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SUPPLEMENTATION WITH UNDENATURED CYSTEINE RICH WHEY PROTEIN ISOLATE REDUCED OXIDATIVE STRESS IN NON-ALCOHOLIC STEATOHEPATITIS PATIENTS

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is becoming a common cause of chronic liver disease in Thailand reflecting the increasing prevalence of obesity and diabetes. Non-alcoholic steatohepatitis (NASH) is part of a spectrum of NAFLD that ranges from pure fatty liver (steatosis) to steatohepatitis and to cirrhosis[1]. Long-standing NASH with cirrhosis has been associated with the development of hepatocellular carcinoma[2]. There are no medicinal drugs approved for the treatment of NASH.

We have shown that supplementation of NASH patients with HMS 90®*, an undenatured cysteine rich whey protein isolate, has a positive effect on steatohepatitis and liver enzymes (see poster 925). The levels of plasma glutathione were also shown to increase significantly. The objective of this poster is to evaluate other markers of oxidative stress to provide support for the hypothesis that the positive effects observed were due to redressing the oxidant-anti-oxidant imbalance in these patients.

METHODS

1. Experimental Design
Dosing - Patients were instructed to take 20 g per day of undenatured cysteine rich whey protein isolate (HMS 90®) for 12 weeks in two equal portions of 10 g mixed with water. No dose adjustments were made for patient weight.

Evaluation - the following information was collected according to the patient visit schedule:
a : Clinical assessment - adverse events, concurrent medication and protocol compliance were assessed. Body weight, height, waist and hip circumference, systolic and diastolic blood pressure was measured.
b : Blood samples - to allow measurement of biochemical parameters.
c : NASH severity was estimated by computed tomography.

PATIENT VISIT SCHEDULE

Week 0	Week 3	Week 6	Week 0	Week 0
a,b,c	a	a	a	a,b,c

2. Glutathione measurements
Plasma glutathione levels were determined by using 5,5'-dithiobisnitrobenzoic acid (DTNB) reagent[3]

3. Total antioxidant capacity (TAC) assay
TAC was determined by a modified protocol for 2,2'-azinobis-(3-ethylbenzo)-thiazoline-6-sulfonic acid (ABTS) decolorization method[4].

4. Malondialdehyde assay
MDA, the end product of lipid peroxidation, was determined by a modified thiobarbituric acid (TBA) procedure[5].

5. Protein hydroperoxide assay
Protein hydroperoxide (PrOOH) was assayed with a modified protocol using ferrous oxidation of xylenol orange (FOX)[6].

6. Erythrocyte haemolysis assay
This protocol was modified from the H_2O_2 induced erythrocyte fragility test[7].

7. Statistical Analysis
All statistical analyses of study data were performed using SPSS statistical software package, version 10.01 (SPSS, Chicago. IL, USA).

RESULTS

The results of the biochemical parameters measured in blood taken from NASH patients before and after supplementation with undenatured cysteine-rich whey protein isolate are tabulated in Table 1.
The assessment of the clinical results of this study are presented in Poster 925.

Table 1: **Biochemical parameters; GSH, TAC, MDA, PrOOH, and Erythrocyte Haemolysis in 38 subjects with non-alcoholic steatohepatits (NASH)**

Parameters	Pre-supplement Mean ± S.E. (range)	Post-supplement Mean ± S.E. (range)
Glutathione (mg/dl)	52.95 ± 11.82 (32-75)	67.80 ± 11.75* (41-85)
Total antioxidant activity (TAC) (mmol/L)	1.26 ± 0.10 (0.50-2.50)	2.03 ± 0.09 * (0.75-3.50)
Malondialdehyde (MDA) (micromol/L)	22.52 ± 0.78 (10-35)	18.10 ± 0.69* (0-28)
Protein hydroperoxide (PrOOH) (micromol/L)	2.28 ± 0.16 (0.75-10.00)	1.68 ± 0.25 * (0.50-7.50)
Erythrocyte Haemolysis (%)	26.47 ± 0.75 (18-35)	24.78 ± 0.61 * (9-35)

(p <0.05 compared to pre-supplement data in each parameter)*

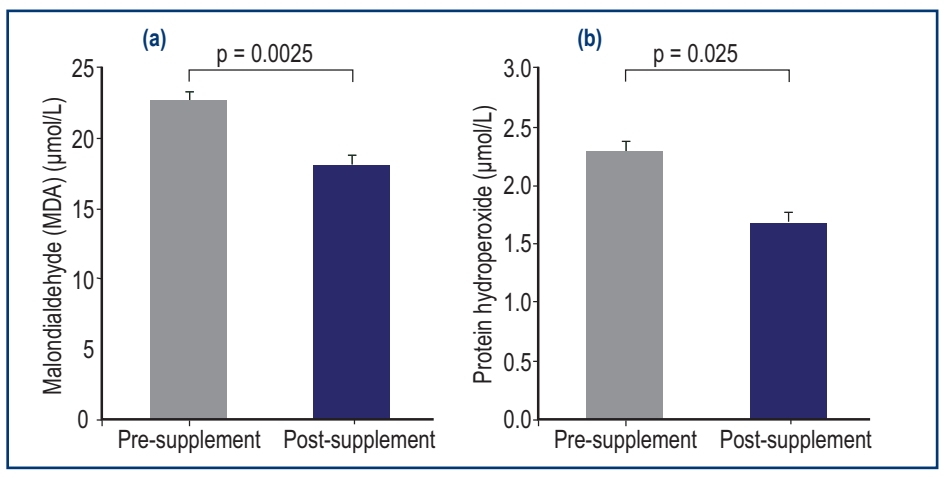

Figure 2: (a) Malondialdehyde (MDA) and (b) Protein hydroperoxide (PrOOH) levels before and after supplementation with undenatured cysteine rich whey protein isolate in 38 patients with non-alcoholic steatohepatitis (NASH). Error bars show the mean with standard error (S.E.).

