Insulin Resistance, Hyperglycemia, and Glucosuria in Intensively Milk-Fed Calves

Robyn Leigh Hostettler-Allen*, Luc Tappy†, and Jürg W. Blum*3

*Division of Nutritional Pathology, Institute of Animal Breeding, University, Berne and †Institute of Physiology, University, Lausanne, Switzerland

ABSTRACT: In intensively milk-fed calves postprandial glucose (G) and insulin (I) concentrations, but not preprandial G concentrations, increased or failed to decrease during the growth period, compared with data from calves that were progressively weaned. This study was, therefore, designed to investigate G and I metabolism in veal calves. Euglycemic-hyperinsulinemic and hyperglycemic clamps in the unfed state demonstrated mutual responsiveness of I and G, but revealed a relative I resistance. After feed consumption, I resistance was exaggerated, as seen by decreased G clearance rates after i.v. G and I administration in fed compared with unfed calves. Milk replacer is a source of readily available lactose, fat, and protein, the intake of which, on a kilogram75 basis, gradually increased with age. Increased substrate availability and effects of nutrients themselves were probably responsible for elevated plasma concentrations of G and I and led to I resistance. Additionally, hyperglycemia > 1.5 g/L was followed by urinary excretion of G.

Key Words: Hyperglycemia, Hyperinsulinemia, Insulin, Glucosuria, Veal Calves


Introduction

In nonruminants and pseudo-nonruminants, such as veal calves, ingestion of feed and absorption of glucose (G) stimulates the secretion of insulin (I), which decreases hepatic glucoseogenesis while simultaneously promoting tissue uptake of G and other blood components (DeFronzo, 1988). Glucose intolerance, characterized by abnormally increased G and sometimes excessively elevated I concentrations, can develop in intensively fed veal calves (Colvin et al., 1967; Webb et al., 1969; Kamula and Trenkle, 1978; Ronge and Blum, 1989a; Doppenberg and Palmquist, 1991; Palmquist et al., 1992). In contrast, in calves raised for beef production or breeding and fed differently than veal calves, G and I concentrations decrease with age (Leat, 1971; Fahey and Berger, 1988).

We hypothesized that owing to the nature of the diet given to veal calves, metabolites, in particular G, are present in the circulation in increased concentrations, resulting, with time, in I resistance and disturbed G homeostasis. Deviation from optimal metabolic functioning would result in reduced biochemical efficiency and, therefore, decreased feed conversion. Experiments were designed to test this hypothesis and to study the etiology of this problem.

Materials and Methods

Animals and Experimental Design

In Exp. 1 (Gr1), 20 Simmental × Red Holstein bull calves were used and housed at the Swiss Federal Research Station for Animal Production, Postieux-CH. On arrival at the station, at the age of 4 to 6 wk, calves were ear-tagged, tethered, and given a 4-d prophylactic antimicrobial treatment. Health of each calf was regularly monitored, and sick calves and those with fever (> 39.5°C) were treated appropriately.

Calves were fed a milk replacer (MR) twice daily according to a feeding plan designed to allow an ADG of 1.3 to 1.4 kg. Per kilogram of DM, the MR (UFA...
Animal Production as calves in Grl, but the calves were taken. Also, i.v. G-tolerance tests, insulin-injection tests, and MR and lactose feeding trials were hyperglycemic clamps, and oral G-tolerance tests. Followed immediately by constant infusion at a rate of the beginning and end of the growth period for the No. CLM-1396-90, Cambridge Isotope Laboratories, the morning feeding for hormone and metabolite fat. The MR contained minerals and vitamins but no antibiotics. Body weight was measured weekly, 2.5 to 3 h after feeding, and the rate of feeding for the entire group was adjusted accordingly. Individual feed refusals were recorded daily.

Weekly blood samples were taken 2.5 to 3 h after the morning feeding for hormone and metabolite determinations. Collection was from the jugular vein using lithium heparin and Na-EDTA Vacutainers® (Becton Dickinson Europe, Meylan/Cedex-F). Additionally, postprandial changes of hormones and metabolites were studied. Furthermore, animals were subjected to euglycemic-hyperinsulinemic clamps, hyperglycemic clamps, and oral G-tolerance tests.

Experiment 2 (Gr2) was conducted with 12 Simmental × Red Holstein bull calves, which were raised and treated at the Swiss Federal Research Station for Animal Production as calves in Gr1, but the calves were housed in two straw boxes and tethered only during the experiments. Blood samples were taken at the beginning and end of the growth period for the determination of hormone, metabolite, hemoglobin, and plasma Fe concentrations. In addition to the postprandial blood samples, which were collected 2.5 to 3 h after the morning feeding, preprandial samples were taken. Also, i.v. G-tolerance tests, insulin-injection tests, and MR and lactose feeding trials were performed.

Euglycemic-Hyperinsulinemic Glucose Clamps. Experiments were performed with 16 calves at a BW of 161 ± 3 kg. On the day before an experiment, catheters were inserted into both jugular veins. One experiment was performed per day. Calves were tethered with freedom to lie or stand. Experiments started after an overnight 14-h feed restriction. Blood samples were collected from the right catheter. The left catheter was used for infusions.

At a relative time of −150 min, administration of [6-13C]glucose ([6-13C]G; D-Glucose, 90% enrichment; No. CLM-1396-90, Cambridge Isotope Laboratories, Woburg, WA) was started. First, a bolus of 200 µg of [6-13C]G/kg of BW was injected over a period of 15 s, followed immediately by constant infusion at a rate of .1 mL/min of [6-13C]G (2 µg/kg BW-min) for 5.5 h (i.e., from −150 to 180 min).

