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Perinatal undernutrition alters intestinal alkaline phosphatase and its main transcription factors KLF4 and Cdx1 in adult offspring fed a high-fat diet

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Abstract

Nutrient restriction during gestation and/or suckling is associated with an increased risk of developing inflammation, obesity and metabolic diseases in adulthood. However, the underlying mechanisms, including the role of the small intestine, are unclear. We hypothesized that intestinal adaptation to the diet in adulthood is modulated by perinatal nutrition. This hypothesis was tested using a split-plot design experiment with 20 controls and 20 intrauterine growth-retarded (IUGR) rats aged 240 days and randomly assigned to be fed a standard chow or a high-fat (HF) diet for 10 days. Jejunal tissue was collected at necropsy and analyzed for anatomy, digestive enzymes, goblet cells and mRNA levels. Cecal contents and blood serum were analyzed for alkaline phosphatase (AP). IUGR rats failed to adapt to HF by increasing AP activity in jejunal tissue and cecal content as observed in controls. mRNA levels of transcription factors KLF4 and Cdx1 were blunted in jejunal epithelial cell of IUGR rats fed HF. mRNA levels of TNF-α were lower in IUGR rats. They also displayed exacerbated aminopeptidase N response and reduced jejunal goblet cell density. Villus and crypt architecture and epithelial cell proliferation increased with HF in both control and IUGR rats. Serum AP tended to be lower, and serum levamisole inhibition-resistant AP fraction was lower, in IUGR than controls with HF. Serum fatty acids and triglycerides were higher in IUGR rats and higher with HF. In conclusion, the adult intestine adapts to an HF diet differentially depending on early nutrition, jejunal AP and transcription factors being blunted in IUGR individuals fed HF.

Keywords: Alkaline phosphatase; Transcription factors; Intestine; IUGR; High-fat diet

1. Introduction

Individuals with intrauterine growth retardation (IUGR) are at higher risk of neonatal morbidity and of developing metabolic diseases later in life, including type-2 diabetes, obesity and hypertension [1]. This has led to the concept of developmental programming supporting the idea that long-term control of metabolism may be determined by nutritional influences during "windows of susceptibility" early in life [2]. Regarding the gastrointestinal tract (GIT), IUGR status is a strong predictor of neonatal necrotizing enterocolitis [3] and may predispose to colorectal cancer at adulthood [4]. Whether IUGR impacts the small intestine is presently unknown, but it has been recently shown to alter colonic postnatal maturation in rats [5]. It has been hypothesized that neonatal nutrition influences GIT status, causing several disturbances such as on GIT structure, metabolism and function [6], but experimental evidence is still lacking. Recent data suggest that epigenetic maturation of the colon continues until adulthood in mice [7].

Metabolic diseases may also result from so-called "low-grade" or "metabolic" inflammation, and it has been reported that increased intestinal passage of gram-negative bacteria lipopolysaccharide (LPS) may be a causal factor in these diseases [8]. Interestingly, LPS can be detoxified by dephosphorylation of its lipid A moiety by intestinal alkaline phosphatase (IAP), and this enzyme is crucial to intestinal function and control of inflammation [9–11]. Besides, IAP inhibits fat absorption, while amino-peptidase N (APN), another intestinal enzyme, enhances cholesterol absorption [12–14].

We hypothesized that intestinal function, including IAP and APN enzyme activities, is affected by IUGR and that it can be disclosed in adults consuming a high-fat (HF) diet. We tested this hypothesis in a rat model of IUGR in which we already observed an exacerbated abdominal fat accumulation when fed an HF diet [15]. Our present data show that IAP was up-regulated by an HF diet in controls, but not in IUGR rats that also displayed blunted mRNA levels of specific transcription factors in their jejunal epithelial cells. Conversely, the activity of APN was exacerbated in IUGR rats fed the HF diet compared with the control rats. Both changes in IAP and APN activities may contribute to explain increased intestinal absorption of fatty acids and cholesterol in IUGR individuals fed an HF diet.

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2. Materials and methods

2.1. Animals and diets

The experiment was carried out in accordance with the European Union regulations for the care and use of animals for experimental procedures (Council Directive No. 86/609/EEC of November 24 1986). IUGR was generated in Sprague–Dawley rats as reported previously [15]. Briefly, female pregnant rats were housed individually and fed either a control diet with a standard protein content (control; 200 g protein/kg) or an isocaloric low-protein diet (80 g protein/kg) (Table 1). From weaning to 240 days of age, 40 pups from the control and low-protein diet groups (n=20 per group) were fed a standard laboratory chow containing a normal amount of fat (NF; 37 g/kg) provided by vegetal oil (Table 1). Animals were housed by two in standard individual cages until the age of 8 months [15]. In total, 20 litters from each control or IUGR group were used in this study.

At 240 days of age, both control and IUGR rats were housed individually and assigned to two subgroups with close mean body weights (n=10 rats per subgroup). While one subgroup of each control and IUGR rats continued to be fed the NF diet, the other subgroups of each experimental group was switched to an HF diet (265 g fat/kg diet). The HF diet was formulated according to the AIN-93G recommendation for rodent diets [16], and its composition is provided in Table 1. The fat in the HF diet was provided by a mixture of vegetal oil and lard (70 and 195 g/kg diet, respectively). Fat accounted for 9% and 47% of total energy in the NF and HF diets, respectively. The rats had *ad libitum* access to the NF or HF diets for 10 days. Rat body weight and food intake were determined regularly throughout the experiment. At 250 days of age, all rats were sacrificed by CO_2 asphyxiation followed by cervical dislocation.

