Veal calves fed by bucket often develop postprandial insulin resistance, hyperglycemia, and glucosuria during fattening. Automatic feeding systems allow feed intake for 24 h, and small ingested portions are expected to decrease postprandial glucose loads. We have studied metabolic and endocrine traits in calves that were either 1) fed identical daily amounts of whole milk plus milk replacer by a computer-programmed automatic feeder (≥6 portions from 0800 to 2400 h) (GrA) or 2) fed by bucket at 0800 and 1630 h (GrB). Calves started at a body weight of 118 kg, and the experiment lasted for 3 wk. During wk 3, lactose was supplemented to stress postabsorptive glucose homeostasis. Feed intake and average daily gains in GrA and GrB were similar. Plasma concentrations during an 8-h period of glucose (in part), lactate, urea, and somatostatin (in wk 3), and of glucagon and insulin (wk 2 and 3) were smaller in GrA than in GrB, whereas growth hormone, insulin-like growth factor I, insulin-like growth factor binding protein-1 (wk 2), and prolactin concentrations (wk 2 and 3) were higher. Lactose supplementation in wk 3 enhanced transient postprandial hyperglycemia and hyperinsulinemia. Thus, there were marked metabolic and endocrine differences when calves sucked their feed in six or more portions during a 16-h period daily or if fed only twice daily at fixed times. Therefore, we fed calves with an automatic feeder or by bucket. Studies were performed with and without stressing glucose homeostasis by oral lactose supplementation.

**INTRODUCTION**

There are metabolic and endocrine differences between calves raised for milk production, suckling calves held with their dams, and veal calves fed intensively by bucket. Thus, during weaning of calves raised for breeding, plasma glucose and insulin concentrations decrease with increasing age (3, 16, 28), whereas in suckling calves glucose and insulin concentrations remain stable to the age of 3 mo (11). In contrast, veal calves fed intensively by bucket frequently develop insulin resistance, hyperglycemia, glucosuria, and galactosuria (9, 15, 17, 18, 22, 27), which is not found in calves raised for potential breeding and calves held with and suckling on their dams. The use of programmable automatic feeders has become increasingly popular in the production of veal calves, which are held in groups and have access to feed for 24 h. The daily ration can be called on during preprogrammed intervals. It can be hypothesized—but has not been specifically investigated—that metabolism and homeostatic regulatory systems are less metabolically stressed when calves have free access to feed in comparison to once or twice daily bucket feeding. Our aim was to test the hypothesis that metabolic and endocrine parameters differ, especially the relationship of glucose with insulin in veal calves with access to feed at least six times during a 16-h period daily or if fed only twice daily at fixed times. Therefore, we fed calves with an automatic feeder or by bucket. Studies were performed with and without stressing glucose homeostasis by oral lactose supplementation.

**MATERIALS AND METHODS**

**Animals, Husbandry, and Experimental Design**

Experimental protocols were approved by the Committee for the Permission of Animal Experiments of the Canton of Freiburg, Granges-Paccot, Switzerland.
Fourteen male veal calves (Simmental × Red Holstein) were studied. They were bought at the age of 5 wk and housed at the Research Station of the Division of Nutritional Pathology, located at the Swiss Federal Research Station for Animal Production, Posieux, Switzerland. For health and growth control, calves were weighed and examined weekly. Calves were kept in loose housing systems on straw litter. On arrival, the calves were ear-tagged and given a prophylactic antibiotic treatment for 5 d.

The study was divided into a preexperimental period (up to 10 wk of age, thus lasting for 6 wk), during which the animals were adapted to two different feeding methods (feeding by automatic feeder and by bucket, respectively) and the 3-wk experimental period (at the age of 11 to 13 wk).

At arrival, calves were divided into group A (GrA, n = 7) and group B (GrB, n = 7). Calves of GrA were fed with an automatic feeder (Stand-Alone II, Forster, Engen, Germany; program: Kalbmanager 4.2), allowing calves to receive their daily ration divided into six meals over a period of 16 h from 0800 until 2400 h, whereas calves of GrB were fed by bucket twice daily at 0800 and 1630 h. Calves during the 3-wk experimental period (at the age of 11 to 13 wk) were fed daily a ration containing 11 L of milk from the milk pool of the research station [DM: 122 g/kg; gross energy (GE): 23 MJ/kg of DM; CP: 274 g/kg of DM; crude fat (CL): 273 g/kg of DM; lactose: 397 g/kg of DM; inorganic matter: 56 g/kg of DM], a milk replacer [UFA 200-Natura, Union Futter AG, Sursee, Switzerland (DM: 950 g/kg; GE: 22 MJ/kg of DM; CP: 230 g/kg of DM; CL: 230 g/kg of DM; nitrogen-free extracts: 475 g/kg of DM; lactose: 455 g/kg of DM; ash 70 g/kg of DM)], a mineral and vitamin premix (#5910.0.7, Prolac S.A., Cossonay-Gare, Switzerland), and water. Immediately before each meal whole milk, milk replacer, and the premix were mixed and then were provided to calves by the automatic feeder or by bucket.

