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Effect of neonatal β_3-adrenoceptor agonist CL 316,243 treatment on body fat accumulation and intestinal alkaline phosphatase activity in rats from reduced nests

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Abstract

Introduction. The aim of this study was to evaluate the effects of early life administration of β_3-adrenoceptor agonist (CL 316,243) on somatic and feeding parameters, obesity development as well as small intestinal enzyme activity in 21- and 40-day-old overfed male Sprague-Dawley rats.

Material and methods. To induce postnatal overnutrition, litter size was reduced to 4 pups/litter (small litters, SL); while in normally-nourished groups (NL) the litter size was adjusted to 10 pups/litter. From days 5 to 15, half of the suckling pups from NL and SL groups received CL 316,243 subcutaneously. From 21st to 40th day, in the post-weaning period, both control (NL, SL) and CL 316,243-treated (NL-CL, SL-CL) rats had free access to standard diet and water. Body composition was determined by magnetic resonance imaging, blood pressure was measured using non-invasive tail-cuff, and intestinal alkaline phosphatase (AP) activity was assessed by histochemistry.

Results. At 21 and 40 days of age the SL rats showed higher body mass, displayed higher adiposity and had significantly increased duodenal and jejunal AP activity compared with NL animals. On day 21, NL and SL rats treated with CL 316,243 showed significantly less fat deposition and jejunal AP activity than the non-treated controls. In contrast, treatment-related changes in adiposity and AP activity were not observed in 40-day-old NL-CL, SL-CL rats.

Conclusions. These results indicate that early pharmacological intervention with CL did not permanently influence physiological processes involved in body weight/fat regulation, which resulted in the development of early-programmed overweight status in SL rats after weaning. *(Folia Histochemica et Cytobiologica 2015, Vol. 53, No. 4, 307–313)*

Key words: pre-weaning nutrition; β_3-adrenergic agonist; obesity; intestinal alkaline phosphatase; fat depots

Introduction

In general, obesity develops when energy intake chronically exceeds energy expenditure, resulting in storage of excess energy in white adipose tissue (WAT). It has been documented that the critical factor influencing physiological processes involved in food intake, body weight regulation and adiposity control throughout life is nutritional status in the early developmental phase [1, 2]. For example, overfeeding due to higher maternal milk fat content and increased milk availability in rats from reduced litters (3–4 pups/litter) resulted during later life in enhanced weight gain, increased body fat stores and a complex of obesity-related alterations, particularly increased plasma insulin, leptin and glucose [3–5]. Moreover, available data suggest that exposure of rat pups to high-fat milk during this critical period of life leads also to alterations in small intestine functionality [2]. These changes include early postnatal adjustment of intestinal enzymes' activity, which participate in energy-balance control in later life and may be con-

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sidered as one of the factors causing obesity. Alkaline phosphatase (AP) is present mainly in the microvilli of small intestine enterocytes, where it is involved in nutrient (fat) absorption. Its activity displays circadian fluctuations, *i.e.* it is dependent on actual feeding conditions [6], becomes significantly decreased after food deprivation [7], and also increases markedly after fat--rich diet feeding [8–11]. Furthermore, some studies have revealed in rat pups subjected to pre-weaning overnutrition *via* litter size reduction significantly higher AP activity in the small intestine in comparison with rats nursed in normal nests on days 15 and 20, as well as on days 40, 50 and 80 [2, 12–14].

Other animal studies showed that the early nutritional imprint in small litter (SL) rats also causes morphological and functional changes in interscapular brown adipose tissue (BAT) in the juvenile and/or adult phase of life, leading to reduced BAT thermogenesis and energy expenditure in SL rats, which also probably increases susceptibility to obesity [1, 15, 16].

Lipolysis in WAT and thermogenesis in BAT is controlled *via* sympathetic nervous system. Direct visual evidence that WAT is indeed innervated by the sympathetic neurons that establish neuro-adipose junctions, directly 'enveloping' adipocytes, has been recently published [17]. The results of this study suggest also that the direct activation of the sympathetic nervous inputs to adipose tissue may represent strategy for fat mass reduction.

In rodents, β_3-adrenergic receptors are predominantly present in BAT and WAT, where their stimulation by selective β-adrenergic agonists results in increased energy expenditure in BAT and elevated lipid mobilization in WAT. It was shown that long--term administration of CL 316,243 (CL), a highly selective β_3-adrenoceptor agonist, largely reduced fat stores and retarded the development of obesity in young rats fed a high-fat diet [18], as well as reversed established diet-induced obesity in older animals [19–21]. Similar fat-reducing effect of CL was also described in adult non-obese rats [22, 23].

