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ORIGINAL ARTICLE

Whey protein, as exclusively nitrogen source, controls food intake and promotes glutathione antioxidant protection in Sprague-Dawley rats

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Abstract The inclusion of whey protein concentrates (WPC) in the diet can lead to a decrease in food intake. Considering that excessive food intake and weight gain are correlated with increased oxidative stress and other risk factors, the anorectic action of WPC may have important clinical implications. The aims of the current study were to verify the effects of WPC in comparison with those of casein on food intake, weight, and oxidized glutathione (GSSG) and total glutathione (GSH) concentrations in the blood and liver with or without oxidative stress induced by oral carbon tetrachloride intoxication. Male Sprague-Dawley rats were fed a balanced liquid diet for 3 weeks. Half of the rats received WPC (group P), while the control group received casein (group C). Group P rats ate significantly less than group C rats ($p < 0.0001$), and their weights decreased significantly. After carbon tetrachloride intoxication, there was a significant increase in GSH in rats of group P compared with the levels in rats of group C both in the liver (GSH group P 4,994 ± 652.6, group C 2,196 ± 323.2 nmol/mg, $p < 0.01$) and in the blood (GSH group P 1,368 ± 69.56, group C 1,088 ± 48.35 nmol/ml, $p < 0.05$). These findings indicate that WPC is effective in reducing food intake and preventing weight gain, and it may also play a protective role against oxidative stress by increasing glutathione synthesis in the liver.

Keywords Whey protein · Glutathione · Appetite inhibition · Energy intake · Antioxidant defences · Oxidative stress · Acute carbon tetrachloride intoxication

Introduction

According to the literature, the most satiating nutrient is protein, while the degree of appetite suppression depends on the source of protein and composition of the meal (as far as digestion and absorption are concerned) [1–3]. The release and subsequent absorption of amino acids in the bowel depends on, among other things, the kind of proteins in the diet. In particular, it is very important to distinguish between (so-called) "slow" and "fast" dietary proteins [4, 5]. Indeed, the duration of digestion of protein and the absorption of amino acids may be relevant factors influencing the sense of satiety [3].

Among the proteins which are currently used as protein sources, whey protein concentrates (WPC) are a heterogeneous group of proteins (including beta-lactoglobulin, alpha-lactalbumin, serum albumin and immunoglobulins) obtained from milk after precipitation of the caseins. In vitro studies with WPC have shown anticancer activity including the ability to inhibit cancer cell growth and an antimutagenic effect [6–8], while in experimental tumour animal models, WPC appear to be able to

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reduce the tumour burden [9, 7]. Moreover, WPC show immunoenhancing properties due to glutathione synthesis by lymphocytes and the liver, which may represent a possible adjuvant therapeutic tool in diseases associated with oxidative stress [10, 11].

In a study of amino acid enteric absorption in healthy adult subjects following consumption of whey protein or casein, consumption of whey protein was associated with significantly greater protein synthesis, with no difference in protein catabolism, compared to consumption of casein [4]. Recently it has been demonstrated in both human and animal studies that a whey protein preload, unlike a casein preload, is able to reduce food intake in normal subjects [3, 12]. Considering that there is a linear correlation between high food intake and oxidative stress, and an inverse correlation between the amount of food taken in and survival in rats [13, 14], the anorexic action of whey protein may be very valuable clinically. As a matter of fact, obesity itself, even in the absence of other risk factors such as smoking, diabetes mellitus, hyperlipidaemia, or renal or liver disease, causes lipid peroxidation and decreased activity of cytoprotective enzymes [15]. This enhanced oxidative stress also underlies the pathophysiology of hepatic steatosis [14].

Moreover, many animal and human studies have shown a correlation between ageing and increased oxidative stress [16], while an altered glutathione status has been reported under conditions of chronic or acute oxidative challenge related to both normal and pathological states including sustained exercise [11]. Recently, Mariotti et al. [17] have demonstrated that acute ingestion of dietary proteins improves liver glutathione levels after exercise in rats in a dose-dependent relationship with cysteine content. In particular, liver glutathione levels after exercise were clearly linked to the amount of cysteine in the last meal, with a curvilinear dose-dependent relationship [17]. Moreover, virtually maximal stimulation is reported to be attained with dietary proteins that are rich in cysteine, such as α-lactalbumin [17].