Saline (.9%; 92 mL/min) was infused through a second catheter from −30 to 0 min, followed by infusion of bovine insulin in amounts of 2 mU/kg min from 0 to 5 min and of 1 mU/kg min at a rate of .6 mL/min from 5 to 240 min. Insulin (1 mg = 26.2 IU, Lot 64F 0349; Sigma Chemical, St. Louis, MO) was dissolved in saline (.9%) and BSA (.1%). The calf’s own plasma was added to the infusate at a rate of 4% to prevent the adsorption of I to glassware and infusion lines. Potassium chloride was infused (12.4 mg of K+/min) to prevent hypokalemia (Janes et al., 1985).

Glucose extracted from sugar beets (Hausmann Laboratories AG, St. Gallen-CH) was used for infusions because its [6-13C]G enrichment (1.083 atom % excess) was similar to basal plasma values. Glucose infusion commenced 4 min after the start of the I infusion at a rate of approximately 1 mg/(kg-min) using two peristaltic pumps, delivering a 10 or 25% G solution depending on needs. Glucose infusion rate (GIR) was continually adjusted according to plasma G measurements. The infusion rate was increased in a step-wise fashion in the stabilization phase (0 to 60 min), and after constant concentrations were reached at 60 to 240 min only small changes in GIR of < .1 mg/(kg-min) were necessary. Clamp experiments were accepted only if > 90% of steady-state G values did not deviate ± 10% from basal concentrations.

Blood samples were taken at −30, −20, −10, and 0 min: 1) to enable calculation of the basal plateau plasma [6-13C]G enrichment and 2) to obtain basal values of I and G. From 0 to 240 min, samples of .25 mL every 5 min and of 10 mL every 10 min were collected. Every 5 min, 25 mL of blood was centrifuged at 12,000 × g for 10 s and 10 µL of plasma was analyzed immediately for G using a Beckman glucose analyzer (Beckman Instruments Int. SA, Zürich-CH). The remainder of the blood collected every 10 min was added to tubes containing sodium-EDTA, cooled, then centrifuged at 3,000 × g for 20 min within 1 h. Plasma was then frozen for later analysis of I and [6-13C]G enrichment.

Rates of G turnover from −30 to 0 min and from 135 to 180 min were calculated as follows: rate of G appearance (Ra) = rate of G utilization (Ru) = (rate of tracer infusion:plasma 13C enrichment) – rate of tracer infusion (Wolfe, 1984). Tracer infusion was the amount of [6-13C]G infused, and plasma [6-13C]G enrichment was in atom % excess/100. Endogenous G production was calculated as follows: Ra – G infusion rate (GIR).

Results of the G clamp during the 1st h were excluded because this was considered the stabilization phase. Of the 20 experimental calves, four calves developed acute fever as a result of the inadvertent infusion of endotoxin (8 ng/mL)-contaminated [6-13C]G solution. These calves were excluded from this part of the trial.
approximately 140 kg BW were infused sequentially for 2 h with 0.1, 1, and 10 mM of I/(kg-min). Basal concentrations of G and I were 1.046 ± 22 mg/L and 23 ± 4 mU/L, respectively. Insulin infusions of .1, 1, and 10 mM/(kg-min) yielded average plasma I plateaus of 19 ± 3, 76 ± 2, and 820 ± 384 mU/L, respectively, and required that 7 ± 3, 4.2 ± .5, and 11.2 ± 2.9 mg of G/ (kg-min), respectively, be infused to maintain hyperglycemia. Glucose infusion rates were correlated with plasma I concentrations (y = 1.8 + .007x, where x = I and y = GIR; r = .87; P < .001).

Hyperglycemic Glucose Clamps. Clamps were performed in 19 calves 7 to 14 d after the euglycemic clamps at a BW of 181 ± 3 kg. Jugular veins were catheterized the previous afternoon. Two experiments were performed per day. After an overnight period without feed, the experiment with the first calf was started at approximately 0800 and with the second calf approximately 3 h later.

Three blood samples were taken at -20, -10, and 0 min to determine basal concentrations of I and G. Our aim was to raise the G concentration to 1,800 mg/L and to keep it clamped at that concentration for 3 h. The G increment was approximately 800 mg/L and was intermediate between the 1,250 mg/L increase used in humans (DeFronzo et al., 1979), 1,000 mg/L in rats (Terrettaz and Jeanrenaud, 1983), and 500 mg/L in cows (Sano et al., 1991) and corresponded to the highest G concentration measured postprandially in veal calves (Blum and Flückiger, 1988; Zimmerli and Blum, 1990; Ceppi, 1992). To achieve the hyperglycemic concentration, a 15-min priming G infusion was given. The priming GIR was adapted from that of DeFronzo et al. (1979); the initial rate was 35.2 mg of G/(kg BW-min) and decreased every minute to 4.7 mg of G/(kg BW-min) by 15 min. From 15 to 180 min, GIR was adjusted every 5 min to maintain the hyperglycemic concentration. Adjustment of GIR was made according to the G concentration, measured every 5 min. Glucose was purchased from Hausmann Laboratories and was infused in concentrations of 100, 250, or 500 g/L as required.

Blood was sampled every 5 min for 180 min and prepared immediately after collection for G measurement, as described for the euglycemic clamps. Plasma from every second sample was frozen for later determination of I. Results of the 1st h were excluded from calculations because this period was used to stabilize G concentration. In one of the 20 calves, we experienced complications with the catheters and could not complete the clamp.

