

# Lactase activity is under hormonal control in the intestine of adult rat

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**SUMMARY** In the adult rat, starvation during 48 hours led to a three fold increase of lactase specific activity in the intestinal brush border membranes. Thyroxine injection during the three days before death (0.5  $\mu\text{g/g}$  daily) inhibited the stimulation of lactase activity induced by starvation without modifying sucrase activity whereas hydrocortisone injections (25  $\mu\text{g/g}$  daily) or thyroidectomy did not modify the stimulatory effect of starvation on lactase activity. These results suggests a specific hormonal control of intestinal lactase activity in the rat.

Functional adaptation in the intestine of the rat occurs simultaneously with the dietary carbohydrate change taking place at weaning, leading to a marked decrease in lactase activity and stimulation of sucrase activity.<sup>1,2</sup>

It has been shown that lactase activity in the intestine of adult rat was enhanced by dietary lactose<sup>3-5</sup> but also by various carbohydrates.<sup>6-9</sup> No specific stimulation however, was obtained under these conditions as the increase of other brush border enzyme activities (sucrase, maltase) paralleled that of lactase activity. Up to the present, the only known condition that led to a specific stimulation of lactase activity was food deprivation.<sup>10,11</sup> As shown previously<sup>11</sup> food deprivation provoked a significant increase in the specific but also in the segmental activities of jejunal lactase during the first 24 hours of starvation, maximum stimulation being reached by 48 hours, whereas the activities of other brush border enzymes either were not modified or were decreased. In contrast, it has been shown that sucrase activity was specifically enhanced by dietary sucrose in the intestine of adult rats,<sup>12-14</sup> sucrose inducing neosynthesis of sucrase molecules.<sup>14,15</sup>

These results suggest that lactase and sucrase are regulated by different mechanisms and we have proposed,<sup>11</sup> that regulation of intestinal lactase activity might be under hormonal control in the adult. In this regard, the most serious candidates seem to be the thyroid hormones as various physiological and experimental conditions have indicated

that lactase activity was related to thyroid function, for example: (1) the normal decrease in lactase activity at weaning paralleled the rise in the serum concentration of thyroxine,<sup>16</sup> (2) jejunal lactase activity is decreased by thyroxine injections in non-starved adult rats,<sup>17,18</sup> (3) starvation, a condition which provoked stimulation of lactase activity, caused reduced circulating level of thyroxine in adult rat.<sup>19</sup>

In order to gain more insight into the mechanisms involved in the regulation of lactase activity in the intestine of adult rats we have investigated the effects of thyroxine or glucocorticoid injections and of thyroidectomy on the stimulation of lactase activity induced by starvation.

## Methods

### ANIMALS

Experiments were performed with adult Wistar rats weighing 300 to 350 g kept in individual metabolic cages. Rats were fed a commercial laboratory chow *ad libitum* before initiation of the experimentation. The animals were starved for 48 hours but received water *ad libitum* and were killed at the same time as normally fed rats. The proximal part of the jejunum was resected, it included the first 10 cm extending from the ligament of Treitz. The length was measured by stretching the intestine with a 3 g weight and the segment was flushed with a cold saline solution.

### HORMONAL TREATMENT

Rats were injected intraperitoneally with hydrocortisone (Sigma) diluted in 0.9% saline or with

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## Hormonal control of lactase activity

DL-thyroxine (Sigma) dissolved in 0.01 M NaOH. One injection was performed daily during the three days before death. Dosages were 25  $\mu\text{g}$  hydrocortisone or 0.5  $\mu\text{g}$  thyroxine per gram body weight.

Thyroidectomy was carried out one month before experiment initiation, the thyroid being dissected out under the dissecting microscope.

