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# Meal cysteine improves postprandial glucose control in rats fed a high-sucrose meal

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**Abstract**

Whey protein, particularly the α-lactalbumin fraction, are rich in cysteine (cys) and could therefore favor postprandial glucose homeostasis by a glutathione-mediated effect. This work investigates the effects of the ingestion of an α-lactalbumin-rich whey concentrate (α-LAC) during a high-sucrose (HS) meal on postprandial glucose homeostasis in healthy rats. In the first experiment, rats received an HS meal containing 14% protein, in which the protein source was either α-LAC ($HS_a$) or total milk proteins, alone ($HS_0$) or supplemented with 17 mg ($HS_1$) or 59 mg ($HS_2$) of *N*-acetylcysteine (NAC). This resulted in a total cys content 3.6-fold higher in the $HS_1$ and $HS_a$ meals and 12-fold higher in the $HS_2$ meal, when compared to the $HS_0$ meal. Postprandial parameters were monitored for 3 h after ingestion of the meal. The same measurements were performed on rats injected with 4 mmol/kg of buthionine sulfoximine (BSO), a specific inhibitor of glutathione synthesis. Increasing the meal's cys content dose-dependently reduced both postprandial glucose and insulin ($P$<.05). The inhibition of glutathione synthesis with BSO injection abrogated the beneficial effects of NAC supplementation on postprandial glucose response but did not affect those of α-LAC. These results show that (1) the substitution of α-LAC for total milk protein reduces glucose response, as does the addition of a cys donor to the meal, (2) but contrary to those of a simple cys donor, the beneficial effects of α-LAC are not entirely mediated by glutathione synthesis, suggesting additional mechanisms.
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*Keywords:* α-lactalbumin; Cysteine; *N*-acetylcysteine; Glutathione; Glucose homeostasis

## 1. Introduction

Whey protein has recently attracted a growing interest because of their promising nutritional properties associated with their high chemical score and good digestibility [1,2]. Compared to many protein sources, whey protein, particularly the α-lactalbumin fraction, are also rich in cysteine (cys), a precursor of the tripeptide glutathione [GSH (γ-glutamyl-cysteinyl-glycine)], which plays a key role in body redox status [3]. Several experimental and clinical studies have reported an improvement in GSH status in response to whey protein ingestion under acute or chronic conditions of oxidative stress [4,5].

A growing body of evidence suggests that GSH status could modulate glucose homeostasis. GSH depletion has been reported to decrease insulin sensitivity and impair insulin signaling in rats [6]. Conversely, intravenous GSH infusion has been shown to increase total body glucose disposal in healthy humans [7] and glucose-induced β cells insulin release in rats [8]. Because the postprandial increase in blood glucose has been shown to induce oxidative stress in diabetics and also in healthy subjects [9], the ingestion of cys-rich protein may help to maintain GSH status during the postprandial oxidative challenge, and this may result in a better postprandial glycemic regulation.

In the current study, we investigated in healthy rats the effects of the ingestion of an α-lactalbumin-rich whey concentrate (α-LAC) on postprandial glucose regulation during a high-sucrose (HS) meal, a condition that challenges the oxidative balance. To ascertain that the effects of α-LAC

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Table 1
Protein and amino acid composition of the experimental meals

| | $HS_0$ | $HS_1$ | $HS_2$ | $HS_a$ |
|---|---|---|---|---|
| *Protein composition*[a] *(g/100 g protein)* | | | | |
| Casein | 80 | 80 | 80 | 0 |
| β-Lactoglobulin | 11 | 11 | 11 | 15.2 |
| α-Lactalbumin | 4 | 4 | 4 | 59.8 |
| Serum albumin | 1 | 1 | 1 | 20 |
| *Amino acids content*[b] *(mg in the experimental 3-g meal)* | | | | |
| Isoleucine | 20.7 | 20.7 | 20.7 | 21.4 |
| Leucine | 37.5 | 37.5 | 37.5 | 42.8 |
| Valine | 27.5 | 27.5 | 27.5 | 18.9 |
| Methionine | 9.3 | 9.3 | 9.3 | 3.6 |
| Cysteine | 3.6 | 3.6 | 3.6 | 16.4 |
| *N*-acetylcysteine | 0 | 17.3 | 58.8 | 0 |
| Equivalent total cys content | 3.6 | 16.4 | 48.1 | 16.4 |

The protein sources were prepared from bovine milk and provided by Armor Protéines SAS.