2.2. Collection and sampling of blood, jejunal tissues, cecal digesta and liver

Blood was collected from the trunk at slaughter of rats, and serum was prepared and frozen at −20°C as previously reported [15]. After laparotomy, the whole GIT was removed, and the small intestine was ligated and separated from the mesentery, visceral fat and the rest of the tract. The length of the small intestine was determined and 20 cm of jejunum from the middle region was dissected. The digestive contents were gently flushed out with cold saline (NaCl 9 g/L), and the tissue was briefly dried with absorbent paper. The collected jejunal segment was divided into four parts: the first 4 cm was fixed in cold-buffered formalin (10%) during 24 h and stored for histological analysis. The following 15 cm was used to determine digestive enzyme activity. The mucosa was gently scraped with a glass slide on ice before being briefly homogenized, aliquoted and snap frozen in liquid nitrogen and stored at −80°C. The remaining 1 cm of jejunal tissue was cut into small pieces, put into tubes containing TRIzol reagent, snap frozen in liquid nitrogen and then stored at −80°C until RNA isolation for RT-qPCR (reverse transcriptase-quantitative polymerase chain reaction) analysis. Similarly, liver samples were collected for RT-qPCR analysis. The last portion of jejunum (20 cm) was taken for epithelial cell isolation. Finally, cecal digesta were collected immediately after removal of the GIT and were frozen at −20°C.

2.3. Villous and crypt morphometry and goblet cell countings

After fixation, the jejunal segment was cut into smaller segments and included in paraffin following usual procedures [17]. Tissue sections of 5-μm thickness were prepared. After paraffin removal and tissue rehydration, two sections per rat were stained with hematoxylin and eosin and mounted under glass cover slips. Jejunal full size, well-oriented villi and crypts (10–15 per section) were measured by image analysis (Lucia software, Laboratory Imaging, Prague, Czech Republic) for their length, width, perimeter and surface area using a microscope (Eclipse E400, Nikon, Champigny-sur-Marne, France) fitted with a color digital camera (DMX 1200, Nikon) and a computer. Morphology parameters were averaged per animal prior to statistical analysis. Jejunal tissue sections were also stained for total goblet cells with a combined staining of Alcian blue 8 GX at pH 2.5 and periodic acid-Schiff reaction and then counted as previously reported [17].

Table 1
Composition of the experimental diets (g/kg) [15]

	Mothers' diets		Offspring diets	
	Control	Low protein	Standard chow	High-fat diet
Ingredients				
Casein	220	90	180	315
Corn starch	631	761	630	106
Dextrin				105
Sucrose				140
Lard				195
Vegetal oil	43	43	37	70
Cellulose	54	52	53	
Vitamin mix[a]	10	10	10	30
Mineral mix[a]	40	40	40	40
Composition (kcal/g or %)				
Energy density	3.8	3.8	3.6	5.4
Protein	23.0	9.5	20.2	25.0
Carbohydrate	67.0	80.3	70.5	27.8
Fat	10.0	10.2	9.3	47.2

[a] Vitamin and mineral mixtures were formulated according to the AIN-93G recommendation for rodent diets [16].

2.4. Jejunal epithelial cell proliferation and apoptosis

Two tissue sections per rat were immunolabeled with a monoclonal antibody against proliferating cell nuclear antigen (PCNA, clone PC10, Sigma, Saint-Quentin-Fallavier, France) to determine intestinal cell proliferation [18]. Two other sections per rat were processed for apoptosis using a colorimetric TdT-mediated dUTP nick-end labeling system (TUNEL) technique [19] following manufacturer's instructions (Promega, Madison, WI, USA). Proliferating cells were counted in 20 full-size well-oriented crypts per section, while apoptotic cells were counted in all the full-size well-oriented villi (mean of 35 villi per section). The number of stained epithelial cells per crypt (proliferation) or villi (apoptosis) was quantified and expressed in relation to the length of the crypt (PCNA+ cells/100 μm) or villus (TUNEL+ cells/1000 μm) epithelium. Mean proliferation and apoptosis values from each animal were averaged prior to statistical analysis.

2.5. Digestive enzyme activity determination

After thawing and homogenization of jejunal mucosa samples in appropriate ice-cold buffers, proteins were extracted and their concentration was determined [20]. The activity of alkaline phosphatase (IAP; E.C. 3.1.3.1) was assayed in jejunal tissue and cecal digesta homogenates with a commercial kit (Sensolyte, Anaspec, San Jose, CA, USA) using *para*-nitrophenyl phosphate as the substrate. The activity of aminopeptidase N (APN; E.C. 3.4.11.2) was assayed in jejunal tissue homogenates using the synthetic substrate L-leucine *p*-nitroanilide (ref. L9125, Sigma) [21]. The activities of disaccharidases sucrase (E.C. 3.2.1.48) and trehalase (E.C. 3.2.1.28) were measured in jejunal tissue homogenates using sucrose (ref. S9378, Sigma) and trehalose (ref. T9531, Sigma) as specific substrates, respectively [22]. The generated glucose was determined using the glucose-6-phosphate dehydrogenase-hexokinase kit (Boehringer, Mannheim, Germany). Enzymes activities were determined from the number of μmoles of hydrolyzed substrate per min (IU) and expressed as specific (/mg protein) and total (/g mucosa) activities.

2.6. Jejunal epithelial cell isolation

Jejunal epithelial cells were isolated immediately after tissue collection following the method of Grossmann [23]. Briefly, 20 cm of mid-small intestine was taken and gently rinsed with cold saline for flushing out digesta contents. The segment was everted, ligated at one end, filled with DMEM/F12 medium containing MEM amino acids (Gibco, Invitrogen) and ligated at the other end. The segment was incubated twice under shaking (30 rpm) in a plastic tube with 30 ml of Ca^{++}/Mg^{++} free (CMF)-Hank's buffered salt solution (HBSS) containing 1 mM dithiothreitol at room temperature for 15 min in order to separate mucus and digesta remnants. The segment was then incubated under shaking (30 rpm) in 15 ml of CMF-HBSS solution containing 1 mM EGTA and 1 mM EDTA for 60 min at +4°C on ice. Afterwards, the segment was vigorously vortexed for 2 min in order to separate epithelial cells. The rest of jejunal tissue was removed, and the cell suspension was centrifuged at 1000 rpm for 5 min at 4°C. The cell pellet was resuspended in new EGTA-HBSS and recentrifuged (same conditions). The cell pellet was resuspended in a small volume (100–200 μl) of phosphate-buffered saline, fractionated in Eppendorf and snap-frozen in liquid nitrogen and then stored at −80°C until processing.

