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Liver gamma-glutamyltranspeptidase activity and glutathione levels in lactating rats and pups: Effect of dietary protein quantity and feed intake

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Liver gamma-glutamyltranspeptidase (GGT—EC 2.3.2.2) activity and reduced glutathione (GSH) levels were measured on the delivery day (day 1) and in the middle of lactation (day 10) in rats. These rats were fed during gestation and up to the 10th day of lactation diets that varied in both protein quantity (25% versus 6% casein) and feeding level (ad libitum versus pair-fed 50% of ad libitum 25% casein intake). Just after birth (day 1 of lactation) there was a reduction ($P < 0.05$) of GGT activity in dams, returning to the highest nonpregnant values at day 10 of lactation, independent of diet. In their pups, the enzyme activity was high at day 1 but decreased sharply at day 10. Not only the effect of protein restriction (6% casein) but also the energy intake restriction (50% of ad libitum 25% casein intake) were significant ($P < 0.01$) at the delivery day, leading to an increase in the liver GGT activity in dams. A significant rise of the pups' GGT activity was promoted by the protein restriction at day 10. GSH was significantly reduced ($P < 0.01$) by protein deprivation at day 10 in both lactating dams and pups. This decrease was associated with alterations in the enzyme activity only for the pups. Although no clear interrelationship could be established between the liver GGT activity and the GSH content, these results indicate a special influence of protein malnutrition on both parameters at lactation. (J. Nutr. Biochem. 7:93–98, 1996.)

Keywords: lactation; protein-calorie malnutrition; food restriction; liver gamma-glutamyltranspeptidase; liver glutathione; protein deficiency

Introduction

Lactation is characterized by widespread changes in the metabolism of different tissues to ensure a sufficient supply of substrates to the mammary gland for milk production.[1] It is well established that starvation, as well as inadequate maternal intake of dietary protein and energy, impair lactation in humans and animals by exerting significant effects on the anabolic processes in mammary tissue such as protein synthesis.[2–6] It has also already been pointed out that short-term starvation in women and rats decreases the free amino acid concentration in milk through a reduction of both the amino acid supply and transportation in mammary gland.[7,8]

Amino acid translocation during the lactation cycle is affected by hormonal factors as well as by the activity of the pathway-initiating enzymes[9] with the most striking changes occurring at both parturition and the onset of lactation.[10] It has been shown[10–12] that not only the enzyme gamma-glutamyltranspeptidase (EC 2.3.2.2—gamma GT or GGT) but also glutathione (GSH) participate in this translocation process thereby supporting Meister's hypothesis which points out that GGT and GSH have an important function in both amino acid translocation and its uptake by the cells.[13,14] Likewise, GGT participates in GSH hydrolysis[13,15,16] and therefore it could mobilize and provide cys-

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teine from GSH pools to perform hepatic synthesis of proteins, such as albumin.[17,18] The GGT activity is associated with both a fast disappearance of the liver GSH content in starved animals and its recovery after refeeding, thus suggesting that not only the enzyme activity but also the GSH level depend on both the amount of food intake and the quality of the diet.[15] This quality includes the protein quantity[19–21] and/or quality and also the addition of sulfur-containing amino acids.[17,22,23] Although there has been much discussion about these physiological aspects of GGT, poor attention has been paid to its role in nutritional adaptation during the critical periods of life.

Considering the above-mentioned aspects of GGT and GSH participation in the lactation process, this study was undertaken to determine the effects of the protein and caloric restriction (during pregnancy and lactation) on both GGT activity and GSH levels in the liver.

Methods and materials

Animal care and diets

Virgin female Wistar rats, 136 to 138 days old and 214.1 ± 14.0 g, were mated with well-fed adult males of the same age and strain. They all came from the same in-house animal colony.

Just after breeding plugs were observed, dam females were randomly sorted into two sets of pregnant and lactating rats. Each set had three different groups, sorted according to the diet pattern (*Table 1*). The diets were formulated using two different protein levels, 25% and 6% (wt/wt) casein supplemented with methionine. These diets were made isoenergetic by balancing the dietary energy with starch. The third group was pair-fed to 50% of the consumption level of the corresponding groups fed with 25% casein ad libitum. Additional groups of nonpregnant and nonlactating rats were submitted to the same diets mentioned above. A total of 85 females were involved in the treatment group with each group having 6 to 8 animals. Every animal was housed individually in a galvanized iron cage with a controlled room temperature (23 to 25°C), 12 hr light–dark cycle, and ad libitum water.

