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Developmental changes in intestinal glycosylation: nutrition-dependent multi-factor regulation of the fucosylation pathway at weaning time

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Abstract

Developmental changes in the fucoglycoproteins of the intestinal brush-border membranes were determined by lectin affinoblotting after electrophoresis. Whereas only two α(1-6)-fucoglycoproteins were detected in brush-border membranes from suckling rats, a large number of *N*-fucoglycoproteins with α(1-2)- and/or α(1-6)-linked fucose residues were detected in rat membranes after weaning. Dietary manipulations at weaning time were used to investigate the effect of nutritional factors in the development of fucosylation in the small intestine of prolonged-nursed rats fed with milk (a high-fat, low-carbohydrate diet) compared to rats weaned normally with a standard high-carbohydrate diet. The fucose content of the mucosa glycoproteins was lower in 22-day-old prolonged-nursed rats than in 22-day-old rats weaned normally with the standard diet. The appearance of fucoglycoproteins in the brush-border membranes, which was delayed by prolonged nursing, was accompanied by a concomitant delay in the increase of intestinal fucosyl-transferase activity and in the decrease of GDP-fucose substrate breakdown. The developmental decrease in the activity of the inhibitory protein which regulates the fucosyl-transferase activity was also delayed by prolonged nursing. The intestinal fucosylation of brush-border membrane glycoproteins (which include many digestive enzymes) displayed ontogenic changes on which were superimposed dietary influences at the time of weaning. The complete maturation of the brush-border membrane glycoproteins, and particularly their terminal fucosylation, is a developmental event which thus seems to be strongly influenced by the manipulation of nutritional factors during the weaning period.

Keywords: Fucoprotein; Fucosylation; Intestine; Development; Weaning; Glycosylation

1. Introduction

The apical membrane of the rat intestinal cells exhibits striking adaptive developmental changes in membrane composition and enzymic equipment which culminate at weaning [1]. In the brush-border membranes, during postnatal development, between the young suckling and adult weaned stages, these changes include marked modifications both in the activities of the glycoprotein enzymes involved in the digestive process (glycosidases, aminopeptidase, alkaline phosphatase [2–4]), and in the glycosylation process. The latter involves a shift from sialylation to fucosylation observed in the membrane-bound glycoproteins [5–8], accompanied by changes in the activity of the enzymes responsible for the transfer of fucose and sialic acid to glycoproteins at the time of weaning [7,9].

Quantitative changes in fucose glycoprotein content in the small intestine during development have been determined, but the fucoglycoproteins in the brush-border membranes (the most functionally important membranes in the enterocyte) are less well known in qualitative terms. Lectin affinoblotting with a lectin – UEA I from *Ulex europaeus*, which recognizes α(1-2)-linked fucose [11] or AAA from *Aleuria aurantia*, which is specific for α(1-6)-linked fucose [12] – can be used to investigate the linkage of fucose with glycoproteins during development, and especially at weaning time.

Fucosyl-transferase activity in adult rat intestine is sensitive to dietary modifications [10]. It is also found to increase markedly during weaning [9]. It may be that nutritional changes, which take place during weaning, cause variations in the fucosyl-transferase activity and in the fucosylation process as a whole. Nutritional effects on other enzymic and regulatory systems involved in the fucosylation pathway are possible. Indeed, the existence of an endogenous intestinal protein that inhibits fucosyl-trans-

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ferase activity [13], combined with changes in its activity during postnatal development, and with contrary changes in fucosyl-transferase activity, have already been demonstrated [14]. In the light of these data, the effect on the fucosylation pathway of dietary manipulations (by prolonged-nursing experiments) at weaning was examined as a way of explaining the drastic changes in glycoprotein fucosylation that are observed at the time of weaning.

This paper describes the appearance of fucoglycoproteins in the brush-border membranes during postnatal development. The effect of variations caused by drastic nutritional manipulation at weaning time on developmental changes in the fucosylation process has been investigated. Changes in fucoglycoproteins, in different enzymes (fucosyl-transferase, enzymes acting on biosynthesis, and breakdown of the fucosylation substrates) and in the regulatory protein (fucosyl-transferase inhibitor) involved in the fucosylation process are discussed.

2. Materials and methods

2.1. Animals

Male rats and pups (with dams) of the Sprague-Dawley strain (IFFA-CREDO, France) were housed in controlled conditions of temperature (21°C) and light (12 h light, 12 h darkness). Just after birth, the pups were divided up into litters of 10 males. For prolonged-nursed groups, litters were placed in special cages between day 14 and 22; the cages were designed to avoid standard-diet intake and coprophagic behavior by the pups, so that they were actually unweaned until 22 days of age. Milk composition is given in Table 1, according to Keen et al. [16]. For weaned groups, litters were placed in similar conditions until the pups were 19 days old, at which point weaning was induced abruptly by separation of pups from dam and feeding with standard diet (Souriffarat®, IFFA CREDO, France), whose composition is described in Table 1.

2.2. Cell fractionation

The animals were killed by decapitation. The small intestines were removed between the gastro-duodenal junction and the ileo-caecal junction, and flushed with cold 0.9% NaCl; the mucosa was then scraped with a microscope glass slide. $100\,000 \times g$ pellets (microsomes) and supernatants (cytosols) were obtained as previously described [15], frozen, and stored at −20°C. The brush-border membranes from the total small intestine were prepared by precipitation with $CaCl_2$ according to the method of Kessler et al. [17], then resuspended in water, aliquoted in small quantities, lyophilized and stored at −20°C.

