Intensive rearing of male calves during the first three weeks of life has long-term effects on number of islets of Langerhans and insulin stained area in the pancreas

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ABSTRACT: Permanent effects of early postnatal nutrition on the development and function of tissues and organs have been previously demonstrated primarily in humans and rodents. The objective of this study in calves was to analyze the impact of rearing conditions during the first 3 wk of life on morphology of insulin-producing pancreatic β-cells. Forty-two male Holstein calves were raised during the first 3 wk of life either intensively (intensively reared [INT]; ad libitum milk feeding and individual hutches; n = 21) or according to an established restrictive rearing protocol (4 L milk/d) during wk 1 in hutches and 720 g/d milk replacer (MR) from d 8 to 21 in group pens (restrictively reared [CON]; n = 21). Thereafter, all calves were housed and fed under comparable conditions. Birth weight and weekly BW up to wk 10 were recorded. Plasma glucose, insulin, IGF-1, and GH levels were assessed in wk 1, 2, 3, and 10 of life. Slaughtering took place after 8 mo and pancreatic tissue from the medium body (corpus pancreatic) was removed. The number of islets of Langerhans and the insulin stained area were examined histologically. Total milk intake of INT calves was nearly double the intake in CON calves in the first 3 wk of life (P < 0.01). Daily starter intake during wk 4 to 10 of life did not differ between groups (P = 0.24). During the first 3 wk, the ADG were up to 9 times higher in INT calves compared to CON calves (P < 0.01), yet BW at time of slaughter did not differ (P = 0.18). Intensive rearing led to increased plasma glucose, insulin, and IGF-1 concentrations after 3 wk of life compared with rearing to the established standard protocol (all P < 0.05), whereas GH was lower in INT calves during the second week of life. At time of slaughter, the mean number of islets of Langerhans was higher in INT calves compared to CON calves (9.1 ± 0.3 vs. 7.8 ± 0.3; P < 0.01). Also, the total insulin stained area per photograph was higher in INT calves compared to CON calves (107,180 ± 4,987 μm² vs. 84,249 ± 4,962 μm²; P < 0.01). Number of islets of Langerhans was negatively associated with birth weight but positively correlated with insulin and in trend with IGF-1 plasma levels during the second week of life. Insulin stained area tended to be linked with IGF-1 concentration during the third week of life. In conclusion, differences in the morphology of pancreatic islets of Langerhans indicate that calves can be programmed metabolically by an altered postnatal rearing intensity.

Key words: ad libitum intake, calves, insulin, islets of Langerhans, metabolic programming, rearing intensity


INTRODUCTION

During the last decade, numerous studies revealed an influence of perinatal environmental and nutritional conditions on long-term health and performance in many species (Srinivasan et al., 2003; Guilloteau et al., 2009; Moallem et al., 2010). During critical periods in fetal and neonatal life, the amount and the composition of nutrients permanently program biological switches such as the hypothalamic neuropeptide regulatory
Rearing of calves affects pancreas morphology (Langley-Evans et al., 2005; Waterland, 2005; Taylor and Poston, 2007). In this regard, it was observed that rat pups fed a high-carbohydrate formula diet in their first week of life had an altered number and size of the islets of Langerhans, a clear shift on the level of insulin secretion, and a higher gene expression of preproinsulin (Srinivasan et al., 2003). These effects were still found during adulthood far after the period of dietary intervention, indicating that pancreatic islet function can be programmed during critical phases of early postnatal development in rats. Insulin produced in the pancreatic β-cells is a key anabolic hormone for glucose homeostasis. In humans, a combination of insufficient pancreatic β-cell secretion of insulin and peripheral insulin resistance results in the development of type II diabetes. In cattle, an impaired metabolism of insulin is also characteristic particularly for dairy cows who display a massive glucose drain toward the udder (De Koster and Opsomer, 2013). It is not known if the morphology of the insulin-producing β-cells of the pancreas can be programmed by early postnatal rearing conditions in cattle.