[a] The protein compositions were determined by gel permeation chromatography on a TSK Gel® column.

[b] The amino acid compositions were estimated based on the protein composition.

were due to its high cys content, we compared the effects of α-LAC with those of *N*-acetylcysteine (NAC) supplementation, an effective cys donor [5]. Then, we examined the implication of GSH synthesis on the effects of α-LAC on glycemic regulation using a specific inhibitor of the rate-limiting step of GSH synthesis, buthionine sulfoximine (BSO) [10].

## 2. Method and materials

### 2.1. Experimental design

All experiments (Exp) fully complied with the guidelines of the French Committee for Animal Care. Male Wistar rats (Harlan, France), 6 weeks old and weighing 192±4 g at the beginning of each experiment, were adapted to the laboratory conditions for the first week, housed in individual cages at 22±2°C under a reverse light-dark cycle (lights on at 2100).

During Exp 1, the rats ($n$=40) were implanted with a soft catheter (Silastic, Perouse, France), fitted in the right jugular vein, which enabled the collection of large blood samples from conscious and unrestrained animals [11]. Rats were allowed a 1-week recovery and fed a standard AIN-93M diet [11]. During this week, they were adapted to semiliquid food (water/powder 1:1) and fed two meals per day, one meal between 09:00 and 09:15 (6 g of moistened diet) and one large meal in the afternoon to complete their daily energy intake. This feeding schedule ensured the prompt consumption of a calibrated meal in the morning. On the experimental day, rats were randomly assigned to a 6 g experimental meal. All meals were AIN-93M modified meals, isoenergetic and contained 2.1 g sucrose and 420 mg protein. The protein source was either α-LAC ($HS_a$) or total milk protein enriched with 17 mg NAC ($HS_1$), 59 mg NAC ($HS_2$) or not enriched ($HS_0$) (Table 1). After the usual 15 min, most rats had completed the meal, and those that had not finished by that time were fed the remaining food with a syringe. Blood samples were collected over the 3 h following the ingestion of the meal.

During Exp 2, the kinetics of the inhibitory activity of BSO (Sigma Aldrich, France) on liver glutamate–cys ligase (GCL), and the subsequent kinetics of liver and blood GSH status were analyzed. Sixteen rats were injected with 4-mmol/kg-body weight (BW) BSO sc (dissolved in 0.9% sodium chloride, with the aid of 0.1 N sodium hydroxide, final pH 8.5), the optimum dose for GCL inhibition [12], and killed immediately ($t$=0) or 1, 2 or 4 h after BSO injection (sodium pentobarbital, 30 mg/kg BW). Blood and liver samples were collected.