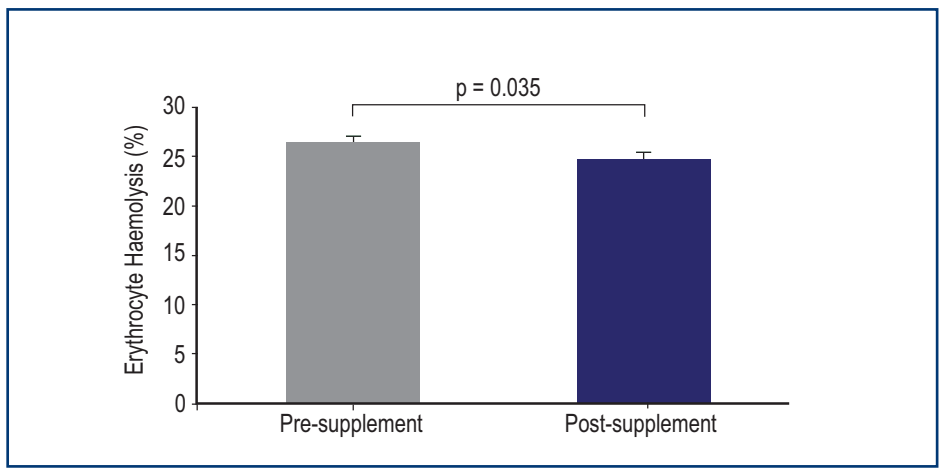

Figure 3: Percentage of haemolysis before and after supplementation with undenatured cysteine rich whey protein isolate in 38 patients with non-alcoholic steatohepatitis (NASH). Error bars show the mean with standard error (S.E.).

DISCUSSION

Oxidative stress has long been recognized as a key mechanism responsible for liver damage and disease progression in NAFLD[8]. This study showed a high level of oxidative stress in all 38 patients with non-alcoholic steatohepatitis (NASH) before supplementation, elevated levels of lipid peroxidation and protein oxidation in blood and low levels of glutathione and total antioxidant capacity (TAC) were observed (Table 1). After supplementation with undenatured cysteine rich whey protein isolate, the results showed that GSH and TAC were increased significantly (see Figure 1) whereas the lipid and protein oxidation were reduced significantly (see Figure 2).

Furthermore, the results of this study showed the potential for supplementation with cysteine rich whey protein isolate to reduce red cell haemolysis (see Figure 3). This effect may be mediated by inhibition of hydrogen peroxide production or scavenged lipid or protein hydroperoxide. A previous report showed that high lipid levels in plasma were related to a high percentage of erythrocyte haemolysis[9].

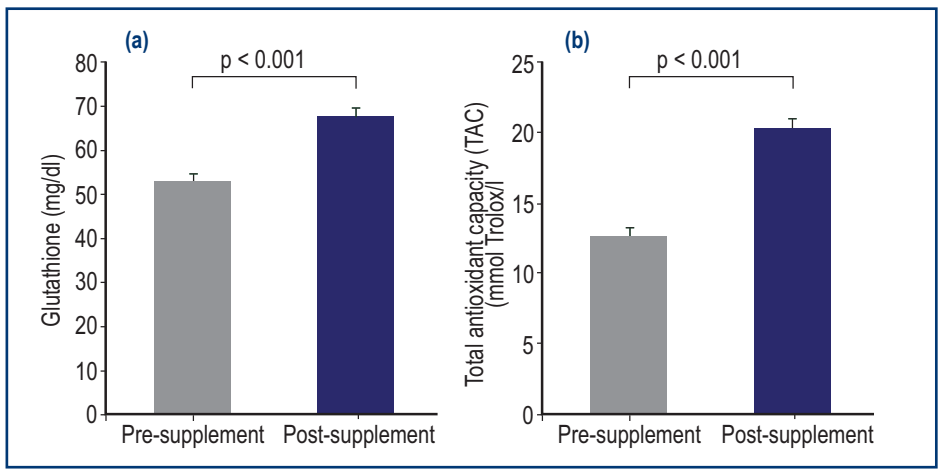

Figure 1: (a) Glutathione (GSH) and (b) total antioxidant capacity (TAC) levels before and after supplementation with undenatured cysteine rich whey protein isolate in 38 patients with non-alcoholic steatohepatitis (NASH). Error bars show the mean with standard error (S.E.).

CONCLUSIONS

It seems reasonable to conclude that the beneficial effects to NASH patients from supplementation with this protein result from an increase in the endogenous anti-oxidant glutathione due to increased availability of cysteine and confirm the hypothesis that oxidative stress is a significant factor in this disease.

Supplementation with this protein might well find other applications for patients where oxidative stress and pathology of glutathione deficiency are implicated.

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