It was, therefore, the aim of this study to analyze the long-term effects of intensified rearing of Holstein bull calves during the first 3 wk of life compared to an established rearing protocol on development of the number and area of pancreatic islets of Langerhans.

**MATERIALS AND METHODS**

**Calves, Management, and Feeding**

The experiment was set up and performed according to strict federal and international guidelines on animal research (accepted by the State of Lower Saxony, Germany; file number 312-7224.11).

The study was conducted at the Research Center Futterkamp (Schleswig-Holstein, Germany; 190 cows and 10,300 kg mean lactation milk yield). The experimental setup has been previously described in detail (Maccari et al., 2014). Male Holstein calves (n = 42) born between May 2010 and September 2010 were separated from their dams’ after birth and moved into individual straw-bedded hutches. The calves were randomly assigned to either the experimental (intensively reared [INT]; n = 21) or control group (restrictively reared [CON]; n = 21). All calves were fed 3 L colostrum from the dam within the first 3 h of life. At the age of 2 d, each calf received injections of 1 g iron (as Fe₃⁺-III-hydroxy-dextran; s.c.; Belfer; belapharm, Vechta, Germany) and vitamins (1,500,000 IU vitamin A, 250 mg α-tocopherolacet, 500,000 IU cholecalciferol; s.c.; Vitamin ADE; aniMedica GmbH, Senden-Bñosensell, Germany). Between the second and eighth day of age, each calf received halofuginon (100 μg/kg per os.; Halocur; Intervet Deutschland GmbH, Unterschleißheim, Germany) once a day for prevention of diarrhea caused by Cryptosporidium parvum. In the second or third week, all calves were vaccinated with an attenuated live vaccine against bovine respiratory syncytial virus and parainfluenza virus 3 (Ripsoval RS PI3 Intranasal; Pfizer Deutschland GmbH, Berlin, Germany). In the second and third month of age, every calf was vaccinated twice with an attenuated live vaccine against ringworm (Trichovac LTF 130; IDT Biologika GmbH, Dessau-Roßlau, Germany).

The INT calves were housed in individual hutches during the first 3 wk of life and were offered 6 to 9 L milk twice a day in nipple buckets (0600 and 1700 h; average energy and composition: 17.3 MJ/kg DM ME, 259 g/kg DM CP, 308 g/kg DM crude fat, 0 g/kg DM crude fiber [CF], 56 g/kg DM crude ash, and 377 g/kg DM lactose). Transition milk and bunk milk were mixed and acidified (Schaumacid Drink C flüssig; Schaumann GmbH, Pinneberg, Germany; 1.5 mL/L and pH approximately 5.5) for feeding. Each calf received 50 g of a supplement in the milk (HaGe Vollmilch Aufwerter; HaGe Nord AG, Rendsburg, Germany; 12.2 MJ/kg DM ME, 122 g/kg DM per kg CP, 19 g/kg DM per kg crude fat, 1 g/kg DM per kg CF, 86 g/kg DM per kg crude ash, 772 g/kg DM per kg lactose, 250,000 IU vitamin A, 25,000 IU vitamin D₃, 1,500 mg vitamin E, 2,000 mg vitamin C, and 2,000 mg iron) once a day. The ingested volume was recorded and the residual amounts were discarded. On the 22nd and 23rd day of life, each calf received a mixture of 2 L acidified milk and 2 L milk replacer (MR; 120 g/L; BRIIO Kälbermilch, Brio BV, Zeegse, Holland; whey powder, whey protein concentrate, and vegetable fat [coconut oil and palm oil]; 15.9 MJ/kg DM ME, 214 g/kg DM CP, 179 g/kg DM crude fat, 2 g/kg DM CF, 63 g/kg DM crude ash, and 542 g/kg DM lactose) twice daily. On d 24, the calves moved into group pens and were fed 6 L MR daily by an automatic feeder (SA 2000; Förster Technik, Engen, Germany) up to d 28. The CON calves received, during their first week of life, 2 L acidified milk twice daily (0600 and 1700 h) using nipple buckets. They were moved into group pens on their eighth day of life and were fed 6 L MR daily by an automatic feeder up to d 28.

