Body Fat Deposition Does Not Originate from Carbohydrates in Milk-Fed Calves\textsuperscript{1,2}

Joost J. G. C. van den Borne,\textsuperscript{3,*} Gerald E. Lobley,\textsuperscript{4} Martin W. A. Verstegen,\textsuperscript{3} Jane-Martine Muijlaert,\textsuperscript{3} Sven J. J. Alferink,\textsuperscript{3} and Walter J. J. Gerrits\textsuperscript{3}

\textsuperscript{3}Animal Nutrition Group, Department of Animal Sciences, Wageningen University, 6700 AH Wageningen, The Netherlands; and \textsuperscript{4}Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB, United Kingdom

Abstract

Milk-fed heavy calves utilize dietary protein with a low efficiency and often develop hyperglycemia and insulin resistance. Distributing the daily nutrient intake over an increasing number of meals increases protein deposition and improves glucose homeostasis. Therefore, we examined effects of feeding frequency (FF) and feeding level (FL) on the diurnal pattern of substrate oxidation and on the fate of dietary carbohydrates in milk-fed heavy calves. Eighteen milk-fed calves weighing 136 ± 3 kg were assigned to FF (1, 2, or 4 meals daily) at each of 2 FL (1.5 or 2.5 times maintenance), except for calves at FF1 (only at a low FL). Urea, leucine, and glucose kinetics were assessed for each treatment by use of \textsuperscript{13}Curea, \textsuperscript{1-13}Cleucine, [U-\textsuperscript{13}C], and [2-\textsuperscript{13}C]glucose, respectively. FF altered the diurnal pattern, but not the total, of urea production production. Although urea production correlated well with nitrogen retention, oxidation of oral L-[1-\textsuperscript{13}C]leucine did not. Dietary glucose was almost completely oxidized (80% based on [\textsuperscript{13}C]glucose and 94% from indirect calorimetry) regardless of FL. Fatty acid synthesis from glucose appeared to be negligible based on similar recoveries of \textsuperscript{13}CO\textsubscript{2} from orally supplied [U-\textsuperscript{13}C]glucose and [2-\textsuperscript{13}C]glucose. The increased fat deposition at the higher FL originated almost exclusively from greater transfer of fatty acids to body lipid stores. These findings contrast with both glucose and lipid metabolism in growing pigs and indicate that alternative adaptive mechanisms operate in heavy milk-fed calves. J. Nutr. 137: 2234–2241, 2007.

Introduction

During the past decades, skimmed milk protein in calf milk replacer has largely been substituted by more rapidly hydrolyzable (i.e., nonclotting) protein sources such as vegetable proteins and whey. This change, coupled with a high feeding level (FL)\textsuperscript{5} and low feeding frequency (FF; twice daily is common practice) results in rapid absorption of nutrients, such as amino acids, glucose, and galactose, soon after ingestion (1). The calves need adaptive mechanisms to cope with these high loads of nutrients, with partition toward either nutrient deposition or oxidation. Importantly, milk-fed heavy calves often develop insulin resistance (2), reflected in hyperglycemia (2) and low efficiency of dietary protein utilization (3). The insulin resistance may impact also on the capability to process large amounts of other rapidly absorbed nutrients toward anabolic outcomes.

Under such conditions, more efficient nutrient utilization may occur if the smaller amounts of nutrient are absorbed over short time intervals and this can be achieved by increasing FF at the same FL. Indeed, increased FF improves insulin sensitivity in calves, as indicated by lower postprandial insulin to glucose ratios and lower glucose excretion in urine (4). Furthermore, fat deposition is enhanced (5) by increasing FF, but whether this is due to either increased channelling of glucose into fatty acid synthesis de novo, as occurs in nonruminants (6), and/or better utilization of dietary fatty acids is not known. In other species, including obese and insulin-resistant human subjects (7,8), improved insulin sensitivity increases conversion of glucose to fatty acids and alters metabolic fuel selection, affecting diurnal patterns of glucose and fatty acid oxidation (O XF). Nonetheless, milk-fed heavy calves are ruminants ontologically but are maintained in a preruminant state by a liquid diet that supplies large amounts of digestible glucose and galactose. Fat synthesis from glucose is not a major route in ruminants, because acetate released by rumen fermentation provides ample precursor and there is not the reliance on either citrate lyase or malate dehydrogenase as observed in nonruminants (9). In the milk-fed heavy calf, however, it is unclear whether nutrient source or developmental physiology has the greater impact on the precur-
Materials and Methods