Table 1
Composition of diet

	Milk composition		Standard diet composition	
	% by weight (wet diet)	% of total energy	% by weight (wet diet)	% of total energy
Proteins	11.1	27.6	21.0	26.2
Carbohydrates	2.9	6.7	53.5	62.9
Fats	12.2	65.7	4.0	10.9

The standard diet Souriffarat® (IFFA-CREDO, France) also contained 11.5% water, 4.5% cellulose, 5.5% minerals and vitamins. Its caloric value was 13600 kJ/kg.

2.3. Enzymic treatment of brush-border membranes

In order to test the specificity of lectin binding to sugars in the glycan chains of glycoproteins, the brush-border membrane glycoproteins were treated with: either 1 U of α-L-fucosidase from bovine kidney (Sigma, USA) per mg of brush-border membrane protein, in a pH 5.5 buffer (75 mM sodium acetate, 23 mM $CaCl_2$, 0.03% NaN_3, 1 mM PMSF) at 37°C for 24 h; 10 U of *N*-glycanase from *Flavobacterium meningosepticum* (Genzyme, USA) per 400 μg of protein in a pH 7.4 buffer (50 mM Tris-HCl, 0.5% Triton X-100) for 24 h at 37°C; 0.5 mg of *O*-sialoglycoprotein endopeptidase from *Pasteurella hemolytica* (Cedarlane, Canada) per 200 μg of protein in 50 mM Hepes, pH 7.4 buffer for 4 h at 37°C, or 250 mU of *Vibrio cholera* neuraminidase (Sigma) per 400 μg of protein in a pH 5.5 buffer (75 mM sodium acetate, 23 mM $CaCl_2$, 0.03% NaN_3, 1 mM PMSF) at 37°C for 3 h followed by the addition of 50 mU of *O*-glycanase from *Diplococcus pneumoniae* (Genzyme) for 15 h at 37°C. For all treatments, the reaction was stopped after incubation by boiling for 4 min. Brush-border membrane samples, treated with *N*-glycanase, *O*-sialoglycoprotein endopeptidase or neuraminidase/*O*-glycanase, were directly aliquoted and lyophilized. For the fucosidase-treated membranes, dialysis was necessary before lyophilization.

2.4. Electrophoresis of the brush-border membrane proteins, transfer onto cellulose nitrate membranes, and lectin affinoblotting

The lyophilized brush-border membranes were analyzed by electrophoresis after sample denaturation by boiling for 4 min in a pH 6.8 buffer (62.5 mM Tris-HCl, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol). The proteins were separated by electrophoresis: for 45 min at 200 V on 3% stacking polyacrylamide gels and on 0.1% SDS-7.5% separating polyacrylamide gels (8.5 × 7 cm), using a Mini-Protean II system (Bio-Rad, UK).

The proteins were electrotransferred overnight at 30 volts, using a Bio-Rad transfer cell, onto 0.45 μm cellulose nitrate membranes (Schleicher and Schuell, Germany). Proteins were either detected using an immunogold staining kit (Biocell, UK) or studied for glycan-lectin binding.

For lectin binding studies, the cellulose nitrate membranes were incubated at room temperature for 30 min

with a 0.5% blocking reagent (Boehringer, Germany) in a pH 7.5 buffer (50 mM Tris-HCl, 150 mM NaCl containing 0.1% Tween 20 (TBST)), then washed twice with TBST for 10 min, and once with a pH 7.5 buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% Tween 20 (TBSTS)) for 10 min. The membranes were then incubated for 1 h with either UEA I-biotin conjugate (Sigma) (0.6 μg/ml), AAA-digoxigenin conjugate (0.1 μg/ml), ConA-digoxigenin conjugate (4 μg/ml) or GNA-digoxigenin conjugate (Boehringer) (1.5 μg/ml) in TBSTS. After three washes with TBST, the membranes were incubated, either – for UEA- or ConA-treated membranes – with an anti-digoxigenin-Fab fragment conjugated with alkaline phosphatase (Boehringer) at 750 mU/ml, in TBST for 1 h or – for AAA-treated membranes – with streptavidin conjugated with alkaline phosphatase (Boehringer) at 750 mU/ml. They were then washed twice for 10 min with TBST and once with TBST without Tween 20 for 10 min. The staining reaction with alkaline phosphatase was performed by incubation of the membranes with 0.5 mM nitro-blue tetrazolium chloride, 0.5 mM 5-bromo-4-chloro-3-indolylphosphate, 4-toluidine salt in a pH 9.5 buffer (100 mM Tris-HCl, 50 mM $MgCl_2$, 100 mM NaCl); it was stopped by rinsing the membrane with deionized water twice for 5 min. In order to verify the specificity of staining, a search was carried out for intestinal alkaline phosphatase according to the method of Komoda et al. [18] but none was detected. The absence of unspecific labeling due to endogenous protein-bound biotin or streptavidin receptors was verified by incubation without lectin. Fucose specificity was confirmed after treatment of the brush-border membranes with α-L-fucosidase, and also by incubating the lectins in the presence of 0.2 M α-L-fucose. A check was also done for possible interference due to lectin detection of the enzymes used for treating the brush-border membranes.