Postprandial Changes of Metabolites and Hormones after Milk Replacer Intake. Experiments were performed with 22 calves in Gr1 and Gr2 at a BW of 148 ± 2 kg to monitor changes in G, triglycerides, cholesterol, I, growth hormone (GH), and cortisol after intake of MR. Basal probes were taken at -20, -10, and 0 min. The MR was then given at the rate and concentration determined by the normal feeding regimen. Postprandial blood samples were taken in Gr1 every 15 min up to 210 min and every 30 min up to 480 min, and in Gr2, every 30 min up to 240 min and every 60 min up to 480 min. Additionally, where possible, mid-stream urine samples were collected for analysis of G. Urine was stored frozen until it was analyzed.

Blood collections in Gr1 were through jugular vein catheters inserted 36 h before commencement of the trial. Blood samples were added to Na-EDTA-containing tubes. Blood samples in Gr2 were taken by jugular venipuncture using evacuated tubes. Samples were cooled and centrifuged within 1 h, and the plasma was stored at -20°C until it was analyzed.

Oral Glucose Tolerance Tests. Tests were performed with eight calves in Gr1 at a BW of 151 ± 3 kg. Catheters were used for blood collection. Basal samples were taken at -20, -10, and 0 min. Calves were then offered a 10% G solution calculated to provide 2 g of G/kg of BW. Total or partial refusal of the G solution resulted in the exclusion of three calves from the trial.

After consumption of the G “meal,” blood samples were collected every 15 min up to 210 min and every 30 min up to 480 min. Blood was added to Na-EDTA-containing tubes, cooled immediately, and centrifuged within 1 h. Plasma was stored at -20°C until it was analyzed for G and I. Urine was collected when possible; the samples were stored at -20°C until they were analyzed for G.

Oral Lactose-Glucose Tolerance Tests. The 12 calves in Gr2 were subjected to an oral lactose-glucose (L-G) intake test at a BW of 151 ± 3 kg. The dose of L-G was chosen to mimic the total sugar content of MR (lactose, 350 g/kg of MR; G, 20 g/kg of MR). From the feeding plan, the individual MR allowance (kilogram of DM/feeding) was noted, and then L-G in quantities equivalent to those in a normal MR feeding were prepared for each calf.

After basal samples were taken at -10 and 0 min, the L-G solution was given. Thereafter, blood samples were taken every 30 min up to 240 min and every 60 min up to 480 min. Collection was by venipuncture using evacuated tubes. Samples were cooled, centrifuged within 1 h, and the plasma stored frozen for later analysis.

Intravenous Glucose Tolerance Tests. Tests were performed at a BW of 131 ± 2 kg at two times relative to feeding (i.e., preprandial after an overnight period without feed, and postprandial 3 h after the intake of normal morning MR diet). Eight calves in Gr2 were used for the i.v. G tolerance test; each calf was tested both pre- and postprandially. On d 1, half of the calves were tested preprandially, the other half postprandially; on d 2, the measurement scheme was reversed.

A catheter was inserted into a jugular vein in each calf the evening before the i.v. G tolerance test began. Pre-glucose infusion samples were drawn at -10 and 0 min. At time 0 the G infusion was begun at a rate of .33 g/(kg·75 min) and lasted for 5 min as used previously (Bossett et al., 1985; Blum and Flückiger, 1988; Zimmerli and Blum, 1990; Grüter and Blum, 1991). Glucose was delivered by a peristaltic pump in
a concentration of 50%. Additional samples were drawn from the catheter at 5, 10, 15, 20, 30, 45, and 60 min. These were added to tubes containing Na-EDTA, cooled, and centrifuged within 1 h. The plasma was frozen for later analysis of G and I.

**Intravenous Insulin Injections.** Eight calves in Gr2 at a BW of 141 ± 3 kg were pre- and postprandially injected i.v. with I. The four calves that underwent an unfed i.v. test on d 1 were subjected to the same i.v. test on d 2, but 3 h after the intake of a normal diet, and vice versa for the second four calves. Pre-insulin-injection samples were drawn from catheters at −10 and 0 min. At time 0, bovine I was injected i.v. at a BW of 61238 kg (Blum and Flückiger, 1988; Zimmerli and Blum, 1990), and the catheter was then thoroughly flushed with a 9% NaCl solution. Blood samples were drawn at 1, 2, 5, 10, 15, 20, 30, 45, and 60 min and added to Na-EDTA-containing tubes. These were cooled and centrifuged, and the plasma was stored frozen until it was analyzed.

**Laboratory Analyses**

Hormones were measured with RIA as described previously (Blum et al., 1980; Blum, 1984; Blum and Flückiger, 1988; Ronge and Blum, 1989a; Zimmerli and Blum, 1990). Metabolites were measured enzymatically using kits from Bio Mérieux (Marcy l’Etoile-F: glucose, PAP 61273; cholesterol, PAP 61224; albumin, 61051; urea, 61981; triglycerides, PAP 61238) and from Wako Chemicals (Neuss-D: nonesterified fatty acids: NEFA C, 994-75409). Total protein was measured with the biuret method.

The determination of plasma [6-13C]G isotopic enrichment was carried out at the Institute of Physiology (University, Lausanne-CH). Approximately 2 mL of plasma was deproteinized with perchloric acid (final concentration of 3%), neutralized with 3.2 M potassium carbonate, and partially purified with sequential anion-exchange chromatography using resins AG 50 W-X8 and AG 1-X8 (BioRad, Richmond, CA). Partially purified samples were evaporated to dryness, resuspended in 120 μL of double-distilled water, and glucose was isolated with HPLC with a Dynamax NH2-80-725-C5 column (Rainin, Woburn, CA). The elution buffer was .0012 M H2SO4, the flow rate was .6 mL/min, and the temperature was 25°C. Plasma G eluted at approximately 6 min. Isolated G was again evaporated to dryness, resuspended with 20 μL of distilled H2O, and the 13C:12C ratio was determined with continuous flow-isotope ratio mass spectrometry (Roboprep CN, Tracermass 13C:15N, Europa Scientific, Crewe, U.K.). Plasma [6-13C]G isotope enrichment (atom percentage excess) was obtained by reading 13C:12C ratios against a [6-13C]G standard curve (0 to .1 atom % excess) prepared by diluting [6-13C]G 90% (Cambridge Isotope) with plasma, thus avoiding possible day to day fluctuations in isotope ratio measurement.