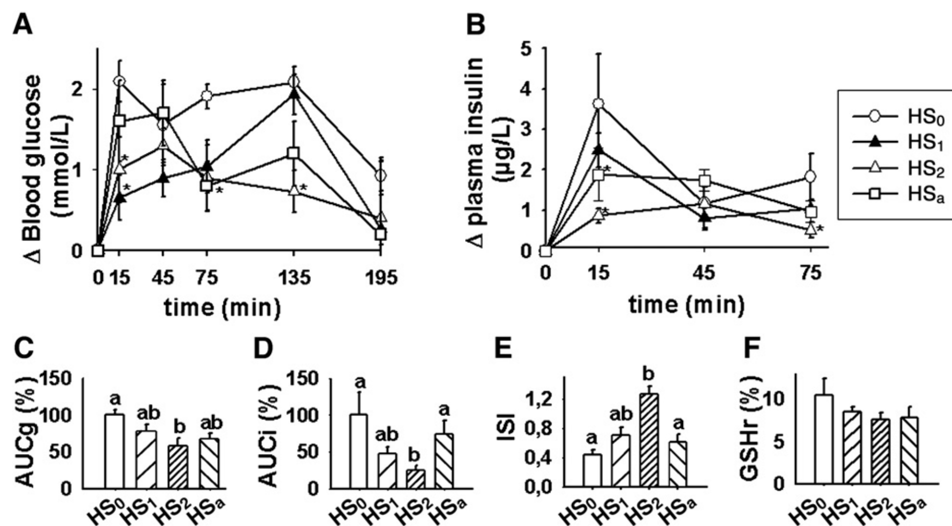


Fig. 1. Postprandial glucose (A), insulin (B), glucose AUC (C), insulin AUC (D), ISI (E) and GSHr at 195 min (F) following the ingestion of the $HS_0$, $HS_1$, $HS_2$ and $HS_a$ meals. Data are means±S.E.M. ($n$=10). *$P$<.05 vs. $HS_0$. Means not sharing a common superscript letter differ ($P$<.05). AUC values, calculated above baseline, are expressed as percentages of the mean in the control group ($HS_0$).

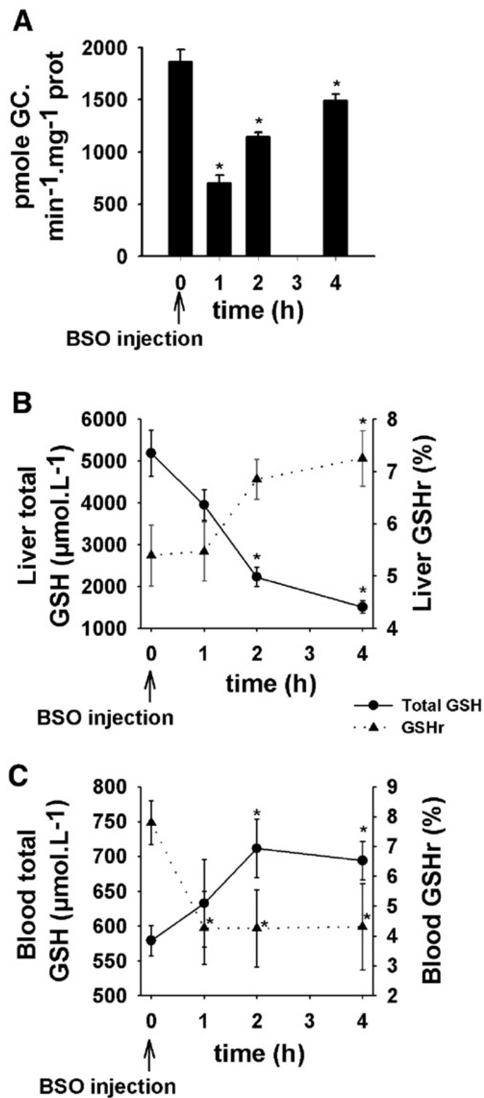


Fig. 2. Kinetics of the inhibitory activity of BSO on liver GCL activity (A) and subsequent evolution of liver (B) and blood (C) GSH status. Data are means±S.E.M. ($n$=4). *$P$<.05 vs. $t$=0.

During Exp 3, the protocol used in Exp 1 was repeated on a new set of 40 rats. On the experimental day, rats were injected with 4-mmol/kg BSO 1 h before the morning meal, the optimum time for GCL inhibition as determined during Exp 2 and fed their experimental meal as described in Exp 1. Blood samples were collected at time points indicated in Figs. 1–3.