Experimental design. The experimental design has been described in detail by Van den Borne et al. (5). Briefly, 18 male Holstein Friesian calves weighing 136 ± 3 kg were used in 9 trials, each with 2 calves of similar age. The trials consisted of 2 consecutive experimental periods each of 6 wk, 4 of which were for adaptation to the treatments. Period 1 was at low FL and period 2 at high FL. Within each trial, 1 particular FF was applied, because the visual and (limited) auditory contact between the individual calves could affect physical activity and cephalic phase reflexes. Animals used in a 2 × 3 factorial arrangement with FL [1.5 × metabolizable energy requirements for maintenance (MEm) and 2.5 × MEm] and FF (1, 2, or 4 meals per day). Six calves were studied per treatment. Animals on the high FL could not consume such an intake within a sensible period if this was offered as 1 meal and so this treatment was not included. Calves at FF 1 served as controls for the effect of treatment. Animals on the high FL could not consume such an intake within a sensible period if this was offered as 1 meal and so this treatment was not included. Calves at FF 1 served as controls for the effect of experimental period, because FL was confounded with period in this study.

Diet and feeding. Calves were fed according to their metabolic body weight (kg0.75). Feed supply was adjusted daily for a projected mean daily gain of 0.5 kg at the low FL and 1.5 kg at the high FL. The MEm was assumed to be 460 kJ/kg0.75 d. Whey was used as the only protein source in the milk replacer; this provides a rapidly hydrolyzable protein source that can be included at high levels in diets for milk-fed calves. The ingredients and analyzed nutrient composition of the experimental milk replacer is presented in Table 1. Nutrient analyses of the milk replacer have been described previously (5). Milk replacer was reconstituted with water (140 g/L) and supplied by bucket at a temperature of ≈40°C. Roughage was not supplied. Feeding times were 0000 (FF 2 and 4), 0600, 1200, and 1800 (FF 1, 2, and 4), and 1800 (FF 4). In addition, calves at FF 1 were supplied with 3 L of warm water (40°C) at 0600 and 1800 to prevent dehydration. Calves were allowed 15 min to consume the meal. Mean energy and protein intakes were 753 ± 3.4 kJ/kg0.75 d and 6.9 ± 0.04 g/kg0.75 d for calves at the low FL and 1228 ± 6.4 kJ/kg0.75 d and 11.4 ± 0.07 g/kg0.75 d at the high FL.