The amounts of milk replacer, premix, and water were adjusted weekly according to the feeding plan, as described for the whole fattening period (22) and shown in Table 1 for the preexperimental period (BW 60 to 110 kg; at the age of 5 to 10 wk) and for the 3-wk experimental period (BW 110 to 170 kg; at the age of 11 to 13 wk). During the 3rd wk of the experimental period (from d 15 to 21), both groups were provided with an additional 300 g of lactose/animal per day.

Blood samples were taken from the jugular vein during fattening when calves were 11, 12, and 13 wk old, i.e., on d 4 before (d −4) and on d 14 and 21 after the start of the 3-wk experimental period. To minimize stress during the blood sampling, catheters were inserted on the afternoon before sampling and a 30-cm-long polyvinyl tube was fitted to the catheter to allow easier sampling.

To measure hemoglobin in blood and 3,5,3'-triiodothyronine (T3) and thyroxine (T4) in plasma, we collected blood samples at 0800 h on d −4, 14, and 21 before and during the 3-wk experimental periods, in tubes containing 1.8 mg of dipotassium-EDTA/ml of blood. Blood was collected with tubes without anticoagulant at 0800 h on d −4, 14, and 21 for analysis of total protein, albumin, and iron in serum. Glucose was determined hourly in blood samples for 8 h (from 0800 to 1600 h) on d −4, 14, and 21 before and during the experimental periods, with tubes containing 1.8 mg of dipotassium-EDTA/ml of blood. To determine the concentrations of lactate, NEFA, triglycerides, urea, insulin, cortisol, IGF-I, and IGF binding protein-1 (IGFBP-1), we collected preprandial blood samples on d −4, 14, and 21 and hourly for 8 h (from 0800 to 1600 h) on d 14 and 21 of the experimental periods, using tubes containing 1.8 mg of dipotassium-EDTA/ml of blood. Tubes for the determination of lactate contained (per ml of blood) 1.8 mg of dipotassium-EDTA and 3 mg of sodium-fluoride. Glucagon was determined in blood samples also collected in tubes containing dipotassium-EDTA, preprandially on d −4, 14, and 21 and every 2 h for 8 h (from 0800 to 1600 h) on d 14 and 21 of the 3-wk experimental periods. Growth hormone (GH) and prolactin (PRL) were determined in blood samples at 0800 h on d −4 and for 8 h (from 0800 to 1600 h) every 20 min on d 14 and 21 of the experimental periods. All samples obtained at 0800 h on d −4, 14, and 21 were taken before feeding. Somatostatin and ACTH were determined in pools made of each calf of plasma samples obtained hourly for 8 h (from 0800 to 1600 h) on d 14 and 21 of the experimental periods. For technical reasons,

### Table 1. Feeding plan for the veal calves from arrival until slaughter

<table>
<thead>
<tr>
<th>Body weight, kg</th>
<th>Whole milk, kg/d</th>
<th>Milk replacer, g/d</th>
<th>Premix, g/d</th>
<th>Water, L/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>60–69</td>
<td>9</td>
<td>. . .</td>
<td>35</td>
<td>. . .</td>
</tr>
<tr>
<td>70–79</td>
<td>10</td>
<td>. . .</td>
<td>35</td>
<td>. . .</td>
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<tr>
<td>80–89</td>
<td>11</td>
<td>. . .</td>
<td>35</td>
<td>. . .</td>
</tr>
<tr>
<td>90–99</td>
<td>12</td>
<td>. . .</td>
<td>35</td>
<td>. . .</td>
</tr>
<tr>
<td>100–109</td>
<td>11</td>
<td>350</td>
<td>35</td>
<td>1.7</td>
</tr>
<tr>
<td>110–119</td>
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<tr>
<td>140–149</td>
<td>11</td>
<td>950</td>
<td>35</td>
<td>3.0</td>
</tr>
<tr>
<td>150–159</td>
<td>11</td>
<td>1090</td>
<td>35</td>
<td>3.4</td>
</tr>
<tr>
<td>160–169</td>
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<tr>
<td>170–179</td>
<td>11</td>
<td>1400</td>
<td>35</td>
<td>4.0</td>
</tr>
<tr>
<td>180–189</td>
<td>11</td>
<td>1570</td>
<td>35</td>
<td>4.4</td>
</tr>
<tr>
<td>190–200</td>
<td>11</td>
<td>1720</td>
<td>35</td>
<td>4.4</td>
</tr>
</tbody>
</table>

*Body weight was measured weekly and feeding adjusted the following day. Calves entered the 6-wk preexperimental period at a BW of about 60 kg (at the age of 5 wk) and they entered the 3-wk experimental period when they reached between 110 to 120 kg.*
concentrations of IGFBP-2 and -3 were only measured
in preprandial samples taken on d −4. Tubes were cen-
trifuged at 1000 × g for 20 min at 4°C, then the sup-
ernatants were partitioned into aliquots and stored at −20°C
until they were analyzed.