**Statistical Analyses**

Data are given as means ± SEM. Differences of values relative to basal values at a few selected times were tested for significance by paired comparisons using Tukey’s Studentized range test. For differences between tests within time (independent measures), an analysis of variance was made using the GLM procedure of SAS (1990). The model used was as follows: \( Y_{ij} = \mu + A_i + e_{ij} \), where \( Y_{ij} \) = one measurement of a certain variable, \( \mu \) = general mean, \( A_i \) = test, and \( e_{ij} \) = residual error.

**Results**

**Growth Performance**

In Exp. 1 (Gr1), calves entered the trial at the age of 5 wk and with an initial BW of 70 ± 1 kg. During 77 ± 1 d on the trial, calves gained 106 ± 1 kg. Daily weight gain increased throughout the trial, especially between 90 and 130 kg of BW, and then it tended to stabilize. The ADG was 1.38 ± .03 kg. Feed refusals were negligible (.037 ± .01 kg of MR/d). The gain:feed ratio was .66 ± .02 kg.

The intake of MR (kilograms of DM/day) increased \( (P < .05) \) from wk 3 to the end of the growth period; however, MR intake rate did not change with time \( (wk 1 \text{ and } 12: 119 \pm 3 \text{ and } 121 \pm 5 \text{ g of MR/kg BW-wk}, \text{respectively}) \). Similarly, intake rates of lactose, crude fat, and crude protein did not change during the growth period \( (wk 1: 42 \pm 1, 25 \pm 1, \text{ and } 26 \pm 1 \text{ g/kg BW-wk}, \text{respectively}; wk 12: 42 \pm 2, 26 \pm 1, \text{ and } 27 \pm 1 \text{ g/kg BW-wk}, \text{respectively}) \). However, the intake of MR, lactose, fat, and protein on a metabolic BW basis increased \( (P < .01) \) from wk 5 to the end of the growth period. Milk replacer, lactose, crude fat, and CP intakes in wk 1 were 348 ± 36, 122 ± 3, 73 ± 2, and 77 ± 2, respectively, and in wk 12, 443 ± 14, 155 ± 5, 93 ± 4, and 97 ± 4 g/kg BW-wk, respectively.

In Exp. 2 (Gr2), calves entered the trial at the age of 7 wk and an average BW of 82 ± 2 kg, and at the completion of experiments in wk 8, they had an average BW of 158 ± 4 kg. Daily weight gain increased throughout the trial, especially between 90 and 135 kg BW, and then tended to stabilize. The ADG was 1.42 ± .06 kg, the gain:feed ratio was .62 ± .04 kg of DM/kg BW. Feed refusals were negligible (.004 ± .01 kg of MR/d).

The MR intake increased weekly from wk 2 to the end of the growth period \( (P < .05) \); however, MR intake per unit of BW did not change \( (wk 1: 112 \pm 3 \text{ and wk 8: } 116 \pm 4 \text{ g/kg BW-wk}) \). Similarly, intakes of lactose, crude fat, and crude protein did not change during the growth period \( (wk 1: 39 \pm 1, 24 \pm 1, \text{ and } 25 \pm 1 \text{ g/kg BW-wk}, \text{respectively}, \text{and wk 8: } 41 \pm 2, 24 \pm 1, \text{ and } 26 \pm 1 \text{ g/kg BW-wk}, \text{respectively}) \). However, the intake of MR, lactose, crude fat, and CP on a metabolic BW basis increased \( (P < .05) \) from wk 4 to the end of the growth period. Milk replacer, lactose,
fat, and protein intakes in wk 1 were 344 ± 5, 120 ± 3, 72 ± 2, and 76 ± 2 g/(kg BW\(^{75}\).wk), respectively and in wk 8 they were 410 ± 6, 144 ± 4, 86 ± 2, and 90 ± 2 g/(kg BW\(^{75}\).wk), respectively.

**Metabolites and Hormones During the Growth Period**

In Exp. 1, G and I concentrations increased up to wk 3 and 4, respectively, and remained elevated (P < .05) thereafter (Figure 1). The concentration of IGF-I steadily increased up to wk 12 (P < .05). Growth hormone concentrations were variable and did not change significantly during the growth period.

In Exp. 2, metabolite and hormone concentrations were measured pre- and postprandially in wk 0 and 8 (Table 1).

Preprandial G concentrations were lower in wk 8 than in wk 0 (P < .05), whereas postprandial concentrations were not different. Preprandial triglyceride and I concentrations in wk 8 and 0 were similar, whereas postprandial concentrations in wk 8 were higher than in wk 0 (P < .05). Pre- and postprandial concentrations of cholesterol, total protein, albumin, urea, and IGF-I were higher in wk 8 than in wk 0 (P < .05). Pre- and postprandial NEFA, GH, and cortisol concentrations were similar in wk 0 and wk 8.

Regardless of weeks on trial, preprandial G and I concentrations were lower, whereas those of NEFA and GH were higher than postprandial concentrations (P < .05). Pre- and postprandial total protein, albumin, triglyceride, cholesterol, urea, cortisol, and IGF-I concentrations were similar.