2.7. RNA isolation and real-time quantitative RT-PCR

Total RNA was isolated using TRIzol reagent (Invitrogen) and treated for 45 min at 37°C with RQ1 DNAse (Promega). One microgram of RNA was reverse transcribed using random primers and the Superscript III-Reverse Transcriptase (Invitrogen) at 50°C for 45 min, according to the manufacturer's instructions. The resulting cDNA was subjected to real-time quantitative PCR in a Bio-Rad iCycler iQ system using the iQ SYBRGreen Supermix PCR kit (Bio-Rad Laboratories) and specific primers for rat IAP, TLR4, IL-1β, TNF-α, KLF4, Cdx1, ATGL, TGAT1, TGAT2 and 18S mRNA. We chose to focus on these genes for the following reasons: IAP gene is a target for the nuclear transcription factors KLF4 (gut-enriched Krüppel-like factor) and Cdx1 (caudal-type homeobox-1) [24,25]; IAP gene expression can be induced by LPS [26] and acts on cells through TLR4 signaling [27]. We focused on ATGL (adipose triglyceride lipase), TGAT1 and TGAT2 (diacylglycerol acyltransferase-1 and -2) in the liver because they are the most predominant tissue enzymes involved in lipolysis and triglyceride synthesis, respectively [28,29]. Quantitative PCR consisted of 45 cycles, each PCR cycle consisting of 30 s at 95°C and 30 s at 60°C. Primers used are listed in Table 2. The expression level of 18S was used as a reference value to normalize IAP and TLR4 genes expression in

Table 2
Sequences of primers for different rat genes used in the study

Genes	Forward	Reverse
18S	GATGCGGCGGCGTTATTC	CTCCTGGTGGTGCCCTTCC
IAP	TCAGCAGACCCCTCCCTGGC	TAAGCCGTGCCCGCA TGGTG
KLF-4	CCTATACGAAGAGTTCTCATCTCA	GTAGTGCCTGGTCAGTTCA
Cdx1	CTGGCTGCTAACTTGGGTCTC	CTGCTGCTGCTGCTGCTG
TLR4	GATTGCTCAGACATGGCAGTTTC	CACTCGAGGTAGGTGTTTCTGCTAA
IL-1β	GTTTCCCTCCCTGCCTCTGAC	GACAATGCTGCCTCGTGACC
TNF-α	GCAGAGCCTTCCAAGCCTACC	GTTACCCAGCCCACCTCCTTTG
ATGL	TGTGGCCTCATTCCTCCTAC	TCGTGGATGTTGGTGGAGCT
DGAT1	GGCATCATACTCCATCATCTTC	CCCACTGACCTTCTTCCC
DGAT2	GGTCATCTCAGTCCTACAG	CTATCAGCCAGCAGTCAG

each sample. Relative quantitative gene expression was calculated for each sample using the comparative ΔC_T method.

2.8. Serum analysis

Serum AP was analysed using the same kit as reported for intestinal AP above. Serum AP analysis was conducted without and with levamisole (.1 M, tetramisole hydrochloride, reference L9756, Sigma, Saint-Quentin Fallavier, France) in order to evaluate the contribution of intestinal AP to total serum AP. Indeed, levamisole inhibits AP isoforms, except intestinal AP [30,31]. Serum fatty acids and triglycerides were analyzed as reported previously [15]. Serum LPS-binding protein (LBP) is considered as a marker of endotoxemia and is associated with obesity and metabolic syndrome [32]. Serum LBP assay was carried out using a commercial kit (reference HK503, Hycult Biotech, Uden, the Netherlands).

2.9. Statistical analysis

Data are given as least square (LS) means and S.E.M. The experimental design was a split-plot with body weight at birth (control vs. IUGR) as the main plot and food regimen at adulthood (NF vs. HF) as the subplot. Data were analyzed using the MIXED procedure of Statistical Analysis Systems (SAS, version 8.1, SAS Institute, Cary, NC, USA). A REPEATED statement was added in the model for serum AP activity measured in the absence or presence of levamisole, and the interactions between levamisole, birth body weight and adulthood diet were considered. When the interactions were significant ($P \leq .10$), differences between means were analyzed using the Bonferroni's test for multiple comparisons. Statistical significance for comparisons between means was set at $P<.05$. Data for KLF4 mRNA levels were log-transformed prior to analysis because of their non-Gaussian distribution. Linear correlations between variables were calculated whenever appropriate.

3. Results

3.1. Rat body weight and food intake

One day after birth, rat pups born to dams fed the low-protein diet exhibited lower body weight than that of pups born to dams fed the control protein diet (5.97±.09 and 7.00±.18 g, respectively; $P<.001$). Body weights of control and IUGR offspring at 240 days of age did not differ significantly (686±18 and 697±20 g, respectively). Daily food intake was essentially similar between groups of rats [16.1±.7 and 14.7±.4 kcal/100 g body weight (BW), respectively]. After switching to the HF diet for 10 days, controls rats consumed 17% more energy than IUGR rats (22.7±.7 and 18.9±.5 kcal/100 g BW, respectively; $P<.01$). Rat body weight at slaughter at 250 days of age was higher in the HF than in the NF group (737±17 and 680±18 g; $P<.01$), with no differences between control and IUGR rats in either group.

3.2. Anatomy of the small intestine, jejunal villus–crypt architecture and dynamics and goblet cell density

The length of the small intestine at slaughter was not influenced by body weight at birth or diet at adulthood (mean small intestinal length across treatments: 111±3 cm) (data not shown). In contrast, jejunal mucosa thickness as well as the length, perimeter and surface area of jejunal villi and crypts increased (or tended to increase for villus surface area, $P=.09$) in response to HF diet intake ($P<.01$ to $P<.001$) (Table 3). Surface areas (μm^2) of villi (y) and crypts (x) were positively correlated ($y=9.69x+5120$; $R^2=.397$, $P<.0001$). However, villus height to crypt depth ratio did not vary significantly (Table 3). None of the villus–crypt architecture parameters was influenced by body weight at birth, and the perinatal diet by diet interaction at adulthood was never significant.

