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# Effects of Abomasal Infusion of Linseed Oil on Responses to Glucose and Insulin in Holstein Cows

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## ABSTRACT

The objective was to study the effects of abomasal infusion of linseed oil, a source rich in n-3 C18:3, on whole-body response to insulin (experiment 1) and on insulin antilipolytic effects during feed restriction (experiment 2). In experiment 1, eight nonlactating, nongestating cows were assigned to a crossover design, fed to meet maintenance requirements, and infused abomasally with either linseed oil (LIN) or tallow (TAL) at a rate of 0.54 g/kg of body weight per d for 5.5 d. Infusions were performed every 8 h during the first 3 d of each period and every 4 h thereafter. Intravenous glucose tolerance tests (IVGTT) were performed on d 5 of each period, followed by i.v. insulin challenges (IC) 12 h later. In experiment 2, six nonlactating, nongestating cows were assigned to a replicated 3 × 3 Latin square design. The experimental protocol included a water (WTR) treatment and feeding was suspended on d 3, leading to 50 and 62 h of feed restriction before IVGTT and IC, respectively. Clearance of glucose during IVGTT and IC was not affected by treatments in either experiment. However, LIN had an insulin sensitizing effect in experiment 1, because a lower insulin concentration led to the same clearance of glucose as TAL. In experiment 1, plasma nonesterified fatty acid (NEFA) concentration was low, reflecting a postprandial state, but NEFA was greater for LIN than TAL during IVGTT (108 vs. 88 ± 4 μEq/L) and IC (133 vs. 83 ± 9 μEq/L). In experiment 2, insulin concentrations during IVGTT did not differ across treatments. Basal plasma NEFA concentration before IVGTT tended to be greater for LIN than for TAL (612 vs. 508 μEq/L). Plasma NEFA clearance rate during IVGTT was greater for LIN than for TAL (2.8 vs. 2.5%/min), leading to a shorter time to reach half NEFA concentration (25 vs. 29 min) and greater absolute value of NEFA response area under the curve [AUC; –64,150 vs. –46,402 (μEq/L) × 180 min]. Data suggest that LIN enhanced the antilipolytic effects of insulin. Yet, other factors could have been involved because plasma NEFA concentration before IVGTT was 104 μEq/L greater for LIN than TAL for unknown reasons.
**Key words:** alpha-linolenic acid, linseed oil, glucose tolerance, insulin challenge

## INTRODUCTION

Previous research has shown that specific fatty acids (**FA**) have the ability to modulate bovine energy metabolism in vitro (Mashek et al., 2002; Mashek and Grummer, 2003) and in vivo (Mashek et al., 2005). Intravenous infusion of an emulsion derived from linseed oil to feed-restricted nonlactating Holstein cows decreased plasma NEFA and BHBA concentrations, and tended to reduce liver triacylglycerol (**TG**) accumulation, compared with tallow. Because there were no differences in hepatic FA oxidation, the authors speculated that modifications in adipose tissue metabolism by linolenic acid originating from linseed oil may have enhanced sensitivity to insulin and increased insulin-mediated inhibition of lipolysis (Mashek et al., 2005).

Insulin resistance develops in the periparturient period and increases the availability of glucose, amino acids, and FA to the mammary gland (Bell, 1995; Bell and Bauman, 1997). Elevated plasma NEFA concentration is common around parturition and is associated with decreased feed intake and energy-related metabolic disorders (Grummer, 1993). We have previously shown that induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in Holstein cows, which was likely due to increased supply of NEFA derived from TG hydrolysis (Pires et al., 2007b). In a follow-up study, lowering plasma NEFA concentration using nicotinic acid as an antilipolytic agent enhanced the response to insulin in feed-restricted cows, thus implicating plasma NEFA as a causal factor of insulin resistance (Pires et al., 2007a). Because the ability of insulin to suppress FA release from adipocytes may be impaired in insulin-resistant states (Frayn, 2002) and in humans with genetic disposition to type 2 diabetes (Lewis et al., 2002), we speculate that elevated plasma NEFA concentrations may

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lead to the onset of insulin resistance in adipose tissue of dairy cows, and that exaggerated insulin resistance may further enhance mobilization of FA.