**Euglycemic Hyperinsulinemic Glucose Clamps**

As shown in Figure 2, I increased rapidly on infusion and then remained steadily elevated (P < .05). Insulin concentrations between 60 and 240 min were tested with linear regression analysis against time for stability; the slopes were not different from zero. Metabolic clearance rate (MCR) of I was 16.1 ± 1.5 mL/(min-kg) and ranged from 9.6 to 22.9 mL/(min-kg).

Glucose concentration from 60 to 240 min was not different from basal G concentration. Linear regression analysis of the G concentration against time during the clamp revealed that the slope was not different from zero. From 60 to 240 min, 7% of the values were > 10%, but < 15%, different from basal values.

The GIR increased with time; GIR in the 1st h of the plateau were less (P < .05) than in the 2nd and 3rd h (4.1, 4.9, and 5.2 mg/[kg-min], respectively). The average GIR (from 60 to 240 min) required to maintain G at euglycemic levels was 4.7 mg/(kg-min).

The rate of appearance of G (Ra) was 2.7 mg/(kg-min) between -30 and 0 min and increased (P < .05) to 5.3 mg/(kg-min) between 135 and 180 min, respectively. The rate of endogenous G output between -30 and 0 min was .27 mg/(kg-min) and decreased (P < .05) to .5 mg/(kg-min) between 135 and 180 min, respectively.

The average atom percentage excess of [6\(^{13}\)C] was .067 and .042 between -30 to 0 min and from 60 and 180 min, respectively, and decreased (P < .05) to .048, .038, and .014 at 60, 180, and 240 min, respectively.

![Figure 1. Glucose, insulin, insulin-like growth factor I (IGF-I), and growth hormone concentrations measured weekly at 2.5 to 3 h after intake of the morning ration in 20 veal calves in Exp. 1.](image)
Figure 2. Plasma glucose concentration, glucose infusion rate, plasma insulin concentration, and atom percentage excess of [6-13C]glucose in 16 veal calves in Exp. 1 during infusions of [6-13C] glucose (200 µg/[kg·min]), insulin [1 mU/[kg·min]], and glucose (varying rate to maintain euglycemia in euglycemic-hyperinsulinemic glucose clamps).

**Hyperglycemic Glucose Clamps**

As shown in Figure 3, G increased to stable concentrations after 20 min of G infusion. From 60 to 180 min, 8% of the measured G values fell outside the 10% allowable range. The stability of the new G plateau was evaluated with regression analysis; the slope did not differ significantly from zero.

The average GIR required to maintain steady-state hyperglycemia was 6.8 mg/(kg·min). Less \( P < .05 \) G was required from 60 to 120 min than from 120 to 180 min (5.4 and 8.2 mg/[kg·min], respectively).

Insulin concentrations increased rapidly \( P < .05 \) after the commencement of G infusion and continued to increase from 60 to 180 min at a rate of .71 mU/(L·min) \( r = .71 \). The average I concentration during the 2-h clamps was 98 mU/L.

**Changes in Insulin, Growth Hormone, Cortisol, Glucose, Triglycerides, and Cholesterol after Milk Replacer Intake**

As shown in Figure 4, I increased \( P < .01 \) to peak concentration at 210 min, then decreased, but at 480 min I was greater \( P < .05 \) than basal concentrations. Concentrations of GH were highly variable. Except for a transient decrease \( P < .01 \) at 90 min, postprandial GH concentrations were similar to basal concentrations. Cortisol did not change significantly from basal concentrations until 480 min, when a slight increase \( P < .01 \) was recorded.

Glucose increased \( P < .05 \) after intake of MR, peaked at 150 min, and decreased to nearly basal concentrations at 480 min. Triglyceride concentrations increased transiently at 30 min, decreased \( P < .01 \) below basal concentrations from 90 to 300 min, and at 480 min concentrations were greater \( P < .05 \) than basal concentrations. Cholesterol concentrations did not change postprandially.

**Glucose and Insulin after Intake of Milk Replacer or Lactose Plus Glucose**

As shown in Figure 5a, G increase \( P < .05 \) in calves fed MR and L-G to peak concentrations at 120 and 60 min, respectively. Concentrations of G after L-G intake from 30 to 120 min were greater \( P < .05 \), but from 240 to 480 min were less \( P < .05 \) than after MR intake.

Insulin increased \( P < .05 \) to peak concentrations at 210 min and 150 min after intake of MR and L-G, respectively. The increase was more marked after
intake of MR than of L-G (P < .05) from 30 min up to 480 min.

Glucose and Insulin after Intake of Milk Replacer or Glucose

As shown in Figure 5b, G increased (P < .01) in calves fed MR and G to peak concentrations at 195 and 75 min, respectively; thereafter, G decreased (P < .05) below basal concentrations from 240 to 480 min in calves fed G, whereas in calves fed MR, G decreased to basal concentrations at 360 min. Concentrations of G from 30 to 120 min were greater (P < .05) and from 180 to 480 min were less (P < .05) in calves fed G than in those fed MR.