### EXPERIMENTAL TECHNIQUES

To obtain sequential cell release from villus tip to the crypt base, the intestinal segment was everted and submitted to successive incubations of 10 minutes at 37°C in phosphate-buffered saline (no  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) containing 1.5 mM EDTA and 0.5 mM dithiothreitol under agitation at 150 rpm in a waterbath shaker, the released cells were collected as described earlier.<sup>14 20</sup>

The cell fractions or the whole mucosa obtained after scraping the entire intestinal segment with a glass slide were homogenised in 50 mM mannitol, 2 mM Tris (pH 7.1). Brush border membranes were isolated from the whole mucosal homogenate as described previously.<sup>21 22</sup> Sucrase (EC 3.2.1.48) was assayed according to Messer and Dahlgvist.<sup>23</sup> Lactase (EC 3.2.1.23) was measured in the presence of p-chloromercuribenzoate according to Koldovsky *et al.*<sup>24</sup>

Enzyme activities were expressed as specific activities: milliunits (mU) per milligram of protein. One unit of activity equals 1  $\mu\text{mol}$  of product formed per minute at 37°C. Proteins were assayed according to the method of Lowry *et al.*<sup>25</sup> Student's *t* test was used to determine significant differences between treatment means.

## Results

### EVOLUTION OF LACTASE ACTIVITY ALONG THE VILLUS-CRYPT AXIS

When compared with the normally fed rats, those fasted during a 48 hour period showed an increase in lactase activity all along the villus axis, maximum stimulation occurred in the mature enterocytes from the villus tip (Fig. 1). A daily injection of thyroxine during the three days before death inhibited the stimulation of lactase activity induced by starvation, the pattern of lactase activity of the starved rat receiving thyroxine was then similar to the one observed for the fed rat (Fig. 1).

### MODIFICATIONS OF SUCRASE AND LACTASE SPECIFIC ACTIVITIES IN BRUSH BORDER MEMBRANES

The specific activity of sucrase was not modified by starvation in the various experimental conditions when compared with the activity measured in the

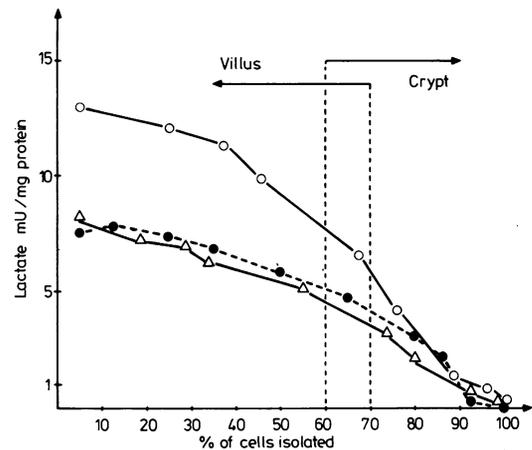


Fig. 1 Representative gradient of lactase activity in intestinal cells isolated along the villus-crypt axis in fed rat ( $\Delta$ - $\Delta$ ), in rats starved for 48 hours ( $\circ$ - $\circ$ ) and in rats starved for 48 hours and injected daily with thyroxine during the three days before death ( $\bullet$ - $\bullet$ ). Hundred per cent of cells isolated corresponds to the sum of the fractions expressed as protein. The percentage of cells isolated in each successive fraction was determined by the proportion of cell protein isolated in a given fraction.

fed animals. Nevertheless, sucrase activity was significantly reduced in the fed rats receiving hormonal injections during the three days. Thus, in the fed rats injected with thyroxine or with hydrocortisone the values for sucrase activity were respectively  $271.80 \pm 15.46$  mU/mg protein and  $238.27 \pm 17.15$  mU/mg protein and of  $330.94 \pm 12.68$  mU/mg protein in the fed controls ( $p < 0.001$ ). In contrast, the fed thyroidectomised animals exhibited similar values for sucrase activity as those of the controls (Fig. 2).