### 2.2. Processing of blood and tissue samples and biochemical measurements

Blood samples were collected in pre-chilled tubes (10% EDTA in Trasylol, Bayer), centrifuged and the resulting plasma stored at −20°C until analysis. Blood glucose concentrations were measured using a refractometer (Glucometer, Bayer Diagnostics). Plasma insulin was detected using an ELISA assay (Mercodia Insulin ELISA, Biovalley). The insulin sensitivity index (ISI) was calculated using the following formula, derived from the Belfiore formula[2] [13]: 2/[(Insp·Glyp)+1]. Oxidized (GSSG) and total GSH concentrations were measured in samples homogenized in 5% trichloroacetic acid using a spectrophotometric method [14]. The GSSG/GSH ratio (GSHr) was used as an indicator of blood and tissue redox status [3]. GCL activity was assessed using a fluorimetric assay [15].

### 2.3. Statistical analysis

Data are given as mean values and standard errors (±S.E.M.). All kinetics were analyzed using mixed models for repeated-measures analysis (SAS Institute, Cary, NC). In a first model, time and meal were used as independent fixed factors. In an alternative model, the meal variable was subdivided into the protein source and the meal's cys content. Multiple comparisons were made using orthogonal contrasts under mixed models. The threshold of statistical significance was set at $P$<.05 and the statistical trend at $P$<.1.

## 3. Results

### 3.1. Effects of α-LAC and NAC supplementation on postprandial glucose control and GSH status

The nature of the meal significantly influenced postprandial blood glucose response (meal effect, $P$<.05; time effect, $P$<.05; time×meal effect, $P$<.1) (Fig. 1A). In the $HS_1$ and $HS_2$ groups, blood glucose levels at 15 min were lower than in the $HS_0$ group. Blood glucose returned to baseline earlier in the $HS_2$ and $HS_a$ groups than in the $HS_0$ group and was significantly lower at 75 or 135 min. Glucose area under the curve (AUC) values were significantly lower in $HS_2$ rats than in $HS_0$ rats ($P$<.05) and tended to be lower in $HS_1$ and $HS_a$ rats than in $HS_0$ rats ($P$<.1) (Fig. 1C). The effect of the meal's cys content on postprandial AUC values was significant. Posprandial insulin response was also influenced by the type of meal (meal effect, NS; time effect, $P$<.05; time×meal effect, $P$<.05). Insulin peaks at 15 min were lower in $HS_a$ and $HS_2$ rats than in $HS_0$ rats ($P$<.05) (Fig. 1B). Insulin AUC values were significantly lower in $HS_2$ rats than in $HS_0$ rats ($P$<.05) and tended to be lower in $HS_1$ rats ($P$<.1), while they did not differ between $HS_a$ and $HS_0$ rats (Fig. 1D). The effect of the meal's cys content on postprandial insulin AUC values was significant. The postprandial ISI was lower in $HS_2$ rats than in $HS_0$ rats ($P$<.05) (Fig. 1E) and was significantly affected by the meal's cys content, indicating higher postprandial insulin sensitivity. Blood GSH concentrations, measured at three time points (at 0, 75 and 195 min), did not vary after the meal ingestion (data not shown). The blood GSHr was not affected by the meal either but tended to be lower in $HS_2$ rats than in $HS_0$ rats 195 min after the meal ($P$<.1) (Fig. 1D).

[2] Insp, area under the insulin curve above baseline; Glyp, area under the glucose curve above baseline.