At gestational day 20, the rats were transferred to plastic cages lined with wood shavings. The litters from the lactating groups were adjusted to eight pups at day 2 postdelivery. Body weight and food intake were controlled every day at 11:00 a.m. All adult female rats plus two pups (if any) were sacrificed by cardiac puncture under diethylether light anesthesia on either the delivery day or day 10 of lactation at 10:00 to 12:00 a.m. The livers were quickly removed, rinsed with cold physiological solution, and weighed prior to homogenization.

Table 1 Diet composition (g/kg of diet)*

Ingredients (g/kg)	Diets	
	25% casein	6% casein
Casein†	321.8	77.2
Day 1-methionine	3.0	3.0
Sucrose	100.0	100.0
Corn oil	80.0	80.0
Salt mixture‡	35.0	35.0
Vitamin mixture§	10.0	10.0
Fiber	10.0	10.0
Cornstarch	440.2	684.8

*Committee on Laboratory Animal Diet (1979). Control of diets laboratory animal experimentation. *Nutr. Abstr. Rev.* **49**, 413–419.
†From Tacrigy, S.A. Diets were analised for nitrogen by the micro-Kjeldahl method.
‡AIN-76. Mineral mixture based on the NAS recommended levels for rats (op.cit. 1).
§AIN-76. Vitamin mixture additioned of choline bitartrate (20 g/100 g mix) (op.cit. 1).

Tissue homogenate/liver extract preparation

A 4% (wt/vol) homogenate was prepared using a medium submitted to 10 up-and-down strokes of a motor-driven potter type homogenizer. The medium was prepared using 0.21 M mannitol, 0.07 M sucrose, and 0.1 mM EDTA and adjusted to pH 7.4.

Aliquots of this homogenate were used to determine the GGT activity. An equal volume of cold 1.2 N percloric acid containing 2 mM EDTA was added to this homogenate, and the mixture was centrifuged (15,000*g* for 10 min) to separate the acid supernatant. The acid supernatant was used on the same day for the estimation of glutathione.

Assays

The GGT (EC 2.3.2.2) activity of the liver homogenate was assayed by following the p-nitroaniline released from gamma-glutamyl-p-nitroanilide in the presence of glycylglycine as a glutamyl acceptor.[24] An enzyme-containing solution (0.1 mL of homogenate) was added to 1.0 mL of the substrate solution consisting of 50 mM Tris buffer (pH 8.25), 2 mM L-gamma-glutamyl-3-carboxi-4-nitroanilide, and 50 mM glycylglycine.[24]

Reduced glutathione was estimated by the Saville method.[25] The sulfhydryl content estimated by this procedure is not specific for reduced glutathione but essentially represents it since the content of other acid-soluble thiols in rat liver is very low.[17]

Chemicals

L-gamma-glutamyl-p-nitroanilide, glycylglycine, and reduced glutathione were from Sigma Chemical Co. (St. Louis, MO USA). The other chemicals were reagent grade.

Statistical analysis

Data are given as mean and standard error of the mean. The *t* statistic, with the significance level of $P < 0.05$, was used to test the difference between means.

Results

Tables 2 and *3* summarize changes in the GGT activity and the GSH level in the liver of dams at day 1 and day 10 of lactation, respectively.

The liver enzyme activity dropped by the end of pregnancy and the beginning of lactation (delivery day) independent of diet. Apart from this fall, it remained basically unalterated at day 10 of lactation and not significantly different when compared with the nonpregnant control values. The effects of both protein and caloric restriction were significant ($P < 0.01$) only at the delivery day. In 6% casein-fed dams and 25% casein-restricted ones, the liver GGT activity increased when compared with the 25% casein-fed rats (control group).

The liver GSH concentration was measured to check whether changes in it could or could not be correlated with changes in the GGT activity. The results showed that the

Table 2 Liver GGT activity and GSH concentration in pregnant and nonpregnant rats at day 1 of lactation*

Protein quantity	Feeding level†	GGT‡ (U/g of liver)	GGT‡ (U/g of protein)	GSH (μmol/g of liver)
Nonpregnant rats				
25% casein	AL	1.13 ± 0.40^a	3.12 ± 1.11^a	4.00 ± 0.53^a
6% casein	AL	1.16 ± 0.18^a	3.42 ± 0.54^a	3.97 ± 1.44^a
25% casein	PF 50%	1.25 ± 0.31^a	3.24 ± 0.81^a	3.60 ± 0.54^a
Pregnant rats				
25% casein	AL	0.10 ± 0.09^b	0.28 ± 0.24^b	3.06 ± 0.94^a
6% casein	AL	0.46 ± 0.09^c	1.37 ± 0.26^c	3.89 ± 1.12^a
25% casein	PF 50%	0.59 ± 0.19^c	1.64 ± 0.53^c	3.05 ± 0.83^a