Laboratory Analyses

Hemoglobin concentrations were measured as de-
scribed (11). Glucose, triglyceride, urea, protein, and iron
concentrations were measured using kits from Hoff-
mann-La Roche, Basle, Switzerland (#07 3679 1, #07
3685 6, #07 3678 3, and #07 5181 2, respec-
tively). Kits from Bio Mérieux, Marcy l’Etoile, France,
were used to determine albumin and L-lactate concen-
trations (#61051 and #61192, respectively). The con-
centration of NEFA was measured with a kit (#994-75409)
from Wako, Neuss, Germany. To analyze these traits we
used the automatic analyzer Cobas-Mira plus, Hoff-
mann-La Roche, Basle, Switzerland. Concentrations of
insulin, glucagon, IGF-I, T₃, T₄, GH, PRL and cortisol
were measured by radioimmunoassay (RIA) as recently
described (11, 14). Concentrations of ACTH were deter-
mined by RIA using a kit (#RB 306 from Euroa-Diagnos-
tics Laboratories, Webster, TX). Concentrations of
somatostatin, after plasma extraction by Sep-Pak 18 car-
tridges (Millipore, Volketswil, Switzerland), were deter-
mined by RIA using a kit (#RB 306 from Euroa-Diagnos-
tica AB, Malmö, Sweden).

Plasma concentrations of IGFBP-1 were measured by
a homologous RIA developed in the laboratory of B.
Breier. Antibodies were raised in rabbits against a syn-
thetic peptide of 16 AA derived from the bovine IGFBP-
1 sequence plus an additional lysine coupled to keyhole
limpet hemocyanin (Sigma, batch #H2133) by conjuga-
tion with 6% gluteraldehyde for 4 h at room temperature.
The antiserum was used in a final dilution of 1:18,000
and showed no cross reactivity with ovine or human
IGFBP-3, IGF-1, and IGF-2. The bIGFBP-1 peptide was
used as standard and as tracer after iodination. The
standard peptide showed very high parallelism with
adult and fetal sheep plasma samples and with native
ovine IGFBP-1 purified by affinity chromatography.
The tracer was prepared by iodinating 5 µg of bIGFBP-1
peptide with 0.5 mCi 125I using the chloramine-T method
(3) and purified by size separation chromatography.
The assay buffer contained 0.05 M sodium phosphate, 0.1 M
NaCl, 0.01 M EDTA, 0.5% BSA, 0.1% Triton X100, 0.05%
NaN₃, pH 7.4 with 100 ng of rhIGF-1 (Pharmacia, Swed-
en, batch #56820A51) per 100 µl of the antibody solu-
tion. The solution of the first antibody (100 µl) was added
to 100 µl standard or sample per tube, followed by 100
µl of tracer in assay buffer after 20 h of preincubation
at 4°C. After a further 20 h of incubation at 4°C, 1 ml of
a second antibody-PEG-complex was added to separate
bound and free ligands as described (3). Recoveries of
bIGFBP-1 peptide or purified ovine IGFBP-1 were 103
± 8.7% (n = 11) and 92 ± 9.6% (n = 8), respectively. The
assay sensitivity was 0.1 ng/tube and the inter- and
intraassay coefficients of variation were 7 and 4%, re-
spectively. The IGFBP-2 and −3 concentrations were mea-
ured based on Western blots (14).

Statistical Analyses

Values of metabolic traits and hormones in blood
plasma or serum are expressed as means ± SEM. Calcula-
tions of areas (area₀−₈h) under concentration curves (8-
h periods) served as a measure of mean concentrations
of metabolites and hormones. After subtraction of basal
(preprandial) values, the areas under the concentration
curves were calculated as a measure of total incremental
or decremental changes (Δarea₀−₈h) of metabolite and
hormone concentrations to evaluate total effects.

Episodic secretion of GH and PRL on d 14 and 21
(mean concentrations, basal concentrations, peak
heights, and peak frequencies) were analyzed as de-
scribed (25).

For time and treatment differences preprandial and
concentrations during the 8-h period, total effects during
the 8-h period on d −4, 14, and 21, and measures of
episodic secretion (GH, PRL) on d 14 and 21 were evalu-
at using the RANDOM and REPEATED methods of
the MIXED procedure (30). Different feeding frequency
and time were used as fixed effects and the individual
calves were used as random effects. P-values were de-
ferred from Student’s t tests using estimates after mixed
model procedure and were considered significant if (P <
0.05). Effects of lactose supplementation could not be
differentiated from age effects, i.e., were confounded.

