Insulin Resistance, Hyperglycemia, Glucosuria, and Galactosuria in Intensively Milk-Fed Calves: Dependency on Age and Effects of High Lactose Intake

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ABSTRACT: Calves intensively fed milk replacers frequently develop postprandial insulin resistance, characterized by excessively elevated plasma insulin concentrations, hyperglycemia, and glucosuria. To test the hypothesis that insulin secretion and insulin-dependent glucose metabolism are modified by age and carbohydrate intake, 20 male calves (Simmental × Red Holstein) were fed a milk replacer containing 290 and 423 g lactose/kg DM from 60−70 to 190−200 kg BW. Responses of insulin and glucose to milk replacer intake and orally administered glucose and pre- and postprandial glucose responses to i.v. infused glucose and i.v. injected insulin were tested at 75−105 and 160−200 kg BW. Urine was collected during 24 h to determine glucose, galactose, dopamine, noradrenaline, and creatinine excretion. Insulin resistance, hyperinsulinemia, hyperglycemia, glucosuria, and galactosuria developed with increasing age and occurred primarily postprandially. High lactose intake enhanced postprandial hyperglycemia but did not significantly increase glucosuria, galactosuria, and hyperinsulinemia. Based on urinary excretion of dopamine and noradrenaline there was a marked age-dependent increase in the activity of the sympathetic nervous system, which was not modified by lactose intake. High feeding intensity and lactose intake, excessive hyperinsulinemia per se and enhanced activity of the sympathetic nervous system possibly contributed to the development of insulin resistance. Glucose-dependent insulinotropic polypeptide, growth hormone and cortisol concentrations, and iron intake were low and comparable in both groups and therefore were etiologically not involved in the development of insulin resistance. Increasing circulating concentrations of insulin-like growth factor I during growth may have in part allowed high growth rates in the presence of insulin resistance.

Key Words: Veal Calves, Growth, Lactose, Glucose, Insulin, Catecholamines

Introduction

Previous studies have shown that veal calves toward the end of their growing period are characterized by postprandial hyperglycemia, glucosuria, hyperinsulinemia, and marked insulin (I) resistance (Doppenberg and Palmquist, 1991; Palmquist et al., 1992; Hostettler-Allen et al., 1994), indicating insufficient metabolic control and inefficient use of glucose (G) as an energy source. In the study of Hostettler-Allen et al. (1994) it was suspected that this problem has a constitutional basis and that its expression is dependent on age or development stage. Growth hormone (GH) and cortisol excess could be excluded as etiological factors of I resistance (Hostettler-Allen et al., 1994). High feeding intensity, energy, and fat intake were suspected as causes, although Palmquist et al. (1992) provided evidence against high-fat diets as factors contributing to the problem. Wijayashinge et al. (1984) have shown that calves fed high-lactose-low-fat milk replacer (MR) diets develop hyperglycemia and glucosuria. Feeding high amounts of lactose, which is often added to high-fat MR, may cause hyperglycemia, hyperinsulinemia, and I resistance. Based on that we tested the hypothesis that hyperglycemia, glucosuria, hyperinsulinemia, and I resistance develop dependent on age or development stage and that high lactose intake contributes to the problem.
Table 1. Composition of milk replacer

<table>
<thead>
<tr>
<th>Item</th>
<th>Group C</th>
<th>Group L</th>
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<tbody>
<tr>
<td>Dry matter, g/kg</td>
<td>962</td>
<td>975</td>
</tr>
<tr>
<td>Total sugar, g/kg DM</td>
<td>310</td>
<td>541</td>
</tr>
<tr>
<td>Lactose, g/kg DM</td>
<td>290</td>
<td>423</td>
</tr>
<tr>
<td>Crude fat, g/kg DM</td>
<td>227</td>
<td>207</td>
</tr>
<tr>
<td>Crude protein, g/kg DM</td>
<td>234</td>
<td>234</td>
</tr>
<tr>
<td>GE, MJ/kg DM</td>
<td>21.3</td>
<td>21.5</td>
</tr>
<tr>
<td>Iron, mg/kg DM</td>
<td>39</td>
<td>37</td>
</tr>
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Materials and Methods

Animals, Husbandry, and Experimental Procedures

Twenty Simmental × Red Holstein bull calves were housed at the experimental station, at Posieux, Switzerland. They were allowed free movement in loose housing systems on straw. During the 1st wk after arrival, calves were fed whole milk and given a 4-d prophylactic antimicrobial treatment. Then calves were weighed, ear-tagged, and divided in two groups of 10 calves each. Calves of both groups initially had comparable mean BW, hemoglobin (Hb), and serum iron (SFe) concentrations.