Therefore, it could be very interesting to evaluate the possible benefits of a reduction in food intake and body weight induced by WPC, which are particularly rich in cysteine, and of their specific antioxidative stress properties, during acute oxidative stress. To this end, carbon tetrachloride (CCl_4) was chosen as the agent to induce acute oxidative stress. CCl_4 is a lipid-soluble potent hepatotoxic substance, whose toxicity has been suggested to be due to the generation of free radicals leading to membrane lipoperoxidation [18–20]. Moreover, cyclooxygenase (COX) catalyses prostaglandin formation from arachidonic acid and its involvement in inflammation is well known. Following CCl_4 treatment, the nonenzymatically and enzymatically catalysed formation of eicosanoids, namely the isoprostanes and prostaglandins F(2α), is enhanced [21].

The primary end-point of this experimental study was to evaluate the effects of medium-term (3 weeks) oral intake of food with different sources of protein (WPC or casein) on food intake and oxidized glutathione (GSSG) and total glutathione (GSH) concentrations in the blood and liver of rats. The secondary end-point was to assess the effects of long-term oral intake of WPC or casein on food intake, and on GSSG and GSH concentrations in the blood and liver after induction of acute oxidative stress by oral CCl_4 intoxication, under strictly monitored and controlled conditions.

Materials and methods

Animals

Male Sprague-Dawley rats (n = 36, age 8 weeks, mean body weight 200 ± 25 g) were obtained from Harlan Italy (San Pietro al Natisone, Udine). The rats were housed in proper wire cages and were maintained in an air-conditioned environment at a temperature of 22°C and 55% humidity, under a 12-h dark/light cycle, according to current official good animal care rules (N. 116/92). The animals were subdivided equally into two groups of 18 rats: control group (group C, casein diet) and group P (whey protein diet). The animals were housed in single cages with ad libitum access to food. By using feeding bottles containing the liquid diet in excess, it was possible, by subtraction, to verify how much food they had consumed. All animals were kept on these special diets for 3 weeks. Food intake was monitored daily and weight weekly.

Diets

The animals were fed a balanced liquid diet for rats (Lieber-De Carli type [22]; Laboratori Piccioni, Gessate, Italy) containing 20% protein, 18% lipids, 62% carbohydrate, vitamins, and micronutrients. Choline, cysteine, minerals, and vitamins were added as recommended in the AIN-93 rodent diet [23]. The group C diet consisted of the balanced liquid diet, without antioxidants, and with 19% of the total protein content as casein. The group P diet consisted of the same balanced liquid diet with 19% of the total protein content as ultrafiltered, nondenatured whey proteins (Prother; Advanced Food Research/AFR, Bollate, Milan, Italy) instead of casein. Minerals provided by the various protein types were balanced in all diets to a constant level as recommended in the AIN-93 diet [23].

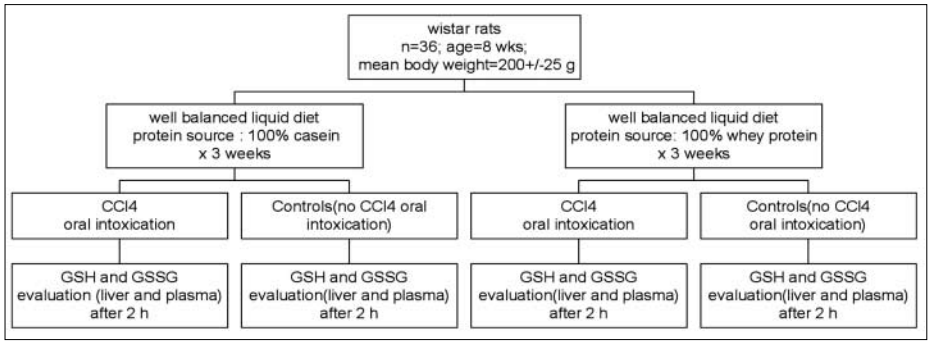

Fig. 1 Study design

Treatment

The study design is shown in Fig. 1. All the rats were fed for 3 weeks. Groups C and P were each then further divided into two subgroups (nine rats without intoxication and nine with CCl_4 intoxication), which were treated and then killed as described below. Blood was collected by heart puncture into heparinized tubes. An aliquot of whole blood was used for glutathione determination, and another aliquot was centrifuged to obtain plasma, which was immediately frozen, for the thiobarbituric acid test. Specimens of liver were removed and immediately utilized for determination of glutathione content. All experimental procedures using rats were approved by the University of Genoa Animal Ethics Committee.