The sensitivity of adipose tissue to the antilipolytic effects of insulin is greater than the sensitivity of whole-body glucose utilization in sheep (Petterson et al., 1993, 1994). Therefore, we expect that strategies to increase insulin sensitivity during the periperturient period would first act on adipose tissue, leading to decreased lipolytic rates and lower NEFA concentrations, while maintaining muscle refractory to insulin-stimulated glucose uptake. The use of insulin sensitizers could decrease lipolytic rates, NEFA-induced insulin resistance in adipose tissue, and plasma NEFA concentration, therefore preventing the onset of energy-related metabolic disorders in periparturient dairy cows.

Dietary FA profile can modulate the whole-body response to insulin. Specifically, long-chain n-3 polyunsaturated FA (derived from fish oil) may prevent development of insulin resistance in humans and rodents (Clarke, 2000; Storlien et al., 2000; Delarue et al., 2004). In agreement with findings from nonruminants, abomasal infusion of fish oil enhanced insulin-induced utilization of amino acid and glucose in the bovine, and increased key intermediaries in the intracellular insulin signaling cascade in muscle (Gingras et al., 2007). The few studies focusing on the effects of n-3 C18:3 on insulin resistance have also shown enhanced response to insulin in rodents receiving fat sources rich in this FA (Storlien et al., 1991; Mustad et al., 2006). We hypothesized that changing the fatty acid profile in blood serum of Holstein cows by enriching its content with n-3 C18:3 would enhance the response to insulin (experiment 1), potentially increasing the antilipolytic effects of insulin and reducing plasma NEFA concentration during periods of negative energy balance (experiment 2).

## MATERIALS AND METHODS

### *Animals and Treatments*

The Animal Care and Use Committee for the College of Agriculture and Life Sciences at the University of Wisconsin-Madison approved all animal procedures. In both experiments, cows were weighed each period on 2 consecutive days before initiation of treatments, 5 to 6 h after morning feed was offered. Body weights were 671 ± 39 and 765 ± 36 kg (mean ± SD) in experiments 1 and 2, respectively. Body weight was used to determine doses of feed, treatments, glucose for i.v. glucose tolerance test (**IVGTT**), and insulin for i.v. insulin challenge (**IC**). Fatty acid composition of tallow (HRR Enterprises Inc., LaPorte, IN) and linseed oil (Virtus Nutrition LLC, Lewisburg, OH) is presented in Table 1 and it is similar to those previously reported (NRC, 2001).

**Table 1.** Fat supplement fatty acid composition (% of total FA) of linseed oil and tallow used in experiments 1 and 2

| | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| Fatty acid | Linseed oil | Tallow | Linseed oil | Tallow |
| C14:0 | 0.0 | 3.3 | 0.0 | 3.4 |
| C16:0 | 5.1 | 25.2 | 5.0 | 25.3 |
| C16:1 | 0.1 | 4.3 | 0.0 | 4.7 |
| C17:0 | 0.0 | 1.2 | 0.0 | 1.4 |
| C18:0 | 4.0 | 16.8 | 3.9 | 13.4 |
| C18:1 | 22.1 | 42.9 | 20.9 | 45.2 |
| C18:2 | 18.0 | 3.1 | 17.0 | 2.6 |
| C18:3 | 50.3 | 0.2 | 50.8 | 0.2 |
| Other | 0.4 | 3.0 | 2.3 | 3.7 |

***Experiment 1.*** Eight nonlactating, nongestating, ruminally cannulated Holstein cows (3.3 ± 0.5 BCS; mean ± SD) were randomly assigned to a sequence of treatments in a crossover design with 2 periods. Treatments were abomasal infusion of linseed oil (**LIN**) or tallow (**TAL**) for 5.5 d at a rate of 0.54 g/d per kg of BW. This rate is equivalent to a 735-kg cow ingesting a diet containing 2.7% TG at 2% of BW (Mashek et al., 2005). Total amount of fat administered in this experiment averaged 369 ± 45 g/d per cow (mean ± SD). Treatments were infused using 60-mL catheter tip syringes via abomasal lines (Gressley et al., 2006) every 8 h during the first 3 d of each period, at 0700, 1500, and 2300 h. Tallow was liquefied by heating to approximately 39°C to allow infusion. The frequency of administration was increased to every 4 h on d 4 and 5 (at 0700, 1100, 1500, 1900, 2300, and 0300 h) to promote a more continuous supply of fatty acids. On d 5 of each period, IVGTT and IC were performed at 0900 and 2100 h, respectively (Figure 1). Tallow infusion was used to maintain isocaloric conditions across treatments to assess the effects of modulation of FA profile on blood metabolites, insulin, and response to challenges. For logistical reasons, animals were divided in 2 groups, each starting the experimental protocol 3 d apart. Four weeks were allowed between experimental periods to prevent potential carryover effects.