2.5. Determination of fucosyl-transferase activity

Fucosyl-transferase (EC 2.4.1.68) activity was determined in microsomal fractions homogenized in a pH 7.4 buffer (10 mM Tris-HCl, 10 mM KCl, 10 mM $MgCl_2$), or in cytosols. Enzymic activity was studied either with an endogenous acceptor or with asialofetuin as a glycoproteinic exogenous acceptor. The membrane-bound fucosyl-transferase assay contained (in 250 μl) between 150 and 250 μg of microsomal proteins, 2 μM of asialofetuin (200 μg), 0.25% Triton X-100, 5 mM $MnCl_2$, 10 mM AMP and 6 μM GDP-fucose (with 2960 Bq of GDP-[^{14}C]fucose/ml of the incubation mixture (spec. act. 10 GBq/mmol, NEN, USA)). The incubations were performed at 23°C for 30 min. For the soluble fucosyl-transferase assay, Triton X-100 was omitted. After stopping the reaction with 20% trichloroacetic acid, the precipitates which were radiolabeled during incubation were collected on GF/B glass fiber filters (Whatman, UK) and the radioactivity was determined by liquid scintillation counting with a Toluene scintillator (Packard, USA).

2.6. Determination of fucosyl-transferase inhibitor activity

10 ml of cytosolic fraction, diluted 4-fold with water, were incubated for 30 min at 4°C with 1 g of DEAE-cellulose (DE 52 Whatman, UK) in 10 ml of 10 mM Tris-HCl, pH 7.6. The DEAE-cellulose, whose purpose was to retain the inhibitor, was poured into the column, and the elution was performed with 30 ml of a pH 5.5 buffer (280 mM KCl, 10 mM Mes). The fucosyl-transferase inhibitor activity was assayed as previously described [14] against a partially-purified fucosyl-transferase [19] obtained from adult rat intestine. One inhibitor unit was defined as the amount of protein giving 50% inhibition of the fucosyl-transferase activity.

2.7. Determination of GDP-mannose and GDP-fucose biosynthesis

The biosynthesis of GDP-mannose was determined by incubation (in 200 μl) of 500 μg of cytosolic proteins with 200 μM [^{14}C]mannose (spec. act. 11.1 GBq/mmol, CEA, France) in 10 mM 2,3-dimercaptopropanol, 10 mM ATP, 10 mM GTP, 10 mM cysteine and 35 μM glucose 1,6-diphosphate at 30°C for 3 h. The reaction was stopped after incubation by boiling for 20 s; this was followed by the addition of 100 μl 1 M $NaClO_4$, pH 4.2, freezing, and the addition of 200 μl of 1 M potassium acetate. The substrate and products were separated in a SAX Partisil column (Waters, USA) by HPLC after elution with a gradient of 20 mM (pH 4.1) to 500 mM (pH 4.6) KH_2PO_4 buffer. For the GDP-fucose biosynthesis study, the transformation of GDP-mannose was estimated after incubation of cytosolic fractions with GDP-[^{14}C]mannose [14] followed by separation of the reaction products by reverse-phase HPLC [19].

2.8. Determination of GDP-fucose breakdown

The degradation of the substrate by GDP-fucose pyrophosphatase was estimated after incubation of GDP-[^{14}C]fucose with cytosol and the separation of GDP-fucose from its degradation products by HPLC, as previously described [19].

2.9. Other chemical determinations

The fucose content of the fucoglycoproteins in the intestinal mucosa was determined by the method of Dische [20] after delipidation according to the method of Folch [21].

Protein content was determined either by the method of Lowry [22] or by that of Schaffner [23].

2.10. Statistical treatment of results

The results were treated, and the means were compared by Student's *t*-test, using the INSTAT biostatistics program (GraphPad, USA).

3. Results

3.1. Developmental changes in the fucoglycoproteins of the brush-border membranes

Fucoglycoproteins in the intestinal brush-border membranes of 7- to 18-day-old suckling rats and 22- to 48-day-old weaned rats were studied by lectin affinoblotting during postnatal development. No α(1-2)-fucoglycoprotein was found in 7- to 18-day-old suckling rats (lines 1 to 3) after detection with UEA I (Fig. 1a), whereas in all the small intestines of 22- to 48-day-old weaned rats (lines 4 to 7) between five and eight fucoglycoproteins were detected, with molecular masses of > 300, 200, 150, 140, 130, 110, 95 and 70 kDa.

AAA detection (Fig. 1b) indicated the progressive appearance of one or two fucoglycoproteins with α(1-6)-linked fucose residues with molecular weight of 95 (lines 1 to 3) and 120 kDa for 18-day-old rats only (line 3) in the brush-border membranes of 7- to 18-day-old suckling rats. In weaned rats (lines 4 to 7), five fucoglycoproteins were revealed as fine bands with molecular masses of between 270 and 200 kDa, and five others were revealed as more strongly marked bands with molecular masses of 150, 130, 110, 95 and 75 kDa.