The subgroups without intoxication received mineral oil only and were then killed after 2 h by intraperitoneal injection of sodium pentobarbital (65 mg/kg). The subgroups with CCl_4 intoxication received a single dose of CCl_4 (250 μl/100 g weight as a 50% solution with mineral oil) and were killed after 2 h by intraperitoneal injection of sodium pentobarbital (65 mg/kg). CCl_4 is an alcan-alogene, which is metabolized in the endoplasmic reticulum producing reactive oxygen species (ROS).

Blood and liver glutathione analysis

In order to calculate the GSSG/GSH ratio as accurately as possible, we used both the method of Fariss and Reed [24], which enables evaluation of GSH, and the method of Asensi et al. [6], which yields very reliable values for GSSG. However, the method of Fariss and Reed gives reliable results for GSH, but does not allow for in vitro or casual GSH oxidation to GSSG which would increase the GSSG value artificially. On the other hand, the method of Asensi et al. allows for GSH oxidation, but does not enable evaluation of the reduced form of glutathione. Briefly, liver specimens for GSH evaluation were homogenized and the GSH was precipitated with perchloric acid (PCA) (10% final concentration), while in the aliquots of whole blood GSH was directly precipitated with PCA. The thiol groups were then blocked with iodoacetic acid at alkaline pH. Analytes were then converted to 2,4-dinitrophenyl derivatives with 1% 1-fluoro-2,4-dinitrobenzene (FDNB) at 4°C in the dark overnight [24]. In the liver and blood specimens used for GSSG evaluation, the thiol groups were immediately blocked with *N*-ethylmaleimide (20 mM) in PCA (6%). After precipitation and alkylation, derivatization was performed with 1% FDNB [6].

Quantitative determination of derivatized analytes was performed by HPLC. The HPLC system was equipped with an NH_2 Spherisorb column and a UV detector set at 365 nm, and the flow rate was 1.5 ml/min. The mobile phase was maintained at 80% A (80% methanol) and 20% B (0.5 M sodium acetate in 64% methanol) for 5 min, followed by a 10-min linear gradient to 1% A and 99% B, and the mobile phase was then maintained at 99% B until GSSG eluted [24]. Total GSH content was evaluated in the chromatograms obtained by the method of Fariss and Reed as the sum of GSH and 2GSSG, as suggested by Furukawa et al. [25], and expressed in GSH equivalents [26, 27].

Statistics

Statistical analyses were performed using SPSS 10.0 for Windows (SPSS, Chicago, IL). The statistical analysis included Student's *t*-test (with Welch's correction when necessary), ANOVA and linear regression. *P* values <0.05 were considered statistically significant. All data are expressed as means ± SEM.

Results

Energy intake and weight gain

The rats of group C regularly ate almost the entire amount of food given (97.25 ± 0.83%). In contrast, rats of group P spontaneously left considerable amounts of the food given, eating only 67.42 ± 2.20%. This difference was highly significant ($p < 0.0001$; Table 1). The

Table 1 Percentage of available food eaten, body weight change, and the body weight expressed as a percentage of the initial weight of rats fed an isocaloric isonitrogen diet containing WPC (group P) or casein (group C) as the source of protein. There was a significant difference between groups P and C after 3 weeks ($p < 0.001$, ANOVA). Values are means ± SEM

	Group C ($n = 18$)	Group P ($n = 18$)	p value
Food eaten (%)	97.25 ± 0.83	67.42 ± 2.20	< 0.0001
Weight change (g)	+35.92 ± 3.98	−6.33 ± 10.34	0.0009
Percentage of initial weight (%)	+12.37 ± 1.3	−1.7 ± 3.1	0.0009