After the 25th day of life, INT and CON calves were similarly housed and fed. Weaning took place between d 29 and 70 by reducing the amount of MR from 6 to 2 L per calf gradually. The intake of MR and calf starter after the third week of life was recorded by the automatic feeding system (KalbManagerWIN, version 1.2.1.0 and 2.0.0.4; Förster Technik).

At all times, all calves had free access to water, hay, and calf starter (30.1% wheat, 20.0% wheat glu-
ten feed, 15.0% linseed extraction meal, 12.5% soy extraction meal, 11.5% dried pulp, 5.0% rapeseed expeller, and 3.0% molasses.

After weaning, all calves moved to fatteners at an average age of 85 ± 11 d. Two fatteners were involved for technical reasons. Thirty-two calves (INT, n = 16, and CON, n = 16) were housed in an open straw-bedded stable and were fed concentrates ad libitum. Ten calves (INT, n = 5, and CON, n = 5) were housed in a closed stable with a slatted floor and were fed corn silage ad libitum and 3 kg concentrates per day and calf. Animals of both groups were slaughtered at an age of 238 ± 1 d.

**Body Weight and Blood Data Acquisition**

The BW of each calf was recorded after birth and weekly up to wk 10 and on day of slaughter. Daily weight gain was calculated from birth to slaughter. At the slaughterhouse, carcass weight was recorded for each animal.

Blood samples were collected by puncture of the jugular vein (1.20 × 40 mm; Sterican; B. Braun Melsungen AG, Melsungen, Germany) approximately 2 to 3 h after fresh feed supply on d 2 to 3 (wk 1 of life), d 10 to 12 (wk 2 of life), d 18 to 21 (wk 3 of life), and d 65 to 70 (wk 10 of live). Due to the high intraday variation of blood metabolites in cattle (Wiedemann et al., 2013) and the unknown period of prior starving, no blood analyses were performed during time of slaughter. The blood was conserved in tubes containing K2 EDTA or sodium fluoride. Centrifugation (3,000 × g for 10 min at 4 °C) took place within 1 h after blood sampling. Plasma was stored in Eppendorf cups at –20°C until analysis. The concentrations of glucose in fluoride plasma were measured using a photometric automatic clinical chemistry analyzer (A11A01667; ABX Diagnostics, Montpellier, France) with a hexokinase method (Passey et al., 1977). Quality control and calibrations were performed daily. The CV of 20 measurements of 1 sample was 3.1%. A RIA validated for bovine insulin (IM3210; Immunotech, Pasadena, CA) diluted with PBS (dilution ratio 300; Merck, Darmstadt, Germany) was applied and incubated for 45 min. An avidin–biotin complex (Vectastain ABC Kit; Vector Laboratories Inc., Burlingame, CA) diluted with PBS (dilution ratio 300; Merck, Darmstadt, Germany) was added and incubated for 45 min. The pancreatic preparations were stained for 10 min with a 3,3’-diaminobenzidine kit to visualize peroxidase activity (Vector Laboratories Inc.). The area containing insulin was stained in brown. The specificity of staining was determined by preabsorbing the primary antibody with purified bovine insulin (>27 units/mg; Sigma-Aldrich, St. Louis, MO). In brief, the anti-insulin antibody (0.05 mg/mL) was preincubated (molar ratio 1:10) with bovine insulin (0.5 mg/mL) at 4°C overnight and the mixture was used for immunohistochemistry. Nonspecific staining was determined by omission of the secondary antibody.