The control glycoprotein thyroglobulin (containing α(1-6)-linked fucose) was detected by AAA (Fig. 1b, line 9) but not by UEA I, whereas erythropoietin (containing α(1-3)-linked fucose) was not detected by either of the two lectins in the given conditions. After α-L-fucosidase treatment of the brush-border membranes, or incubation of the lectins with L-fucose, no more fucoglycoprotein was detected; this indicating a high degree of specificity of the two lectins for fucose. After *N*-glycanase treatment of the brush-border membranes, nearly all the fucoglycoproteins appeared as *N*-glycoproteins, no glycoprotein being detected by AAA (not shown) and only one (molecular mass about 110 kDa) by UEA (Fig. 2a, line 4). The mannosylated *N*-glycans were detected at normal levels by ConA in the same samples of untreated brush-border membranes (lines 1, 2 in Fig. 2b), whereas the disappearance of the *N*-glycans in *N*-glycanase-treated brush-border membranes (lines 3, 4, Fig. 2b) indicated that the action of the *N*-glycanase was complete. Similar results were obtained after GNA detection, indicating that all the high-mannose chains were degraded. After treatment with *O*-sialoglycoprotein endopeptidase, which degrades protein chains at the linkage sites of *O*-glycan chains (lines 3, 4 in Fig. 2c), all the bands of glycoproteins were again detected by AAA, and no degradation was found, thus confirming that the α(1-6)-fucose residues were linked to *N*-glycan chains. For UEA detection, difficulties in the interpretation of blots were induced by a weak reaction of the commercial sialoglycoprotein endopeptidase with UEA I (probably due to the presence of serum proteins in the enzyme preparation). However, after fucoglycoprotein detection of brush-border membranes treated with neuraminidase from *Vibrio cholera*, and then with *O*-glycanase from *Diplococcus pneumoniae* (active only on asialoglycoproteins), neither

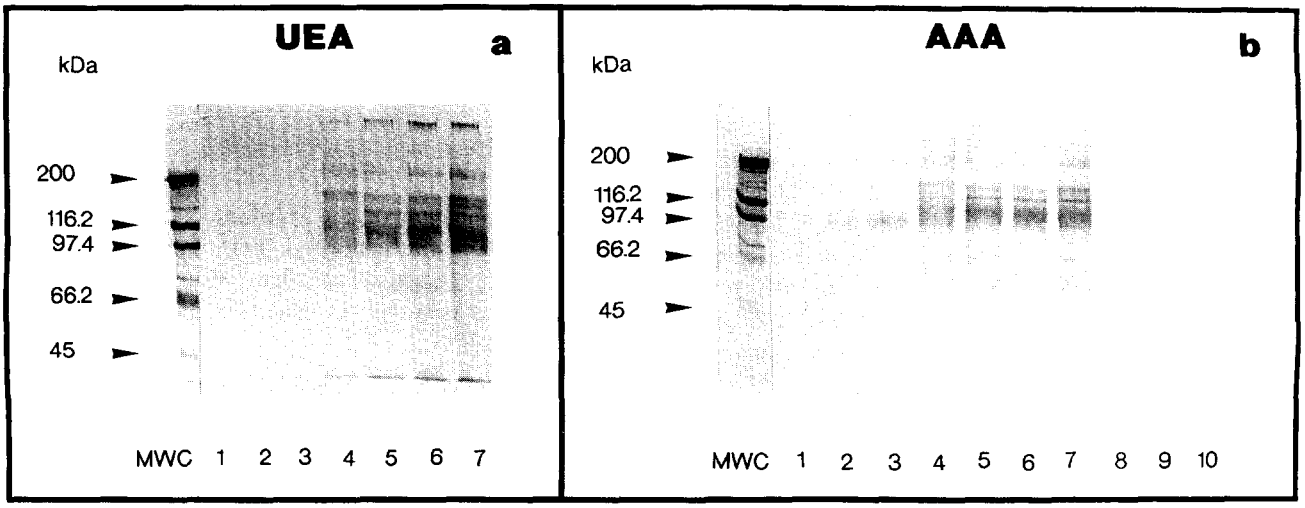

Fig. 1. Developmental changes in brush-border membrane fucoglycoproteins. (a) UEA I detection of α(1-2)-fucoglycoproteins in 5 μg of brush-border membrane proteins from suckling rats (1, 2, 3 = respectively 7, 14 and 18 days of age) and from weaned rats (4, 5, 6, 7 = respectively 22, 28, 38 and 48 days of age). MWC = molecular weight control proteins stained by gold immunorevelation. (b) AAA detection of α(1-6)-fucoproteins in 0.2 μg of brush-border membrane proteins from 7-, 14-, 18-day-old rats (lines 1, 2, 3) and from 22-, 28-, 38-, and 48-day-old rats (lines 4, 5, 6, 7). 8 = serotransferrin (0.5 μg), 9 = thyroglobulin (0.5 μg), 10 = erythropoietin (0.5 μg).