Table 2 Total GSH and GSSG in rats killed with oral CCl_4 intoxication or without CCl_4 intoxication following 3 weeks receiving an isocaloric isonitrogen diet with casein or WPC. GSH and GSSG are significantly higher in the liver and blood of rats of group P after CCl_4 intoxication. Values are means ± SEM

	Group C		Group P	
	Without CCl_4 ($n = 9$)	With CCl_4 ($n = 9$)	Without CCl_4 ($n = 9$)	With CCl_4 ($n = 9$)
Liver				
Total GSH (nEq/g)	2043 ± 258.0	2196 ± 323.2	3250 ± 256[a]	4994 ± 652.6[a]
GSSG (nmol/g)	30.73 ± 5.399	77.57 ± 10.22[e]	27.95 ± 5.250	231.1 ± 90.65[b]
Blood				
Total GSH (nEq/ml)	1528 ± 86.36[f]	1088 ± 48.35	1188 ± 40.04	1368 ± 69.56[c]
GSSG (nmol/ml)	6.388 ± 1.039	9.227 ± 1.629	7.565 ± 0.8313	13.18 ± 3.006[d]

[a] $p < 0.001$ *vs.* group C without CCl_4; $p < 0.01$ *vs.* group C with CCl_4; $p < 0.001$ *vs.* group P without CCl_4
[b] $p < 0.05$ *vs.* group C without CCl_4 and *vs.* group C with CCl_4
[c] $p < 0.001$ *vs.* group C without CCl_4; $p < 0.01$ *vs.* group C with CCl_4; $p < 0.001$ *vs.* group P without CCl_4
[d] $p < 0.05$ *vs.* group C without CCl_4; $p < 0.01$ *vs.* group P without CCl_4
[e] $p < 0.01$ *vs.* group C without CCl_4
[f] $p < 0.01$ *vs.* group C with CCl_4 and *vs.* group P without CCl_4

rats of group C grew regularly, gaining a mean of 35.92 ± 3.98 g (12.37 ± 1.3% of the initial weight) during the 3 weeks of treatment. Among the rats of group P, some gained weight and some lost weight, and the mean weight change was negative (−6.33 ± 10.34 g, i.e. −1.7 ± 3.1% of the initial weight). Again, the difference was highly significant ($p = 0.0009$; Table 1). There was a significant linear correlation, as expected, between the percentage of food eaten and weight change ($r^2 = 0.6122$; $p < 0.0001$).

Blood and liver glutathione concentration after casein or whey protein consumption

The total amount of blood GSH was significantly reduced in rats of group P compared with those of group C (group P 1,188 ± 40.04, group C 1,528 ± 86.36 nmol/ml; $p = 0.0017$), but there was no significant difference between the groups for blood GSSG (group P 7.56 ± 0.83, group C 6.39 ± 1.04 nmol/ml; $p = 0.3860$; Table 2, Fig. 2). The total amount of liver GSH was significantly increased in rats of group P compared with those of group C (group P 3250 ± 256, group C 2043 ± 258.0 nEq/g protein; $p = 0.0031$), but there was no significant difference between the groups for liver GSSG (group P 7.565 ± 0.8313, group C 6.388 ± 1.039 nmol/g protein; $p = 0.6555$).

There was a significant negative linear correlation between total liver GSH content and weight change ($r^2 = 0.2690$; $p = 0.0112$). There were significant positive correlations between blood GSH and the percentage of food eaten ($r^2 = 0.4296$; $p = 0.0005$) and weight change ($r^2 = 0.2102$; $p = 0.0242$).

Blood and liver glutathione concentrations after casein and whey protein consumption following CCL_4 intoxication

After CCl_4 intoxication there was a dramatic increase in both GSSG and GSH in rats of group P compared with the levels in rats of group C both in the liver (GSH group P 4,994 ± 652.6, group C 2,196 ± 323.2 nmol/mg, $p = 0.011$; GSSG group P 231.1 ± 90.65, group C 77.57 ± 10.22 nmol/mg, $p < 0.05$) and in the blood (GSH group P 1,368 ± 69.56, group C 1,088 ± 48.35 nmol/ml, $p < 0.079$; GSSG group P 13.18 ± 3.006, group C 9.227 ± 1.629 nmol/ml, p = NS; Table 2, Fig. 2).