At present, however, there is a lack of information about the impact of neonatal β_3-adrenergic agonist treatment (increasing energy expenditure and lipolysis) on the later occurrence and/or attenuation of obesity and related health complications (increased food intake, blood pressure and AP activity) in rat pups nursed either in reduced or in normal litters. Using litter-size adjustment as an experimental design allowed us to evaluate whether early-life CL 316,243 intervention could counteract accumulation of body fat and reverse early programming of obesity in rat offspring after weaning. The purpose of this study was, therefore, to determine the differences in body growth parameters as well as brush border-bound duodenal and jejunal alkaline phosphatase activity in 21- and 40--day-old postnatally overfed (4 pups/nest) and normally fed (10 pups/nest) CL 316,243 treated rats, as well as in their saline-treated control littermates submitted to the same postnatal nutritional manipulations.

Material and methods

Animals. All animal experiments were reviewed and approved by the Ethical Committee for animal experimentation of the Institute of Animal Physiology, approved by the State Veterinary and Food Administration of the Slovak Republic, and were performed in accordance with Slovak legislation (Law No. 377/2012) on the protection of animals used for experimental and other scientific purposes.

Sprague-Dawley virgin rat dams (Charles River Laboratories, Prague, Czech Republic) weighing 240–260 g, mated at 10 weeks of age, were individually housed in Plexiglas cages and kept under conditions of constant room temperature (22 ± 2°C), relative humidity (55 ± 10%) and 12 h light/dark cycle (light on 06:00 to 18:00 h) with free access to a standard laboratory diet (containing 13.4 kJ/g, with 26.3% energy as protein, 9.5% as fat and 64.2% as carbohydrate; Laboratory diet M1, Řicmanice, Czech Republic) and tap water. Within 24 h of parturition, the litters with less than 8 or more than 12 pups were excluded from the experiment. To induce early postnatal normal nutrition or overnutrition, the litter size in one group of dams (n = 5) was adjusted after parturition to 10 pups per nest (normal litters, NL) and in the other group of dams (n = 11) to 4 pups per nests (small litters, SL). Half of the number of pups in each nest (CL groups) was injected subcutaneously with β_3-adrenoreceptor selective agonist, CL 316,243 (5-[(2R)-2-[[(2R)-2-(3-chlorophenyl)-2-hydroxyethyl]amino] propyl]-1,3-benzodioxole-2,2-dicarboxylate, Sigma-Aldrich, Deisenhofen, Germany) at a dose of 1 mg/kg of body weight per day, or an equivalent volume of saline solution (control groups), daily from day 5 to 15 after birth. After weaning (on day 21), the male rats were individually housed under the standard laboratory conditions (water, temperature, light-dark regime) with free access to a standard laboratory diet up to day 40.

To determine the growth and post-weaning feeding performance in all four mentioned groups, the body weight was measured at birth and then every 5 days, and from day 21 to 40 their food intake was recorded every 48 hours. On days 21 and 40, duodenal and jejunal segments were taken for enzyme assay after decapitation of the animals (between 08:00 and 09:00 h). Bilateral epididymal and perirenal WAT as well as interscapular BAT samples were removed and weighed.

Determination of milk composition. The individual dams were milked on day 10 of lactation. Two hours before milking the dams were separated from their pups, which were kept

in a constant temperature chamber 34 ± 1°C, and 10 min before milking the dams received 2 IU oxytocin (Sigma-Aldrich) intraperitoneally. Milk fat was measured using the crematocrit method of Lucas *et al.* [24] and its concentration was expressed in g/100 mL milk using the formula given by Nagasawa *et al.* [25].

Measurement of alkaline phosphatase activity. For enzyme assay, small (0.5 cm) segments of the duodenum and middle part of the jejunum were immediately removed; the lumen was rinsed in distilled water and samples were frozen in liquid nitrogen. Segments of frozen tissue were cut (8 μm) in a cryostat at –25°C, and the tissue slices were transferred to glass slides and air-dried. The analysis of alkaline phosphatase activity was performed using a modified simultaneous azocoupling method [26]. The incubation medium contained 2.0 mmol/L naphthol AS-BI phosphate (Sigma-Aldrich), 0.8 mmol/L hexazotized new fuchsin (Serva, Heidelberg, Germany), and 0.05 mol/L veronal acetate buffer. The sections were incubated at 37°C for 10 min at pH 8.9 [27].

The histochemically-stained slides were illuminated with white light after filtering with a 520 nm monochromatic filter and visualized with image analysis tool (Ellipse program ViDiTo, Košice, Slovakia), where the gray level of each pixel was given a value in the range 0–255. The correspondence between the gray level values and the known integrated absorbance values of the same section points was determined by calibration. A special semi-interactive algorithm was used to find relevant pixels along the villus length, which density was measured [28]. Quantification of the enzyme activity (pixel intensities) was carried out along the villus length in a whole section of at least four samples, and the mean values recorded were referred to one animal [29].