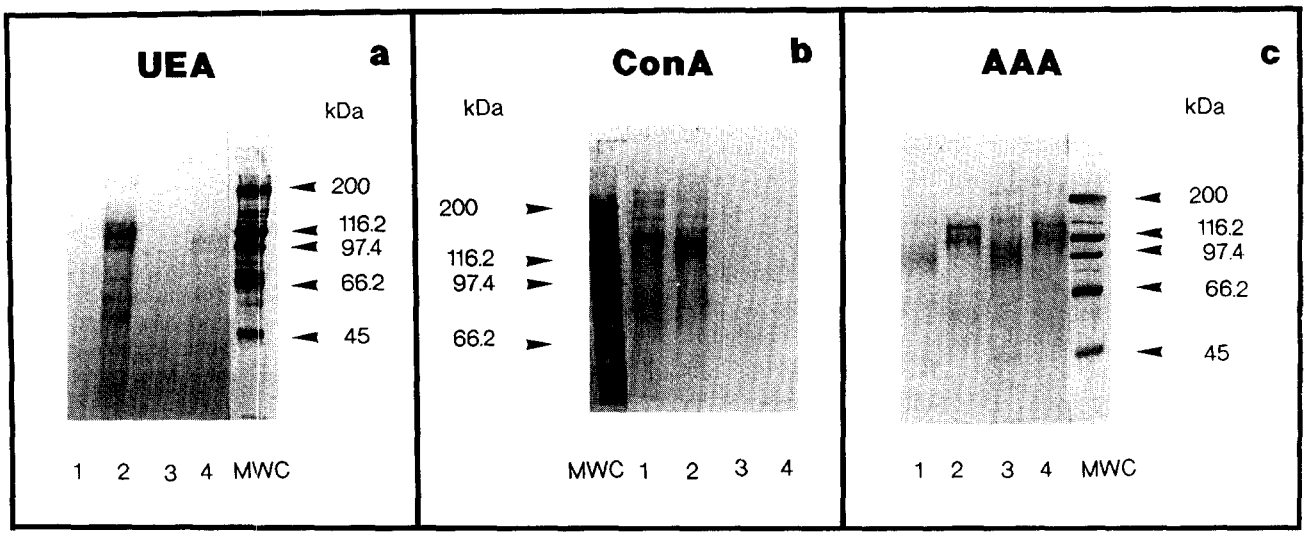

Fig. 2. *N*-Glycanase and *O*-sialoglycoprotein endopeptidase treatment of fucoglycoproteins. (a) UEA I detection of fucoglycoproteins in 3 μg of *N*-glycanase-treated brush-border membranes: 1, 2 = control BBM from small intestine of 14- and 48-day-old rats; 3, 4 = *N*-glycanase-treated BBM from the same 14- and 48-day-old rats. (b) *N*-glycan detection with ConA in 3 μg of the same samples than in a. (c) AAA detection of fucoglycoproteins border membranes treated with *O*-sialoglycoprotein endopeptidase: 1, 2 = control BBM from small intestine of 14- and 48-day-old rats; 3, 4 = BBM (treated with *O*-sialoglycoprotein endopeptidase) from the same 14- and 48-day-old rats.

UEA I nor AAA detection revealed any variation in treated brush-border membranes as compared to untreated brush-border membranes (not shown).

3.2. *Nutrition-dependent changes of fucoglycoproteins at the time of weaning*

The effect of drastic nutritional changes on fucoglycoproteins at weaning was studied, using groups of 22-day-old rats either submitted to prolonged nursing (PN) or weaned when they were 19-day-old (W).

The fucose content of the glycoproteins was significantly lower in the entire intestinal mucosa of the 22-day-old prolonged-nursed rats (71.5 ± 10.9 μg/g intestine, $n = 8$) than in the mucosa of the age-matched weaned rats (120.8 ± 16.6 μg/g intestine, $n = 8$, $P < 0.025$).

As shown in Fig. 3b for UEA I detection, in brush-border membranes from the 22-day-old prolonged-nursed rats

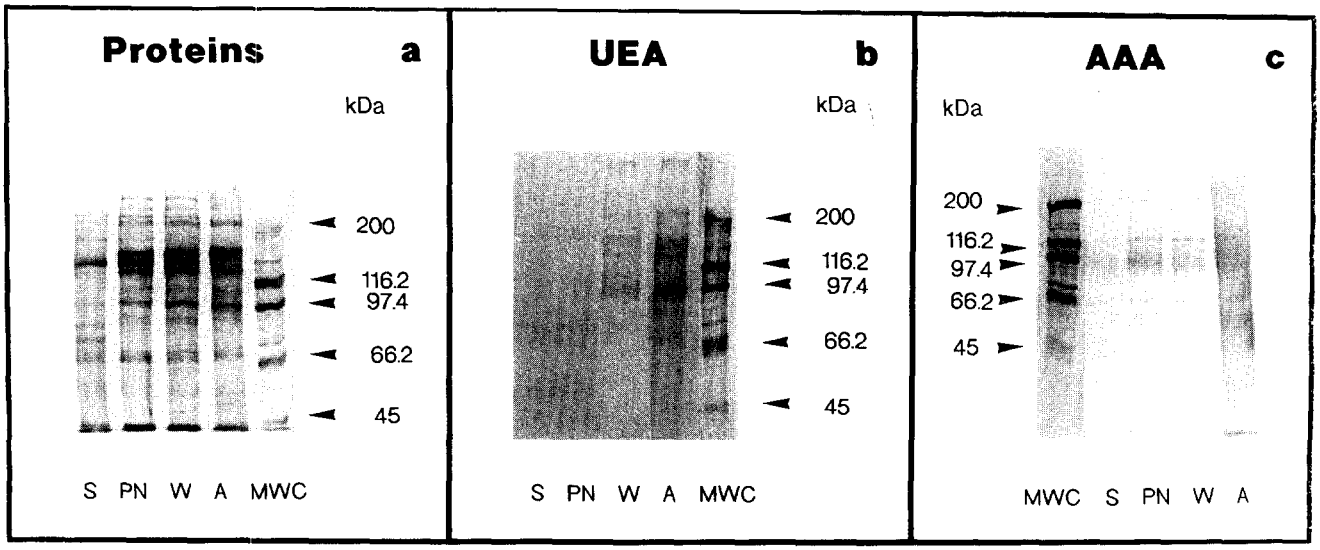

Fig. 3. Effect of nutritional changes at weaning on the appearance of brush-border membrane fucoglycoproteins. (a) Protein detection by immunogold reaction in 4 μg of brush-border membrane from the small intestine of 7-day-old suckling (S), 22-day-old prolonged-nursed (PN), 22-day-old weaned (W) and 48-day-old adult (A) rats. MWC = molecular weight control proteins stained by gold immunorevelation. (b) UEA I fucoglycoprotein detection in 7 μg of brush-border membranes from the same samples as in a. (c) AAA fucoglycoprotein detection of 0.5 μg of brush-border membranes from the same samples as in a.