**Histological Analyses**

A hematoxylin eosin overview stain (Fischer et al., 2008) was performed to determine the quality of the pancreatic tissue samples. Immunohistochemistry was performed to analyze the number of islets of Langerhans and the insulin stained area. First, a rabbit polyclonal antibody to insulin (BioLogo, Kronshagen, Germany) was diluted with 1% BSA (0.75 to 1 g BSA on 100 mL PBS; dilution ratio 10) and incubated overnight. Subsequently, a biotinylated goat anti-rabbit antibody (Vectastain ABC Kit; Vector Laboratories Inc., Burlingame, CA) diluted with PBS (dilution ratio 300; Merck, Darmstadt, Germany) was applied and incubated for 45 min. An avidin–biotin complex (Vectastain ABC Kit; Vector Laboratories Inc.) was added and incubated for 45 min. The pancreatic preparations were stained for 10 min with a 3,3’-diaminobenzidine kit to visualize peroxidase activity (Vector Laboratories Inc.). The area containing insulin was stained in brown. The specificity of staining was determined by preabsorbing the primary antibody with purified bovine insulin (>27 units/mg; Sigma-Aldrich, St. Louis, MO). In brief, the anti-insulin antibody (0.05 mg/mL) was preincubated (molar ratio 1:10) with bovine insulin (0.5 mg/mL) at 4°C overnight and the mixture was used for immunohistochemistry. Nonspecific staining was determined by omission of the secondary antibody.

**Number of Islets of Langerhans and Insulin Stained Area**

For the determination of the number of islets of Langerhans, the immunostained preparations were used. The Axiophot photo microscope (4-mm lens; Carl Zeiss Inc., Thornwood, NY) and the associated camera (Axio Cam; Carl Zeiss Inc.) were applied to take pictures of the histological sections in a standardized field (Taniyama et al., 1993). The exposure time was adjusted at 16 ms. All pictures were filled with tissue. Particular care was given to avoid double photographing of 1 tissue section.
The Axio Vision 4.5 computer program (Carl Zeiss Inc.) was used to take and process the pictures. As many as possible tissue sections of all calves (6–18, depending on cross-section size of preparation) were photographed. An islet of Langerhans was defined as a unit of at least 6 insulin-positive immunoreactive cells to avoid counting single and newly formed nonislet cells (Petrik et al., 2001). The number of islets of Langerhans was randomly counted twice for each photograph and an arithmetic mean per photograph was calculated for each animal. At first, a validation of the method with samples of 6 randomly selected calves was performed. The mean CV of all calves between the 6 to 18 cross-sections was 31.2%. For 6 randomly selected calves, 2 different sections of pancreatic tissue were stained and the mean values of the cross-sections differed by 16.1%.

The determination of the immunohistochemically stained area (insulin stained area) was performed with the Eclipse E600 photo microscope (Nikon, Düsseldorf, Germany) and the associated camera (DS-Fi1; Nikon). A 4-mm lens was used. The NIS-Elements Basic Research 3.2 software program (Nikon) was used to take and process the pictures. The distribution of the 5 pictures on the section was chosen as follows to prevent an influence of the photographer: top left, top right, central, bottom left, and bottom right. All pictures were filled with tissue. The quality of resolution was defined at 2,560 × 1,920 pixels and the focus at 1,280 × 960 pixels. The exposure time was adjusted at 1 ms. Quantitative threshold values of the color intensity were individually defined for each picture to mark the brown insulin stained area by the program. Different correcting functions were used to best assess the brown stained area. On the basis of a field measurement, the computer program calculated the area of all marked districts with a minimum size range of 20 μm at 1 picture. The mean CV of all calves between the 5 photographs of 1 section was 25.8%. For 6 randomly selected calves, 2 different sections of pancreatic tissue were stained and the mean values of the sections differed by 11.2%.

**Statistical Analyses**

All data were analyzed using SAS (version 9.3; SAS Inst. Inc., Cary, NC). For analyses of total feed intake, birth weight, and weight at slaughter, generalized linear models were applied (GLM procedure). The “group” was included in all models as fixed effect. “Birth weight” and “age at slaughter” were further included as covariates in the model to calculate weight at slaughter.

Simple correlations between number of islets of Langerhans and insulin stained area with plasma metabolites, ADG, birth weight, and weight at slaughter were calculated (CORR procedure in SAS).