Calves were fed by bucket at 0730 and 1630. **Group C** was fed a commercial milk replacer (MR), manufactured by Provimi AG, Cossonay, Switzerland, and **group L** the same MR enriched with lactose. The composition of the MR is shown in Table 1. The rate of feeding was adjusted weekly based on individual BW. The feeding plan is shown in Table 2. Possible MR rests after each meal were weighed daily for each calf to calculate feed intake and gain:feed ratios.

The health status of each animal was recorded twice weekly (on Tuesday and Friday). Behavior, rectal temperature, heart rate, respiratory rate, pulmonary sounds, coughing, nasal discharge, eye discharge, fecal consistency, and appetite were scored and the health index calculated from the sum of these scores (Moser et al., 1994). All animals with fever (≥39.5°C) were treated with antibiotics and supporting medications.

Meal tolerance tests, oral G tolerance tests, i.v. G tolerance tests, and i.v. I tolerance tests were performed to measure postprandial changes of G, NEFA, urea, I, GH, cortisol, and GIP. Insulin-like growth factor I was measured only in preprandial samples. Blood samples (10 mL) were taken at −30 and 0 min and every 30 min after MR intake up to 480 min.

In oral G tolerance tests calves were offered a 10% hand-warm solution of D(+)-G-monohydrate in amounts of 2 g G/kg BW. Blood samples (5 mL) to determine G and I concentrations were taken at −30 and 0 min before and every 30 min up to 480 min from the catheterized jugular vein. Three calves (one at the start and two at the end of growth period) refused to drink the G-meal and were excluded from the study.

Intravenous G tolerance tests were performed on two consecutive days. Each calf was tested pre- and postprandially. On d 1, half of the calves (10 animals, 5 of each group) were tested preprandially (after an overnight period of 15 h without feed) and half of the calves (10 animals; 5 of each group) postprandially (3 h after a.m. MR intake). On d 2, the measurement scheme was reversed. Blood samples (5 mL) were collected at −10 and 0 min before and at 5, 10, 15, 20, 30, 45, and 60 min after the infusion of G (.33 g/[kg75´min] for 5 min) for determinations of G and I.

Intravenous I tolerance tests were performed on two consecutive days. All calves were tested pre- and postprandially. On d 1, half of the calves (10 animals,
Table 3. Overview of tests performed during the growth trial

<table>
<thead>
<tr>
<th>Body weight, kg</th>
<th>Tests</th>
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<tbody>
<tr>
<td></td>
<td>Early growth period</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>76 ± 2</td>
<td>Oral glucose tolerance tests</td>
</tr>
<tr>
<td>83 ± 2</td>
<td>Meal tolerance tests</td>
</tr>
<tr>
<td>93 ± 2</td>
<td>Insulin tolerance tests</td>
</tr>
<tr>
<td>99 ± 3</td>
<td>Intravenous glucose tolerance tests</td>
</tr>
<tr>
<td>109 ± 3</td>
<td>Urine collection</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>159 ± 5</td>
<td>Oral glucose tolerance tests</td>
</tr>
<tr>
<td>166 ± 5</td>
<td>Meal tolerance tests</td>
</tr>
<tr>
<td>176 ± 6</td>
<td>Insulin tolerance tests</td>
</tr>
<tr>
<td>180 ± 6</td>
<td>Urine collection</td>
</tr>
<tr>
<td>192 ± 8</td>
<td>Intravenous glucose tolerance tests</td>
</tr>
</tbody>
</table>

5 of each group) were tested preprandially (after an overnight period of 15 h without feed) and half of the calves (10 animals, 5 of each group) postprandially (3 h after a.m. MR intake). On d 2, the measurement scheme was reversed. Blood samples (5 mL) for G and I determinations were taken at −10 and 0 min before and at 1, 2, 5, 10, 15, 20, 30, 45, and 60 min after i.v. injection of bovine insulin (27 IU/mg, lot. 112HOH353, Sigma Chemical, St. Louis, MO) at .1 U/kg BW.