Table 3
Villus–crypt architecture in the midjejunum of control and IUGR rats fed a standard chow (normal-fat diet) or a high-fat diet in adulthood

	Control		IUGR		S.E.M.	P^a		
	Normal fat	High fat	Normal fat	High fat		BW	Diet	BW*diet
Mucosa thickness[b] (μm)	544	699	541	658	32	.50	<.001	.56
Villi								
Height (μm)	426	550	422	520	28	.55	<.001	.64
Width (μm)	137	130	131	127	7	.54	.39	.86
Perimeter (μm)	1136	1364	1112	1313	64	.56	.002	.83
Surface area (×1000 μm^2)	55.8	65.3	52.8	60.7	5.0	.45	.09	.87
Crypts								
Depth (μm)	119	148	119	138	7	.49	.002	.45
Width (μm)	45.4	43.9	46.1	47.9	1.6	.14	.92	.30
Perimeter (μm)	314	370	313	348	14	.42	.002	.44
Surface area (×1000 μm^2)	5.06	6.06	5.02	5.95	.29	.79	.003	.91
Villus height to crypt depth ratio	3.59	3.78	3.56	3.84	.22	.94	.29	.84

[a] BW, birth weight; diet, in adulthood; BW*diet=interaction between birth weight and diet in adulthood.
[b] Estimated as the sum between villus height and crypt depth.

Epithelial cell proliferation in the crypts increased in response to HF diet intake ($P<.01$) (Table 4, Fig. 1). Epithelial cell apoptosis per villus was higher with the HF diet than with the normal diet ($P<.01$). However, this effect was no longer significant when apoptosis data were expressed per unit of epithelium length. Birth weight status had no significant effect on epithelial cell proliferation and apoptosis (Table 4). The density of total goblet cells per jejunal villi was not influenced by the diet in control rats, but it was lower with the HF compared to NF diet in IUGR rats ($P=.0445$) (Table 5). When expressed per unit of epithelium length, both villi and crypt goblet cell densities were influenced by offspring diet only ($P<.05$ to $P<.001$). The birth weight status by offspring diet interaction was not significant.

3.3. Jejunal enzyme activities

Specific and total activities of IAP of the jejunal mucosa were influenced by body weight at birth ($P<.05$) and adult diet ($P<.01$), and

Table 4
Epithelial cell proliferation and apoptosis in the midjejunum of control and IUGR rats fed a standard chow (normal-fat diet) or a high-fat diet in adulthood

	Control		IUGR		S.E.M.	P		
	Normal fat	High fat	Normal fat	High fat		*BW*	*Diet*	*BW*diet*
Crypts								
Proliferation (cell/crypt)	12.4	19.0	13.6	18.1	1.7	.93	.003	.51
Proliferation[a] (cell/100 μm)	3.97	5.11	4.33	5.25	.45	.59	.031	.82
Villi								
Apoptosis (cell/villus)	1.75	2.36	1.70	2.25	.33	.56	.002	.83
Apoptosis[a] (cell/1000 μm)	1.60	1.67	1.66	1.74	.26	.82	.78	.97

[a] Per 100 or 1000 μm of crypt or villus epithelium length.

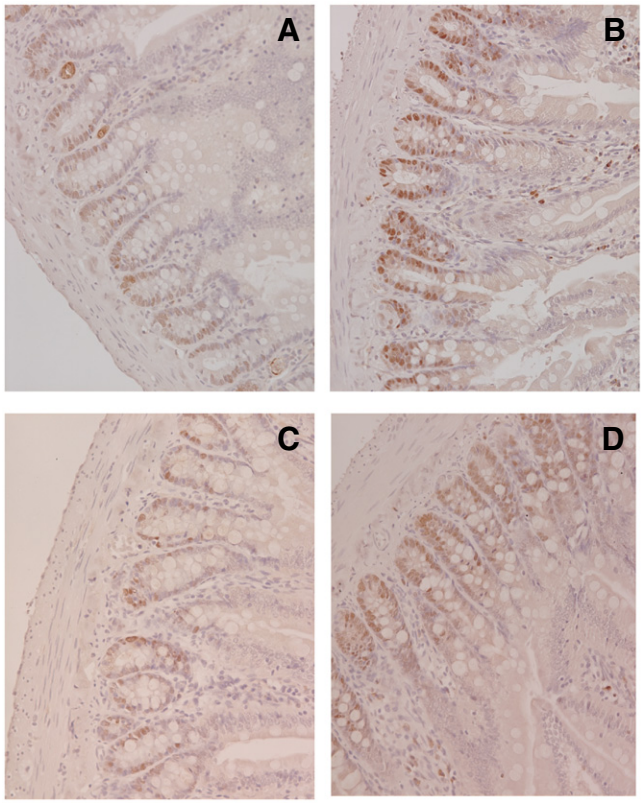

Fig. 1. Effects of body weight at birth and later diet on jejunal epithelial cell proliferation in rats. Two-hundred-forty-day-old rats born to control or protein-restricted dams (IUGR) were fed standard chow (NF) or a high-fat diet (HF) for 10 days before sacrifice. Photomicrographs of representative jejunal tissue sections from control (A, B) and IUGR (C, D) rats fed standard chow (A,C) or a high-fat diet (B, D). Sections were immunostained with anti-PCNA antibody. PCNA-positive cells appear stained in brown. Note that control rats, but not IUGR animals, exhibit a higher number of proliferating cells in response to the high-fat diet (A, C) ($P<.01$). Magnification: ×20.

the interaction was significant ($P\leq.10$) (Table 6). Control and IUGR rats displayed similar IAP activities when fed the NF diet, while IUGR rats showed much lower IAP activities than control rats under the HF diet ($P<.05$). Specific and total activities of APN were higher with the HF than with the NF diet ($P<.01$ to $P<.0001$). APN total activity was influenced by body weight at birth ($P<.01$), and the interaction was significant ($P<.10$). Control and IUGR rats displayed similar APN total activities under conditions of exposure to the NF diet, while IUGR rats had a higher APN total activity than controls when fed the HF diet ($P<.05$). The IAP to APN activity ratio in jejunal tissue was calculated (Fig. 2) as an "anti-inflammatory to proinflammatory" ratio (see Discussion). It revealed an effect of body weight at birth ($P<.05$), a tendency for a diet effect ($P=.091$) and a significant interaction between body weight at birth and diet composition ($P<.05$). This ratio doubled in control rats fed the HF diet ($P<.05$) while remaining essentially unaffected in IUGR rats.