Concerning lactase, starvation during 48 hours provoked a significant stimulation of its specific activity from  $29.61 \pm 2.28$  mU/mg protein in the fed rat to  $79.80 \pm 7.76$  mU/mg protein in the starved animal. Neither hydrocortisone injections nor thyroidectomy did modify the stimulatory effect caused by starvation as the values for lactase activity obtained under these conditions were similar to those measured in the starved controls and were respectively of  $70.22 \pm 4.10$  mU/mg protein and  $73.17 \pm 7.29$  mU/mg protein. In contrast, thyroxine injections completely inhibited the stimulatory effect of starvation on lactase activity. It was noteworthy that, in the fed rats, lactase activity was significantly increased after thyroidectomy, values reaching  $40.67 \pm 2.70$  mU/mg protein ( $p < 0.001$ ) in the fed animal one month after thyroidectomy.

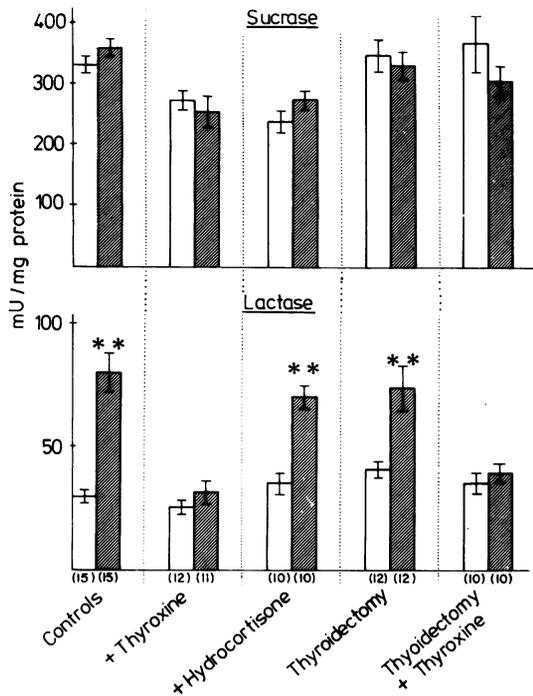


Fig. 2 Specific activities of intestinal sucrase and lactase in purified brush border membranes of fed rats ( $\square$ ) and of rats starved for 48 hours ( $\blacksquare$ ). Various experimental conditions are described under Methods. Results shown are the mean  $\pm$  SE. Number of animals is indicated in parentheses. \*\*\* $p < 0.001$  (one tailed Student's *t* test).

## Discussion

This study clearly showed the dependency of intestinal lactase activity on the thyroxine concentration in the rat. Indeed, it was only by lowering the thyroxine level either by thyroidectomy or by starvation<sup>19</sup> that a specific increase of lactase activity was obtained in the intestine. On the other hand, thyroxine injections inhibited completely the stimulatory effect induced by starvation on lactase activity without modifying sucrase activity, whereas hydrocortisone exhibited no effect on the activities of intestinal disaccharidases.

The thyroxine mediated effects on lactase activity did not result from modification occurring at the level of villus architecture or of protein mass. Thus, starvation during 48 hours caused a 20% shortening of the villus height in all conditions. Furthermore, the brush border protein yield was not significantly modified by the various treatments. In addition we can assume that the modifications observed in lactase activity were not mediated by changes in cell

kinetics as it has been shown that a daily injection of tetraiodothyronine for 12 days provoked a marked decrease in jejunal brush border lactase which was caused by a direct effect and not mediated through variations in cell kinetics.<sup>18</sup>

Of interest was the finding that starvation produced a stimulatory effect on lactase activity essentially in the mature cells of the villus and that this stimulatory effect was inhibited by thyroxine. Measurements of lactase activity along the villus-crypt axis performed 24 hours after the initiation of starvation led to similar results although the concentrations of lactase activity were lower all along the villus. The present results confirmed that two different cellular compartments were involved in lactase stimulation and in sucrase stimulation along the villus-crypt axis. Indeed lactase stimulation mediated by starvation or by dietary factors<sup>26</sup> occurred in the mature cells of the villus whereas sucrase stimulation induced by dietary carbohydrates occurred mainly in the immature cells of the villus base.<sup>14 26 27</sup>