For urine collection calves were placed in individual pens at least 12 h before the test. Urine was sampled using a urinal formed of elastic silicone (Rhodorsil RTV 585, Aseol AG, Berne, Switzerland) and fixed on the calf around the abdomen. Containers were ice-chilled to keep the temperature at 4°C. Volume and pH measurements were done immediately after closure of the test and the urine was then frozen at −20°C until it was analyzed for G, galactose, creatinine, DOPAC, DA, NA, and A. One blood sample was taken for the determination of creatinine and later calculation of creatinine clearance rates.

For histopathological analyses, samples of rumen, jejunum, and colon walls of slaughtered calves were fixed in formaldehyde (4%). For the determination of mucosal lactase activity samples of jejunum were frozen in liquid nitrogen immediately after slaughter and stored at −80°C until they were analyzed. Studies indicated that lactase activity does not decrease during storage.

**Laboratory Analyses**

Concentrations of components and GE in milk replacer were measured as previously described (Moser et al., 1994).

Concentrations of SFe, creatinine, G, and urea were measured using kits from Hoffmann La Roche Diagnostic, Basle, Switzerland, NEFA using a kit from Wako Chemicals, Neuss, Germany, and galactose using a kit from Böhringer, Mannheim, Germany. Serum Fe following liberation of Fe from transferrine (using guanidine chloride) was reduced by ascorbic acid to Fe²⁺, which was chelated by Ferrozine®, thus forming a red color, whose intensity was directly concentration-related and measured photometrically. Creatinine was measured colorimetrically by a kinetic method based on the formation of a yellow-red complex of creatinine with picric acid, whose rate of formation is concentration-related. Glucose dehydrogenase converted β-D-glucose to D-gluconate in the presence of NAD⁺, combined with the formation of NADH, which was directly related to G concentration. Galactose in the presence of NAD⁺ by galactose dehydrogenase was converted to galactonic acid and NADH, which was directly related to galactose concentration. Urea was first converted by urease to NH₄⁺, which by GIDH in the presence of 2-oxoglutarate and NAD⁺ was further converted to L-glutamate and NAD⁺; thereby the decrease of NADH served as a measure of the urea concentration. Nonesterified fatty acids were converted by acyl CoA synthetase to acyl-CoA, which was converted by acyl CoA oxidase to hydrogen peroxide, which in the presence of 3-methyl-N-ethyl-N(β-hydroxyethyl)-aniline with 4-aminoantipyrine formed a purple color that was concentration-related and photometrically measured. Hemoglobin was converted by cyanoferricyanate to cyanmethemoglobin, which was measured photometrically. Concentrations of I, IGF-I, GH, and cortisol were measured by RIA (Hofstettler-Allen et al., 1994). Inter- and intraassay coefficients of variation for the determination of these hormones were below 15 and 10%, respectively.

The concentration of GIP was determined by RIA (by C. Eberle, Div. of Gastroenterology, Dept. of Medicine, University Hospital, Zürich) using human GIP (whose 42 amino acids differ from bovine GIP only at positions 18, 34, and 37). The antiserum was kindly provided by S. R. Bloom, Dept. of Medicine, Royal Postgraduate Medical School, London. Human
GIP (purchased from Bachem AG, Bubendorf, Switzerland) was used for iodination and for standards. Mixtures of pre- and postprandial blood plasma containing low and high amounts of GIP from a calf paralleled the standard curve and behaved identically to human plasma, indicating close immunological similarity of bovine and human GIP. The sensitivity was 30 pmol/L. Intra- and interassay coefficients of variation were <4 and <18%, respectively.

Adrenaline, DOPAC, DA, and NA in urine were measured by HPLC. Urine was first diluted 1:50 before extraction of catecholamines with aluminium oxide using extraction tubes (ESA, Bedford, MA). Extracted urine (20 μL), standards (1 mg/L), and dihydroxyphenylalanine (5 mg/L) were injected into the mobile phase (Cat-A-Phase, ESA, composed of phosphate buffer, methanol, and an ion pairing agent) and passed (at a rate of 1 mL/min) an HR-80 column (3 μm), which was heated to 37°C. Signals were measured using an electrochemical detector (Coulonchem Mod. 5100 A, Esa). Recoveries were between 60 and 80%.