Insulin increased (P < .01) in response to MR and G intake to peak concentrations at 210 and 135 min, respectively; then I decreased to basal concentrations at 240 min in calves fed G and at 480 min in calves fed

Table 1. Metabolite and hormone concentrations in 12 veal calves after an overnight period without feed and after intake of feed during the growth period

<table>
<thead>
<tr>
<th>Item</th>
<th>Week of trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/L</td>
<td>0</td>
</tr>
<tr>
<td>Preprandial</td>
<td>974 ± 33</td>
</tr>
<tr>
<td>Postprandial</td>
<td>1,420 ± 237c</td>
</tr>
<tr>
<td>Triglycerides, mg/L</td>
<td>184 ± 26</td>
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<tr>
<td>Preprandial</td>
<td>96 ± 9abc</td>
</tr>
<tr>
<td>Cholesterol, mg/L</td>
<td>991 ± 128a</td>
</tr>
<tr>
<td>Postprandial</td>
<td>975 ± 128a</td>
</tr>
<tr>
<td>Nonesterified fatty acids, μEq/L</td>
<td></td>
</tr>
<tr>
<td>Preprandial</td>
<td>227 ± 24</td>
</tr>
<tr>
<td>Postprandial</td>
<td>106 ± 8c</td>
</tr>
<tr>
<td>Total protein, g/L</td>
<td>62 ± 1a</td>
</tr>
<tr>
<td>Preprandial</td>
<td>61 ± 1a</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>37 ± .4a</td>
</tr>
<tr>
<td>Preprandial</td>
<td>37 ± .4a</td>
</tr>
<tr>
<td>Urea, mg/L</td>
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</tr>
<tr>
<td>Preprandial</td>
<td>176 ± 15a</td>
</tr>
<tr>
<td>Insulin, μL/L</td>
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<tr>
<td>Preprandial</td>
<td>132 ± 25abc</td>
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<tr>
<td>Postprandial</td>
<td>226 ± 26a</td>
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<tr>
<td>Postprandial</td>
<td>217 ± 23a</td>
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<td>Growth hormone, μg/L</td>
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<tr>
<td>Preprandial</td>
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<td>Cortisol, μgL</td>
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<tr>
<td>Preprandial</td>
<td>2.1 ± .8</td>
</tr>
</tbody>
</table>

Values with different superscript letters differed between weeks (P < .05). Postprandial values differed from preprandial values (P < .05).

MR. Concentrations of I from 45 to 480 min in calves fed MR were greater than those in calves fed G (P < .05).

Intravenous Glucose Tolerance Tests

As shown in Figure 6, G concentrations increased (P < .01) up to the end of the 5-min G infusions, thereafter they decreased (P < .01) to concentrations above basal after 60 min. Glucose concentrations before and after i.v. G infusions were higher (P < .05) postprandially than preprandially. Half-lives of G after preprandial G infusions were shorter (P < .05) than after postprandial G infusions (44 ± 5 and 78 ± 7 min, respectively).

During pre- and postprandial G infusions, I increased (P < .05) to peak values at 15 and 60 min, respectively. Insulin concentrations throughout the experiment were higher (P < .05) when G was infused postprandially than when it was infused preprandially.
Figure 4. Plasma concentrations on insulin, growth hormone, cortisol, glucose, triglycerides, and cholesterol in 22 calves from Exp 1 and 2 after intake of milk replacer.
Figure 5. Plasma concentrations of glucose and insulin after 5a) intake of a normal ration of milk replacer or lactose (3.18 g/kg BW) plus glucose (.18 g/kg BW) by 12 veal calves in Exp. 2 and 5b) a normal ration of milk replacer or glucose (2 g/kg BW) by eight veal calves in Exp. 1. Milk replacer = ■; lactose/glucose = △; glucose = O.

**Intravenous Insulin Injections**

As shown in Figure 7, G decreased \( (P < .01) \) by 510 mg/L at 45 min after preprandial I injection. After postprandial I injection, G decreased \( (P < .05) \) by 144 mg/L at 20 min, and at 45 and 60 min, G was similar to the preinjection concentrations. At all times, preprandial G concentrations were lower \( (P < .01) \) than postprandial G concentrations. Half-lives of G preprandially were shorter \( (P < .05) \) than those postprandially \( (25 \pm 4 \text{ and } 112 \pm 10 \text{ min, respectively}) \).

After pre- and postprandial I injections, I concentrations increased \( (P < .01) \) and then returned after 60 min to preinjection concentrations. Preinjection I concentrations in postprandial tests were higher \( (P < .01) \) than in preprandial experiments. At 1 min after injection, I concentration in unfed calves was higher \( (P < .01) \) than in fed animals; however, by 2 min, the concentrations were again similar. Thereafter, postprandial I concentrations remained higher \( (P < .01) \) than preprandial I concentrations.

**Urinary Glucose**

As shown in Figure 8, before and after intake of MR, L-G, G, or after infusion of G, when plasma G concentration was < 1,500 mg/L, G was detectable in urine in amounts of .12 g/L. After MR intake and when plasma G was > 1,500 mg/L, average urinary G was 4.7 g/L (range .02 to 23.7 g/L). After intake of L-G, G, or after infusion of G, and when plasma G was > 1,500 mg/L, urinary G concentrations were 12.2, 6.9, and 4.3 g/L, respectively (range for all urine G at plasma concentration > 1,500 mg/L was .1 to 23.6 g/L).

**Discussion**

**Growth Performance**

On a kilogram of BW basis, DM intake, and therefore, intake of all feed components, was constant throughout the growth period, as planned. In our intensively fed calves, ADG, feed intake, and efficiency of feed utilization were high, and feed refusals were minimal. An additional factor contributing to the high growth rates was the relatively good health status maintained in both experimental groups. There were few incidences of diarrhea and pneumonia. Concentrations of hemoglobin \( (84 \pm 3 \text{ g/L}) \) and plasma Fe \( (6 \pm \text{ g/L}) \) were normal.
3 μmol/L) toward the end of the growth period were within the expected ranges for veal calves.