Fat mass measurements. Body composition was determined using magnetic resonance imaging (Echo MRI-700 TM, Echo Medical Systems, Houston, TX, USA). Scans of male rats were performed from day 10 to 40 with the Whole Body Analysis protocol in the scanner software package at 10-day intervals. Body composition data were reported as absolute mass of fat in grams. The percentage of body fat was calculated from the body weight in grams.

Blood pressure measurements. Blood pressure was non-invasively measured by determining the tail blood volume using a volume pressure recording sensor and occlusion tail-cuff method (CODA System, Kent Scientific, Torrington, CT, USA). Before recording, the rats were adapted to the measurement conditions for at least 10–15 min over a period of 3 days. For each animal, the mean systolic and diastolic values were calculated from 10 measurements on day 40.

Statistical analysis. Statistical analyses were performed using the Statistica AXAZ software package (StatSoft CR, Prague, Czech Republic). Two-way ANOVA followed by Fisher's LSD *hoc* test were used for detecting significant differences between SL and NL groups and for evaluating their responses to the CL 316,243 treatment. Data were summarized as means ± SEM. A $p < 0.05$ level was considered statistically significant.

Results

Milk composition

Reduction of litter size resulted in a significant increase in milk fat concentration on day 10 *postpartum*. In SL nursing dams as compared with NL mothers the mean values of milk fat concentration were 17.56 ± ± 0.8 *vs.* 13.0 ± 0.05 g/100 mL, respectively ($p < 0.001$).

Effects of litter size manipulation

There was no significant difference in the average body weight between NL pups (10 pups/dam) and SL (4 pups/dam) on postnatal day 1 (6.5 ± 0.06 *vs.* 6.5 ± ± 0.11, respectively). The male pups from SL litters compared with the NL controls showed significantly higher final body weight and weight gain, as well as significantly higher accretion of epididymal plus perirenal fat pads weight expressed as the percentage of body weight on day 21 and on day 40 (Table 1).

These somatic differences persisted in SL rats despite post-weaning access to identical diets as consumed by the controls. In addition, the data from magnetic resonance imaging (Echo MRI-700 TM) demonstrated in SL rats relative body fat mass (% b.w.) significantly exceeding the values for NL rats by 48% on day 10, 70% on day 20 and 19% on day 40 (Figure 1). During the post-weaning period (from day 21 to 40), in SL rats significantly higher food intake by 8.93% was recorded than in NL controls (Table 1).

The effect of overfeeding induced by small litter rearing resulted in different functional maturation of the small intestine; in particular, significantly increased ($p < 0.01$) duodenal and jejunal alkaline phosphatase activity was recorded on days 21 and 40 (Table 1, Figure 2). Moreover, SL rats compared with the NL controls had significantly increased systolic (149.30 ± 5.83 *vs.* 128.80 ± 2.70 mm Hg, respectively) and diastolic (110.50 ± 5.31 *vs.* 95.50 ± ± 3.83 mm Hg, respectively) blood pressure.

Effect of β_3-adrenoceptor agonist — CL 316,243 treatment

We found that 10 days of repeated CL 316,243 administration did not influence final body weight and weight gain on postnatal day 21 (Table 1). Even though the control and the CL-treated rats had identical final body weight at sacrifice, the

Table 1. Effect of CL 316,243 treatment on somatic parameters and intestinal alkaline phosphatase activity in 21- and 40-day-old SL and NL pups

	21st day				40th day			
	NL	SL	NL-CL	SL-CL	NL	SL	NL-CL	SL-CL
Body mass [g]	43.73 ± 0.39	50.35 ± 0.64***	41.03 ± 0.68	51.97 ± 1.46$$$	158.90 ± 5.25	172.30 ± 3.06*	170.00 ± 2.27	178.10 ± 4.53
Body mass gain [g] from day 1 to day 21	37.23 ± 0.36 117.46%	43.84 ± 0.66*** 114.85%	34.18 ± 1.53 120.04%	45.03 ± 1.64$$$ 115.41%	NA	NA	NA	NA
Body mass gain [g] from day 1 to day 40	NA	NA	NA	NA	151.96 ± 5.13 104.57%	165.59 ± 3.05* 104.05%	162.88 ± 2.15 104.37%	171.21 ± 4.50 104.02%
Food intake [g/day] from day 21 to day 40	NA	NA	NA	NA	12.76 ± 0.36	13.90 ± 0.33*	13.78 ± 0.21	14.27 ± 0.50
Fat pads' mass (% b.w.)	0.17 ± 0.01	0.35 ± 0.02***	0.08 ± 0.02##	0.27 ± 0.02††, $$$	0.42 ± 0.03	0.57 ± 0.04**	0.49 ± 0.02	0.57 ± 0.02$
Duodenal AP activity (arbitrary units)	17.03 ± 0.57	18.94 ± 0.26**	17.29 ± 0.38	18.49 ± 0.23$	15.96 ± 0.38	17.82 ± 0.33**	16.54 ± 0.49	17.36 ± 0.58
Jejunal AP activity (arbitrary units)	16.79 ± 0.19	17.73 ± 0.27**	16.05 ± 0.25#	16.48 ± 0.21†††	13.12 ± 0.28	14.39 ± 0.27**	13.44 ± 0.28	14.36 ± 0.44