For the determination of lactase activity the jejunum after slaughter was excised, washed with ice-cold saline, transferred to liquid nitrogen, and kept frozen at −80°C until analyzed. Immediately before lactase determination the partially thawed intestinal mucosa were scratched off with a scalpel, added to 4 mL of cold distilled water, homogenized for up to 30 s, and centrifuged at 2,500 × g for 10 min at 4°C. After addition of substrate (lactose) samples were incubated for 60 min in a water bath at 37°C in phosphate buffer (.2 molar, pH 6). After the reaction was stopped, the concentration of G resulting from lactose breakdown was determined by a glucose-oxidase-peroxidase method using a kit from BoÈhringer Mannheim, Germany). One lactase unit (U) corresponds to the release of 1 μmol of glucose/min at 37°C. Lactase activities were expressed per gram of mucosal protein, which was determined by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL) based on the ability of BCA to react with peptide bonds, cysteine, cystine, tryptophan, and tyrosin and to form in the presence of Cu2+ a color whose intensity is proportional to the protein concentration and that can be measured photometrically.

Histological analyses were performed by G. Bestetti at the Inst. of Veterinary Pathology, Univ. of Berne using tissue slices stained with Hemalaun-Eosin.

Statistical Analyses

Values are expressed as means ± SE. Within groups, differences (Δ) between mean basal values and maximal (peak) or minimal (nadir) values, between mean basal values and total incremental or decremental changes (Δ0−360 min or Δ0−480 min; based on calculation of the area under the concentration curves), and between mean basal values and mean concentrations in case of stable concentrations after the start of meal tolerance tests, oral G tolerance tests, i.v. G tolerance tests, and I tolerance tests were calculated. These measures were compared for experiments performed at the start and end of the growth trial and between groups. The half-lives (t1/2) of G and I in I tolerance tests were calculated as described previously (Hostettler-Allen et al., 1994).

Data were analyzed by ANOVA using the GLM procedure of the SAS System for Windows (Release 6.08, SAS Inst. Inc., Cary, NC). The model used was Yiijk = μ + treatmenti + periodj + eijk, where Yiijk = measured value, μ = general mean, treatmenti = feeding milk replacer with or without supplemental lactose, periodj = early or late period of the growth trial, and eijk = residual error. Paired t-test was used to evaluate differences of values within groups and Student’s t-test was used to evaluate the significance of differences (P < .05) between groups.

Results

Feeding and Growth Performance

As shown in Figure 1, initial BW were similar in groups C and L, but final BW tended to be higher (P < .1) in group L than in group C toward the end of the trial. The ADG was numerically but not significantly lower (P < .15) in group C than in group L (1.26 ± .07 and 1.39 ± .05 kg/d, respectively). Feed intake (on a DM basis) increased (P < .001) during the growth trial (from .91 ± .03 to 2.48 ± .05 and .87 ± .01 to 2.67 ± .01 kg/d in group C and L, respectively; means: 2.02 ± .13 and 2.1 ± .14 kg/d in group C and L, respectively), whereas gain:feed ratio slightly decreased (P < .1). Feed refusals were negligible. Crude protein, crude fat, and lactose intake increased (P < .05) from wk 1 to wk 2 when calves were changed from the cow milk to the milk replacer diet. During the whole growth trial gain:feed ratios (means: 1.7 ± .2 and 1.68 ± .1 kg/kg in groups C and L, respectively), DM intakes (means: 1.69 ± .1 and 1.63 ± .08 g/kg BW.75), crude protein intakes (means: 12.5 ± .3 and 12.8 ± .4 g/kg BW.75 in groups C and L, respectively), crude fat intakes (12.1 ± .3 and 11.5 ± .4 g/kg BW.75 in groups C and L, respectively), and GE intakes (1.09 ± .03 and 1.12 ± .03 MJ/kg BW.75 in groups C and L, respectively) were similar, whereas lactose intakes were much higher (P < .001) in group L than in group C (means: 23.1 ± .6 and 15.4 ± .4 g/kg BW.75, respectively).

Health Status

Fecal consistency was lower (P < .05) in group L than in group C, but there were no significant differences with respect to the other traits (behavior, rectal temperature, heart rate, respiratory rate, pul-
Figure 1. Body weight (a), average daily gain (b), feed intake (c), gain: feed ratio (d), crude protein intake (e), crude fat intake (f), lactose intake (g), and gross energy intake in veal calves fed normal (group C, ○) or high (group L, ■) amounts of lactose (290 and 423 g/kg DM, respectively) during the growth trial.
monary sounds, coughing, nasal discharge, and eye discharge) and the health index. One calf of group L and two calves of group C needed treatment (for 3 d) because of pneumonia and two calves (of group L) were treated (for 3 and 5 d, respectively) because of diarrhea.