Measurements. The calves were housed for 10 d in a metabolic cage within respiration chambers followed by 4 d when the calves were maintained in a metabolic cage within a climate-controlled stable (Fig. 1). Oxidation of leucine was quantified from 13CO2 excretion after an oral bolus dose of 25.2 μmol i-1[1-13C]leucine (99.0 atom%; Cambridge Isotope Laboratories) per gram dietary protein was added to the milk replacer offered at 1200. Glucose oxidation was determined from the amount of 14CO2 exhaled after an oral bolus dose of 2.5 μmol [U-13C]glucose (99.0 atom%; Cambridge Isotope Laboratories) per gram dietary lactose was added to the milk replacer supplied at 1200. The [2-13C]glucose (99.0 atom%; Cambridge Isotope Laboratories) was supplied similarly but at 15 μmol/kg dietary lactose. Differences in oxidation of [U-13C]glucose and [2-13C]glucose would reflect, indirectly, fatty acid synthesis from glucose, with a greater reduction in the amount of 13CO2 released from [2-13C]glucose if this is used for fatty acid synthesis (11). The labeled C-atom from [2-13C]glucose is not released if acetate is formed and used for fatty acid synthesis. Bicarbonate sequestration was determined from expired 13CO2 after injection of a bolus dose of 4.7 mmol [13C]bicarbonate (99.1 atom%; Mass Trace) into an ear vein. The infused bolus was prepared in 10 mL sterile 0.15 mol/L NaCl and was injected within 2 min at 1400, i.e., 2 h after feeding for each treatment. During the 10 d in respiration chambers, nitrogen (N) and energy balances were performed (5) and exchanges of oxygen, carbon dioxide, and methane were measured over 6-min intervals, as described by Verstegen et al. (12). In addition, during the tracer studies, 13C02 production was measured during 6-min intervals by nondispersive infrared spectrometry (Advance Optima Uras 14, ABB) as described by Alferink et al. (13). Sequestration of labeled bicarbonate was measured on d 3, leucine oxidation on d 6, and oxidation of [U-13C]glucose and [2-13C]glucose on d 4 and 8, respectively.

After the 10-d period in a respiration chamber, the calves were prepared with a venous catheter (16 gauge/1.70 mm; Secalon T, Becton Dickinson) in each jugular vein. The catheters were attached to the skin using Vicryl suturing (Ethicon) and were extended with a 200-ml Becton Dickinson) and stored on ice until plasma was collected. Blood was immediately transferred into lithium heparin tubes (Vacutainer, Becton Dickinson) and stored on ice until plasma was collected after centrifugation at 1500 × g, 10 min. Plasma samples were stored at −20°C pending analyses.

Urea concentration was measured spectrophotometrically by the linked urease and glutamate dehydrogenase procedure (Cobas Integra 800; Roche Diagnostics). The enrichment of 13C in urea was measured as CO2 released by urease. Briefly, plasma was deproteinized by adding 5 mL methanol, mixing, and storing the tubes at −20°C for 2 h. After centrifugation at 1500 × g for 10 min, the supernatant was transferred

<table>
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<th>Ingredient</th>
<th>Nutrient</th>
<th>g/kg</th>
<th>g/kg DM</th>
</tr>
</thead>
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<tr>
<td>Whey</td>
<td></td>
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<td>978.0</td>
</tr>
<tr>
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<td></td>
<td>360.0</td>
<td>73.1</td>
</tr>
<tr>
<td>Decolactose whey</td>
<td></td>
<td>70.0</td>
<td>190.5</td>
</tr>
<tr>
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<td></td>
<td>144.0</td>
<td>195.6</td>
</tr>
<tr>
<td>Coconut oil</td>
<td></td>
<td>36.0</td>
<td>470.3</td>
</tr>
<tr>
<td>α-Methionine</td>
<td></td>
<td>1.32</td>
<td>20.6</td>
</tr>
<tr>
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<td></td>
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<td>13.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td></td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td></td>
<td>11.4</td>
<td></td>
</tr>
</tbody>
</table>

1 Provided per kilogram of the experimental diet: 7.8 g calcium; 6.5 g phosphorous; 1.4 g magnesium; 7.5 mg ribitol; 0.05 mg cholecalciferol; 80 mg dl-tocopherol; 84 mg zinc; 9 mg copper; 48 mg iron; 0.1 mg selenium; and 1.9 mg cobalt.
2 Analyzed content, unless indicated otherwise. DM, dry matter.
3 Calculated content.