Abbreviations: AP — alkaline phosphatase; NL — normal litter reared rats; SL — small litter reared rats; NL-CL and SL-CL — rats from normal and small litters treated s.c. from day 5 to 15of life with CL 316,243. Values are expressed as means ± SEM (8 animals/group on day 21, 10 animals/group on day 40). Mass of fat pads is a sum of epididymal and perirenal fat mass. Brush border-bound duodenal and jejunal alkaline phosphatase (AP) enzyme activity is given as density values (pixel intensities) at wavelength 520 nm. Significant differences between NL *vs.* SL groups (*p < 0.05, **p < 0.01, ***p < 0.001), between NL *vs.* NL-CL (#p < 0.05, ##p < 0.01), between SL *vs.* SL-CL groups (††p < 0.01, †††p < 0.001) and between NL-CL *vs.* SL-CL ($p < 0.05, $$$p < 0.001) based on Fisher's LSD comparison test after two-way ANOVA

adiposity determined by whole-body MRI scans was significantly, and to a similar extent, reduced in both groups treated with CL: on day 20 the body fat mass was reduced by 20% and 19% in NL-CL and SL-CL rats, respectively, as compared to non-treated NL and SL animals (Figure 1B). Moreover, exposure of NL and SL rats to CL treatment led on day 21 to a significant reduction of epididymal and perirenal fat pads expressed as the percentage of body weight (NL-CL 53% *vs.* SL-CL 26%) as compared to NL and SL controls. The interscapular brown adipose tissue expressed as the percentage of body weight was not affected by CL administration in normal litter reared pups (0.47 ± 0.01 for NL *vs.* 0.44 ± 0.02 for NL-CL) as well as in small litter reared pups (0.62 ± 0.03 for SL *vs.* 0.58 ± 0.02 for SL-CL).

The administration of β_3-adrenoceptor agonist significantly decreased intestinal alkaline phosphatase activity in 21 day-old rats both in NL-CL and SL-CL animals (Table 1).

However, this processed did not persist for longer time, since on day 40, at the end of the experiment; rats treated with CL 316,243 displayed a non-significant tendency toward body fat reduction and intestinal functional changes (Table 1).

There were no apparent differences in food intake between both NL and SL rats treated with the CL 316,243 and their respective controls. Furthermore, in both CL 316,243-treated groups, we did not observe differences in systolic and diastolic blood pressure parameters compared with the NL and SL controls on day 40 (data not shown).

Discussion

It is generally known that nutritional changes during the critical initial period of life contribute to the development of various diseases in adulthood, including obesity and obesity-related health complications. The findings of the present study show that early life overfeeding due to higher milk fat content in SL rats resulted in an accelerated overall growth persisting throughout experiment despite transition to standard laboratory feeding conditions after weaning. Similarly, data from magnetic resonance imaging and our monitoring of epididymal plus perirenal fat pad mass at the end of the experiment revealed significantly higher fat mass in rats from small litters than from normal litters. Moreover, our data also indicate that altered nutritional background during the pre-weaning period significantly increased duodenal and jejunal brush-border-bound alkaline phosphatase activity in 21- and 40-day-old small litter rats in comparison with NL rats. It can be assumed that these acquired