For statistical analyses of data on feed intake and BW generalized linear mixed models (MIXED procedure in SAS) were used. Models for milk and concentrate intake as well as BW between wk 1 and 10 included “group”, “week of life”, and “group × week of life” as fixed effects and “animal” as a random effect.

The analyses of number of islets of Langerhans and insulin stained area per photograph (MIXED procedure in SAS) included “group” as fixed effect. “Birth weight” and “weight at slaughter” were highly correlated ($P = 0.0003$). Because “birth weight” resulted in a better fit of the model, it was chosen as covariate. The “animal” was considered a random effect.

For plasma metabolite concentrations the fixed effects were “group”, “age at sampling,” and “group × age at sampling” (MIXED procedure in SAS). The random error term was “animal”.

If an overall significant effect was found, a subsequent Bonferroni post hoc analysis was performed. A $P$-value of $<0.05$ was considered significant and a $P$-value of $<0.1$ was considered a trend for all models. Data are presented as Least square means ± SE.

**RESULTS**

**Feed Intake and BW Development of Calves**

Mean ad libitum intake of milk increased during the first 3 wk of life up to 10.5 L/d in INT calves (Fig. 1). Until the end of wk 3 of life, total milk consumption was almost twice as high in INT calves compared with the consumption of CON calves (196 vs. 102 kg of milk/MR per calf during the first 3 wk), whereas no differences were obtained between wk 4 and 10 of life ($4.3 ± 0.05$ vs. $4.2 ± 0.05$ kg MR/d). Energy and protein intakes did not differ from those reported by Maccari et al. (2014), who studied all animals of this experiment and an additional 6 calves. In short, in the first 3 wk of life, intake of ME was more than twice as high in INT calves compared to CON calves and an even more pronounced difference between the groups was found for intake of CP. Average daily intake of calf starter was negligible during the first 3 wk of life. Thereafter, starter intake increased and was comparable between groups ($0.94 ± 0.06$ vs. $0.84 ± 0.06$ kg/d; $P = 0.28$).

Birth weights in INT and CON calves were similar ($P = 1.0$; Table 1). Successive weekly BW increased in all calves with exceptions during the time periods of the transfer of calves from individual hutches into group pens. Average daily gain during the first week of life was 2.5 times higher in the INT calves compared with the CON calves ($P < 0.05$; Fig. 1). During the
second and third week, an increased rearing intensity resulted in almost 9 times higher ADG compared with a standard rearing protocol. During wk 5, a tendency for lower weight gains was observed in INT calves (P < 0.1). However, the INT calves remained heavier than the CON calves until weaning (P < 0.01; Table 1). Average daily gain, BW, and carcass weight at time of slaughter did not differ significantly between both treatment groups (Table 1).

**Blood Parameters**

The basal plasma concentrations of glucose, insulin, IGF-1, and GH on d 2 were comparable for both treatment groups (Fig. 2). Ad libitum feeding of milk was accompanied by higher glucose concentrations in the INT calves compared to the CON calves during the third week of life (P < 0.01). During the second and third week, the INT calves exhibited 3- to 5-fold higher plasma insulin and IGF-1 concentrations compared to the CON calves (P < 0.05), whereas GH was found to be 2-fold higher in the CON calves compared to the INT calves during wk 2 (P < 0.01). In wk 10 of life, glucose, insulin, GH, and IGF-1 concentrations in plasma did not differ.

**Number of Islets of Langerhans and Insulin Stained Area**

The preabsorption of the primary anti-insulin antibody performed to ensure the specificity of the insulin-staining resulted in a disappearance in staining intensity in comparison to the immunostaining with the non-preincubated antibody (Fig. 3c and 3d). The omission of the primary antibody yielded no further detectable specific staining. However, both experiments showed a faint brown unspecific background staining in the exocrine part of the pancreas, which was not included in the analysis of the endocrine pancreas.

At time of slaughter with an approximate age of 8 mo, INT calves had a higher number of islets of Langerhans per field of view compared to CON calves (+17%; P < 0.01; Table 2; Fig. 3a and 3b).