RESULTS

Growth Performance and Feeding during the
3-wk Experimental Period

Intakes of DM, GE, CP, CL, lactose, and inorganic
matter were higher (P < 0.05) on d 14 than on d −4 and
were higher (P < 0.05) on d 21 than on d 14, as planned,
but there were no significant differences between groups,
as planned (Table 2). Body weights increased (P < 0.001)
from d −4 to 21 (Table 2) and the average daily gain in
GrA and GrB (1.64 ± 0.06 and 1.58 ± 0.05 kg/d, respec-
tively) and the absolute gains from d −4 to 21 (41.1 ±
1.6 and 39.4 ± 1.6 kg, respectively) were similar. Calves
fed by automatic feeder used their possibility to obtain
feed (6 times/d from 0800 to 2400 h), as shown in Fig-
ure 1.
Blood Metabolite and Iron Concentrations

Preprandial plasma glucose concentrations (Figure 2) remained stable during experiments and were not different between groups. Concentrations transiently increased (P < 0.01) in the 8-h period on each experimental day. In both groups mean concentrations and responses after feed intake increased (P < 0.05) from d −4 to 21. In GrB the response after feed intake additionally increased (P < 0.05) from d −4 to 14. Responses after feed intake were smaller (P < 0.05) in GrA than in GrB on d 14 and on d 21. On d −4 and 14 values at 2 and 3 h after feed intake were lower (P < 0.05) in GrA than in GrB, and on d 21 values from 2 to 4 h after feed intake were lower (P < 0.05) in GrA than in GrB, whereas at 7 h after feed intake were higher in GrA than in GrB. Mean concentrations on d −4, 14, and 21 did not significantly differ between groups, but postprandial peak values tended to be higher (P < 0.15) on d 21 than on d 14 and −4 in both groups.

Preprandial plasma lactate concentrations (Figure 3) decreased (P < 0.001) in GrA and GrB from d −4 to 14 and then remained low up to d 21. Concentrations in GrB increased (P < 0.05) after feed intake on all days. Responses after feed intake were smaller (P < 0.01) in GrA on d 14 and tended to be smaller (P < 0.1) in GrA on d 21 than in GrB. Values 2 h after feed intake on d 14 and 3 h after feed intake on d 21 were lower (P < 0.05) in GrA than in GrB. Mean concentrations did not differ.

Preprandial plasma NEFA concentrations (Figure 4) in GrB were lower (P < 0.01) on d 21 than on d −4 and 14. Concentrations on all days in both groups decreased (P < 0.01) during the 8-h period. Values 1 h after feed intake on d 21 were higher (P < 0.01) in GrA than in GrB. Mean concentrations and responses after feed intake were smaller (P < 0.01) in GrB on d 14 than on d 21. The decrease after feed intake on d 21 tended to be greater (P < 0.1) in GrA than in GrB.

Preprandial plasma triglyceride concentrations (Figure 5) did not change during the 3-wk experiment and were not different between groups. Concentrations decreased (P < 0.05) transiently after feed intake. On d 14 at 6 and 7 h and on d 21 at 7 h after feed intake, values were higher (P < 0.05) in GrB than in GrA, and on d 21, at 1 h after feed intake were lower (P < 0.05) in GrB than in GrA. The decrease on d 21 tended to be greater (P < 0.1), and mean concentrations on d 14 tended to be lower (P < 0.1) in GrA than in GrB.

Preprandial plasma urea concentrations transiently increased (P < 0.01) in both groups on d 14, and in GrA on d 21 were lower (P < 0.01) than on d −4. Concentrations decreased (P < 0.05) in the 8-h period on d 14 (Figure 6). On d 14 values at 1 and 2 h tended to be lower (P < 0.1) in GrA than in GrB, and on d 21 preprandial and values up to 5 h after feeding as well as mean concentrations were lower (P < 0.05) in GrA than in GrB.

Preprandial serum total protein concentrations in GrA were lower (P < 0.05) on d −4 and on d 21 (55 ± 1 g/L) than on d 14 (57 ± 0.5 g/L) and were lower (P < 0.05) in GrB on d −4 than on d 14 and 21 (53 ± 1, 56 ± 0.4, and 56 ± 0.4 g/L, respectively). Preprandial serum albumin concentrations on d 21 were lower (P < 0.01) in GrA than in GrB (34 ± 0.4 and 36 ± 1 g/L, respectively), but were similar on d −4 and 14 (GrA: 34 ± 1 and 35 ± 1 g/L, respectively; GrB: 35 ± 0.2 and 36 ± 1 g/L, respectively). Blood hemoglobin concentrations in GrA were higher (P < 0.001) on d −4 than on d 14 and 21 (GrA: 6.6 ± 2.5, 5.7 ± 2.2, and 5.5 ± 2.1 mmol/L, respectively; GrB: 4.6 ±
Figure 1. Means of feed intake of calves fed by automate (GrA) on d −4, d 14 and d 21 of the 3-wk experiments, i.e. at the age of 10, 12, and 13 wk, respectively. Feed was available from 0800–2400 h, during which time calves could ingest ≥ 6 meals.