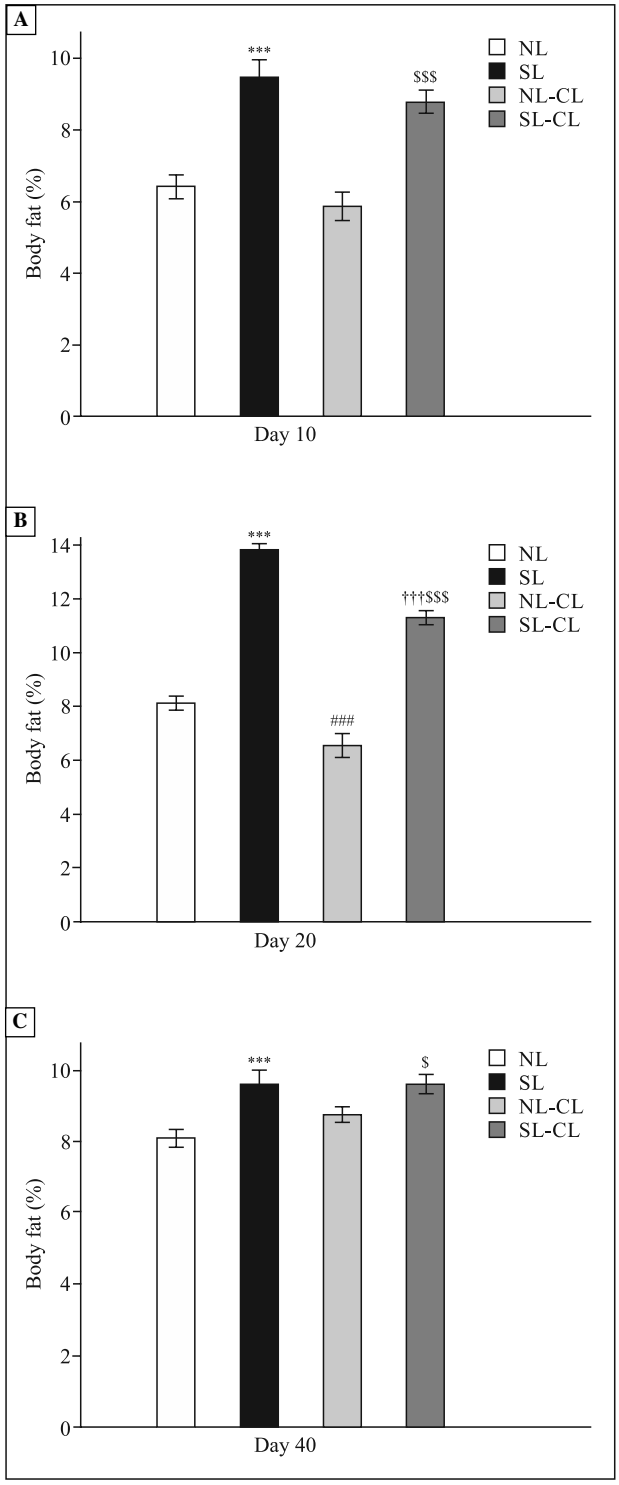

Figure 1. Whole body fat mass (% b.w.) on day 10 (**A**), on day 20 (**B**) and on day 40 (**C**) in normal litter (NL), small litter (SL) and CL 316,243-treated rats NL-CL, SL-CL was determined using magnetic resonance imaging. Values are means ± SEM (18 animals/group on days 10 and 20; 10 animals/group on day 40). Significant differences between NL *vs.* SL groups (***$p < 0.001$), between NL *vs.* NL-CL (###$p < 0.001$), between SL *vs.* SL-CL groups (†††$p < 0.001$), between NL-CL *vs.* SL-CL ($^{\$}p < 0.05$, $^{\$\$\$}p < 0.001$) based on Fisher's LSD comparison test after two-way ANOVA

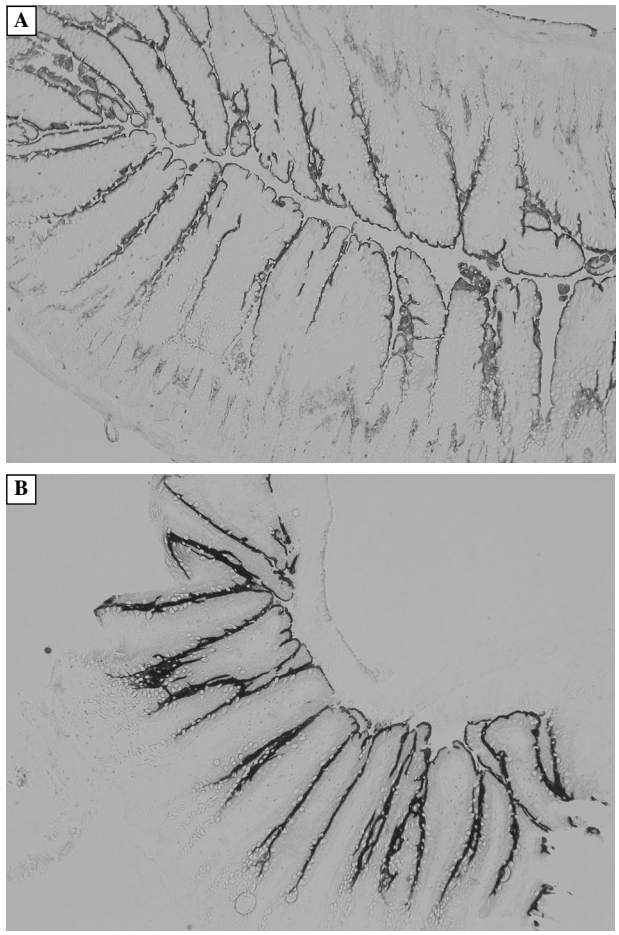

Figure 2. Histochemical demonstration of jejunal alkaline phosphatase activity in cryosections of 40-day-old NL and SL rats. The enzyme activity is expressed in the brush-border of absorption cells of the intestinal villi in NL (**A**) and SL (**B**) rats