*Means ± SEM; $n > 6$. Means within columns with common superscripts are not significantly different ($P > 0.05$).
†Abbreviations: AL, ad libitum; PF, 50% AL intake.
‡U = μmol of p-nitroaniline released/min/g of liver or protein at 37°C.

day 1 liver GSH level in the above-mentioned groups was not significantly altered, maintaining the same values as the nonpregnant rats fed the same diets. However, when compared with its first-day value, the liver GSH level was significantly increased on day 10, except for the protein-restricted group (6% casein) of lactating rats. In this case, the GSH concentration was significantly reduced ($P < 0.01$).

The possibility that differences in the diet account for the observed different GSH concentrations was examined. The analysis of consumption data, shown in *Table 4*, revealed that the 6% casein groups had both feed intakes (g/day) and energy intakes (kcal/day) similar to those of the control groups. Yet the protein intake (g/day) of the 6% casein groups was lower than that of the calorie-restricted ones, but both were significantly lower than that of the 25% casein control groups.

The results obtained for the pups are presented in *Table 5*. They show that the protein-restricted group of pups at day 10 of lactation, as had happened to their mothers, presented a liver GSH concentration significantly lower than both the control ($P < 0.05$) and the calorie-restricted groups ($P < 0.01$). Furthermore, the GSH concentration in the liver of pups rose on day 10 although only for the calorie-restricted group was it significantly higher ($P < 0.05$) than day 1 values. In contrast to the dams, however, the pups had significantly ($P < 0.01$) higher liver GGT activity at delivery day than at day 10, when they presented a significant increase of GGT with the protein restriction. Thus, just after birth there was an abnormal decrease of the liver GGT in dams while it increased sharply in pups. Only in the pups were the changes in the GGT caused by the protein deprivation accompanied by the corresponding GSH alterations.

Discussion

A significant induction of the liver GGT in the gestation/lactation cycles should be expected since there is a high requirement for amino acids for new tissues and/or milk protein biosynthesis in these periods. However, the results reported here have demonstrated a reduction of the liver GGT in dams following delivery, returning to nonpregnant levels at day 10 of lactation. These data confirmed those of Puente et al.[10]: studying the GGT activity in normal pregnant and lactating rat liver, a decline in the activity of the enzyme during pregnancy was observed. It reached its minimum value at the time of parturition, recovering the gestational level at day 5 of lactation and then progressing to the virgin level.

According to the results of Puente et al.[10] and other authors,[26,27] the GGT activity in the mammary gland, in contrast to the liver GGT activity, increases slowly through the pregnancy and sharply by its end, reaching maxi-

Table 3 Liver GGT activity and GSH concentration in lactating and nonlactating rats at day 10 of lactation*

Protein quantity	Feeding level†	GGT‡ (U/g of liver)	GGT‡ (U/g of protein)	GSH (μmol/g of liver)
Nonlactating rats				
25% casein	AL	0.80 ± 0.21^a	2.00 ± 0.56^a	6.88 ± 0.76^a
6% casein	AL	0.85 ± 0.26^a	2.27 ± 0.69^a	7.66 ± 2.57^a
25% casein	PF 50%	1.00 ± 0.17^a	2.59 ± 0.43^a	$5.90 \pm 0.78^{a,b}$
Lactating rats				
25% casein	AL	0.87 ± 0.39^a	2.33 ± 1.05^a	$6.03 \pm 0.50^{a,b}$
6% casein	AL	0.94 ± 0.23^a	2.66 ± 0.58^a	1.51 ± 0.14^c
25% casein	PF 50%	1.31 ± 0.35^a	3.59 ± 0.95^a	$6.51 \pm 0.87^{a,b}$

*Means ± SEM; $n > 6$. Means within columns with common superscripts are not significantly different ($P > 0.05$).
†Abbreviations: AL, ad libitum; PF, 50% AL intake.
‡U = μmol of p-nitroaniline released/min/g of liver or protein at 37°C.

