, is provided by G6PD activity (Halliwell & Gutteridge, 2007). Identification and characterization of early events that contribute to and/or regulate the expression of chromosome 21 gene products that are triplicated in DS is crucial to understand the ways in which neurodegenerative cycles and the mechanisms implicated in promotion of the neuropathophysiological progression in DS. In the brain, three early events, neuritic amyloid- $\beta$  plaques ( $A\beta$ ), neurofibrillary tangles and glial activation, have been reported in DS fetuses and each one is related to the others as they induce, and are induced by each other and also by cytokines subsequent to neuroinflammatory changes (Wilcock & Griffin, 2013).

We recently demonstrated the efficacy of an antioxidant supplementation during 6 months in attenuating a systemic OS in DS patients (Parisotto et al., 2014). This is a prospective study carried out to verify the persistence effect of the antioxidant intervention in 1.5 years of follow-up in biomarkers of inflammation and OS in the blood of DS children and teenagers.

## 2. Subjects and methods

### 2.1. Subjects

The control group was constituted of 18 healthy children (average age of  $6.7 \pm 3.0$  years, all showing inflammatory markers within normal ranges) without DS (10 males and 8 females; 3–12 years), who were recruited from the Joana de Gusmão Children's Hospital in Florianópolis, State of Santa Catarina, South Brazil. The DS participants were between 3 and 14 years old, with an average age of  $7.7 \pm 3.18$  years ( $n = 21$ ; 12 males and 9 females). Most of the patients were concentrated in the age group 6–10 years (65%). The inclusion criteria were: age between 3 and 14 years without illnesses associated with systemic diseases and not participating in other studies. Patients taking medication or food supplements were excluded. The 21 DS individuals were recruited from two local organizations for DS children ("Associação Amigos Down" and "Associação de Pais e Amigos dos Excepcionais – APAE"). The study protocol was previously approved by the Ethics Committee of UFSC, according to the national and international guidelines for research involving human subjects (Resolution No. 1996 of the National Health Council), which regulate experiments involving human subjects (local Protocol CEP N. 2112/2011). All patients received information about the study and their parents signed a free and informed consent form.

### 2.2. Study design

In two recent accompanied papers by Garlet et al. (2013) and Parisotto et al. (2014), both from our laboratory, biomarkers of OS were evaluated in blood samples collected from children and teenagers with DS, using the same protocol and experimental design of the present study. However, now the biomarkers of OS were evaluated in the blood of DS patients ( $n = 21$ ) before ( $t_0$ ) and after a daily antioxidant intervention (vitamin E 400 mg, C 500 mg, E-TABS<sup>®</sup> and Energil C<sup>®</sup>, respectively) during 6 months ( $t_1$ ), as well as after an interruption of the supplementation (also after 6 months) ( $t_2$ ) and after a new supplementation of further 6 months ( $t_3$ ). The dose was lower (less than half) of that recommended by Nathens et al. (2002) for adults, which is based in the upper intake level (UL), showing that it is safe and presenting no adverse effect (Kappus & Diplock, 1992).

### 2.3. Sample preparation

Whole blood was obtained from the antecubital vein in chilled tubes containing EDTA as anticoagulant, or without EDTA to obtain serum. Immediately after blood collection a blood fraction (200  $\mu L$ ) was precipitated in trichloroacetic acid (TCA 12%, 1:4 v/v) for reduced glutathione (GSH) assays. The remaining blood was centrifuged at  $1500 \times g$  for 10 min to separate red cells from plasma. For enzymatic assays, red cells were diluted in distilled water (1:4) and hemolysis was carried out by freezing and thaw procedure. After this, plasma, serum and the acid extracts were stored in liquid nitrogen ( $-170^\circ C$ ) until analysis of the parameters. Enzymatic evaluations were carried out in hemolysates, while the contents of GSH were obtained in whole blood acid extracts. Thiobarbituric acid reactive substances (TBARS), protein carbonyl and vitamin E contents were examined in plasma. The myeloperoxidase (MPO) activity and the uric acid (UA), TNF- $\alpha$  e IL-1 $\beta$  contents were analyzed in serum.

### 2.4. Reduced glutathione (GSH) assay

Reduced glutathione (GSH) was measured at 412 nm (Beutler, Duron, & Kelly, 1963) by using the reagent DTNB (2-dithionitrobenzoic acid). After centrifugation at  $3000 \times g$  for 5 min, the supernatants from the acid extracts were added

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