intestinal changes represent significant determinants for future physiological processes regulating metabolism and body composition. Alkaline phosphatase is a crucial brush-border enzyme functionally involved in nutrient (fat) absorption in the intestinal mucosa [30]. For this reason the diet can be considered as a major factor influencing AP activity. It has been previously documented that elevated alkaline phosphatase activity occurs in rats [8, 12] and mice [31] in response to increased fat content in the diet. Current data also suggest that the rat's intestine adapts to a high-fat diet differentially depending on early nutrition. This is supported by the finding that whereas in adulthood jejunal alkaline phosphatase activity doubled in response to high-fat diet feeding in control rats, this effect was not recorded in perinatally malnourished rats [11]. Recently, it has been revealed that inactivation of the AP gene (Akp3) resulted in faster weight gain and fat accumulation in mice fed a high-fat diet in comparison with wild-type controls, suggesting

that AP may negatively regulate fat absorption [31]. Nevertheless, this phenomenon probably resulted from the concomitant up-regulation of Akp6 gene in distal regions of the intestine involved in accelerated fat absorption in these mice [32].

Moreover, changes in early postnatal diet modified not only the somatic and enzymatic/functional status of our rats, but also their blood pressure, *i.e.* in over-nourished SL rats significantly elevated systolic/ /diastolic blood pressure was found in comparison with NL rats, even when they were subjected to consumption of standard laboratory diet after weaning.

Since the prevalence of obesity worldwide continues to increase, and the current strategies involved in the prevention and treatment of obesity have been minimally effective, interest in novel therapeutic interventions has burgeoned. It was previously suggested that β_3-adrenoceptor, the predominant subtype of adrenoceptor expressed mainly in white and brown adipose tissue play an important role in the regulation of lipolysis, thermogenesis and energy homeostasis. Earlier studies reported that β_3-adrenergic receptor stimulation in rats and mice fed high-fat diets is very effective in normalization of obesity [20, 21]. Moreover, 4 weeks treatment of obese Zucker rats with CL 316,243 resulted in an increase metabolic rate by 96% per rat as well as in decrease and remodeling of retroperitoneal white fat depots [33]. In contrast, mice lacking β_3-adrenergic receptor had modestly increased fat stores [34].

The aim of this study was therefore to determine whether early-life β_3-adrenergic agonist administration leads to prevention and/or attenuation of obesity development in male rats subjected to nutritional manipulation during the suckling period. To this end we examined the effects of different nutritional conditions *via* litter size reduction and 10 days of treatment with selective β_3-adrenergic agonist CL 316,243 on adjustment of the future energy balance control and body fat regulation in NL-CL and SL-CL groups compared to the non-treated controls. Our results confirm the fat-reducing effect of the β_3-adrenergic agonist CL in 21 day old pre-weaning SL rats. These rats showed apparent reduction of whole body fat as well as epididymal and perirenal fat pads despite their higher dietary energy intake due to significantly higher milk fat concentration. Moreover, these fat-reducing changes were accompanied with significantly decreased brush border-bound AP activity of jejunal enterocytes in the SL-CL-treated group as compared to SL rats. In this regard, it seemed that the reduction in intestinal AP activity recorded in the CL-treated groups on day 21 could have considerable effect on energy balance control and might perhaps contribute to obesity prevention.

On the other hand, our results indicate that this anti-obesity impact of early-life CL treatment disappeared in 40 day old SL rats, *i.e.* in SL-CL rats similarly sustained elevations in adipose depots and increased intestinal parameters of enzyme activity were found as in their overfed but non-treated littermates. According to our results this lack of long-term impact of β_3-agonist on energy balance control and body weight/ /fat regulation in rats with different nutritional background (excess fat intake) during the critical period led to the development of similar overweight status as in the case of non-treated SL rats. CL treatment was not essential for apparent reduction of body fat reserves, and previously acquired somatic and functional differences continued until the end of the experiment.

Conclusions

The results of our study indicate that early-life β_3-adrenoceptor agonist CL 316,243 administration did not permanently protect against development of adiposity in rat pups subjected to obesogenic diet (high fat milk) during the suckling period. Moreover, adjustment of the intestinal-bound AP activity to a lower level in pre-weaning SL rats was only transitory and withdrawal of previous pharmacological intervention (CL 316,243 treatment) resulted in similarly increased post-weaning intestinal alkaline phosphatase activity and body fat accretion as found in control rats. Therefore, additional information focused on developmental and enzymatic adaptability in rats under different nutritional conditions will be needed for better understanding of the mechanisms involved in body fat regulation and adiposity control.