***Experiment 2.*** Six nonlactating, nongestating, ruminally cannulated Holstein cows (3.9 ± 0.5 BCS; mean ± SD) were assigned to treatments in a replicated 3 × 3 Latin square design. Cows in each square initiated the experimental protocol 2 d apart. Treatments were abomasal infusions of water (**WTR**), LIN, or TAL for 5.5 d, at the same rate as in experiment 1 (0.54 g/d per kg of BW). Total amount of treatments was 409 ± 22 g/d per cow (mean ± SD). The experimental protocol was similar to experiment 1, except that WTR treatment was included and feeding was suspended on d 3,

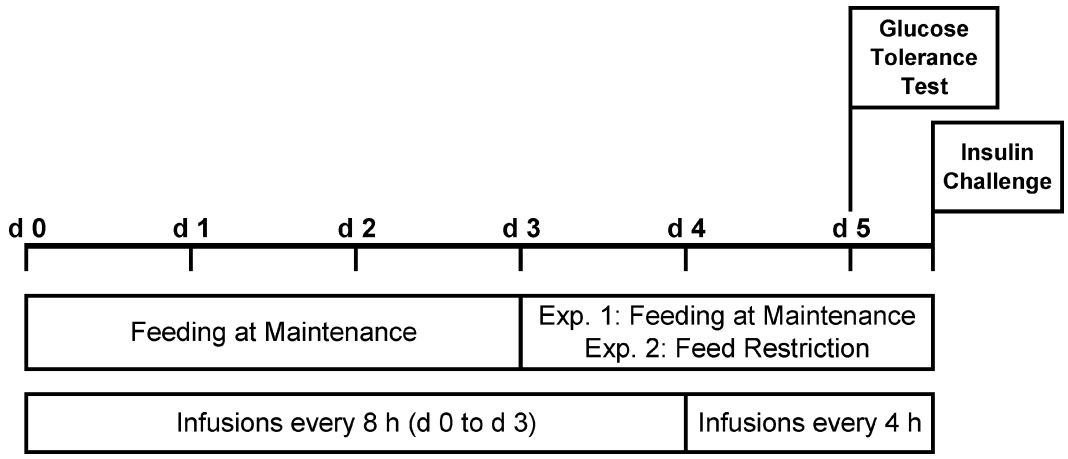


**Figure 1.** Schematic representation of experiments. Abomasal infusion of treatments were performed every 8 h during the first 3 d of each period, at 0700, 1500, and 2300 h, and every 4 h on d 4 and 5 (at 0700, 1100, 1500, 1900, 2300, and 0300 h). Cows were fed at a rate to meet individual maintenance requirements (NRC, 2001) in experiment 1. Feeding was suspended at 0700 h on d 3 of experiment 2 to promote mobilization of body reserves. Blood samples were collected daily before 0700 h infusions and feeding. Intravenous glucose tolerance tests were performed at 0900 h and i.v. insulin challenges at 2100 h on d 5.

leading to 50 and 62 h of feed restriction before IVGTT and IC, respectively (Figure 1). Feed restriction was used to stimulate body reserve mobilization, increase plasma NEFA concentration, and allow the assessment of treatment effects on clearance of plasma NEFA after IVGTT and IC. Three weeks were allowed between experimental periods.

### *Diets and Feeding*

Cows were fed at a rate to meet individual maintenance requirements (NRC, 2001) and were allowed free access to water and trace mineralized salt block. Daily feed was divided into equal allotments that were offered immediately after each infusion. Between experimental periods, the amount of feed offered was based on average BW of all cows.

Feeding was suspended on d 3 of each period in experiment 2. Cows were offered a feed dose corresponding to one-third of daily maintenance requirements at 0700 h and forage was withdrawn until the end of the period. During feed restriction, cows were supplemented with vitamins and minerals to meet requirements (NRC, 2001) using wheat middlings as carrier (total of 1 kg/d). After termination of the sampling period, cows were offered straw ad libitum for 2 d and a TMR was gradually increased to 150% of maintenance requirements for 5 d to allow replenishment of body reserves. Cows were fed at maintenance thereafter.