Table 5
Density of goblet cells in the midjejunum of control and IUGR rats fed a standard chow (normal-fat diet) or a high-fat in adulthood

	Control		IUGR		S.E.M.	*P*		
	Normal fat	High fat	Normal fat	High fat		BW	Diet	BW*diet
Number per villus	34.2	32.3	34.4	24.9[a]	3.2	.27	.084	.25
Number per mm villous epithelium	30.3	23.6	31.6	18.7	2.4	.46	.0003	.21
Number per crypt	9.4	9.6	9.8	9.2	.6	.98	.66	.49
Number per mm crypt epithelium	30.4	26.4	31.5	26.9	2.0	.70	.044	.89

[a] Different from control and IUGR rats fed the normal-fat diet ($P=.0445$).

Table 6
Digestive enzyme activities of the mucosa of the midjejunum of control and IUGR rats and fed a normal- or a high-fat diet in adulthood

	Control		IUGR		S.E.M.	*P*		
	Normal fat	High fat	Normal fat	High fat		BW	Diet	BW*diet
Alkaline phosphatase								
Specific activity*	63[b]	131[a]	53[b]	71[b]	15	.023	.0056	.10
Total activity†	4.8[b]	11.0[a]	4.4[b]	6.8[b]	1.0	.038	.0003	.087
APN								
Specific activity*	85	106	89	113	7	.44	.002	.82
Total activity†	5.8[c]	7.3[b]	6.3[bc]	9.8[a]	.5	.006	<.0001	.058
Sucrase								
Specific activity*	164	86	170	61	10	.34	<.0001	.11
Total activity†	11.2	6.1	12.1	5.3	.8	.94	<.0001	.29
Trehalase								
Specific activity*	68	65	64	62	9	.70	.74	.96
Total activity†	4.6	4.5	4.5	5.6	.7	.47	.50	.40

*In μmol/min/mg protein for IAP and nmol/min/mg protein for APN, sucrase and trehalase.
†In mmol/min/g mucosa for IAP and μmol/min/g mucosa for APN, sucrase and trehalase.
[a,b,c]Means in the same row with different letters are different ($P<.05$).

Specific and total activities of sucrase in the jejunal mucosa were lower with the HF than the NF diet ($P<.0001$), with no effect of body weight at birth (Table 6). Finally, the activities of trehalase were influenced by none of the factors tested.

3.4. AP activities in cecal contents and serum

The effects of body weight at birth and diet on AP activity in cecal contents were significant ($P<.05$ and $P<.01$, respectively), as was the interaction between these factors ($P<.05$). Cecal AP activity increased fourfold in control rats in response to HF diet intake, while it did not vary significantly in IUGR rats (Fig. 3A). AP activity in serum was influenced by the diet ($P<.0001$), while body weight at birth and the interaction were not significant (Fig. 3B). However, the intestinal component (resistant to levamisole inhibition) of AP serum activity was lower in IUGR than in control rats when fed the HF diet ($P<.05$). The highest positive and linear correlation was found between serum AP and jejunal IAP activity (Fig. 4A, B and C).

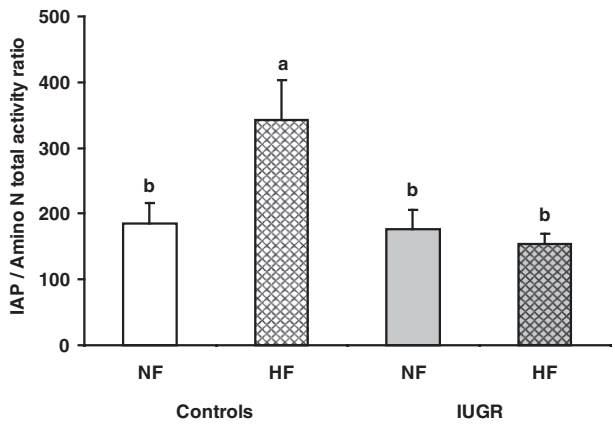

Fig. 2. Effects of body weight at birth and later diet on IAP to APN total activity ratio in jejunal mucosa. Two-hundred-forty-day-old rats born to control or protein-restricted dams (IUGR) were fed standard chow (NF) or a high-fat diet (HF) for 10 days before sacrifice. IAP to APN total activity ratio is increased in controls but not in IUGR rats fed a high-fat diet as compared to standard chow (NF diet) for 10 days ([a,b]$P<.05$). Results are expressed as LS means±S.E.M., $n=10$.

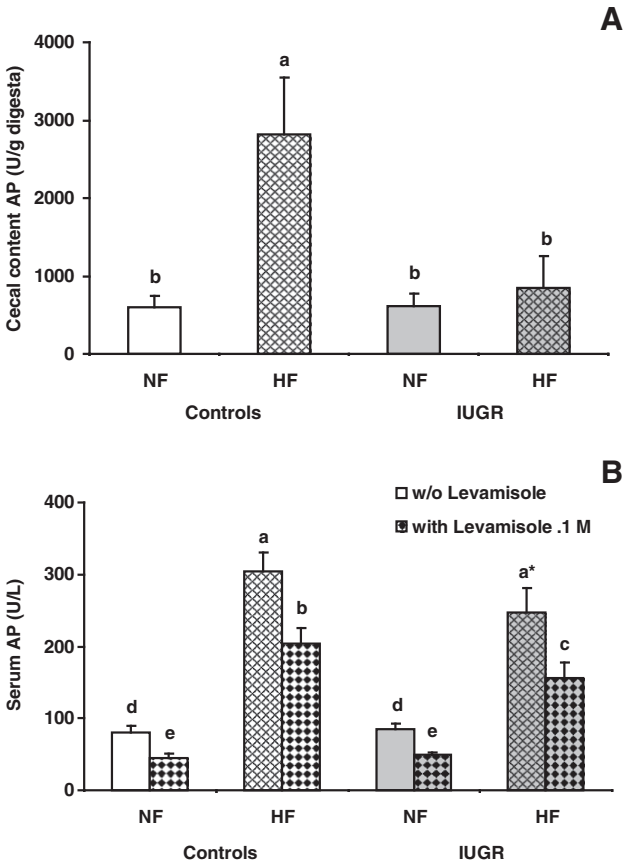

Fig. 3. Effects of body weight at birth and later diet on AP activity in cecal content (A) and serum AP activity determined without or with addition of levamisole .1 M (B). Two-hundred-forty-day-old rats born to control or protein-restricted dams (IUGR) were fed standard chow (NF) or a high-fat diet (HF) for 10 days before sacrifice. AP activity in cecal content is increased in control but not in IUGR rats in response to the high-fat diet ($P<.05$). Serum AP activity (B) is increased in both control and IUGR rats fed a high-fat diet as compared with animals fed standard chow (NF diet) for 10 days ($^{a,b}P<.05$). However, serum AP tended to be ($P=.066$), and serum AP fraction not inhibited by levamisole was lower in IUGR than in control rats when fed the HF diet ($P<.05$). Results are expressed as LS means±S.E.M., $n=10$.