1.8, 5.1 ± 1.9, and 5.0 ± 1.9 mmol/L, respectively), and on d −4 were higher (P < 0.001) in GrA than in GrB. Preprandial serum iron concentrations did not change during the experiments and mean concentrations in GrA and GrB groups were similar (means: 13 ± 2 and 13 ± 4 (µmol/L, respectively).

Blood Hormone Concentrations

Preprandial plasma insulin concentrations (Figure 7) did not change during experiments and were not different between groups. Concentrations transiently increased (P<0.01) during the 8-h period. Mean concentrations and responses after feed intake were higher (P < 0.01) in both groups on d 21 than on d 14. In GrA mean concentrations and responses after feed intake on d 14 and 21 were lower (P < 0.05) than in GrB. Postprandial concentrations up to 5 h on d 14 and 21 were lower (P < 0.05) in GrA than in GrB. Insulin/glucose ratios much more markedly increased in the 8-h period in GrB than

Figure 2. Plasma glucose concentrations (means ± or – SEM) in veal calves on d −4 before and on d 14 and d 21 of the 3-wk experiments, i.e. at the age of 11, 12, and 13 wk, respectively. Calves were fed by automate (GrA ▲) or by bucket (GrB ○). In calves of GrA ≥ 6 meals were available from 0800–2400 h. In GrB calves were fed at 0800 and 1630 h. On a daily basis GrA and GrB received the same amounts of feed. From d 15 to d 21 of the 3-wk experiments calves were supplemented with lactose [300 g/(animal × d)]. Data are means ± SEM, n = 7 per group. Means with different lowercase letters (a, b, c, x, y) are significantly different (P < 0.05) preprandial values within a group on d −4, 14, and 21. Means with different uppercase letters (A, B) are significantly different (P < 0.05) between groups on d −4, 14, and 21. Means with star(*) or cross (⁺) are significantly different (P < 0.05) from preprandial values on the same day. □ represent differences of areas of GrA and GrB under the concentration curves measured from 0800–1600 h on d −4, 14, and 21.

Figure 3. Plasma lactate concentrations of calves fed by automate (GrA ▲) or by bucket (GrB ○). For further details see legend to Figure 2.
Preprandial plasma glucagon concentrations (Figure 8) decreased \( (P < 0.05) \) in GrA from d 14 to 21, whereas in GrB increased \( (P < 0.01) \) from d 4 to 14. Concentrations decreased \( (P < 0.05) \) slightly and slowly after feed intake in GrB on d 14 and 21 and in GrA on d 14. Values at 2 h after feed intake on d 14 and up to 6 h after feed intake on d 21 were higher \( (P < 0.05) \) in GrB than in GrA. Mean concentrations decreased \( (P < 0.01) \) during the experimental period in GrA, and on d 21 mean concentrations were higher \( (P < 0.01) \) in GrB than in GrA.

Preprandial plasma cortisol concentrations in GrA \( (7.1 \pm 3.2, 5.5 \pm 0.9, \text{ and } 3.8 \pm 1.0 \text{ nmol/L on } d 4, 14, \text{ and } 21, \text{ respectively}) \) decreased numerically but not significantly, in GrB concentrations were higher \( (P < 0.05) \) on d 14 than on d 4 and 21 \( (8.3 \pm 1.8, 3.8 \pm 0.8, \text{ and } 3.4 \pm 1.3 \text{ nmol/L, respectively}) \), but there were no group differences. Cortisol concentrations did not significantly change in the 8-h period (not shown).

Mean plasma concentrations of ACTH (in pools of samples obtained for 8 h) on d 14 and 21 in GrA \( (144 \pm 12 \text{ and } 133 \pm 8 \text{ ng/L, respectively}) \) and in GrB \( (120 \pm 12 \text{ and } 108 \pm 6 \text{ ng/L, respectively}) \) were similar.

Mean plasma concentrations of somatostatin (in pools of samples obtained for 8 h) on d 14 and 21 tended to be lower \( (P < 0.1) \) and on d 21 were significantly lower \( (P < 0.02) \) in GrA than in GrB.
Figure 8. Plasma glucagon concentrations of calves fed by automate (Gr A) or by bucket (GrB ○). For further details see legend to Figure 2.