(PN), no fucoglycoprotein was detected, as was also the case for brush-border membranes from the 7-day-old suckling rats (S). In membranes from the 22-day-old weaned rats, α(1-2)-fucoglycoproteins appeared with a pattern similar to what was found for 48-day-old adult rats (A), but at a lower intensity. Only one α(1-6)-fucoprotein was detected by AAA in the youngest suckling rats (S), many α(1-6)-fucoproteins being found in adult rats (A) (Fig. 3c), as also shown in Fig. 1b. The α(1-6)-fucoglycoproteins detected in brush-border membranes from 22-day-old prolonged-nursed rats (PN) had similar molecular weights to those found in membranes from 18-day-old suckling rats (Fig. 1b, line 3) and from 22-day-old weaned rats (W in Fig. 3c).

3.3. Nutrition-dependent changes in fucosyl-transferase activity at weaning

To determine the sites of regulation of the fucosylation pathway involved in nutrition-dependent quantitative and qualitative modifications in fucoglycoproteins, the different enzymic and regulatory systems involved in this metabolic pathway were studied at weaning.

The activity of the main enzyme involved in glycoprotein fucosylation (fucosyl-transferase) was greatly increased during the weaning period. Table 2 shows the effect of dietary changes on the specific activity of this enzyme, whose membrane-bound form was present at a significantly higher level in the intestine of 22-day-old weaned rats than in the intestine of the age-matched rats that were submitted to prolonged nursing, on endogenous acceptor as well as on asialofetuin. On the other hand, the specific activity of soluble fucosyl-transferase was not significantly modified.

3.4. Influence of nutritional variations on fucosyl-transferase inhibitor activity at weaning

A decrease in the activity of a soluble proteinic inhibitor of intestinal fucosyl-transferase during postnatal development, particularly pronounced between days 18 and 23 days of life has been described [14]. The present study revealed a higher level of inhibitor activity in the intestine of 22-day-old prolonged-nursed rats than in the intestine of age-matched weaned rats (Table 2).

Table 2
Effect of nutritional changes, at weaning, on the activity of fucosyl-transferase and on that of its inhibitor

	Prolonged-nursed rats	Weaned rats
Membrane-bound fucosyl-transferase		
Endogenous	0.246 ± 0.025	0.360 ± 0.037 **
Asialofetuin	1.996 ± 0.163	3.247 ± 0.276 ***
Soluble fucosyl-transferase		
Asialofetuin	0.259 ± 0.047	0.186 ± 0.016
Fucosyl-transferase inhibitor	213.7 ± 32.2	123.1 ± 22.0 *

Results are given as means ± S.E., in pmol/mg protein per min for 18 independent values of fucosyl-transferase, and in U/mg protein for 13 values of the inhibitor activity. Means were compared by Student's *t*-test: * $P < 0.050$, ** $P < 0.025$, *** $P < 0.001$.

Table 3
Effect of nutritional changes on the GDP-fucose metabolism at weaning

	Prolonged-nursed rats	Weaned rats
GDP-mannose biosynthesis	951.3 ± 163.5	773.6 ± 66.9
GDP-fucose biosynthesis	211.1 ± 28.3	234.3 ± 32.3
GDP-fucose breakdown	272.0 ± 28.1	51.2 ± 14.6 *

Results are given as means ± S.E., in pmol. GDP-Man synthesized/mg protein per min for 6 independent values of GDP-mannose biosynthesis, in pmol. GDP-Fuc synthesized/mg protein per min for 16 values of GDP-fucose biosynthesis, and in pmol. GDP-Fuc degraded/mg protein per min for 16 values of GDP-fucose breakdown. Means were compared by Student's *t*-test: * $P < 0.001$.

3.5. Influence of nutritional variations on fucosylation substrate biosynthesis and breakdown at weaning

The active substrate for fucose transfer to protein by fucosyl-transferase is GDP-fucose, whose biosynthesis from GDP-mannose involves a complex enzymic system [21], which is particularly active in the intestine [19]. An increase in the biosynthesis of this substrate (in 18-day-old animals) prior to the rise in fucosyl-transferase activity has been described [14]. Table 3 shows that GDP-fucose biosynthesis from GDP-mannose is quite similar in 22-day-old prolonged-nursed and weaned rats. It was also verified that the previous step, the biosynthesis of GDP-mannose from mannose, was not a limiting step for GDP-fucose biosynthesis, since there was a higher level of GDP-mannose biosynthesis than of GDP-fucose biosynthesis in both groups of animals (Table 3).

The breakdown of the GDP-fucose substrate by GDP-fucose pyrophosphatase, which progressively decreases during postnatal development [14], was significantly lower in weaned than in prolonged-nursed rats (Table 3).

4. Discussion

Some years ago, a large degree of enhancement of the global fucose content of glycoconjugates was reported in the case of brush-border membranes [6,24,25] and mucins [26] in adult rats as compared to young suckling ones. Here, UEA I and AAA detection indicated that the appearance of fucoglycoproteins with α(1-6) residues (generally linked to the *N*-acetyl-glucosamine of the *N*-glycan core) preceded that of fucoglycoproteins with α(1-2) residues (linked at the external part of the *N*-glycan chains). Indeed, whereas α(1-2)-fucoproteins were absent after UEA

I binding in suckling rats, one to two α(1-6)-fucoproteins with molecular masses 120 and 95 kDa were detected after AAA binding. By contrast, in adult rats, several fucoglycoproteins were detected both with AAA and UEA I lectins; this supporting the hypothesis of fucose with α(1-2) or/and α(1-6) linkages. Two glycoproteins (molecular masses: > 300, and 140) were determined with only UEA, and four fucoproteins (molecular masses: 270, 250, 230, 210) were determined with only AAA.