Table 4 Food, energy, and protein consumption during the gestation and lactation periods

Protein quantity	Feeding level†	*n*	Food intake (g/day)	Protein intake (g/day)	Energy intake (kcal/day)
Nonpregnant rats					
25% casein	AL	8	11.89 ± 1.65[a]	2.97 ± 0.41[a]	49.92 ± 6.92[a]
6% casein	AL	8	11.93 ± 1.38[a]	0.72 ± 0.08[b]	50.11 ± 5.81[a]
25% casein	PF 50%	8	6.44 ± 0.64[b]	1.61 ± 0.16[c]	27.06 ± 2.70[b]
Pregnant rats					
25% casein	AL	8	16.26 ± 1.37[c]	4.06 ± 0.34[d]	68.28 ± 5.77[c]
6% casein	AL	6	14.50 ± 0.94[d]	0.87 ± 0.06[e]	60.90 ± 3.93[d]
25% casein	PF 50%	7	8.00 ± 0.00[e]	2.00 ± 0.00[f]	33.60 ± 0.00[e]
Nonlactating rats					
25% casein	AL	7	12.50 ± 1.29[a]	3.13 ± 0.32[a]	52.51 ± 5.42[a]
6% casein	AL	7	13.37 ± 1.26[a,d]	0.80 ± 0.08[b,e]	56.14 ± 5.30[a,d]
25% casein	PF 50%	7	6.30 ± 0.00[b]	1.58 ± 0.00[c]	26.46 ± 0.00[b]
Lactating rats					
25% casein	AL	7	15.95 ± 0.91[c]	3.99 ± 0.23[d]	66.92 ± 6.92[c]
6% casein	AL	6	14.76 ± 1.39[c,d]	0.89 ± 0.08[e]	61.99 ± 5.83[c,d]
25% casein	PF 50%	6	8.04 ± 0.09[e]	2.01 ± 0.02[f]	33.77 ± 0.38[e]

*Means ± SEM. Means within columns, with common superscripts are not significantly different ($P > 0.05$).
†Abbreviations: AL, ad libitum; PF, 50% AL intake.

mum activity during lactation,[26] with a peak at day 10, after which it starts to decline,[10] returning rapidly to basal level at weaning.[27] One explanation for these findings is the time course of changes in the protein synthesis in both the mammary gland and the liver. At the end of the pregnancy and as lactation proceeds, the protein absolute synthesis rate increases in the gland[5] but remains unchanged in the liver, as demonstrated by Jansen and Hunsaker[4] in casein-fed dams. The mammary growth takes place most intensively during late gestation and early lactation (day 4), reflecting a higher proportion of tissue protein and casein synthesis at this stage.[4,5] Thereafter it remains constant. Correspondingly the cysteine requirements may accompany these synthesis changes in the lactation cycle.

An additional possibility to justify why the liver activity of GGT is lower than that of the mammary gland might be the GGT significance to fetuses and neonatal animals as described by Tateishi et al.[28] In fetal livers they observed alterations similar to those described in mammary glands. The fetal liver GGT greatly increases at the end of gestation, with a peak at birth, but then it decreases rapidly within 1 week after birth, that is during the beginning of lactation. These authors[28] also observed an increasing level of GSH in pup livers that stopped just before birth. They concluded that this temporary pause in the GSH elevation seemed to be due to the marked increase of GGT activity thus suggesting the turnover of GSH is stimulated by induction on the enzyme activity.[28]

In the present work, the data for pups are in general accordance with the results of Tateishi et al.,[28] as mentioned above, that the GSH level is lower after birth than at day 10 of lactation, in contrast with higher values of the GGT activity just after birth and lower values at day 10. Instead no similar GGT–GSH relationship in dams after pregnancy (day 1) could be detected. Thus the dams maintained unchanged GSH levels at parturition despite significant reduction in the GGT activity. The most plausible justification for this is a reduction of the liver GSH resynthesis[19,20] caused by a decrease in the maternal concentration of essential amino acids (including cysteine),[28] probably due to the continuous amino acid transference to the fetuses or to other tissues, such as the mammary gland.

Table 5 Liver GGT activity and GSH concentration in pups at day 1 and day 10 of lactation*

Protein quantity	Feeding level†	*n*	GGT‡ (U/g of liver)	GGT‡ (U/g of protein)	GSH (μmol/g of liver)
Day 1 of lactation					
25% casein	AL	16	0.614 ± 0.196[a]	1.39 ± 0.44[a]	3.47 ± 0.88[a,b]
6% casein	AL	12	0.671 ± 0.120[a]	1.44 ± 0.26[a]	3.20 ± 0.83[a]
25% casein	PF 50%	14	0.690 ± 0.125[a]	1.40 ± 0.25[a]	3.05 ± 1.14[a]
Day 10 of lactation					
25% casein	AL	14	0.013 ± 0.008[b]	0.04 ± 0.02[b]	3.98 ± 0.38[b]
6% casein	AL	12	0.025 ± 0.004[c]	0.07 ± 0.00[c]	3.45 ± 0.63[a]
25% casein	PF 50%	12	0.006 ± 0.001[b]	0.02 ± 0.01[b]	6.07 ± 1.22[c]