An important aspect to be discussed in relation to our present findings is the fact that dietary carbohydrates have, under given conditions, a stimulatory effect on intestinal lactase activity as has been shown by many authors.<sup>3-9 26</sup> These results seem at first sight in conflict with the present report indicating a specific hormonal effect on lactase activity in the adult. Mainly two types of experimental procedures have been used to investigate the influence of dietary carbohydrates on brush border enzymes. In the first experimental procedure, rats were starved for several hours and then re-fed a high carbohydrate diet, under these conditions only sucrase and maltase activities were markedly increased by the diet whereas lactase activity was not modified.<sup>13 14 27</sup> In this case, lactase activity which was already enhanced by starvation was not further increased by the dietary carbohydrates which stimulated specifically sucrase and maltase activities.

In the second experimental procedure rats were kept for several weeks on a low carbohydrate diet and thereafter fed with increasing amounts of carbohydrates<sup>7-9 26</sup> leading to a stimulation of not only sucrase and maltase, but also of lactase activities. This latter procedure led to a non-specific increase in lactase activity which might result from a general increase of cellular metabolism. A key role of thyroxine on the regulation of lactase activity can be proposed during the weaning period. At weaning the rat switched from a relative low-carbohydrate diet (milk) to a relative high-carbohydrate diet (50-60% carbohydrates) and this dietary modification led to increased activities of maltase and sucrase whereas lactase activity at the same time was

lowered and remained at a low level in the adult.<sup>1</sup> It is noteworthy that during weaning increased intake of dietary carbohydrates will have no effect on lactase activity. In fact, it is now well known that during the same period increased concentration of circulating thyroxine is detected<sup>16</sup> and may explain the lowering of lactase activity. In adult rat, starvation by reducing the thyroxine concentration<sup>19</sup> will cause increased lactase activity, a situation which has similarities with the suckling period by the fact that during this period thyroxine concentration is very low and associated with high lactase activity.

Our present results suggest that the regulatory processes involved in lactase activity in adult rat are similar to those operating during the postnatal period both involving thyroxine. The molecular mechanisms of the regulation of lactase activity by thyroxine remain entirely to be elucidated.