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References

1. Stefanidis A, Spencer SJ. Effects of neonatal overfeeding on juvenile and adult feeding and energy expenditure in the rat. *PLoS One.* 2012;7:e52130. doi: 10.1371/journal.pone.0052130.
2. Mozeš Š, Šefčíková Z, Raček Ľ. Long-term effect of altered nutrition induced by litter size manipulation and cross-fostering in suckling male rats on development of obesity risk and health complications. *Eur J Nutr.* 2014;53:1273–1280. doi: 10.1007/s00394-013-0630-7.
3. Plagemann A, Harder T, Rake A et al. Perinatal elevation of hypothalamic insulin, acquired malformation of hypothalamic galaninergic neurons, and syndrome x-like alterations in adulthood of neonatally overfed rats. *Brain Res.* 1999;836:146–155. PMID: 10415413.
4. Rodrigues AL, de Moura E, Passos MC, Dutra SC, Lisboa PC. Postnatal early overnutrition changes the leptin signaling pathway in the hypothalamic-pituitary-thyroid axis of young

and adult rats. *J Physiol.* 2009;587:2647–2661. doi: 10.1113/jphysiol.2009.169045.
5. Rodrigues AL, de Moura EG, Passos MC et al. Postnatal early overfeeding induces hypothalamic higher SOCS3 expression and lower STAT3 activity in adult rats. *J Nutr Biochem.* 2011;22:109–117. doi: 10.1016/j.jnutbio.2009.11.013.
6. Martinková A, Lenhardt Ľ, Mozeš Š. Effect of neonatal MSG treatment on day-night alkaline phosphatase activity in the rat duodenum. *Physiol Res.* 2000;49:339–345. PMID: 11043921.
7. Raček Ľ, Lenhardt Ľ, Mozeš Š. Effect of fasting and refeeding on duodenal alkaline phosphatase activity in monosodium glutamate obese rats. *Physiol Res.* 2001;50:365–372. PMID: 11551142.
8. Kaur J, Madan S, Hamid A, Singla A, Mahmood A. Intestinal alkaline phosphatase secretion in oil-fed rats. *Dig Dis Sci.* 2007;52:665–670. doi: 10.1007/s10620-006-9384-x.
9. Mozeš Š, Bujňáková D, Šefčíková Z, Kmeť V. Developmental changes of gut microflora and enzyme activity in rat pups exposed to fat-rich diet. *Obesity.* 2008;16:2610–2615. doi: 10.1038/oby.2008.435.
10. Scoaris CR, Rizo GV, Roldi LP et al. Effects of cafeteria diet on the jejunum in sedentary and physically trained rats. *Nutrition.* 2010;26:312–320. doi: 10.1016/j.nut.2009.04.012.
11. Lallès JP, Orozco-Solís R, Bolaños-Jiménez F, de Coppet P, Le Dréan G, Segain JP. Perinatal undernutrition alters intestinal alkaline phosphatase and its main transcription factors KLF4 and Cdx1 in adult offspring fed a high-fat diet. *J Nutr Biochem.* 2012;23:1490–1497. doi: 10.1016/j.jnutbio.2011.10.001.
12. Mozeš Š, Šefčíková Z, Lenhardt Ľ, Raček Ľ. Obesity and changes of alkaline phosphatase activity in the small intestine of 40- and 80-day-old rats subjected to early postnatal overfeeding or monosodium glutamate. *Physiol Res.* 2004;53:177––186. PMID: 15046554.
13. Šefčíková Z, Bujňáková D, Raček Ľ, Kmeť V, Mozeš Š. Developmental changes in gut microbiota and enzyme activity predict obesity risk in rats arising from reduced nests. *Physiol Res.* 2011;60:337–346. PMID: 21114359.
14. Mozeš Š, Šefčíková Z, Bujňáková D, Raček Ľ. Effect of antibiotic treatment on intestinal microbial and enzymatic development in postnatally overfed obese rats. *Obesity.* 2013;21:1635–1642. doi: 10.1002/oby.20221.
15. Xiao XQ, Williams SM, Grayson B et al. Excess weight gain during the early postnatal period is associated with permanent reprogramming of brown adipose tissue adaptive thermogenesis. *Endocrinology.* 2007;148:4150–4159. doi: 10.1210/en.2007-0373.
16. de Almeida DL, Fabrício GS, Trombini AB et al. Early overfeed-induced obesity leads to brown adipose tissue hypoactivity in rats. *Cell Physiol Biochem.* 2013;32:1621–1630. doi: 10.1159/000356598.
17. Zeng W, Pirzgalska RM, Pereira MM et al. Sympathetic neuro-adipose connections mediate leptin-driven lipolysis. *Cell.* 2015;163:84–94. doi: 10.1016/j.cell.2015.08.055.