The different feeding regime also influenced the total insulin stained area per photograph (+27% in INT calves; P < 0.01), whereas the number of insulin stained areas per photograph did not differ (+11% in INT calves; P = 0.4). The mean area of continuous insulin stained cells tended to be higher in the INT calves compared to the CON calves (+18% in INT calves; P = 0.079; Table 2; Fig. 4).

**Correlations of Histological Findings with Weight Parameters and Blood Metabolites**

Simple correlation procedures revealed a correlation in trend between number of islets of Langerhans and insulin stained area (r = 0.46, P = 0.06). The

<table>
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<tr>
<th>Parameter</th>
<th>INT</th>
<th>CON</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Birth weight, kg</td>
<td>43.9 ± 1.5</td>
<td>44.1 ± 1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Weight at weaning, kg (d 70)</td>
<td>109 ± 1.5</td>
<td>94 ± 1.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age at slaughter, d</td>
<td>238 ± 1</td>
<td>238 ± 1</td>
<td>0.90</td>
</tr>
<tr>
<td>BW at slaughter, kg</td>
<td>319 ± 5</td>
<td>309 ± 5</td>
<td>0.18</td>
</tr>
<tr>
<td>Carcass weight, g</td>
<td>162 ± 3</td>
<td>155 ± 3</td>
<td>0.14</td>
</tr>
<tr>
<td>ADG birth to slaughter, g/d</td>
<td>1,159 ± 21</td>
<td>1,114 ± 20</td>
<td>0.15</td>
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number of islets of Langerhans was negatively correlated with birth weight of calves \( r = -0.4, P < 0.01 \) and weight at slaughter \( r = -0.3, P < 0.01 \). However, birth weight and weight at slaughter are not independent of each other \( r = 0.6, P < 0.01 \) and a multicollinearity with the number of islets is assumed. No relationship was observed between mean insulin stained area with weights at birth or slaughter \( P = 0.9 \) and \( P = 0.8 \). The ADG during the first 3 wk of life was weakly correlated to the number of islets of Langerhans \( r = 0.21, P = 0.01 \) but not with the insulin stained area \( P = 0.6 \) and with birth weight \( P = 0.3 \). Insulin concentration during the second week of life was associated with the insulin stained area in trend \( r = 0.5, P = 0.08 \). The IGF-1 concentrations during the second week was related to the number of islets of Langerhans \( r = 0.4, P < 0.05 \) and during the third week to the insulin stained area in tendency \( r = 0.5, P < 0.08 \). No further relationships between insulin, glucose, IGF-1, and GH concentrations with number of islets of Langerhans and insulin stained area could be established.

**DISCUSSION**

In agreement with our findings, intensive rearing previously has been shown to increase postnatal nutrient intake and BW development of calves in comparison with calves reared according to a conventional protocol (Khan et al., 2011). However, the transfer of calves from individual hutches into group pens and the associated change of the feeding system (from nipple buckets to an automatic feeding system) and of the feedstuff (from whole milk to MR) resulted in reduced feed intakes in both groups. As a consequence, the respective change was associated to a depression in weight gains in CON calves and INT calves after the first and fourth week of life, respectively. In this context, it is recommended to avoid a very early change of housing and feeding. Intensively reared calves seemingly adapted to the new housing and feeding system much easier and faster compared with CON calves. The utilization of the great growth potential by unrestricted feeding lasted up to the 70th day of life in our
study and to the 90th day of life in a previous study (Khan et al., 2007b). Yet differences in BW at slaughter were not as great as expected (10.6 kg). This was inter alia attributed to diseases during the subsequent rearing period (Maccari et al., 2014).