Discussion

Anorectic effect of WPC

Among the macronutrients, proteins are considered the most satiating dietary component. Their anorectic effect

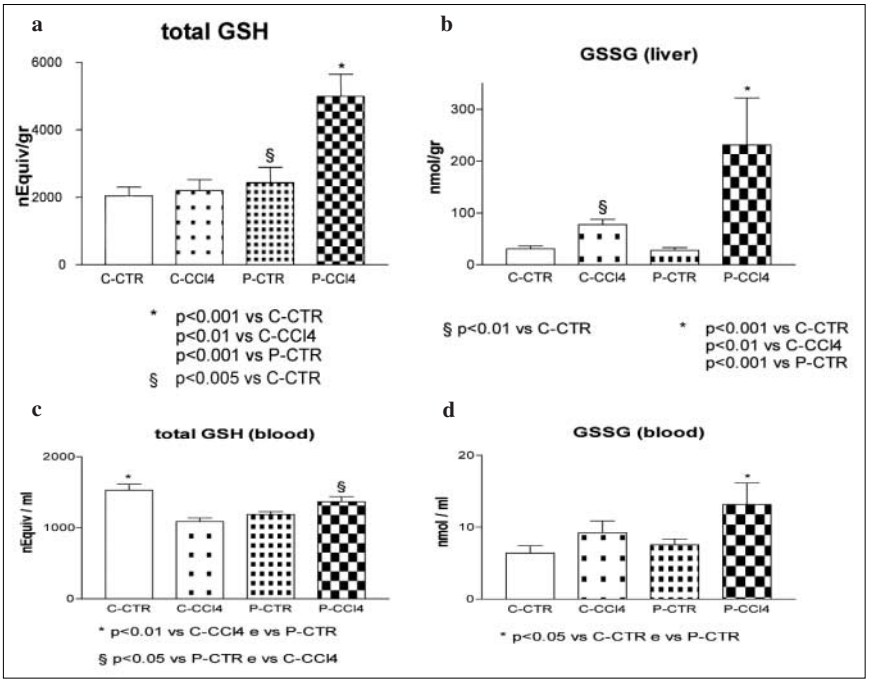

Fig. 2 Concentrations of GSH in the liver (**a**) and blood (**c**) and GSSG in the liver (**b**) and blood (**d**) from rats killed with or without oral CCl_4 intoxication following 3 weeks receiving an isocaloric isonitrogen diet with casein or WPC. GSH and GSSG are significantly higher after CCl_4 intoxication in the liver (**a**, **b**) and blood (**c**, **d**) of rats receiving WPC (group P). The data are presented as means ± SEM (*C-CTR* group C without CCl_4 intoxication, *C-CCl4* group C with CCl_4 intoxication, *P-CTR* group P without CCl_4 intoxication, *P-CCl4* group P with CCl_4 intoxication)

on appetite depends on their source, and the different ways they are digested and absorbed as amino acids from the gut.

The interaction of nutrients with intestinal receptors regulates both gastric emptying and appetite as well as stimulating the release of gastrointestinal hormones, including cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) [28]. According to Boirie et al., we can distinguish "slow" from "fast" proteins by the rate of digestion and absorption from the gut [4]. The authors studied the effects of two milk proteins, casein and WPC, on postprandial whole-body protein metabolism in humans. They combined oral and intravenous administration of ^{13}C-leucine-labelled and unlabelled specific proteins using dual tracer methodology. When WPC was administered to adult patients, a high, rapid and transient peak in plasma amino acids was observed. This event was associated with a significant increase (+68%) in protein synthesis, with no change in protein catabolism. On the contrary, after casein administration, the plasma amino acid surge proved lower and slower, but more prolonged. As a consequence, the protein synthesis was only moderately increased (+31%), whereas a marked inhibition of protein breakdown (i.e. leucine oxidation) was noted, and in particular leucine intake was identical in both meals [4]. WPC, therefore, could be defined as a fast protein, and casein as a slow protein.