18. Himms-Hagen J, Cui J, Danforth E Jr et al. Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. *Am J Physiol.* 1994;266:R1371–R1382. PMID: 7910436.
19. Ghorbani M, Claus TH, Himms-Hagen J. Hypertrophy of brown adipocytes in brown and white adipose tissues and reversal of diet-induced obesity in rats treated with a beta3-adrenoceptor agonist. *Biochem Pharmacol.* 1997;54:121–131. PMID: 9296358.
20. Inokuma K, Okamatsu-Ogura Y, Omachi A et al. Indispensable role of mitochondrial UCP1 for antiobesity effect of beta3-adrenergic stimulation. *Am J Physiol Endocrinol Metab.* 2006;290:E1014–E1021. doi: 10.1152/ajpendo.00105.
21. Ferrer-Lorente R, Cabot C, Fernández-López JA, Alemany M. Comparative effects of oleoyl-estrone and a specific beta3-adrenergic agonist (CL316, 243) on the expression of genes involved in energy metabolism of rat white adipose tissue. *Nutr Metab (Lond).* 2010;7:15. doi: 10.1186/1743-7075-7-15.
22. Ferrand C, Redonnet A, Prévot D, Carpéné C, Atgié C. Prolonged treatment with the beta3-adrenergic agonist CL 316243 induces adipose tissue remodeling in rat but not in guinea pig: 1) fat store depletion and desensitization of beta-adrenergic responses. *J Physiol Biochem.* 2006;62:89–99. PMID: 17217163.
23. Suárez J, Rivera P, Arrabal S et al. Oleoylethanolamide enhances β-adrenergic-mediated thermogenesis and white-to-brown adipocyte phenotype in epididymal white adipose tissue in rat. *Dis Model Mech.* 2014;7:129–141. doi: 10.1242/dmm.013110.
24. Lucas A, Gibbs JA, Lyster RL, Baum JD. Creamatocrit: simple clinical technique for estimating fat concentration and energy value of human milk. *Br Med J.* 1978;1:1018–1020. PMID: 638579.
25. Nagasawa H, Naito T, Kataoka K. Relationship between milk composition and pup's growth in mice. *Proc Soc Exp Biol Med.* 1989;191:78–81. PMID: 2717628.
26. Lojda Z, Gossrau R, Schibler TH. Enzyme histochemistry. Berlin: Springer; 1979:59–70.
27. Mozeš Š, Lenhardt Ľ, Martinková A. A quantitative histochemical study of alkaline phosphatase activity in isolated rat duodenal epithelial cells. *Histochem J.* 1998;30:583–583. PMID: 9792277.
28. Mozeš Š, Šefčíková Z, Lenhardt Ľ. Functional changes of the small intestine in over- and undernourished suckling rats support the development of obesity risk on a high-energy diet in later life. *Physiol Res.* 2007;56:183–192. PMID: 16555945.
29. Šefčíková Z, Hájek T, Lenhardt Ľ, Raček Ľ, Mozeš Š. Different functional responsibility of the small intestine to high fat/high-energy diet determined the expression of obesity-prone and obesity-resistant phenotypes in rats. *Physiol Res.* 2008;57:467–474. PMID: 17552870.
30. Lalles JP. Intestinal alkaline phosphatase: multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. *Nutr Rev.* 2010;68:323–332. doi: 10.1111/j.1753-4887.2010.00292.x.
31. Nakano T, Inoue I, Koyama I et al. Disruption of the murine intestinal alkaline phosphatase gene Akp3 impairs lipid transcytosis and induces visceral fat accumulation and hepatic steatosis. *Am J Physiol Gastrointest Liver Physiol.* 2007;292:G1439–G1449. doi: 10.1152/ajpgi.00331.2006.
32. Narisawa S, Hoylaerts MF, Doctor KS, Fukuda MN, Alpers DH, Millán JL. A novel phosphatase upregulated in Akp3 knockout mice. *Am J Physiol Gastrointest Liver Physiol.* 2007;293:G1068–G1077. doi: 10.1152/ajpgi.00073.2007.
33. Ghorbani M, Teimourian S, Farzad R, Asl NN. Apparent histological changes of adipocytes after treatment with CL 316,243, a β-3-adrenergic receptor agonist. *Drug Des Devel Ther.* 2015;9:669–676. doi: 10.2147/DDDT.S73891.
34. Susulic VS, Frederich RC, Lawitts J et al. Targeted disruption of the beta 3-adrenergic receptor gene. *J Biol Chem.* 1995;270:29483–29492. PMID: 7493988.

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