*Means ± SEM. Means within columns, with common superscripts are not significantly different ($P > 0.05$).
†Abbreviations: AL, ad libitum; PF 50%, pair-fed 50% of AL intake.
‡U = μmol of p-nitroaniline released/min/g of liver or protein at 37°C.

Results that corroborate the referred hypothesis of the amino acid transference and reinforce the possibility of a poorer proportion of protein synthesis in the liver of dams than in the mammary gland have been presented by Araya et al.[26] They detected GSH responses to the elevation of the GGT activity in the mammary gland at the end of rat gestation.

Similarly, Tateishi et al.[28] demonstrated a rise in the liver activity of the enzymes involved in the GSH synthesis without any significant increase in the GSH level in pups. From these results, they concluded that there is a high probability that once synthesized the GSH would turn over rapidly, supporting the idea of the cysteine mobilization from the liver GSH to supply others plus emergency GSH or protein tissue synthesis.

Concerning the data about the hepatic GGT activity in the protein- and calorie-restricted groups of animals and similar to our previous study[29] that showed the effect of protein malnutrition on growing rats, we observed in this research a significant increase in the hepatic GGT activity on the postpartum day. This result supports the statement that the protein-deficient diets could not supply the cysteine requirements for protein synthesis during gestation thus triggering an increased cysteine mobilization from liver GSH pools, by the GGT increased activity,[15–17,22] and consequently lowering the GSH levels. Other studies on growing rats fed with a protein-poor diet have also shown a decrease in various tissues' GSH content that could be prevented by an addition of cysteine or methionine to the diet.[20–23]

Despite the significant increase in the hepatic GGT activity after delivery, this elevated GGT activity was not accompanied by corresponding GSH reduced levels. The maintenance of a sizable and stable GSH pool after rapid depletion of the labile GSH pool would justify these results.[17,23] It seems likely that the labile GSH pool was depleted earlier during the pregnancy period, since liver GGT increases with the protein deficiency,[26] preferentially maintaining the stable pool of GSH. This hypothesis was suggested by Cho et al.[22] in whose work a further decrease of GSH concentration in growing rats was not demonstrated after day 15 of a cysteine-deficient diet.

Although the GSH concentration has not been altered with the GGT increase at day 1, it was significantly decreased at day 10 in dams and pups submitted to a 6% casein diet. But those animals submitted to caloric restriction did not present reduction in the GSH concentration at day 10 of lactation. To justify the discrepancy between the effect of the protein and caloric restriction on GGT and GSH, the following arguments are presented:

- The 6% casein group had significantly the lowest ingestion of protein and the lowest GSH level. Moreover, it has been shown that the hepatic GSH concentration is sensitive to the protein content of the diet.[19–21]
- In protein restriction, the nonavailability of amino acids limits protein synthesis.[6,30] In this situation, low GSH levels might be the result of an attempt to increase the protein synthesis. Instead, in energy malnutrition, the levels of plasma amino acids are normal,[30] and the rate of protein synthesis is not directly affected,[6] justifying the maintainance of the GSH level.
- The liver fat infiltration, a characteristic feature only of protein deficiency but not of caloric deficiency, either leads to an impaired hepatic function, as observed by Atinmo et al.,[31] or depletes the GSH level in a protective attempt to avoid hepatocellular damage. A suggested explanation to support this argument is based on the critical role of GSH in the detoxification of the reactive intermediates of the oxidative metabolism, including those generated from endogenous molecules, drugs, and carcinogens.[32–34] When GSH depletion reaches certain threshold values in animals fed a low protein diet, these animals become more susceptible to tissue damage caused by free radical–generating compounds and carcinogenic xenobiotics.[32–34] It has also been suggested that the GGT activity may be mechanistically related to the neoplastic process[35]; the enzyme is considered the most common histochemical marker for altered foci in hepatocarcinogenesis.[35]

Because both the GGT-increased activity and the GSH depletion caused by protein malnutrition reported here may make the rats vulnerable not only to the potentially toxic effects of drugs or pharmacological substances but also to carcinogenic xenobiotics, the required follow-up to this present work is to test this relationship.

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