**Insulin and Glucose in the Unfed State**

Concentrations of G in blood samples taken after feed was withheld overnight were in the normal range for veal calves. In calves raised for beef or breeding (i.e., not solely milk fed) and in lambs in the unfed state (Jarrett et al., 1964; Leat, 1971; Fahey and Berger, 1988), unfed G concentrations decrease with age. Because G concentrations when feed was withheld in our trial remained stable, development of hyperglycemia relative to beef calves was detected. In a veal calf trial by Webb et al. (1969), G concentrations increased with age (i.e., an absolute hyperglycemia was seen). Concentrations of I in our unfed calves did not change with age, which is in contrast to gradually increasing I concentrations in the unfed calves of Breier et al. (1988).

Euglycemic-hyperinsulinemic clamp studies revealed that our veal calves responded in an expected manner and that there were typical G and I interactions. The hyperinsulinemic steady-state achieved

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**Figure 6.** Plasma glucose and insulin concentrations after infusion of .33 mg of glucose/(kg BW .75 .min) for 5 min in unfed calves and in calves 3 h after intake of a normal milk replacer ration in i.v. glucose tolerance tests. Experiments were performed with eight calves from Exp. 2. Preprandial = ○; postprandial = ■.

**Figure 7.** Plasma glucose and insulin concentrations after injection of .1 U of insulin/kg BW in unfed calves and in calves 3 h after intake of a normal milk replacer ration in i.v. insulin injection tests. Experiments were performed with eight calves from Exp. 2. Preprandial = ○; postprandial = ■.
suppressed hepatic G production only by 82%, thereby demonstrating hepatic I resistance. Terrettaz and Jeanrenaud (1983) reported a more severe hepatic resistance to hyperinsulinemia during clamp studies in obese rats. During the euglycemic clamp, the rate of infusion of G needed to maintain euglycemic is a measure of the rate of G utilization and is an index of the sensitivity of peripheral tissues to I (Weekes et al., 1983). A comparison of euglycemic clamps in our calves and in adult ruminants (Weekes et al., 1983; Janes et al. 1985; Metcalfe and Weekes, 1990; Sano et al., 1991) revealed a higher I sensitivity in calves. However, G utilization rates in calves were similar to those in adult sheep fed corn (Janes et al., 1985), but they were only 50% of the rates for human beings (DeFronzo et al., 1979; Thiébaut et al., 1983) and ≤ 30% of the rates for rats (Terrettaz and Jeanrenaud, 1983; Farrell et al., 1988). Therefore, intensively fed veal calves (i.e., pseudo-nonruminants) are apparently more sensitive to I than adult ruminants are, but they are not as sensitive as true nonruminants. Euglycemic clamps described by various researchers cannot exactly be compared because they have been performed under somewhat different experimental conditions.

In hyperglycemic clamps, G metabolism was studied in relation to endogenous I. A greater I response to a doubling of plasma G was seen compared with hyperglycemic clamps under similar conditions in human beings (DeFronzo et al., 1979). However, the G infusion rate required to maintain hyperglycemia under the influence of this endogenous hyperinsulinemia was lower in calves than in human beings. From this we concluded that there is no basic deficiency in the response of I to G. Instead, as seen in the euglycemic clamps, there is a relative reduction in tissue sensitivity to I compared with human beings (i.e., although absolute concentrations of I were higher, the G utilization rate, as indicated by the G infusion rate, was lower than in human beings).

Additionally, the clearance rate of G in the unfed veal calves after a 5-min i.v. G load was slower than that in dogs (Church, 1980; Kaneko, 1980) or in 1-wk-old calves (Grütter and Blum, 1991), and it was similar to that in grazing steers and adult cattle (Kaneko, 1980; Bossart et al., 1985). These results support our findings of a relatively decreased I sensitivity in veal calves in the unfed state toward the end of the growth period.

**Insulin and Glucose in the Postprandial State**

In our first experiment, in blood samples taken 2.5 to 3 h after the intake of the normal diet, concentrations of G and I increased continuously with age. Postprandial I responses were often in the abnormal range (i.e., up to 1,400 mU/L). Development of postprandial hyperglycemia during the growth period was also reported by Fisher (1976), whereas Doppenberg and Palmquist (1991) recorded hyperglycemia and hyperinsulinemia. In our second experiment, postprandial G concentrations did not increase with age, but postprandial I concentration increased.

During Exp. 1 and 2, individual variations in postprandial G and I concentrations were considerable, indicating differences in the completeness of closure of the esophageal groove (i.e., in nutrient

![Plasma Glucose](https://example.com/plasma-glucose.png)

**Figure 8.** Urinary glucose concentrations in midstream samples collected after 8a) oral intake of milk replacer and 8b) intake of glucose or lactose-glucose or after i.v. infusion of glucose, plotted against the plasma glucose concentration of the corresponding blood sample. Milk replacer = ○; glucose (oral) = ●; lactose/glucose (oral) = ▽; glucose (i.v.) = □.
absorption) and/or genetic differences of G and I responses to feed intake.

Postprandially, the relative I resistance already present in the unfed state was exaggerated. Thus, 3 h after intake of a normal diet, the G clearance rate after a 5-min i.v. G infusion was reduced compared with G clearance after G infusion before feeding. A further indication of I resistance postprandially was demonstrated by I injection experiments, in which 3 h after intake of a normal diet plasma G concentration was barely decreased. The same dose administered to unfed calves caused a typical and rapid decrease in plasma G, as was seen in younger calves (Blum and Flückiger, 1988; Zimmerli and Blum, 1990; Grütter and Blum, 1991).