Overall, the different behaviour of these two proteins probably reflects the different emptying times from the stomach into the duodenum, which is much faster for WPC, causing a rapid influx of amino acids, which are ready to use for protein synthesis [3]. In addition, it must be kept in mind that, although the percentage of amino acids able to stimulate insulin secretion is similar in both proteins, WPC possesses a higher proportion of branched-chain amino acids, which permit a synergistic effect with insulin on protein metabolism (i.e. anabolism). On the other hand, the percentage of amino acids that stimulate glucagon secretion is lower in WPC than in casein, consequently blunting the catabolic action of this hormone [5]. The addition of carbohydrates and lipids to WPC and casein do not significantly modify the protein digestion rate [29].

The type of protein appears not only to influence the gastric emptying time, but also the degree of satiety. The "fast protein" WPC, in fact, not only induces a more rapid gastric emptying than the "slow" protein casein, but also provides a greater subjective feeling of satiety, in response to the whey test meal ($p < 0.05$) [3]. Moreover, in the same study, plasma CCK was increased by 60% (iAUC, $p < 0.005$) and GLP-1 by 65% (iAUC, $p < 0.05$) following WPC administration, compared with casein [3]. Similarly, Bowen et al. also reported a trend for lower ad libitum energy intake at lunch after a whey preload, but the results of this study were unfortunately not statistically significant [30].

These experimental data are confirmed by ours, in a rat model, suggesting that appetite can be partly suppressed by whey protein. According to the results of Bowen et al., whey protein inhibits appetite by means of a rapid postabsorptive increase in plasma amino acids, which stimulate the secretion of both CCK and GLP-1, as potential mediators of the increased satiety response. Moreover, it must be considered that the ad libitum

modality of food intake in the present experimental design demonstrates that whey protein inhibits appetite even when consumed not only as a preload, but also as a continuous administration together with carbohydrates and lipids. It could be argued that WPC could be used in very low calorie diets (VLCD) in substitution for a complete meal, providing a low-calorie/high-fibre intake.

Effect of WPC on blood and liver glutathione concentrations

Recent reports suggest that systemic oxidative stress correlates with BMI [13, 15]. It has been demonstrated that in obesity, elevated ROS appear to upregulate mRNA expression of NADPH oxidase, establishing a vicious cycle that increases oxidative stress in the blood [25].

It is well known that the two major barriers against ROS are located close to the mitochondria, where the oxidative process takes place. Glutathione (L-gamma-glutamyl-L-cysteinylglycine) can spontaneously, or with the help of peroxidase, deliver the hydrogen atoms necessary for the reduction of the radicals. The redox system is represented by GSH in the cells and by the albumin cysteine in the plasma. In both cases, the active element is the SH (thiol) group of cysteine which, when performing its antioxidant activity, is oxidized to cysteine or cysteine disulphide. The cysteine/cysteine ratio therefore defines the redox state which is the major determinant of optimal cell function.

Our data partially agree with those of other authors, according to whom, liver glutathione levels are similar following consumption of WPC and casein diets, because the methionine content of casein is sufficient to produce physiological levels of liver glutathione [7, 31], but differs when oxidative stress is provoked by CCl_4. The effect of the dietary source of glutathione is slight, and excess dietary protein or sulphur amino acids do not enhance the maximum hepatic glutathione amount beyond the normal physiological maximum level obtained with adequate dietary components [32–34].

The tripeptide glutathione is largely distributed inside the cells where it acts as a substrate for two classes of enzymes, the selenium-dependent glutathione peroxidase and a family of glutathione transferases, which catalyse detoxification by means of two different reactions: spontaneous and enzyme-associated. The involvement of glutathione in oxidative stress protection (in particular, against DNA damage) includes reduction by gamma-glutathione peroxidase of hydrogen peroxide, free radicals and ROS. Glutathione transferases catalyse the glutathione conjugation of electrophilic compounds biotransformed from xenobiotics (mutagens and carcinogens), which are thus easily eliminated from the body [27, 35].