**Blood Hemoglobin and Serum Iron Concentration**

Concentrations of Hb decreased (P < .001) during the growth trial from 5.5 ± .4 to 4.3 ± .2 mmol/L in group C and from 5.3 ± .4 to 4.0 ± .1 mmol/L in group L. Concentrations of SFe decreased from 3.6 ± .4 to 2.1 ± .7 μmol/L in group C and from 3.3 ± .6 to 2.7 ± .5 μmol/L in group L. Hb and SFe concentrations were not different between groups.

**Meal Tolerance Tests at the Start and End of the Growth Trial**

As shown in Figure 2, mean basal G concentrations were higher (P < .01) at the end than at the start of the growth trial in both groups, but there were no group differences. Postprandial concentrations of G increased (P < .001) during 90 to 150 min and then decreased toward basal concentrations. Peak G concentrations were reached at 90 min in group C and at 120 min in group L at the start of the growth trial and at 120 min in group C and at 150 min in group L at the end of the growth trial (i.e., always later [P < .05] in group L than in group C). Total postprandial G responses (Δ0-480min) were greater (P < .01) at the end than at the start of the growth trial in both groups and greater (P < .01) in group L than in group C at the start and at the end of the growth trial.

Basal values of NEFA were similar at the start and end of the growth trial. Concentrations of NEFA decreased immediately postprandially (P < .05) during 90 to 150 min and then increased again. Postprandial NEFA concentrations, including nadir concentrations and total responses (Δ0-480min), were similar at the start and end of the growth trial and in both groups.

Basal and postprandial urea concentrations were higher (P < .05) at the end than at the start of the growth trial in group C but not in group L and were higher (P < .05) in group C than in group L at the end of the trial.

Basal I concentrations were higher (P < .001) at the end than at the start of the growth trial in group C as well as in group L, but there were no group differences. Concentrations of I increased postprandially (P < .001) during 120 to 150 min and then decreased toward basal concentrations. Peak concentrations were reached at similar times at the start and end of the growth trial and at comparable times in both groups. Peak I concentrations were much higher (P < .001) at the end than at the start of the trial, but there were no group differences. Total I responses (Δ0-480min) were markedly greater (P < .001) at the end than at the start of the growth trial but were similar in both groups. Postprandial (Δ0-480min) responses of I relative to G (I/G ratios) at the end were greater than at the start of the growth period (4.2 and 1.1, respectively).

Postprandial GH concentrations (not shown) fluctuated considerably, but not in a systematic manner. Mean concentrations were numerically higher, but not significantly (P > .1) at the start (means: 14.4 ± 1.3 and 13.5 ± 1.7 μg/L in group C and group L, respectively) than at the end of the growth trial (means: 5.8 ± .1 and 5.6 ± .4 μg/L in group C and L, respectively), and there were no group differences.

Postprandial cortisol concentrations (not shown) changed considerably, but not in a consistent manner. Basal and mean concentrations of cortisol were similar at the start (means: 8.22 ± 1.5 and 8.93 ± 1.5 μmol/L in groups C and L, respectively) and end of the growth trial (means: 7.2 ± 1.1 and 7.95 ± 1.2 μmol/L in groups C and L, respectively) and similar in both groups.

As shown in Figure 2, basal values of GIP were similar at the start and end of the growth trial. Concentrations increased (P < .05) within 90 to 120 min after MR intake and remained higher than basal concentrations up to 360 min. Total postprandial responses (Δ0-360min) at the start and end of the growth trial in group L and group C were not significantly different.

Preprandial concentrations of IGF-I at the end were higher (P < .05) than at the start of the growth trial (313 ± 13 and 243 ± 18 μg/L, respectively, in group C; 389 ± 24 and 192 ± 14 μg/L, respectively, in group L), but there were no significant group differences.

**Oral Glucose Tolerance Tests at the Start and End of the Growth Trial**

As shown in Figure 3A, basal G concentrations at the start (A) and end (B) of the growth trial and in both groups were similar. Concentrations rapidly and transiently increased (P < .01) after G loads. Peak concentrations were reached between 90 and 120 min and were higher (P < .01) in group C, but not in group L, at the end than at the start of the growth trial. Total responses (Δ0-480min) were more similar at the end than at the start of the growth trial and there were no group differences.

As shown in Figure 3B, basal I concentrations at the start (A) and end (B) of the growth trial and in both groups were similar. Concentrations increased (P < .05) after the oral G load and reached peak concentrations at around 120 min. Peak concentrations and total I responses (Δ0-480min) were more similar at the end than at the start of the growth trial and there were no group differences.