TABLE 1 Ingredient and analyzed nutrient composition of the experimental diet

Substrate oxidation in milk-fed calves 2235
into a 10-mL Ex提质 tube (Labco) and 50 μL of 12 mol/L HCl was added and mixed. After evaporation to dryness in a water bath at 40°C under a gentle stream of nitrogen, 1 mL of (boiled) water and 300 μL of 1 mol/L HCl were added, mixed, and completely evaporated again. Then, 1.5 mL of a cold (4°C) buffer solution (27 g KH2PO4 and 53.5 g Na2HPO4/L, pH 7.0) containing urease (50 kU/L; Sigma Chemical) was added on ice. The tubes were covered with rubber lids, flushed with argon for 10 s to replace the air in the headspace, and then incubated at 37°C with gentle shaking in a water bath for 45 min. The reaction was stopped by injection of 0.5 mL 7 mol/L phosphoric acid and the 13C enrichment in CO2 measured in the headspace using a Finnigan Delta C continuous-flow isotope ratio mass spectrometer (Finnigan MAT). The enrichment was expressed as atom% 13CO2.

Calculations. The derivative of a generalized Michaelis-Menten equation, as proposed by López et al. (14) for growth curves, was used to express 13CO2 production in time. The model was fitted to the 30-min means of 13CO2 excretion in breath (corrected for background enrichment) after ingestion of i-[1-13C]leucine, [U-13C]glucose, and [2,13C]glucose and after infusion of 13C sodium bicarbonate:

\[ y = (b_2 \cdot e^{-t/\tau}) \cdot b_1 t / [1 + (b_1 t / \tau)^c], \]  

where \( y \) = 13CO2 production (micromoles per minute) at time \( t \) (mature); \( b_1, b_2, \) and \( c \) (all > 0) are parameters that define the curve.

The nonlinear least squares regression procedure (PROC NLIN, SAS Institute) was used for curve fitting. Oxidation of the tracer metabolites was calculated by integration of the area under the 13CO2 excretion curve over a period of 24 h after administration of the isotope, \( b_0/(1+b_2 \cdot e^{-4400 \cdot t}) \) and expressed relative to the amount of ingested or calculated from hourly values as areas under the curve over a 6-h period and after feed intake (AUC12–18). As the basal values could be affected by FL and FF, the responses in excess of preprandial values (i.e. 2 h before feeding) were also calculated (ΔAUC12–18).

Statistical analysis. All data were analyzed for the effect of FF, FL, the interaction between FL and FF, and period by ANOVA using PROC MIXED in SAS. The factorial design was analyzed by a mixed model for the fixed effects of FF, FL, and period and the random effect of each calf by ANOVA using PROC MIXED in SAS. The SAS software package version 9.1 (SAS Institute) was used for all statistical evaluations.

Results

General. Two calves at FF 1 were excluded from the experiment because of illness and feed refusals. Another calf, at FF 2, was excluded from 1 of the experimental periods (at a low FL). The results were not affected (\( P > 0.10 \)) by the experimental period. Therefore, the effect of experimental period was not included in the results.

Urea production. FF had limited effect on hourly urea production rates, except that a greater FF increased (\( P < 0.05 \))
urea production rates at 1330, whereas OXF increased (P < 0.001) with the higher FL (Table 2). The AUC12–18 for urea production increased (P = 0.004) with higher FL and tended to decrease (P = 0.053) with greater FF (Table 2), whereas the ΔAUC12–18 decreased with FF (P = 0.016) but was not affected by FL.

**OXCHO and OXF: indirect calorimetry.** At higher FF, the hourly OXCHO values were greater (P < 0.001) until 1330 but lower (P < 0.001) thereafter (Fig. 3). All hourly OXCHO values (except at 1330) were greater (P < 0.001) at the higher FL. Hourly OXF values were greater (P < 0.01) at the lower FL until 1330, whereas OXF increased (P < 0.05) with greater FF after 1630. All hourly OXF values (except 0730 and 1230) were lower (P < 0.05) at the higher FL.

Daily OXP, as a percentage of the protein intake, decreased (P = 0.038) with greater FF but was not affected by FL (Fig. 4).