3.5. RT-qPCR analysis of mRNA expressions of various genes in jejunal epithelial cells and liver

The effect of body weight at birth on IAP and TLR4 mRNA levels in jejunal epithelial cells approached significance ($P=.052$ and $P=.065$, respectively), with a reduced expression in IUGR under normal diet (Table 7). Significant interactions ($P<.05$) between birth body weight and diet in adulthood were observed for IAP transcription factors KLF4 and Cdx1 in jejunal epithelial cells. IUGR rats displayed lower KLF4 and Cdx1 mRNA levels than controls with the HF diet. IL-1β mRNA levels were not influenced significantly by the factors tested. However, differences between NF and HF diets for IUGR rats approached significance ($P=.075$). By contrast, TNF-α mRNA levels were significantly lower in IUGR rats compared to controls ($P<.01$).

RT-qPCR analysis of the expression of liver key enzymes involved in triglyceride catabolism (ATGL) or synthesis (DGAT-1 and 2) did not show any significant differences between groups (data not shown).

3.6. Serum levels of fatty acids, triglycerides and LBP

Serum fatty acids and triglycerides were higher in IUGR rats than in controls with the normal diet ($P<.001$) (Fig. 5). They both increased in control and IUGR rats in response to the HF diet ($P<.001$) and were still higher in IUGR than in control rats ($P<.05$). Finally, serum LBP increased with the HF diet in both groups [414, 513, 394 and 497

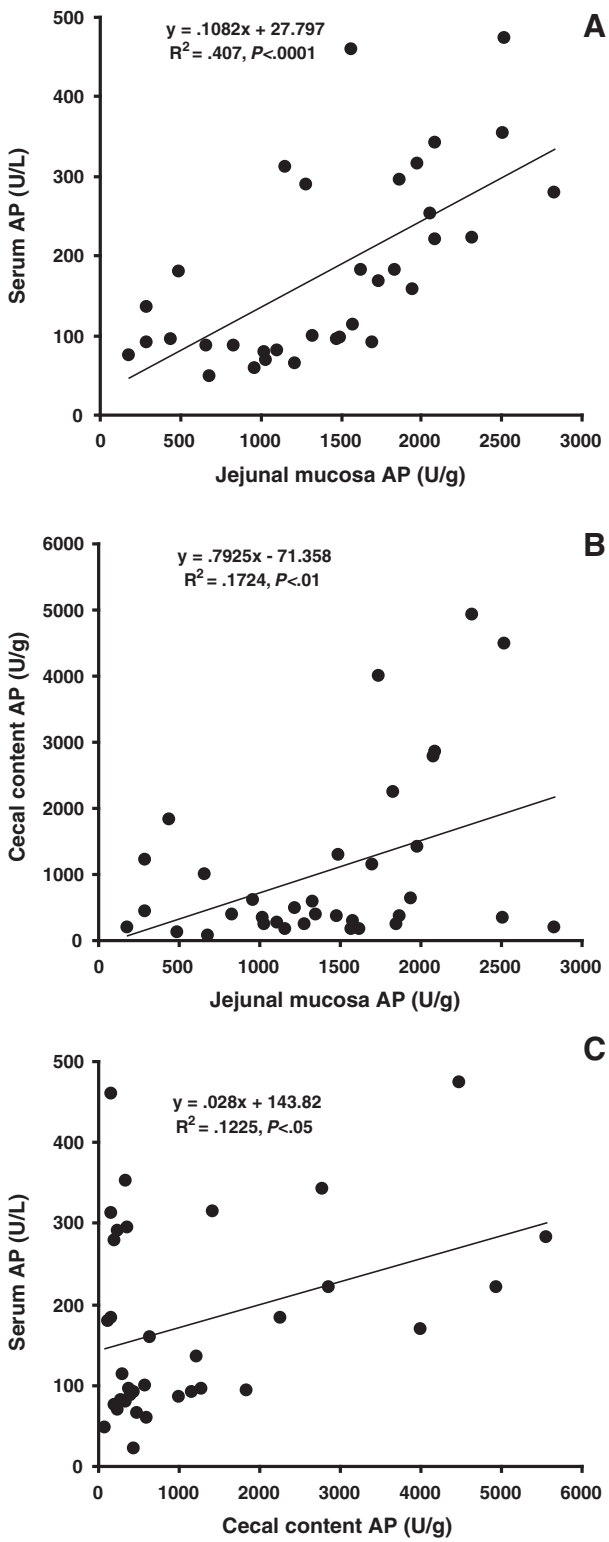

Fig. 4. Relationships between AP activities in jejunal mucosa, cecal content and serum observed in rats at sacrifice. (A) Serum AP activity is linearly and positively correlated with jejunal mucosa AP activity ($P<.0001$). (B) Cecal content AP is linearly and positively correlated with jejunal mucosa AP activity ($P<.01$). (C) Serum AP is linearly and positively correlated with cecal content AP activity ($P<.05$). Results are expressed as LS means±S.E.M., $n=10$.