**Intravenous Glucose Tolerance Tests at the Start and End of the Growth Trial**

As shown in Figure 4, in experiments performed preprandially, basal G concentrations were similar at
Figure 2. Plasma glucose nonesterified fatty acids (NEFA), urea, glucose-dependent insulinotropic polypeptide (GIP), and insulin concentrations in meal tolerance tests before and after milk replacer intake (at time 0) in veal calves of group C (○) and group L (■) in the early phases and late phases of the growth trial. For further details see legend to Figure 1.
the start and end of the growth trial. In experiments performed postprandially (i.e., at 3 h after MR intake), basal G concentrations in group L at the end were higher \((P < .01)\) than at the start of the growth trial, but were similar in group C. In postprandial experiments, basal concentrations at the end of the trial were higher \((P < .001)\) in group L than in group C. Concentrations of G rapidly increased during G infusions \((P < .001)\) to peak concentrations that were higher \((P < .05)\) in group L at the end of the growth trial in experiments starting 3 h postprandially. Total responses \(\Delta_{0-60\text{min}}\) at the start and end of the growth trial were similar, but slightly smaller \((P < .05)\) in group L than in group C at the start of the growth trial in experiments performed preprandially.

Basal concentrations of I in postprandial experiments at the end of the growth trial were particularly high. Concentrations of I increased

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Figure 3. Plasma glucose and insulin concentrations in oral glucose tolerance tests, starting at time 0, in calves of group C (○) and group L (●) in the early phases (A) and late phases (B) of the growth trial. For further details see legend to Figure 1.
moderately ($P < .05$) after preprandial G infusions at the start and the end of growth trials. The rise of I was more marked ($P < .05$) after postprandial infusions than after preprandial G infusions. There were no significant differences between groups either at the start or end of the growth trial. Incremental and decremental changes ($\Delta_{0-60\text{ min}}$) of I relative to G (i.e., the I/G ratios) in group C at the start and end of the growth trial in preprandial experiments were 3 to 14 times smaller ($P < .05$) than in postprandial experiments.

**Intravenous Insulin Tolerance Tests at the Start and End of the Growth Trial**

As shown in Figure 5, basal G concentrations in preprandial experiments in group C at the start and end of the growth trial were in a comparable range but

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**Figure 4.** Plasma glucose and insulin concentrations in the intravenous glucose tolerance test, starting at time 0, in calves of group C (○) and group L (■) realized preprandially in the early phases (A) and late phases (C) of the growth trial, and postprandially (3 h after milk replacer intake) in the early phases (B) and late phases (D) of the growth trial. For further details see legend to Figure 1.
were higher ($P < .01$) in postprandial experiments at the end of the growth trial in group L than in group C and higher ($P < .001$) in postprandial experiments than in preprandial experiments. Concentrations of G decreased ($P < .05$) after I injections during 30 to 40 min, but differences between preinjection values and nadir concentrations as well as the total decremental changes ($\Delta_{0-60\ min}$) were similar in experiments performed post- and preprandially, and there were no significant differences between experiments performed at the start and end of the growth trial and between groups. The $t_{1/2}$ of I, shown in Table 4, in postprandial tests were therefore much longer ($P < .01$) than in preprandial tests in both groups, whereas differences of $t_{1/2}$ of G, also shown in Table 4, between groups were minor.

Figure 5. Plasma glucose and insulin concentrations in the intravenous insulin tolerance tests, starting at time 0, in calves of group C (○) and group L (■) in experiments realized preprandially (A, B; in early and late phases of growth trial, respectively) and postprandially (C, D; in early and late phases of growth trial, respectively). For further details see legend to Figure 1.
Basal concentrations of I in group C and L in preprandial experiments at the start of the growth trial were lower (P < .01) than at the end of the growth trial. Concentrations of I markedly increased (P < .001) after I injections, then rapidly decreased with similar $t_{1/2}$ (shown in Table 4) and reached basal concentrations after 60 min. Peak concentrations reached immediately after I injection in both groups were lower (P < .001) at the start than at the end of the growth trial but similar in postprandial experiments at the end of the growth trial. The incremental changes of I ($\Delta_{0-60\text{ min}}$), relative to the decremental changes of G ($\Delta_{0-60\text{ min}}$), that is, the I/G ratios, were similar in postprandial and in preprandial experiments and more similar at the end than at the start of the growth trial.