Daily OXCHO expressed as percentage of the lactose intake was high with 94.2 and 91.5% oxidation at the low and high FL, respectively, but was not affected by either FF or FL. Daily OXF, as a percentage of the fat intake, tended to be affected by FF (P = 0.058) and was greater for the lower FL (77.7 vs. 30.6%; P < 0.001).

**L-[1-13C]leucine oxidation.** Curve fits for [13CO2] from L-[1-13C]leucine were good and the MSPE averaged 17.6% (r = 0.94), with the majority of the prediction error (> 95%) attributable to random variation. For [13C]bicarbonate, curve fits were also good (MSPE 16.3%; r = 0.97), with 72% of the injected dose recovered as [13CO2] for both FL. The proportion of the L-[1-13C]leucine oxidized was greater with increasing FF at the low FL but decreased with increasing FF at the high FL (FF × FL; P < 0.001; Table 3). The maximum of [13CO2] excretion tended to increase (P = 0.068) with greater FF. The time until maximum [13CO2] excretion was reached decreased (P < 0.001) with greater FF.

**Glucose oxidation; [U-13C]glucose and [2-13C]glucose.** Curve fits were good and gave an MSPE of 17.0% for [U-13C]glucose (r = 0.95) and 17.6% for [2-13C]glucose (r = 0.92), with the majority of the prediction error (> 95%) attributable to random variation. Recoveries of the [U-13C]glucose dose as [13CO2] were high (mean 79.5%) and were unaffected by either FF or FL (Table 3). The maximum of [13CO2] production was greater (P < 0.001) with increasing FF. The time to maximum decreased (P < 0.001) substantially with greater FF. Recoveries of the [2-13C]glucose dose as [13CO2] averaged 78.8%, similar (P > 0.10) to [U-13C]glucose, and were unaffected by FF or FL (Table 3). The maximum [13CO2] production was greater (P < 0.001) with increasing FF, whereas the time to maximum decreased (P < 0.001).

**Discussion**

This study showed that urea production increased after milk intake in calves, was less at the lower FL and tended to decrease with greater FF. Extrapolated daily rates of urea production did not significantly decrease with greater FF. This contrasts with an increased protein deposition from N balance measurements and may indicate that longer-term (i.e. 24 h or longer) measurements of urea production are needed to detect differences in diurnal kinetics of amino acid oxidation. Oral glucose was almost completely oxidized and this was unaffected by both FF and FL, indicating that fatty acid synthesis de novo from glucose was negligible in milk-fed calves. The increase in fat deposition with increased feed intake originates almost exclusively from a reduced oxidation of fatty acids.

**Urea production.** Amino acid oxidation was assessed by 2 approaches: ureagenesis based on [13C]urea kinetics and catabolism of oral L-[1-13C]leucine, in addition to the previously reported N balance data (5). Urea production rates showed marked diurnal fluctuations but, in general, increased after meal ingestion at 1200. Postprandial urea production (AUC12–18) was greater at the higher FL and tended to be greater with decreasing FF (larger meal size). In rats, larger meals also tended to increase amino acid oxidation (and thus urea production) in the post-prandial state (19). After the 1200 meal, the hourly urea production rate approximately doubled in calves fed 1 or 2 meals daily, similar to findings in pigs (20) and humans (21). Although
we hypothesized that urea production rates over 12 h would decrease with greater FF, this was not observed and apparently contrasts with the improvement in N retention found in this study (5). This may be due to differences in time scale between measurements (12 h vs. 10 d), diurnal changes in urea pool size, or the higher variance associated with the isotope-related measurements. Nonetheless, the daily urea-N production showed a strong positive correlation ($r = 0.98$; $P < 0.001$) with the 10-d urinary N excretion across FF.