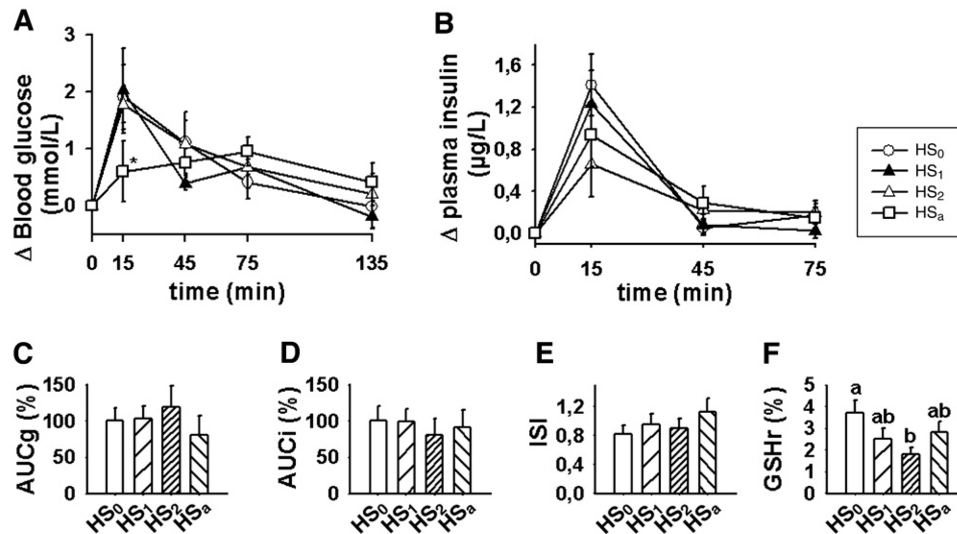


Fig. 3. Postprandial glucose (A), insulin (B), glucose AUC (C), insulin AUC (D), ISI (E) and GSHr at 195 min (F) following the ingestion of the $HS_0$, $HS_1$, $HS_2$ and $HS_a$ meals with inhibition of GSH synthesis. Data are means±S.E.M. ($n$=10). *$P$<.05 vs. $HS_0$. Means not sharing a common superscript letter differ ($P$<.05). AUC values, calculated above baseline, are expressed as percentages of the mean in the control group ($HS_0$).

### *3.2. Effects of BSO injection on liver GCL activity and liver and blood GSH concentrations*

One hour after the BSO injection, liver GCL activity decreased dramatically to 37% of the initial activity ($P$<.001), but tended to recover thereafter, reaching 80% of initial activity 4 h after the BSO injection (Fig. 2A). In the liver, total GSH concentrations declined persistently after the BSO injection ($P$<.05), leading to a significantly lower GSH level 2 h after the injection (Fig. 2B). In parallel, the liver GSHr was significantly increased 2 h after the BSO injection (Fig. 2B). Conversely, blood total GSH levels increased significantly between 0 and 2 h, and the blood GSHr significantly decreased between 0 and 1 h (Fig. 2C).

### *3.3. Effects of α-LAC and NAC supplementation on postprandial glucose control during the inhibition of GSH synthesis.*

Following the BSO injection, neither postprandial glucose and insulin responses, nor glucose or insulin AUC values or postprandial ISI differed between $HS_1$, $HS_2$ and $HS_0$ groups (Fig. 3). In contrast, postprandial blood glucose kinetics in $HS_a$ rats significantly differed from that of $HS_0$ rats. Blood glucose levels at 15 min were lower in $HS_a$ than in $HS_0$ rats ($P$<.05), and glucose AUC was lower in $HS_a$ rats than in $HS_0$ rats, although the difference did not reach statistical significance. The protein source affected blood glucose kinetics ($P$<.05) and tended to alter AUC values ($P$<.1). Postprandial insulin response did not significantly differ according to the meal. Blood GSH concentrations were measured at four time points (before the BSO injection and at 0, 75 and 135 min). After an initial 30% decrease during the first hour following the BSO injection, blood total GSH levels remained stable throughout the study period (data not shown). Blood GSHr significantly decreased over time in $HS_1$, $HS_a$ and $HS_2$ rats, but remained stable in the $HS_0$ rats, resulting in a lower GSHr in $HS_2$ rats than in $HS_0$ rats at 135 min ($P$<.05) (Fig. 3).