To our knowledge, this is the first study in cattle that demonstrates an effect of early postnatal weight gain on the permanent morphology of the pancreas. The analysis of pancreatic tissue in a standardized field of a light microscope revealed a higher number of pancreatic islets of Langerhans in 8-mo-old bulls that were raised with an increased intensity during the first 3 wk of life in comparison with bulls that were raised according to an established rearing protocol. This finding in cattle is in agreement with previous experimental results on long-term effects of early postnatal nutrition and environment on the histology of the islets of Langerhans, which were obtained primarily in rodents (Laychock et al., 1995; Aalinkeel et al., 2001; Petrik et al., 2001; Srinivasan et al., 2001). In rats, the nutrient availability in the late fetal and/or the early postnatal period determines the pancreatic ontogeny at the cellular, biochemical, and molecular level (Waterland and Garza, 1999; Aalinkeel et al., 2001). During this critical time frame, the rate of replication and regeneration of β-cells from the ductal pancreatic epithelium is particularly high and feeding intensity possibly influences the balance of β-cell proliferation and death.

Table 2. Effect of rearing intensity of Holstein bull calves during the first 3 wk of life on number of islets of Langerhans as well as on number, total area, and mean area of insulin stained areas per photograph (Least square means ± SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>INT</th>
<th>CON</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of calves/no. of preparations</td>
<td>21/188</td>
<td>21/191</td>
<td></td>
</tr>
<tr>
<td>No. of islets per field of view</td>
<td>9.1 ± 0.3</td>
<td>7.8 ± 0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>No. of calves/no. of photographs</td>
<td>21/105</td>
<td>21/105</td>
<td></td>
</tr>
<tr>
<td>No. of insulin stained areas per photograph</td>
<td>27.3 ± 2.2</td>
<td>24.6 ± 2.2</td>
<td>0.40</td>
</tr>
<tr>
<td>Total insulin stained area per photograph, μm²</td>
<td>107,180 ± 4,987</td>
<td>84,249 ± 4,962</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean area of insulin stained cells, μm²</td>
<td>5,425 ± 341</td>
<td>4,574 ± 340</td>
<td>0.079</td>
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1INT = intensively reared; CON = restrictively reared (according to a standard protocol).
Rearing of calves affects pancreas morphology

between β-cell proliferation and β-cell death (Hill and Hogg, 1991; Kaung, 1994; Petrik et al., 1999).

The differences in the number of islets were also reflected by differences in the insulin stained area between both feeding strategies. The NIS-Elements Basic Research 3.2 morphometric analysis software was used for the first time to determine the immunohistochemically stained area. Settings had to be made manually for each picture, which might cause a subjective influence of the researcher on the values of the insulin stained area. As a result, absolute values of the insulin stained area have to be interpreted with caution. In this study, only 1 person performed the investigation without knowledge of calves’ belonging to a feeding group. In contrast to the manual determination of the number of pancreatic islets in this study, a differentiation between pancreatic islet cells and other insulin-producing cells such as newly formed single cells and small cell groups was not possible. That could explain the greater percentage difference between INT and CON calves for insulin stained areas than for islets per field of view. Also, cells belonging to 1 islet could be counted as 2 insulin stained areas, which might be the reason for the percentage difference between the manual counting of islets and the automatic counting of insulin stained areas. Previous studies used NIS Elements Basic Research as morphometric analysis software for different tissues (Zhang et al., 2009; Phipps et al., 2012; Walton et al., 2013). For image analysis, individual islets were circled and selected by red, green and blue threshold (Petrik et al., 2001) or by gray-level thresholds (Petrik et al., 1999), which was not feasible in this study.

Until now, the control mechanisms of regeneration and cell death of the islets of Langerhans are not fully understood. During neonatal life, apoptotic islet cells are replaced via neogenesis of β-cells from the ductal epithelium (Scaglia et al., 1997; Petrik et al., 1998). In this developmental stage, the number and size of the newly formed islets are influenced by feeding due to the influence of nutrition on the β-cell neogenesis and replication (Petrik et al., 2001). Neonatal rats fed a high-carbohydrate diet displayed an increased number of smaller islets compared to rats fed normal ra-