As expected, urea production increased with increasing FL, as commonly observed across species (21–23). The urea-N production exceeded the urinary N excretion by 39%. Urea-N production is usually higher than the urinary N excretion due to entry and hydrolysis of urea in the gut lumen. In ruminants, 40–80% of the produced urea is returned to the gastrointestinal tract (24), but this recycling is usually lower in nonruminants. In exclusively milk-fed calves, hydrolysis of urea is still ~30% higher than urinary urea excretion in these heavy calves. A similar phenomenon was seen in human neonates (also milk-fed) in that only 20% of produced urea was excreted in urine (25).

### Dietary amino acid oxidation

The metabolic fate of dietary amino acids has often been studied by adding $^{13}$C- or $^{14}$C-labeled free amino acids to a meal (26–29). From breath $^{13}$CO$_2$ dose as $^{13}$CO$_2$ increased at the low FL but decreased at the high FL (26–29). From breath $^{13}$CO$_2$ dose as $^{13}$CO$_2$ increased at the low FL but decreased at the high FL (26–29). Indeed, the correlation between recovery as exhaled $^{13}$CO$_2$ from $^{13}$C-leucine and OXP calculated from N balance was considerably better at the high FL ($r = 0.77$; $P = 0.003$) than at the low FL ($r = 0.34$; $P = 0.149$). Several explanations may account for the apparent discrepancy between leucine oxidation and urea production. First, to obtain a quantitative value for leucine oxidation requires measurement of the flux through the free leucine pool, but this was not determined in the current study as it would not extend over a meal interval (12 h) with the dietary bolus dose method employed. Changes in leucine flux can influence markedly the net oxidation. For instance, Motil et al. (35) reported fractional leucine oxidation rates in response to protein intake in meal-fed humans of 16.3, 18.2, and 29.3% of the dose, whereas absolute oxidation rates were 11.8, 21.6, and 46.3 mmol/(kg·h), respectively. Second, ingestion of labeled leucine creates an isotopic gradient with the higher leucine enrichment in the gastrointestinal tract and this may overemphasize the contribution of enterocytes to whole body amino acid oxidation. Leucine can be extensively oxidized by enterocytes (36,37), but the proportion oxidized decreases with increasing FL (38). This would make a larger fraction of the dose available for peripheral tissue metabolism and may explain the better correlation with N balance at the higher FL. Third, a time lag between the absorption of free amino acids, such as the added l-[1-13C]leucine, and those originating from protein digestion may have resulted in a temporal amino acid imbalance.

### Glucose and fatty acid oxidation

Calculation of substrate oxidation from indirect calorimetry in fast-growing animals is often complicated by a high protein-free respiratory quotient (RQ), indicating de novo fatty acid synthesis from glucose (41,42). In our study, however, this RQ did not exceed unity. On the other hand, gluconeogenesis from noncarbohydrate precursors (e.g. alanine) results in a low RQ (42) and would lead to an overestimation of OXF and underestimation of OXCHO. Therefore, the estimates of OXCHO in this study should be considered as minimal values.

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**TABLE 2** Influence of FF (1, 2 or 4 meals/d) and FL (high vs. low) on the 12-h urea production rate, the AUC$_{12-18}$, and the AUC$_{12-18}$ in excess of preprandial values ($\Delta$AUC$_{12-18}$)$^{1,2}$

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<th>Item</th>
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<th>FF 4</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>FL low</td>
<td>FL low</td>
<td>FL high</td>
</tr>
<tr>
<td>Urea production</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 h rate, mmol/(kg$^{0.75}$·12 h)</td>
<td>11.2</td>
<td>10.6</td>
<td>16.7</td>
</tr>
<tr>
<td>AUC$_{12-18}$, mmol/(kg$^{0.75}$·6 h)</td>
<td>7.1</td>
<td>6.6</td>
<td>10.5</td>
</tr>
<tr>
<td>$\Delta$AUC$_{12-18}$, mmol/(kg$^{0.75}$·6 h)</td>
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<td>4.6</td>
<td>5.5</td>
</tr>
<tr>
<td>OXCHO$^1$</td>
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<tr>
<td>12 h rate, g/(kg$^{0.75}$·12 h)</td>
<td>7.2</td>
<td>7.5</td>
<td>11.5</td>
</tr>
<tr>
<td>AUC$_{12-18}$, g/(kg$^{0.75}$·6 h)</td>
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<td>OXP$^2$</td>
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<td>0.5</td>
<td>0.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