## 4. Discussion

The major outcome of this work was that increasing the cys content of an HS meal, either by substituting α-LAC for total milk protein or by adding a cys donor, reduced postprandial blood glucose response. However, although the effect of α-LAC can be mimicked by the addition of cys to the meal, our data suggest that the underlying mechanisms are not entirely mediated by GSH synthesis.

NAC supplementation, which was used to control the effect of meal cys, dose-dependently improved postprandial glucose regulation following a HS meal through a mechanism that involved GSH synthesis. The latter point is mainly supported by the observation that the substantial impacts of NAC supplementation on glucose control were abrogated after inhibition of GSH synthesis. When comparing the results of the third experiment with those of the first experiment, it was possible to speculate that BSO injection per se lowered postprandial blood glucose and insulin responses. Although such an effect cannot be completely ruled out, it is unlikely that this effect could account for the lack of difference between groups during the third experiment, in view of the substantial effect of NAC evidenced in the first experiment. Although we did not observe any modification of postprandial total GSH concentrations and GSHr corresponding to the meals, an effect of NAC supplementation on GSH turnover and hepatic efflux cannot be precluded, though these data were not obtainable in the current study. Indeed, liver and blood GSH pools are not interrelated simply. The liver is the main site of GSH

synthesis and storage, and hepatic GSH turnover and output are high in order to maintain extra-hepatic GSH levels, which are tightly regulated. Therefore, liver GSH levels are an indication of body GSH stores, whereas blood GSH values reflect GSH delivery to peripheral tissues [16,17].

Previous studies have suggested the beneficial effects of NAC on glucose homeostasis. NAC infusion has been reported to improve insulin sensitivity in healthy subjects during a hyperglycemic clamp [18], and more recently, the infusion of 0.35 mg/kg per minute NAC during a hyperglycaemic clamp was shown to lower insulin concentrations and C-peptide levels and prevent hyperglycemia-induced insulin resistance in rats [19]. In both studies, the authors concluded that NAC improved glucose regulation through a direct antioxidant effect. However, because both studies used intravenous NAC delivery, they are indeed more relevant to the direct effects of NAC than to its role as a cys donor when given orally. In the latter physiological context, NAC is known to undergo an extensive first-pass deacylation and has been reported to improve post-meal liver GSH status in rats [5]. It has been suggested that GSH per se could modulate glucose tolerance and favor postprandial peripheral glucose disposal through its contribution to the hepatic insulin sensitizing substance (HISS) [20]. According to the HISS hypothesis, the effect of insulin on glucose disposal after a meal is accounted for in approximately equal measures by the direct action of insulin and the action of HISS released from the liver acting on skeletal muscle [21]. Guarino et al. [20] suggested that HISS release depends upon the hepatic production of GSH. The positive effects of oral NAC on postprandial glycemic control could then arise from its contribution to HISS production.

Although previous studies demonstrated that GSH depletion impairs glycemic regulation [6], our study is the first to demonstrate that increasing a meal's cys content promotes postprandial glucose disposal by a GSH-mediated effect.

The current study also suggests that the effect of α-LAC on postprandial glucose regulation cannot be solely accounted for by its high cys content, even though a role for α-LAC as a cys donor and GSH precursor has been previously documented [5] and cannot be excluded from our results. The observation that the inhibition of GSH synthesis abrogated the effect of NAC but not that of α-LAC provides support for the involvement of an alternative mechanism. Previous studies have reported the ability of whey, when compared to other protein sources such as milk protein or cod protein, to reduce postprandial glycemic response in both healthy and type 2 diabetic subjects [22,23]. They concluded that specific amino acids, in particular leucine and isoleucine, which have been shown to increase skeletal muscle glucose uptake [24,25], were good candidates to explain this observation.

In conclusion, our results show that increasing the content of cys in the meal dose-dependently decrease postprandial glucose response, in a way similar to a cys donor or a cys-rich protein. However, in contrast to the effect of a simple cys donor, our data suggest that the effect of α-lactalbumin is not entirely mediated by GSH synthesis.

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