The liver, a key organ for xenobiotic detoxification and elimination, is the major site of glutathione synthesis. Indeed, this organ has the unique and particular ability to convert the sulphur amino acid methionine to cysteine required for glutathione synthesis [27, 36]. The biosynthesis of glutathione is strictly dependent on the concentration of the precursor amino acids (glutamate, glycine, cysteine) and competes with albumin synthesis for the available cysteine [7, 32]. The kinetic characteristics expressed by the Km rate for amino acid activating enzyme (the rate-limiting enzyme for protein synthesis) is 0.003 mmol/l, whereas that for gamma glutamyl cysteine synthetase (the rate-limiting enzyme for GSH synthesis) it is 0.35 mmol/l. This means that the biosynthetic pathway for proteins works maximally at a concentration approximately 166-fold lower than for GSH synthesis, whose production is consequently impaired more than that of proteins at low availability of cysteine [32].

An important source of cysteine is whey protein, whose amino acid content is six times greater than that of casein. It could be argued that WPC allows a larger amount of cysteine to be available for GSH liver synthesis under conditions of oxidative stress, thus representing an important means of defence (Table 3). In obese patients, the levels of oxidative stress are remarkable, just as during ageing, when higher rates of membrane lipid peroxidation occur, along with DNA and protein oxidation [37–43]. In humans, a conspicuous prooxida-

Table 3 Amino acid percentage content of whey protein and casein protein sources used in the diet administered to rats in experiments comparing blood and liver total GSH and GSSG with and without CCl_4 intoxication

Amino acid	Whey protein	Casein
Essential		
Alanine	4.6	3.0
Arginine	2.8	3.6
Aspartame	6.8	7.1
Cysteine	2.9	0.5
Glycine	2.0	1.8
Glutamate	10.9	23.7
Histidine	1.9	2.9
Isoleucine	5.0	5.1
Leucine	12.3	9.2
Lysine	9.7	8.0
Methionine	2.0	2.9
Nonessential		
Phenylalanine	3.6	5.1
Proline	4.1	10.3
Serine	4.9	5.7
Threonine	5.3	4.3
Tryptophan	2.1	1.1
Tyrosine	3.5	5.6
Valine	4.8	6.6

tive shift in the plasma thiol/disulphide redox state has been documented between the third and the tenth decade, while the levels of hydrogen peroxide scavenging enzymes catalase and glutathione peroxidase inversely correlate with ageing [43, 44].

The increases in total and oxidized GSH to high concentration in the rats receiving WPC compared with those receiving casein could be explained by the fact that 2 hours passed between intake of CCl_4 and the rat being killed. This period allowed a faster way of utilizing the cysteine derived from WPC in the rats of group P, in accordance with the hypothesis that WPC are fast proteins. Therefore more glutathione could be synthesized following WPC intake than following casein intake.

Therefore, the use of whey protein ultrafiltrates could represent a very helpful tool in controlling appetite in the elderly who are prone to diabetes and oxidative stress. Moreover, according to the data of Dangin et al. [45], postprandial protein net gain induced by casein and whey protein differs with age: in young men, protein gain seems to be higher with casein, and in older men, protein gain is higher with whey protein. The strong, fast and transient hyperaminoacidaemia observed after whey protein intake, against the slower and more prolonged increase in plasma amino acids after casein intake, may also be a beneficial factor in the elderly to limit protein loss [45].

ROS-induced damage in diabetes could be prevented by dietary modulation of the plasma thiol/disulphide ratio, which may be an easy target for therapeutic intervention by oral *N*-acetylcysteine or other thiol compounds such as whey protein [8]. In particular, *N*-acetylcysteine protects against age-related decreases in oxidative phosphorylation in liver mitochondria [46].

The results of the present study suggest that WPC are effective in reducing food intake, and in preventing body weight gain, while they are also able to increase liver glutathione concentrations, exerting a protective effect against oxidative stress. WPC may thus play an important role in the management of obese patients with increased levels of oxidative stress, particularly in the supportive therapy of morbid obesity (metabolic syndrome) in the elderly. Nevertheless, it is mandatory to evaluate dietary integration with WPC in humans in the long-term.

Conflict of interest statement The authors declare that they have no conflict of interest related to the publication of this article.

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