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## References

- Doell RG, Kretchmer N. Studies of small intestine during development. I. Distribution and activity of  $\beta$ -galactosidase. *Biochim Biophys Acta* 1962; **62**: 353-62.
- Rubino A, Zimbalatti F, Auricchio S. Intestinal disaccharidase activities in adult and suckling rats. *Biochim Biophys Acta* 1964; **92**: 305-11.
- Girardet P, Richterich R, Antener H. Adaptation de la lactase intestinale à l'administration de lactose chez le rat adulte. *Helv Physiol Acta* 1964; **22**: 7-14.
- Cain GD, Moore P, Patterson M, McElveen MA. The stimulation of lactase by feeding lactose. *Scand J Gastroenterol* 1969; **4**: 545-50.
- Bolin TD, McKern A, Davis AE. The effect of diet on lactase activity in the rat. *Gastroenterology* 1971; **60**: 432-7.
- McCarthy DM, Nicholson JA, Kim YS. Intestinal enzyme adaptation to normal diets of different composition. *Am J Physiol* 1980; **239**: 445-51.
- Bustamante S, Gasparo M, Kendall K, Coates P, Brown S, Somawane B, Koldovsky O. Increased activity of rat intestinal lactase due to increased intake of  $\alpha$ -saccharides (starch, sucrose) in isocaloric diets. *J Nutr* 1981; **111**: 943-53.
- Koldovsky O, Bustamante S, Yamada K. Adaptability of lactase and sucrase activity in jejunoileum of adult rats to changes in intake of starch, sucrose, lactose, glucose, fructose and galactose. In: Robinson JW, Dowling RH, Riechen EO, eds. *Mechanisms of intestinal adaptation*. Lancaster: MTP Press, 1982: 153-68.
- Raul F, Doffoel M, Marescaux J, Bockel R, Grenier JF. Effects of chronic alcohol administration and a high carbohydrate-low protein diet on the activities of the jejunal brush border enzymes in the rat. *Gut* 1982; **23**: 962-7.
- Lichtenberger L, Welsh JD, Johnson LR. Relationship between the changes in gastrin levels and intestinal properties in the starved rat. *Am J Dig Dis* 1976; **21**: 33-8.
- Raul F, Doffoel M, Noriega R, Grenier JF, Haffen K. Modifications of brush border enzyme activities during starvation in the jejunum and ileum of adult rats. *Enzyme* 1982; **28**: 328-35.
- Deren JJ, Broitman SA, Zamcheck N. Effect of diet upon intestinal disaccharidases and disaccharide absorption. *J Clin Invest* 1967; **46**: 186-95.
- Kimura T, Seto A, Yoshida A. Effect of diets on intestinal disaccharidase and leucine aminopeptidase activities in refed rats. *J Nutr* 1978; **108**: 1087-97.
- Raul F, Simon PM, Kedinger M, Grenier JF, Haffen K. Effect of sucrose refeeding on disaccharidase and aminopeptidase activities of intestinal villus and crypt cells in adult rats. Evidence for a sucrose dependent induction of sucrase in the crypt cells. *Biochim Biophys Acta* 1980; **630**: 1-9.
- Cezard JP, Broyart JP, Cuisiner-Gleizes P, Mathieu H. Mechanisms of sucrase-isomaltase regulation by dietary sucrose in the rat. (Abstract.) *Gastroenterology* 1981; **80**: 1121.
- Henning SJ. Postnatal development: coordination of feeding, digestion and metabolism. *Am J Physiol* 1981; **241**: 199-214.
- Celano P, Jumawan J, Koldovsky O. Thyroxine evoked decrease of jejunal lactase activity in adult rats. *Gastroenterology* 1977; **73**: 425-8.
- Gutschmidt S, Emde C. Early changes in brush border disaccharidases kinetics in rat jejunum following subcutaneous administration of tetraiodothyronine. *Histochemistry* 1981; **73**: 151-60.
- Goodman MN, Larsen PR, Kaplan MM, Aoki TT, Young VR, Ruderman NB. Starvation in the rat. II. Effect of age and obesity on protein sparing and fuel metabolism. *Am J Physiol* 1980; **239**: 277-86.
- Raul F, Simon P, Kedinger M, Haffen K. Intestinal enzymes activities in isolated villus and crypt cells during postnatal development of the rat. *Cell Tiss Res* 1977; **176**: 167-78.
- Schmitz J, Preiser H, Maestracci D, Ghosh BK, Cerda JJ, Crane RK. Purification of the human intestinal brush border membrane. *Biochim Biophys Acta* 1973; **92**: 305-11.
- Raul F, Simon PM, Kedinger M, Grenier JF, Haffen K. Separation and characterization of intestinal brush border enzymes in adult rats and in suckling rats under normal conditions and after hydrocortisone injections. *Enzyme* 1978; **23**: 89-97.
- Messer M, Dahlqvist A. A one step ultramicro method for the assay of intestinal disaccharidases. *Ann Biochem Biophys* 1966; **14**: 376-92.

- 24 Koldovsky O, Asp NG, Dahlqvist A. A method for the separate assay of "neutral" and "acid"  $\beta$ -galactosidase in homogenates of rat small intestinal mucosa. *Anal Biochem* 1969; **27**: 409–18.
- 25 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265–74.
- 26 Yamada K, Bustamante S, Koldovsky O. Dietary induced rapid increase of rat jejunal sucrase and lactase activity in all regions of the villus. *FEBS Lett* 1981; **129**: 89–92.
- 27 Ulshen MH, Grand RJ. Site of substrate stimulation of jejunal sucrase in the rat. *J Clin Invest* 1979; **64**: 1097–102.