**Etiology of the Development of Insulin Resistance**

One or several factors could contribute to the development of hyperglycemia, hyperinsulinemia, and I resistance in veal calves. Feeding intensity and effects of individual nutrients may have been responsible. In accordance, the unfed concentrations of I, which had been increasing during the time calves were receiving milk, rapidly and markedly decreased upon weaning (Breier et al., 1988). Additionally, impaired pancreatic β-cell function and peripheral I resistance often coexist to disrupt the feedback pathways. A change in plasma G concentration causes I release. Therefore, when a relative β-cell insensitivity to G exists, hyperglycemia would be enhanced. Any simultaneous reduction in peripheral tissue sensitivity to I will increase plasma G and possibly amino acid concentrations, thereby stimulating further I secretion to overcome the resistance. The inverse correlation between circulating I concentrations and I sensitivity due to down-regulation of I receptors has been reported (Porte and Halter, 1981; Etherton, 1982; DeFronzo, 1988).

Excessive intake of lactose, which is converted during digestion into G and galactose, could have caused hyperglycemia and hyperinsulinemia. Guilhemet and Toullec (1983) reported exaggerated G and I concentrations when large amounts of lactose were fed. When lactose intake was halved, the G and I concentrations increased less and lasted a shorter time. In our trial, lactose intake on a metabolic BW basis increased during the growth period, possibly leading to an overload of lactose that could no longer be handled properly. Administration of lactose and G in amounts as present in the MR, or of G alone, caused a more exaggerated hyperglycemia than when calves were fed MR, but I responses were smaller. These data indicate that lactose and G components of MR are partially responsible for postprandial hyperinsulinemia. However, hyperglycemia itself can induce I resistance by reducing the sensitivity of G transport proteins (Yki-Järvinen, 1992). The relative unfed hyperglycemia and the absolute postprandial hyperglycemia that we and others recorded could have had an effect on the development of I resistance.

Absorption of amino acids after excessive protein intake may have caused enhanced I secretion. The relative potency of various amino acids to stimulate I secretion was investigated in sheep (Kuhara et al., 1989). Corresponding studies in prerninant calves and cattle seem to be lacking. Protein intake on a metabolic BW basis increased continuously during the growth period. The increase of plasma protein and albumin concentration during the growth period in our calves was probably the consequence of a decrease of body water. However, the increase of plasma urea concentrations during the growth period may have been due to increased protein intake and amino acid absorption, which facilitated the use of amino acids for energy production and urea formation. Increasing protein intake during the growth period, therefore, could be partially responsible for the relative I resistance.

Enhanced fat intake may also contribute to I resistance (Harris and Kor, 1992). Fat intake in calves fed MR is usually high, but fat content of MR fed to our calves was relatively low. Nevertheless, in our experiments, fat intake on a metabolic BW basis increased continuously during the growth period and an overload with fat was possible. In support, concentration of cholesterol, which increases when fat intake is increased, was higher at the end than at the beginning of the growth period. Indeed, Ferrannini et al. (1991) reported reduced G tolerance in conjunction with hypercholesterolemia in humans. Thus, increasing preprandial cholesterol concentrations in the calves may have influenced the relative unfed I resistance. However, after intake of feed, there was no change in concentration of cholesterol. Preprandial triglyceride concentrations did not change during the growth period. Postprandial triglyceride concentrations were initially lower, then, toward the end of the growth period, they were higher than the preprandial concentrations. Elevated NEFA concentrations have also been implicated in the development of G intolerance in human beings (Jarrett, 1974). The concentrations of NEFA during the growth period did not increase, but they typically decreased postprandially, as reported previously (Blum and Flückiger, 1988). Doppenberg and Palmquist (1991) concluded that serum lipid changes were not important in the transient development of I resistance.

The combined effects of several nutrients, if provided in high amounts as in our veal calves toward the end of the growth period, a high energy intake, and a direct or indirect stimulation of I secretion by gastrointestinal hormones (Porte and Bagdade, 1970), possibly contributed to hyperglycemia and hyperinsulinemia. In our study, the increase of IGF-I concentrations during the growth period, as observed previously (Blum and Flückiger, 1988; Ronge and

Hormones such as GH and cortisol could cause hyperglycemia, decreased G clearance, hyperinsulinemia, and insulin resistance if secreted in high amounts (Rizza et al., 1982; Sherwin et al., 1983; Brockman and Laarveld, 1986). However, pre- and postprandial determinations of GH and cortisol did not reveal abnormal concentrations or behavior. Growth hormone concentrations tended to decrease and cortisol concentrations tended to increase, but not significantly after MR intake.

**Glucosuria**

To our knowledge, this is the first report that G is excreted in high amounts in the urine of veal calves, if the plasma G concentration after MR intake was > 1.4 to 1.6 g/L. The renal threshold for G excretion was in the same range as described by Scholz and Hoppe (1987) but less than the threshold documented by Wijayasinghe et al. (1984). Glucosuria was of the same magnitude as after oral G or lactose and G intake, and after i.v. G administration. Although urinary G loss was probably only a transient phenomenon, it indicated the loss of energy. Not knowing the total daily quantity of urinary G loss after intake of MR, one cannot calculate the extent to which feed and energy utilization is decreased. Despite urinary G losses, ADG and the gain:feed ratio were as expected.

**Implications**

Although abnormalities in insulin disturb energy metabolism, the consequences of insulin resistance for growth performance in veal calves are presently not known. A normal insulin status, characterized by normal plasma insulin concentrations and normal sensitivity to insulin, are generally considered essential for anabolism and growth. Development of insulin resistance would be expected to reduce feed efficiency and growth performance, as in the case of hypoinsulinemia. Prevention of the development of insulin resistance would be expected to improve feed utilization and growth performance.

**Literature Cited**