Table 7
Relative mRNA levels of various genes in epithelial cells isolated from jejunal mucosa of 240-day-old rats born to control or protein-restricted dams (IUGR) that were fed standard chow (NF) or a high-fat diet (HF) for 10 days before sacrifice

	Control		IUGR		S.E.M.	*P*		
	Normal fat	High fat	Normal fat	High fat		BW	Diet	BW*diet
IAP	28	24	14	13	6	.052	.45	.99
KLF4 (log)	.72[a,*]	.86[a]	.91[a]	.47[b]	.14	.47	.29	.044
Cdx1	14.6[ab,*]	23.8[a]	18.1[ab]	12.4[b]	3.3	.24	.60	.030
TLR4	1.43	1.42	.80	.59	.37	.065	.78	.80
IL-1β	.11	.11	.16	.08[†]	.03	.69	.16	.25
TNFα	.95[a]	1.01[a]	.53[ab,‡]	.31[b]	.18	.0034	.65	.45

* Differences between normal-fat and high-fat diet in control rats: KLF4 (log), $P=.054$; Cdx1, $P=.059$.

† Difference between normal-fat and high-fat diet in IUGR rats: $P=.075$.

‡ Difference between control and IUGR rats fed normal-fat diet: $P=.10$.

[ab] Means in the same row with different letters are different ($P<.05$).

(S.E.M. 45) ng/ml for C-NF, C-HF, IUGR-NF and IUGR-HF groups, respectively; $P<.05$], with no significant effects of birth BW nor significant interaction.

3.7. Major conclusions of the experiment

The major conclusions of our experiment are that IUGR rats fed an HF diet in adulthood displayed reduced intestinal AP associated with reduced gene expression of key nuclear transcription factors for IAP. Intestinal TLR4 and TNF-α gene expressions were also blunted in IUGR rats when fed an HF diet.

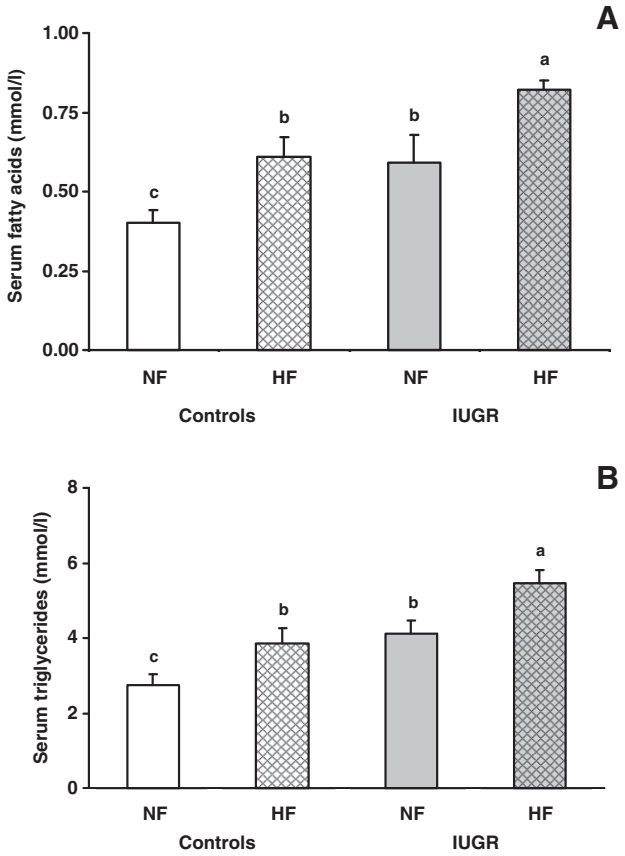

Fig. 5. Serum fatty acid (A) and triglyceride (B) concentrations in the serum of 240-day-old rats born to control or protein-restricted dams (IUGR) were fed standard chow (NF) or a high-fat diet (HF) for 10 days before sacrifice. Both serum fatty acids and triglyceride concentrations were higher in IUGR than in control rats fed either the NF or the HF diet ($P<.001$). Results are expressed as LS means±S.E.M., $n=10$.

4. Discussion

We report for the first time that the activity of two important intestinal enzymes, namely, IAP and APN, in adult rats are influenced by perinatal malnutrition, and this is unveiled only under a high-fat diet. These enzymes have crucial functions in the control of several biological processes in the intestine such as LPS detoxification, fatty acid and cholesterol transport, and inflammation. Therefore, our findings provide new insights into the mechanisms involved in intestinal and metabolic disturbances in adult IUGR individuals in the context of Western diet and obesity.

Besides its implication in the hydrolysis of various dietary substrates, IAP plays a major role in the regulation of intestinal homeostasis through at least three distinct pathways: (a) by dephosphorylating the bacterial endotoxin LPS, thus reducing its toxicity for epithelial cells; (b) by controlling LPS-induced intestinal inflammation and (c) by limiting bacterial translocation from intestine to lymphoid organs [11]. In the present study, we show that IUGR rats fed a high-fat diet displayed a 50% lower jejunal IAP activity than control rats. Hence, adult IUGR rats were unable to increase their IAP activity in response to a high-fat diet. Intestinal epithelial IAP mRNA level tended to be reduced in IUGR rats, and, in parallel, the expression of KLF4 and Cdx1, the major factors involved in the regulation of IAP gene transcription, was blunted in IUGR rats fed the HF diet. Both KLF4 and Cdx1 have been shown to activate IAP transcription in intestinal epithelial cell lines [24,25]. Therefore, perinatal undernutrition interacts with nutrition in adulthood to affect the expression of these factors durably. Other regulatory pathways, including epigenetic modifications, cannot be excluded because transcriptional activation of IAP gene is also associated with histone acetylation changes in its promoter region [33]. Furthermore, IAP expression can be also stimulated by LPS through the TLR pathway [26]. Although we found a trend toward a reduction of TLR4 expression in enterocytes of IUGR rats, this decrease may be low enough to down-regulate LPS-stimulated gene expression. Indeed, the gene expression of the proinflammatory cytokine TNF-α was lower in IUGR rats when fed an HF diet.

Since the anti-inflammatory role of IAP in various intestinal diseases is now well established [34–37], our data suggest that adult IUGR intestine may be more susceptible to inflammation. Importantly, a reduced IAP activity may contribute to chronically increased plasma LPS concentrations and therefore to metabolic endotoxemia [38], which might be at the origin of the metabolic disorders associated with IUGR [1]. Although there were no significant differences in serum levels of LBP (a marker of endotoxemia) between control and IUGR rats, high-fat diet increased serum LBP levels in both groups. These data are in accordance with the fact that high-fat diet is known to increase serum LPS concentration [38,39]. However, the consequences of IUGR in LPS responses warrant further investigation.