Diet was composed of 41% alfalfa silage, 41% corn silage, 6% wheat straw, and 12% wheat middlings as carrier for vitamins and minerals (DM basis) in both experiments. Diet in experiment 1 had 40% NDF, 30% ADF, 14.1% CP, and 1.46 Mcal of $NE_L$/kg of DM. Diet in experiment 2 had 37.1% NDF, 26.3% ADF, 13.8% CP, and 1.49 Mcal of $NE_L$/kg of DM, based on near-infrared reflectance spectroscopy from alfalfa silage and corn silage (UW Soil and Forage Feed Analysis Laboratory, Marshfield, WI) and tabular values (NRC, 2001) for the remaining diet components.

### *Glucose Tolerance, Insulin Challenge, and Blood Sampling*

Catheters (experiment 1: 14-gauge × 14-cm, Abbocath-TAL catheter; Abbott Laboratories, Abbott Park, IL; experiment 2: polyurethane, 14-gauge × 13-cm; MILA International Inc., Erlanger, KY) were fitted into a jugular vein of each cow and attached to an extension set (Baxter International Inc., Deerfield, IL; 86 cm, 3.9 mL volume) on the day before IVGTT and IC. Patency was maintained by flushing catheters with 8 mL of heparinized saline (100 IU/mL) every 8 h or with diluted heparinized saline (10 IU/mL) during frequent sampling. Cows were given penicillin G (10,000 IU/d per kg of BW; G.C. Hanford Mfg. Co., Syracuse, NY) following the insertion of catheters as a prophylactic procedure. Blood samples were collected once daily immediately before the 0700 h infusion. Blood was drawn by venipuncture from the coccygeal vein or artery using Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and from catheters on d 5 using disposable 20-mL syringes.

Intravenous glucose tolerance tests were performed by administering 0.25 g/kg of BW of glucose i.v. (dextrose 50%, wt/vol; Phoenix Scientific Inc., St. Joseph,

MO) followed by 50 mL of sterile saline to flush catheters and prevent contamination of subsequent samples. Glucose infusion was done at 0900 h on d 5 of each period, which corresponded to 2 h after abomasal infusion of treatments in both experiments. Blood samples were collected at –15, –5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, and 180 min relative to administration of glucose. An additional sample was collected at min 7 in experiment 2. Glucose was infused over 4.2 ± 1.1 and 4.6 ± 0.8 min (mean ± SD) in experiments 1 and 2, respectively. Treatments were suspended during IVGTT sampling in both experiments. The 1000 h abomasal infusion was postponed until the end of sampling for IVGTT at 1100 h, and the regular schedule was followed thereafter. The same adjustment was used for feeding in experiment 1. Insulin challenges were performed 12 h after initiation of IVGTT by administering i.v. 0.1 IU of insulin/kg of BW (100 IU/mL, human insulin rDNA origin; experiment 1: Humulin R, Eli Lilly Co., Indianapolis, IN; experiment 2: Novolin R; Novo Nordisk, Clayton, NC) followed by 50 mL of sterile saline. Blood samples were collected at –15, –5, 5, 10, 15, 20, 25, and 30 min relative to administration of insulin in experiments 1 and 2. Sampling continued at 45, 60, 75, 90, and 120 min in experiment 2. Insulin was infused over 1.0 ± 0.2 min (mean ± SD) in both experiments. An IC was not performed in one cow receiving WTR in the last period of experiment 2 because this animal became released from the stanchion and had access to feed before scheduled IC.

### Estrous Synchronization

Cows were synchronized each period with an intravaginal progesterone releasing device (CIDR containing 1.38 g of progesterone; Eazi-Breed, Hamilton, NZ) for 7 d, which was removed on d 4 of treatment. Prostaglandin $F_{2\alpha}$ (Lutalyse, 25 mg; Pfizer Animal Health, Kalamazoo, MI) was administered, i.m., 36 and 24 h before CIDR withdrawal to induce luteolysis and decrease progesterone concentration. To minimize endogenous estradiol production, all follicles with diameter greater than 5 mm were aspirated on d 4, employing an ultrasound-guided transvaginal approach, using a 17-gauge × 55 cm needle and a 7.5-MHz convex array transducer (Aloka SSD-900V, Aloka Co., Wallingford, CT). This protocol produces low concentrations of progesterone and estradiol across treatments and experimental periods (Pires et al., 2007b) and prevents potential interference of reproductive hormones with IVGTT and IC.