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1 Values are means, $n = 8$ (FF 1), $n = 5$ (FF2, FL low), or $n = 6$.
2 Calculated from gas exchange rates and correction based on urea production rates.
A milk-fed calf of 150 kg usually ingests >500 g of lactose in a single meal and portal glucose and galactose appearance is virtually complete within a few hours after feeding (1). Temporary storage of glucose (as additional glycogen) in well-fed animals is very small and therefore glucose is either oxidized or used as a precursor for de novo fatty acid synthesis. From the high $^{13}$C recovery of $[U^{13}C]glucose$ as $^{13}CO_{2}$ (mean 80%) and the high OXCHO as a percentage of the lactose intake (mean 94%), it appears that ingested carbohydrates are predominantly oxidized independent of either FF or FL. The lower glucose oxidation derived from the tracer compared with the gas exchange method may be a consequence of the label sequestration through glucose-glycogen interchange, thus reducing the number of $^{13}$C glucose molecules that enter the glycolytic pathway. The gas exchange method does not discriminate between oxidation of exogenous and endogenous glucose, so this more closely represents net catabolism of glucose. The net result of an increase in FL was 53–59% more OXCHO. At the same time, OXF decreased markedly at the higher FL. In energetic terms, this could be advantageous, because the efficiency of triglyceride synthesis from fatty acids is considerably greater than from glucose [90 vs. 74%, respectively; (43)]. Nonetheless, this is not simply a direct substitution, because regression analysis revealed that every 1 kJ increase in heat production from glucose resulted in only 0.5 kJ decrease in heat production from fatty acids ($r = -0.79$, $P < 0.001$). Partly, this is due to the additional energy utilized by the animal at the higher FL, possibly related to additional demands to support absorption or to drive elevated protein synthesis and urea production (44,45).

**Fatty acid synthesis from glucose.** Other species generally tend to have increased rates of de novo fatty acid synthesis from glucose in the immediate postprandial period with decreasing FF, especially when carbohydrate-rich diets are offered (6). This is accompanied by increased enzyme activities associated with glucose and lipid metabolism and improved sensitivity of adipose tissue to insulin [reviewed in (6)]. This scenario is not true for milk-fed heavy calves, however, because OXCHO is virtually complete, regardless of the FF. Indeed, fatty acid synthesis de novo from glucose can be calculated from the difference between OXCHO plus urinary glucose excretion and digestible lactose intake plus gluconeogenesis from amino acids [as described in (41)]. This produced low values, 5.6 kJ/(kg$^{0.75}$ d), independent of both FF and FL and was <2% of total fat deposition at a FL of 2.5 × $ME_{f}$ (from 5), contrary to our hypothesis that more glucose is used for de novo fatty acid synthesis with increasing FF in milk-fed calves. This finding does not correspond with the higher activities of ATP citrate lyase observed in liver and adipose tissue from milk-fed calves compared with ruminant calves (46) and is also contrary to findings in pigs, where increasing the FL resulted in almost 700 g of the ingested starch retained as fat (41). Even at close to energy maintenance, pigs use substantial amounts (~150 g) of carbohydrates for fatty acid synthesis while also oxidizing body fat (41).

We speculate that the low level of de novo fatty acid synthesis from glucose in milk-fed calves relates to: 1) the (onto)genetic background of the calves belonging to a herbivorous species and may lack enough of the necessary enzyme systems for converting dietary glucose to fat; or 2) the high fat intake and the impact this exerts on the metabolic fate of dietary glucose. Potential mechanisms for the high OXCHO and low utilization...
of glucose for fatty acid synthesis remain to be studied in milk-fed calves.

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