The identity of these glycoproteins, which were prematurely fucosylated in α(1-6) has not been determined. But it is possible that, among the glycoprotein enzymes known to be present in brush-border membranes, there are some which, in view of their molecular weights, could correspond to these proteins (trehalase [27] and lactase [28]), although Büller et al. have shown (but only by means of UEA) that lactase is fucosylated just after weaning [28]. Using a different technical approach, Torres-Pinedo et al. demonstrated an increase in ^{125}I-UEA I lectin binding to all the brush-border membranes after weaning [6]. By cytochemical studies, Taatjes and Roth have shown that UEA I binds to the apical and basolateral plasma membranes in rat jejunum, with intense staining after 23 days of age [29]. These observations are in good agreement with the results of the present study. However, the authors in question did not study the involvement of nutritional factors at weaning.

The causes of the developmental glycoprotein fucosylation changes that are particularly marked at weaning were studied in terms of the possible role of nutritional variations in the fucosylation pathway caused by dietary manipulations at weaning time.

For all the glycoproteins in the intestinal mucosa, the difference in fucose content must have been largely due to dietary changes at weaning, since the 22-day-old weaned rats had glycoproteins that were more fucosylated than those of the age-matched suckling rats that were submitted to prolonged nursing. This result was confirmed by fucosylation of the intestinal brush-border membrane proteins; here, the 22-day-old normally-weaned rats presented a fucoprotein pattern similar to that of adult rats, whereas the age-matched suckling rats submitted to prolonged nursing presented the same fucoprotein pattern as the younger suckling rats.

It has been suggested that the regulation of the expression of some glycosyl-transferase activities takes place at the level of transcription [30]. Although this mechanism could also be at work in the intestine during development, it would not be the only one, and the different systems involved in the metabolic fucosylation pathway may have considerable influence on the fucosylation of glycoproteins. This assumption led us to the hypothesis that modifications of these different systems could occur after dietary manipulations at weaning, and that the inhibitor of fucosyl-transferase could play a role in dietary regulation. The large increase in membrane-bound fucosyl-transferase activity observed at the weaning period can be supposed to be highly influenced by dietary modifications, since the fucosyl-transferase activity of 22-day-old suckling rats submitted to prolonged nursing is seen to be lower than that of weaned rats, and similar to that of younger suckling rats. GDP-fucose biosynthesis, which is greatly enhanced just prior to the increase in fucosyl-transferase activity [14], is not modified by prolonged nursing. Concerning systems opposed to fucosylation, the decrease in GDP-fucose breakdown observed during development is delayed by prolonged nursing. Moreover, the role of the endogenous inhibitor of fucosyl-transferase in the metabolic regulation of glycoprotein fucosylation appears to be important in the nutritional regulation of this process at weaning, given that the decrease in its activity that is observed during postnatal development is delayed in the intestines of 22-day-old rats submitted to prolonged nursing, compared to 22-day-old weaned rats.

Little is known about the effect of dietary changes on intestinal glycosylation in young rats, though there has been a study showing a decrease in the overall fucose content of brush-border membrane in 21-day-old rats submitted to undernutrition by reduced access to milk [31]. The critical difference between milk [16,32] and a standard solid diet is likely to lie in the relative proportions of fats (which are high in milk) and of carbohydrates (which are high in the standard diet). It has already been demonstrated that, in the adult rat intestine, a high-carbohydrate diet has the opposite effect, producing an increase in the fucosyl-transferase activity, whereas a high-fat diet decreases this activity [10]. The present study specifies the role of the nutritional factors involved in the developmental evolution of glycoprotein fucosylation by demonstrating a weaning-dependent concerted regulation of a number of enzymic and regulatory systems involved in this metabolic pathway, including the fucosyl-transferase inhibitor (a recently discovered regulatory protein). However, it is not known whether diet acts on fucosylation in the small intestine in a direct way, or via endocrine factors; this point currently being studied.

The relation between glycosylation and the biological activity of intestinal glycoproteins (maltase, isomaltase, sucrase, alkaline phosphatase,...) which are of great importance in the digestive process is not well known, though the effect of altered weaning on the activity of these enzymes has been studied in some cases. Lactase activity, which normally decreases from birth to weaning time, is maintained at a significantly higher level after prolonged nursing than after normal weaning [33]. In the duodenum and jejunum of prolonged-nursed rats, Lee and Lebenthal found general reductions in enzymes for sucrase, maltase and enterokinase whose activities are normally increased at weaning [34]. Concerning the relation between glycosylation changes in brush-border membranes and variations in their biological activities, Büller et al. established that the lactase oligosaccharide core remains constant during devel-

opment, but that there is an alteration in terminal glycosylation, with a shift from sialic acid during suckling to fucose in adulthood [28]. Naim et al. have suggested that the difference in lactase activity during development could be due to differences in the *O*-glycosylation of lactase [35]. On the other hand, Nsi-Emvo et al. make the assumption that the decline in lactase activity is associated with changes in lactase protein glycosylation occurring at weaning, and that these changes could induce the intracellular accumulation of an inactive 300 kDa lactase protein in a highly mannosylated form, whereas the active form could contain complex glycan chains [36]. In pig intestine, the existence of a high-mannose glycosylated form of sucrase-isomaltase with a lower specific activity than the complex glycosylated form has been found by Sjöström et al. [37]. But fucose is an external sugar linked to glycan chains of a complex type, and its presence in these chains may be very significant. Studies on the relation between changes in glycan characteristics and the biological activity of enzymes involved in the digestive process are of great interest, but remain controversial for the moment.