IAP detoxifies bacterial LPS by dephosphorylating its lipid A moiety [9,10,26]. Therefore, we reasoned that the expression of LPS receptor TLR4 in intestinal epithelial cells might be influenced by IUGR. We found a tendency for TLR4 mRNA ($P=.065$) expression to be reduced in jejunal epithelial cells of IUGR rats (Table 6). An inhibitory effect of IUGR on intestinal IAP activity has been observed in rat neonates, but to date, no data are available on the effects of IUGR on IAP gene expression [40]. A down-regulation of TLR4 mRNA levels in the liver but not in the intestine or spleen of adult IUGR rats has been reported [41]. We found that IUGR induced a strong reduction of

TNF-α mRNA expression in enterocytes. One possibility could be that intestinal reduction of TLR-4 mRNA expression in this group is lower enough to down-regulate LPS response (i.e., cytokine expression). Clearly, more work is needed in this area to assess the sensitivity of the IUGR intestine to LPS stimulation and to investigate the underlying signaling mechanisms.

Intestinal IAP has been shown to limit intestinal absorption of fat since IAP gene deficiency increases fat intestinal absorption and favors visceral fat accumulation and obesity in mice [12,14]. In our study, we found that IAP mRNA levels were lower in IUGR enterocytes than in controls. Interestingly, even under normal-fat diet, IUGR rats had higher serum levels of fatty acids and triglycerides and more abdominal adipose tissue than control rats [15]. Moreover, the inability of IUGR rats to up-regulate IAP activity in response to a high-fat diet in adulthood may contribute to higher intestinal fat absorption and obesity. This is supported in the present work by the observations that when fed the HF diet, IUGR rats consumed 17% less energy but had higher serum concentrations of triglycerides and free fatty acids than control rats [15]. Recent investigations have shown that obesity-prone, but not obesity-resistant, rats fed a high-fat diet for 8 to 12 weeks become obese and display reduced IAP activity [39]. However, the causal relationships between sensitivity to obesity and IAP activity are unclear in this model, and IAP modulation may be a cause or a consequence of intestinal inflammation [39].

The IAP activity of cecal contents and the jejunal mucosa were positively correlated (Fig. 4). This observation is of interest because it shows that IAP, which is mainly produced in the upper jejunum of rat [42], flows to the large intestine in substantial concentrations reflecting jejunal tissue IAP activity. Intestinal IAP is probably responsible for LPS detoxification in the colon due to very low IAP activity in this tissue [10]. Thus, our data suggest that IUGR individuals fed an HF diet may be at increased risk of colonic stimulation and inflammation by bacterial LPS because of reduced IAP activity in cecal contents. However, further work will be necessary to test this hypothesis.

IUGR rats responded to the HF diet by increasing their jejunal APN activity more (+34%) than control rats fed the same diet. The actual significance of this observation is unclear at present. Interestingly, APN is suspected to be implicated in intestinal transport of cholesterol [13,43,44]. In the present experiment, serum concentrations of cholesterol were numerically higher in IUGR rats than in controls fed the HF diet [15]. A positive linear correlation was found between serum cholesterol and jejunal APN activity ($y=.7784\times+4.173$, $R^2=.2326$, $P<.001$), supporting the involvement of APN in intestinal transport of cholesterol [13]. APN (also known as CD13) is involved in immune responses and antigen presentation [45,46]. An interesting point is that inhibitors of peptidases, including APN and dipeptidyl-peptidase IV, reduce colitis in mice [46], suggesting a link between APN and intestinal inflammation. Also, it was suggested that increased APN activity could suppress T-cell regulator function [46]. Therefore, we calculated the jejunal IAP to APN activity ratio as an "anti-inflammatory to proinflammatory" index (Fig. 2). It clearly showed the lack of adaptive response of IUGR to a high-fat diet, suggesting again a potentially higher sensitivity of IUGR intestine to inflammation.

Epidemiological data have demonstrated that there is a positive correlation between low birth weight and obesity in later life [47,48]. Our data suggest that intestinal IAP and APN may contribute to this process because IAP limits fatty acid absorption while APN increases that of cholesterol [12–14].

Feeding a high-fat diet to rats, regardless of their perinatal nutritional status, had significant effects on different facets of intestinal architecture and function, in broad agreement with the literature. Elongated villi and crypts together with increased jejunal proliferation in rats fed the high-fat diet were observed, as previously reported [49,50]. Apoptosis in jejunal villi was not influenced by the high-fat diet fed for 10 days in the present work, in contrast with reported increased intestinal apoptosis [51]. This discrepancy might arise from large differences in the duration of HF treatment (30–90 days vs. 10 days here). In accordance with our observations, rats (presumably born with a normal body weight and) fed with oil were shown to display increased IAP activity [52]. Jejunal sucrase activity was reduced by high-fat diet intake in the present study, in agreement with the literature [53,54]. Finally, TLR4 gene expression in jejunal epithelial cells did not respond to the HF diet in the present study. Increased activation of ileal TLR4 in obesity-prone, but not obesity-resistant, rats fed a high-fat diet was reported recently [39]. Reasons for discrepancies with our work may be a longer exposure to the high-fat diet (8–12 weeks) and the subsequent development of ileal inflammation in that study [39].

We observed a reduced goblet cell density in adult IUGR rats fed the HF diet compared to the normal chow in the present study. This would suggest increased risk for lower intestinal mucus production. In contrast with this observation, decreased crypt depth and augmented goblet cells densities in the proximal colon have been reported in adolescent (40 days old) IUGR rats fed standard chow [5]. Therefore, IUGR may impact the small intestine and the colon differently.

In conclusion, we report important data showing that the adult intestine adapts to a high-fat diet differentially depending on early nutritional status. The long-term effects of perinatal malnutrition on intestinal function appear to be selective. IUGR individuals were unable to increase jejunal IAP, while their APN response was exacerbated. Reduced IAP was associated with reduced gene expression of key nuclear transcription factors for IAP. Further work is needed to unveil other underlying molecular mechanisms participating in these long-term alterations, including epigenetic regulation [6], and to ascertain the susceptibility of IUGR intestine to inflammation in adulthood.

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