Figure 9. Plasma growth hormone (GH) concentrations of calves fed by automate (Gr A) or by bucket (GrB ○). For further details see legend to Figure 2.

Preprandial plasma IGF-I concentrations (Figure 10) in both groups were lower ($P < 0.001$) on d −4 than on d 14 and 21. Mean IGF-I concentrations increased ($P < 0.01$) in GrB from d 14 to 21 and on d 14 and 21 were higher ($P < 0.01$) in GrA than in GrB. There were no significant postprandial changes.

Preprandial plasma concentrations of IGFBP-1 (Figure 11) decreased ($P < 0.05$) during the experimental period in GrA and decreased ($P < 0.05$) from d −4 to 14 in GrB. Concentrations rapidly decreased ($P < 0.05$) postprandially in both groups, but remained low ($P < 0.05$) during the 8-h period only in GrA. Concentrations were lower ($P < 0.05$) in GrB than GrA on d −4, before and until 4 h after feed intake on d 14, and 1 h after feed intake on d 21.
feed intake on d 21, but were higher \((P < 0.05)\) in GrB than GrA 8 h after feed intake on d 14 and 21. Concentrations of plasma IGFBP-2 and IGFBP-3 on d −4 were similar in GrA and GrB (not shown).

Preprandial plasma PRL concentrations (Figure 12) did not change significantly during the 3-wk experiments, but were higher \((P < 0.05)\) in GrA than in GrB on d −4. Mean and basal concentrations in both groups were similar on d 14 and 21. However, mean concentrations on d 14 and 21 (105 ± 10 and 120 ± 22 µg/L in GrA; 42 ± 7 and 42 ± 7 µg/L in GrB) and basal concentrations (78 ± 12 and 91 ± 15 µg/L in GrA; 25 ± 3 and 30 ± 5 µg/L in GrB) were higher \((P < 0.01)\) in GrA than in GrB, whereas peak amplitudes and frequencies were not different on d 14 and 21 and between groups.

Preprandial plasma concentrations of T3 and T4 did not change during experiments and there were no significant differences between groups (means of T3 and T4: 2.6 ± 0.3 and 81 ± 5 nmol/L, respectively).

**DISCUSSION**

The objective of the study was to compare in veal calves the supply of whole milk (combined with milk replacer) on a continuous basis by an automatic feeder (six or more major feeds per day) with that of two large meals provided by traditional bucket feeding. Both feeding systems lead to identical responses in feed consumption and growth rates in both groups. However, some of the major metabolic and endocrine parameters differed markedly with respect to their profile and mean concentration during an 8-h sampling period. Thus, milk supply by the automatic feeder led to significant increases in plasma concentrations of GH, IGF-I, and IGFBP-1, whereas plasma concentrations of insulin and glucagon were significantly lower in comparison with the group fed twice daily by bucket. This change in endocrine traits may represent a shift towards an enhanced anabolic profile in the group fed by the automatic feeder and improved metabolic homeostasis, which may ultimately result in an increase in protein deposition.

This rationale is supported by our observation of a significant reduction in plasma urea concentrations in the group fed by the automatic feeder. The traditional twice daily bucket feeding led to a large postprandial increase in plasma glucose and to hyperinsulinism. The supply of the same amount of milk during a 24-h period into ≥ 6 portions per day by the automatic feeder did not stimulate this marked postprandial pattern. Furthermore, the insulin area during the 8-h sampling period was lower in the group fed by the automatic feeder, suggesting that the automated feeding system leads to enhanced insulin sensitivity such that calves fed by this system require less insulin to establish euglycemia. Whether the hyperinsulinism observed in the group fed by bucket may lead to insulin resistance or other metabolic complications later in life warrants further investigation.

Additional lactose was fed to enhance postabsorptive glucose loads and thereby to stress glucose homeostatic systems (15, 18, 20). During lactose supplementation, feces became slightly looser, but this could not be classified as diarrhea. Calves were tested on d 14 and 21 (after being fed additional lactose for 7 d). Plasma glucose concentrations in GrA remained always lower than 8.3 mmol/L, which is the kidney threshold for plasma glucose in calves (15), except for values at 2 and 4 h on d 21. This was in marked contrast to GrB, in which postprandial glucose concentrations were above the kidney threshold on d 14 and especially on d 21, when lactose was additionally fed. Mean glucose concentrations did not differ between groups, but responses after feed intake were smaller and maximal concentrations were lower in GrA than in GrB. This can be interpreted to be the result of distributing feed intake in smaller portions over 16 h/d and consequently repeated moderate absorptive glucose loads. It cannot be excluded that suckling at the automatic feeder instead of drinking from the bucket also contributed to the difference. Nevertheless, glucose concentrations returned to basal levels in a shorter time in GrB than in GrA, possibly due to higher insulin concentrations in GrB than GrA. The present results are consistent with previous studies (15, 17, 18), in which we found hyperglycemia and glucosuria in veal calves fed by bucket, whereas excessive hyperglycemia never occurred in calves fed with an automatic feeder (19). Taken together these data indicate that glucose
homeostasis is maintained in calves fed by automatic feeder, even if fed high amounts of lactose.