Urinary Volume and pH and Excretions of Glucose, Galactose, Noradrenaline, Dopamine, Dihydroxyphenylacetic Acid, and Creatinine

As shown in Table 5, urinary volume tended to be greater (P < .1) at the end than at the start of the growth trial and was not significantly different between groups. The pH was lower (P < .01) in group C than in group L at the end of the growth trial. The urinary concentrations of G and galactose were higher (P < .05) at the end than at the start of the growth trial and the amounts of G and galactose excreted during 24 h on a kilogram BW basis were also higher (P < .05) at the end than at the start of the growth trial, but there were no group differences.

Urinary concentrations of NA, DA, and DOPAC and amounts excreted during 24 h on a kilogram BW basis were greater (P < .05) at the end than at the start of the growth trial. At the start of the growth trial concentrations and amounts of NA and DOPAC excreted were greater (P < .05) and those of DA tended to be greater in group C than in group L, whereas there were no group differences at the end of the growth trial. Adrenaline was not detectable.

Creatinine excreted during 24 h and creatinine clearance rates (determined based on plasma concentrations of 126 ± 4 and 92 ± 4 $\mu$mol/L in group C and 122 ± 3 and 92 ± 3 $\mu$mol/L in group L at the start and end of the growth trial) were higher at the end than at the start of the growth trial, but there were no group differences.

Lactase Activity in Jejunum

Lactase activity was similar in groups C and L (8.2 ± 1.2 and 8.4 ± 1.4 U/g protein, respectively).

Histology of Rumen, Jejunum, and Colon

There were no gross alterations in groups C and L.

Discussion

This study was performed with calves with a similar health index and under conditions as planned (i.e., different intakes of lactose, but otherwise similar intakes of feed, crude protein, crude fat, and GE). Although weight gain in the group fed the lactose-supplemented MR tended to be (insignificantly) enhanced toward the end of the growth trial, gain:feed ratios and ADG were very similar in both groups.

High lactose intake did not cause histological changes in the intestinal tract, although the lower fecal consistency was a sign of a slight digestive disturbance. However, in calves fed MR with added lactose this did not significantly affect feed utilization. Because jejunal lactase activity was nearly identical in both groups, high lactose intake did not enhance the synthesis of this enzyme. The potential to digest lactose was therefore expectedly similar in both groups and was not limiting in calves receiving the additional lactose.

Pre- and postprandial G concentrations in meal tolerance tests became higher during the growth period and thus mirrored the markedly increased urinary excretion of G and galactose. Plasma galactose concentrations, which are dependent on intestinal lactase activity and on liver function, increased in calves after oral lactose loads (Gutzwiller and Blum, 1996). The progressive hyperglycemia with increasing age was in marked contrast to age-matched breeding
calves fed roughage and concentrates, in which pre- and postprandial plasma G concentrations decrease with increasing age (Breier et al., 1988; Hugi and Blum, unpublished data). Plasma G increased more markedly and the postprandial rise lasted longer in calves fed MR with than in those fed MR without lactose intake and high oral G loads, for which there is an age- or development-dependent decrease in the ability to cope with long-term oral lactose intake and high oral G loads, for which ruminants are genetically not prepared at older ages. If so, effects on NEFA were not affected, in contrast to effects on G.

Plasma urea concentrations under conditions of identical protein intakes and digestion and normal kidney function can provide information on tissue protein breakdown. Protein intake/kilogram BW was constant during the growth trial and nearly identical in both experimental groups, and there was no indication of kidney failure based on creatinine clearance rates. Therefore, the lower urea concentrations at the start than at the start of the growth trial and at the end of the growth trial and there were no group differences. Because the postprandial fall of NEFA expresses inhibition of lipolysis and(or) stimulation of fatty acid tissue uptake primarily by I, this indicates that these effects of I were not modified by age and by different lactose intake. If so, I effects on NEFA were not affected, in contrast to effects on G.

There were no differences in basal NEFA concentrations and in postprandial NEFA responses at the start and at the end of the growth trial and there were no group differences. Because the postprandial fall of NEFA expresses inhibition of lipolysis and(or) stimulation of fatty acid tissue uptake primarily by I, this indicates that these effects of I were not modified by age and by different lactose intake. If so, I effects on NEFA were not affected, in contrast to effects on G.