Figure 4. Immunohistochemical presentation of brown stained β-cells of Langerhans of 2 Holstein bull calves reared in the first 3 wk of life either restrictively or intensively and the respective red marked districts for the calculation of the area. (a) Immunohistochemical presentation of pancreatic tissue of a restrictively reared calf. (b) Figure 4a and the respective red marked districts for the calculation of the area (area: 39,793 μm²). (c) Immunohistochemical presentation of pancreatic tissue of an intensively reared calf. (d) Figure 4c plus the respective red marked districts for the calculation of the area (area: 100,151 μm²).
tions (Petrik et al., 1999, 2001; Srinivasan et al., 2001). Furthermore, there is evidence that growth, maturation, and function of β-cells is dependent on IGF because IGF-1 and IGF-2 can protect islet cells against cytokine-induced apoptosis (Bryson et al., 1989; Petrik et al., 1998, 2001). In line with this finding in humans and rats, 2 to 3 times higher IGF-1 plasma concentrations during the second and third week of life in INT calves could at least partly explain differences in the morphology of pancreatic islets between both groups in this study. The assumption is further supported by the observed correlation between IGF-1 plasma concentration during the second and third week of life with the number of islets of Langerhans and insulin stained area, respectively. Interestingly, the lower levels of IGF-1 in CON calves were accompanied by higher GH levels. This pattern indicates a decoupling of the basically functioning somatotropic axis in calves (Sauter et al., 2003) and has been previously reported for poor-growing piglets (Saleri et al., 2001).

The synthesis and storage of insulin and its precursors in β-cells of the endocrine pancreas is regulated by nutrient, hormonal, and neuronal stimuli. In mice (age 1 to 18 mo), a close positive relationship was demonstrated between islets area and total content of pancreatic insulin as well as between pancreatic insulin and the insulin secretory capacity in simultaneously performed perfusion and morphological analyses of the endocrine pancreas (Bonnevie-Nielsen, 1986). In contrast to our results, they also revealed a strong relationship between BW and islet area and between insulin secretory capacity and total pancreatic insulin but no relationship between BW and pancreatic islet number.

Increased plasma glucose concentrations and plasma insulin concentrations in INT calves during the time of different rearing intensity compared with calves fed restrictively were already established in suckling Simmentaler calves and in intensively fed Holstein calves (Egli and Blum, 1998; Smith et al., 2002). Like in monogastric species, glucose derived from intestinal absorption plays the most important role in the regulation of insulin secretion in young calves (Khan et al., 2007a). Therefore, the plasma glucose and insulin levels are associated with the amount of lactose intake during the first week of life (Hugi et al., 1997). In monogastric animals, changes in glucose levels modify the rate of translation of pre-existing mRNA and thereby the rate of exocytosis of insulin in a matter of minutes. Long-term effects on insulin secretion are modulated through adaptations in the preproinsulin gene transcription rate (Doeherty and Clark, 1994). In rats fed high amounts of carbohydrates during the neonatal period, the plasma insulin levels are also increased (Srinivasan et al., 2000). In such rats, the higher demands of insulin lead to compensatory molecular adaptions of the pancreatic islets (Srinivasan et al., 2001, 2003). The higher lactose levels of INT calves during the first 3 wk of life could be considered as a cause of a similar adaptive reaction of the pancreatic islet. Our results indicate that this effect continues after the development of a viable rumen fermentation and replacement of glucose by short-chain fatty acids and level of vagotone as the primary trigger for insulin secretion (Bloom and Edwards, 1981; Peters and Elliot, 1984; Baldwin et al., 2004).

In conclusion, intensive rearing during the first 3 wk of life results in an enhanced nutrient uptake and body development in comparison with a conventional rearing protocol. Differences in plasma insulin, glucose, IGF-1, and GH concentrations occurred during the intensive rearing period but were not found thereafter. However, the observed alterations in pancreatic tissue imply that the rearing intensity during the neonatal period has long-term consequences. This result supports the theory of a possible metabolic programming in INT calves. Future studies need to address the detailed mechanisms and influencing factors. Consequences for future performance particularly in dairy cows also warrant further investigations.

**LITERATURE CITED**


Rearing of calves affects pancreas morphology


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