In conclusion, the present study shows how nutritional factors at weaning contribute to the regulation of enzyme activities (fucosyl-transferase and substrate degradative enzymes), and of the activity of the fucosyl-transferase inhibitor involved in the fucosylation pathway, leading to the appearance of fucoglycoproteins in the brush-border membrane. The maturational changes in enzymes of the brush-border membranes may be related to developmental differences in the chronology of the synthesis of the proteins and that of their fucosylation pathway. Certain dietary manipulations may modify the natural chronological evolution of small intestine ontogeny.

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References

[1] Henning, S.J. (1985) Annu. Rev. Physiol. 47, 231–245.
[2] Rubio, A., Zimbalatti, F. and Auricchio, S. (1964) Biochim. Biophys. Acta 92, 305–311.
[3] Auricchio, S., Stellato, A. and De Vizia, B. (1981) Pediatr. Res. 15, 991–995.
[4] Yeh, K.Y. and Moog, F. (1975) Dev. Biol. 47, 156–172.
[5] Yeh, K. and Moog, F. (1984) Dev. Biol. 101, 446–462.
[6] Torres-Pinedo, R. and Mahmood, A. (1984) Biochem. Biophys. Res. Commun. 125, 546–553.
[7] Chu, S.H.W. and Walker, W.A. (1986) Biochim. Biophys. Acta 883, 496–500.
[8] Taatjes, D.J. and Roth, J. (1991) Int. Rev. Cytol. 126, 135–193.
[9] Biol, M.C., Martin, A., Richard, M. and Louisot, P. (1987) Pediatr. Res. 22, 250–256.
[10] Biol, M.C., Martin, A., Oehninger, C., Louisot, P. and Richard, M. (1981) Ann. Nutr. Metab. 25, 269–280.
[11] Pereira, M.E.A., Kisailus, E.C., Gruezo, F. and Kabat, E.A. (1978) Archs. Biochem. Biophys. 185, 108–115.
[12] Yamashita, K., Kochibe, N., Ohkura, T., Ueda, I. and Kobata, A. (1985) J. Biol. Chem. 260, 4688–4693.
[13] Martin A, Ruggiero-Lopez, D., Biol, M.C. and Louisot, P. (1990) Biochem. Biophys. Res. Commun. 166, 1024–1031.
[14] Ruggiero-Lopez, D., Biol, M.C., Louisot, P. and Martin, A. (1991) Biochem. J. 279, 801–806.
[15] Biol, M.C., Pintori, S., Mathian, B. and Louisot, P. (1991) J. Nutr. 121, 114–125.
[16] Keen, C.L., Lönnerdal, B., Clegg, M. and Hurley, L.S. (1981) J. Nutr. 111, 226–230.
[17] Kessler, M., Acuto, O., Storelli, C., Murer, H., Müller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 506, 136–154.
[18] Komoda, T., Koyama, I., Nagata, A., Sakagishi, Y., De Schriver-Kecskemeti, K. and Alpers, D. (1986) Gastroenterology 91, 277–286.
[19] Martin, A., Ruggiero-Lopez, D., Broquet, P., Richard, M. and Louisot, P. (1989) J. Chromatogr. 497, 319–325.
[20] Dische, Z. (1955) Methods of Biochemical Analysis, Vol. 2, pp. 313–358, Interscience, New York.
[21] Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509.
[22] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[23] Schaffner, W. and Weissmann, C. (1973) Anal. Biochem. 56, 502–514.
[24] Mahmood, A. and Torres-Pinedo, R. (1983) Biochem. Biophys. Res. Commun. 113, 400–406.
[25] Bresson, J.L., Herscovics, A. and Walker, W.A. (1982) Gastroenterology 5, 1025.
[26] Shub, M.D., Pang, K.Y., Swann, D.A. and Walker, W.A. (1983) Biochem. J. 215, 405–411.
[27] Seetharam, B., Yeh, K.H., Moog, F. and Alpers, D.H. (1977) Biochim. Biophys. Acta 470, 424–436.
[28] Büller, H.A., Rings, E.H.H.M., Pajkrt, D., Montgomery, R.K. and Grand, J. (1990) Gastroenterology 98, 667–675.
[29] Taatjes, D.J. and Roth, J. (1990) Eur. J. Cell Biol. 53, 255–266.
[30] Paulson, J.C. and Colley, K.J. (1989) J. Biol. Chem. 264, 17615–17618.
[31] Jaswall, V.M.S., Babbar, H.S. and Mahmood, A. (1990) Ann. Nutr. Metab. 34, 155–162.
[32] Chalk, P. and Bailey, E. (1979) J. Dev. Physiol. 1, 61–79.
[33] Lebenthal, E., Sunshine, P. and Kretchner, N. (1973) Gastroenterology 64, 1136–1141.
[34] Lee, P.C. and Lebenthal, E. (1983) Pediatr. Res. 17, 645–650.
[35] Naim, H.Y. (1992) Biochem. J. 285, 13–16.
[36] Nsi-Emvo, E., Launay, J.F. and Raul, F. (1987) Cell. Mol. Biol. 33, 335–344.
[37] Sjöström, H., Norén, O. and Danielsen, E.M. (1985) J. Ped. Gastroenterol. Nutr. 4, 980–983.