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Pre- and postprandial I concentrations, too, increased during the growth trial. Because total (Δ₀ \(_{480\text{min}}\)) postprandial I responses at the end relative to the start of the growth trial increased much more than total (Δ₀ \(_{480\text{min}}\)) G responses (6.6-fold compared to 1.5-fold, respectively), postprandial hyperglycemia was not the only factor causing hyperinsulinemia. This was supported by the finding that I responses were not enhanced by feeding MR with added lactose, hence by the more marked hyperglycemia. In accordance, in oral G tolerance tests, despite markedly greater hyperglycemia than in meal tolerance tests, I responses were much smaller. Furthermore, in preprandial i.v. G tolerance tests, I responses were much smaller than in meal tolerance tests.

Hyperinsulinemia, observed in meal tolerance tests, can be induced by one or several factors. Basal G and I concentrations before a challenge seem to be important. Thus, basal G and I concentrations and I responses in postprandial tests were considerably greater than in preprandial tests, particularly when tests were performed at the end of the growth trial, despite comparable incremental hyperglycemic changes. These effects can be explained by enhanced availability of newly synthesized I for rapid release in response to acute hyperglycemia, induced by i.v. G infusions. Hyperglycemia is one, but not the only, factor stimulating I secretion, as discussed above. In this study GH and cortisol did not rise with increasing age and were not elevated in calves fed high amounts of lactose and hence unlikely caused hyperinsulinemia, in accordance with our previous studies (Hostettler-Allen et al., 1994). Glucose-dependent insulinotropic polypeptide, which markedly stimulates I secretion in response to meals in humans, is not insulinogenic in ruminants (Guilloteau et al., 1995). Although GIP increased after MR intake in our calves, the rise was similar at the start and end of the growth trial and was comparable in both groups.

The greater I:G ratios at the end than at the start of the growth period (4.2-fold compared to 1.1-fold, respectively, for Δ₀ \(_{480\text{min}}\) responses) in meal tolerance tests can be considered an expression of I resistance (i.e., reduced I-dependent tissue G uptake). In contrast, in meal tolerance tests the I:NEFA ratio was similar at the start and end of the growth trial and I responses (Δ₀ \(_{480\text{min}}\)) relative to the decremental change of urea were smaller in group L than in group C at the end of the growth trial. This suggests that there was a dichotomy in the relationship of G, NEFA, and urea to I. The I:G ratios were increased and t₁/₂ of G in I tolerance tests were prolonged if I was administered 3 h after than before MR intake, thus confirming previous studies (Hostettler-Allen et al., 1994) that I resistance is primarily a postprandial phenomenon.

Reduced I-dependent G utilization may be the consequence of one or several factors. This study shows that GH and cortisol, which are well known to induce I resistance, can be excluded as etiological factors because concentrations were in the normal range for calves, GH tended to decrease, cortisol did not change during the growth trial, and neither hormone exhibited a secretory pattern associated with changes of G or I, in accordance with previous studies (Hostettler-Allen et al., 1994). However, catecholamines, especially through their effects on β-adrenergic receptors, are well known to cause I resistance, as also shown in calves (Blum and Flückiger, 1988; Zimmerli and Blum, 1990). The markedly greater urinary excretions of NA, DA, and DOPAC at the end than at the start of the growth trial, a finding to the best of our knowledge not shown previously, can be taken as evidence for enhanced activity of the sympathetic nervous system. The enhanced activity of the sympathetic nervous system thus may have caused reduced G utilization and I resistance with increasing age.

Insulin is the hormone primarily stimulating anabolic processes. An adequate I status is therefore a prerequisite for high ADG. Insulin resistance in intensively fed veal calves with very high ADG is therefore surprising. A rise of circulating IGF-I, which occurs in parallel with the rise of I (Hostettler-Allen et al., 1994) and which was also observed in the present study, may allow high ADG despite I resistance. The rise of circulating IGF-I, as I, was probably also a consequence of the high feeding intensity, because IGF-I concentrations decrease in breeding calves fed less intensively with roughage and concentrates (Breier et al., 1988; Hugi and Blum, unpublished data).

Implications

Insulin resistance, hyperinsulinemia, hyperglycemia, glucosuria, and galactosuria in veal calves develop age-dependently and are primarily a postprandial phenomenon. High lactose intake (423 g/kg milk replacer) enhances postprandial hyperglycemia but does not significantly increase glucosuria and hyperinsulinemia and does not affect growth performance. An age-dependent rise in the activity of the sympathetic nervous system may be causally associated with changes in and effects of insulin on glucose metabolism.

Literature Cited


