### Introduction

- Glutathione (GSH) is the most abundant intracellular antioxidant with many additional functions including detoxification & maintenance of immune function.
- Interindividual variation in tissue GSH is high.
- One approach to maintaining health and reducing risk for toxicity and disease involves maintaining optimal GSH levels.
- Laboratory animal studies have demonstrated the bioavailability of oral GSH and its effectiveness at enhancing tissue GSH levels.
- Objectives: To determine the impact of chronic oral GSH supplementation on body stores of GSH and biomarkers of immune function.

### Methods

#### Study Design:
Randomized double-blind, placebo-controlled study

#### Subjects:
54 Healthy adults (Table 1)

#### Arms:
- Placebo, GSH* (250 mg/day), GSH* (1000 mg/day), Setria® (Kyowa Hakko, USA).

#### Study Period & Samples: 6 mo supplementation followed by 1 mo washout - blood and exfoliated buccal mucosal cells collected at 0, 1, 3, 6 & 7 mo.

#### Sample processing:
Blood fractionated into plasma, lymphocytes and lymphocytes by Ficoll-Hypaque density gradient centrifugation.

#### Glutathione levels:
- Measured in metaphosphoric acid (MPA) extracts prepared as follows: 0.4 ml 5% (w/v) MPA added to 0.1 ml blood, ~5x10^6 packed cells (RBC or buccal cells), or 0.4 ml plasma (after reduction with KBH4). For 2 min, free GSH (supernatant) and protein-bound GSH (pellet) were determined by DTNB-enzymatic recycling method.

#### Lymphocyte proliferation:
- Assessed by 51Cr release after incubation with T cell mitogen phytohemagglutinin (PHA) by 3H-thymidine incorporation.

#### Natural Killer Cell cytotoxicity:
- Assessed by ^51Cr release after incubation of lymphocytes with ^51Cr-labeled human K562 cells.

### Results

- Compliance of study subjects was high (>90%) based upon pill count and diary.
- GSH was not associated with side-effects.
- GSH administration enhanced free but not protein-bound GSH in a dose and time-dependent fashion in whole blood (Fig. 1A) & erythrocytes (Fig. 1B).
- GSH administration enhanced GSH levels in a time-dependent fashion in plasma, lymphocytes & buccal mucosal cell GSH (Fig. 2).
- Maximum increases in GSH occurred at 6 mo.
- Increases in erythrocyte GSH were greatest in individuals with low baseline blood GSH levels (<0.89 mmol/ml) (P<0.003).
- GSH administration (1 g/day) enhanced NK cell cytotoxicity (Fig. 3).
- Enhancement of GSH was not a result of changes in GCL or GST activity (Table 2).

### Conclusions

Oral GSH administration is an effective means of chronically enhancing body stores of GSH and may also enhance immune function. Thus, GSH supplementation may represent an effective intervention strategy for disease prevention.

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