Plasma lactate concentrations markedly, albeit transiently, increased in GrB after feed intake, as shown previously (17). The data show that the rise of plasma lactate concentrations was not enhanced by lactose supplementation, i.e., was not solely the consequence of glucose absorption. Because lactate concentrations barely changed in GrA, the pattern of feed intake modifies lactate profiles.

Concentrations of NEFA decreased after feed intake in GrB, as repeatedly shown before (15, 18, 23). This was most likely a consequence of the effects of insulin, whose concentrations increased. Values were similar in both groups, except at 1 h after feed intake on d 21. Thus, there was no sign for insulin resistance concerning NEFA metabolism, in accordance with previous studies (15, 18).

Concentrations of triglycerides decreased during the 8-h period, as shown before (18), and likely are a result of insulin-stimulated tissue uptake of triglycerides. This effect lasted longer in GrA, even though insulin levels from 6 to 8 h after feed intake were not significantly higher than in GrB. Factors other than insulin were probably responsible for reduced triglyceride concentrations.

The increase of serum or plasma protein, albumin, and urea concentrations from d −4 to 14 did not mirror incremental protein intake because CP per kilogram of BW decreased in both groups, but the decrease of urea concentrations from d 14 to 21 may have been associated with lactose supplementation and increased insulin secretion. The lower plasma urea concentrations in calves fed by automatic feeder than by bucket point to higher protein synthesis or lower protein breakdown. Relatively high GH and IGF-I, and low glucagon concentrations in GrA, compared with GrB, possibly contributed to lower urea concentrations. There were group differences of hemoglobin concentrations at the beginning of experiments but values were within the physiological range (24), which could be expected because iron intake and plasma iron concentrations were sufficient for adequate hemoglobin synthesis.

Normal preprandial insulin concentrations in calves range from 0.3 to 1.5 µg/L (5, 11, 15, 19, 23). Preprandial insulin concentrations in our experiment generally remained within this range. Insulin concentrations increased transiently during the 8-h period in both groups, but the rise was greater on d 21 than on d 14, most likely because of a more marked rise of glucose concentrations after additional lactose feeding on d 21 than on d 14, in accordance with previous studies (15, 18). Insulin responses during the first hours after feed intake were markedly greater in GrB than in GrA, in part as a consequence of higher glucose concentrations in GrB than in GrA. However, because mean glucose concentrations were not different between GrB and GrA, factors other than plasma glucose probably contributed to the marked difference of insulin concentrations between GrA and GrB. The much greater insulin/glucose ratios in GrB than GrA indicated that calves of GrB required a markedly increased insulin release to establish euglycemia, i.e., expressed decreased sensitivity and responsiveness of glucose to insulin or insulin resistance.

Glucagon concentrations slightly decreased during the 8-h period in GrB, but remained stable in GrA. This was possibly the result of smaller increases of glucose in GrA than GrB. Surprisingly, mean glucagon concentrations were lower in GrA after additional lactose loads than in GrB, whereas glucagon concentrations were similar in GrB with and without lactose supplementation. Even more intriguing were the higher glucagon concentrations in GrB than in GrA on d 21, when glucose concentrations were higher in GrB than in GrA from 2 to 4 h after feed intake. Factors other than plasma glucose possibly contributed to this unexpected finding. As a consequence of lower glucagon levels in GrA than in GrB, protein breakdown was expected to be smaller in GrA than in GrB, thus possibly contributing to low urea concentrations.

Cortisol concentrations did not significantly change in a consistent manner and were neither affected by feeding systems nor by feeding lactose. In our experiment, cortisol was therefore probably neither involved in metabolic differences induced by lactose supplementation nor involved in differences between GrA and GrB. Because concentrations of cortisol were in a low, but normal, range (33, 34) and cortisol secretion is enhanced under stress conditions, lack of differences between the two groups and a lack of time-dependent changes suggests that the calves in both groups did not experience any major physiological stress. Data on cortisol were in accordance with those of ACTH, which stimulates cortisol secretion. Because ACTH concentrations were similar in GrA and GrB, ACTH behaved different from the two other pituitary hormones (GH and PRL), whose concentrations were different in GrA and GrB.

Growth hormone treatment can cause hyperglycemia and induce insulin resistance, followed by hyperglycemia and hyperinsulinemia, if secreted in high amounts (6, 29). This was not the case in our study, because concentrations of GH were higher and insulin concentrations were lower in GrA than in GrB. Nutritional status plays a major role in determining circulating GH concentrations. Periods of nutritional deficit are characterized by elevated concentrations of GH in sheep (10) and cattle, whereas intensive feeding decreases circulating GH concentrations (2, 4, 26). The transient absence of GH secre-
tory peaks for the first 2 h on d 21 in GrB was possibly the consequence of abrupt, high lactose and energy intakes and marked hyperglycemia, in accordance with studies in sheep (10) and in humans (35). Because calves of GrA and GrB ingested the same amounts of feed during 24-h periods, factors other than energy intake were responsible for group differences in plasma GH concentrations. It cannot be excluded that sucking as such on the nipple of the automatic feeder enhanced GH secretion. This may be associated with changes of the release of neurotransmitters and of the α-adrenergic tone, which, too, modifies GH release in veal calves (31). Importantly, GH concentrations were low in GrB and can therefore not be considered to be responsible for or related to hyperglycemia, hyperinsulinemia, and insulin resistance, in accordance with previous studies (15, 18, 19).

Concentrations of IGF-I did not consistently change in the 8-h period, as shown previously for newborn calves (14) and veal calves (8). Calves fed by automatic feeder had higher IGF-I concentrations than calves fed by bucket. The higher GH concentrations in GrA than in GrB may at least in part explain higher plasma IGF-I concentrations. Uptregulation of plasma IGF-I by increased plasma concentrations of GH could represent an increased anabolic drive. IGF-I concentrations also depend markedly on protein and energy supply (4, 12, 13). Because feed, energy, and protein intakes were nearly identical in GrA and GrB, higher IGF-I concentrations in GrA than in GrB cannot be explained on this basis. Sucking per se, possibly mediated by changed release of neurotransmitters (7), may have been responsible for higher GH concentrations in GrA than in GrB, and may have caused increased IGF-I concentrations. Increased circulating amounts of IGF-I have a negative feedback on GH release, but the higher IGF-I concentrations in GrA than in GrB obviously did not reduce GH release in our study. The lower somatostatin concentrations in GrA than GrB were in accordance with higher GH in GrA than GrB.

Plasma concentrations of IGF-I depend on concentrations of IGFBP. In calves, as in other species IGFBP-3 is the main IGF-I carrier (32), as also shown in this paper. Concentration changes of IGFBP-1 and IGFBP-2, which in contrast to IGFBP-3 can leave blood capillaries, can contribute to rapid alterations of plasma IGF-I levels (14). However, IGFBP-2 and -3 concentrations are postprandially relatively stable. In contrast, this study shows that IGFBP-1 concentrations rapidly decrease postprandially. A decrease of the IGFBP-1 concentration in other species is known to be due to a rise of plasma insulin concentrations, as was the case in this study. Because plasma concentrations of IGFBP-1 were higher in GrA than GrB, these differences may have contributed to differences of IGF-I concentrations. Increased IGFBP-1 concentrations, while IGF-I concentrations are increased, could mean that more IGF-I is available for fast delivery when it is needed, such as during tissue injuries or bacterial infections.

Prolactin concentrations were higher in calves fed by automatic feeder than in calves fed by bucket. As in the case of GH, differences in the release of neurotransmitters between GrA and GrB may have been responsible. It has been shown in calves that exogenous opioids increase PRL secretion through inhibition of dopamine release (21), that dopaminergic antagonists also increase PRL concentrations (37), whereas dopaminergic stimulation reduces plasma PRL concentrations (1). Sucking per se may stimulate the release of endogenous opioids, which would inhibit dopamine release, and enhance PRL secretion.

Concentrations of both T3 and T4 are markedly dependent on energy intake (2, 13). Concentrations of T3 and T4 did not change in time, did not differ between groups, and remained in the physiological range (24). The absence of group differences reflected nearly identical energy intakes.

In conclusion, homeostasis of blood metabolites and especially of glucose and lactate in veal calves is improved when daily feed intake is distributed into more than two portions, as observed in the present study when calves are fed by automatic feeder. Development of hyperinsulinism, seen in calves fed twice daily by bucket, could be avoided if calves were fed by automatic feeder. This also lowered the stress on endocrine systems, especially insulin. Furthermore, sucking on the nipple of an automatic feeder, which is more natural than drinking twice daily from a bucket, may per se be responsible for increased PRL, GH, and IGF-I status and low circulating glucagon. These endocrine changes may contribute to improved postabsorptive homeostasis of glucose and lactate. The resulting endocrine pattern is basically favorable for anabolic metabolism, expressed by low plasma urea concentrations. Whether this results in enhanced daily gains and higher gain:feed ratios in calves fed by automated feeding systems than by bucket has to our knowledge not yet been demonstrated.

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