### Blood Plasma and Serum Analysis

Tubes for collection of plasma (6 mL; containing 12 mg of potassium oxalate and 15 mg of sodium fluoride as a glycolytic inhibitor) were kept on ice until centrifugation at 920 × *g* at 4°C for 20 min in both experiments. For collection of serum, tubes (15 mL; additive-free) were allowed to clot at room temperature and centrifuged at 2,050 × *g* at 20°C for 30 min. Samples collected during IC of experiment 2 were kept overnight at –4°C before separation of serum.

Blood plasma was analyzed for glucose and NEFA, and serum was analyzed for insulin as described previously (Pires et al., 2007b). Intra- and interassay coefficients of variation were 2.8 and 2.5% for glucose, 4.7 and 5.4% for NEFA, and 4.3 and 8.2% for insulin, respectively. Fatty acid profile was analyzed from serum collected 15 min before IVGTT. Total lipids were extracted (Folch et al., 1957), followed by FA transmethylation (Palmquist and Jenkins, 2003) and separation by gas chromatography.

### Calculations and Statistical Analysis

Statistical analysis was performed separately for each experiment using SAS version 9.1 (SAS Institute Inc., Cary, NC). The NLIN procedure was used to fit exponential curves for glucose and NEFA concentration during the first 60 min of IVGTT, and for glucose, insulin, and NEFA concentration during the first 30 min of IC. Glucose clearance (**CR**; %/min), time to reach half maximal concentration ($\mathbf{T_{1/2}}$; min), time to reach basal concentration ($\mathbf{T_{basal}}$; min), area under the curve during the first 60 ($\mathbf{AUC_{60}}$) and 180 ($\mathbf{AUC_{180}}$) min of IVGTT, and area under the curve during first 30 ($\mathbf{AUC_{30}}$) and 120 min ($\mathbf{AUC_{120}}$) of IC were calculated as previously described (Pires et al., 2007b). Basal glucose, insulin, and NEFA concentrations were calculated by averaging values from samples taken 15 and 5 min before IVGTT or IC.

The measurements from IVGTT and IC were analyzed using the MIXED procedure of SAS. For experiment 1, the statistical model included the fixed effects of treatment and sequence, and the random effects of period and cow within sequence. Body condition score was used as covariate in all the models for experiment 1, and was removed whenever its significance level was greater than 0.25. Group was included in the models for all variables but it was not significant ($P > 0.25$) and was removed. For experiment 2, the statistical model included treatment as fixed effect, and the random variables of square, cow within square, and period within square. In both experiments, the concentrations of metabolites and insulin over time were studied by adding time and treatment by time interaction to the models, using repeated measures in time and Kenward-Rogers adjustment for calculation of denominator degrees of freedom. First-order autoregressive covariance struc-

**Table 2.** Serum fatty acid profile (% of total fatty acids) in experiment 1

| | Treatment | | | *P*-value | |
|---|---|---|---|---|---|
| Fatty acid | Linseed oil | Tallow | SEM | Sequence | Treatment |
| C14:0 | 0.96 | 1.33 | 0.11 | 0.46 | 0.02 |
| C16:0 | 8.86 | 14.96 | 0.40 | 0.65 | <0.001 |
| C16:1 | 0.13 | 1.18 | 0.09 | 0.20 | <0.001 |
| C17:0 | 2.27 | 2.11 | 0.20 | 0.40 | 0.50 |
| C18:0 | 16.80 | 17.44 | 0.62 | 0.41 | 0.49 |
| C18:1 | 5.23 | 15.98 | 0.47 | 0.58 | <0.001 |
| C18:2 | 37.59 | 32.68 | 1.72 | 0.78 | <0.01 |
| C18:3 | 18.94 | 4.48 | 0.47 | 0.25 | <0.001 |
| Other | 9.23 | 10.83 | 1.97 | 0.92 | 0.38 |

ture was used for data collected before IVGTT on d 5; spatial power covariance structure was used for data collected during IVGTT and IC to allow for unequal sampling intervals. Heterogeneous variance across treatments was used whenever it provided better fitting according to Schwarz's Bayesian criterion. Values reported are least squares means and SEM, unless otherwise stated. Logarithmic transformation was used whenever needed to comply with the assumptions of normality and homoscedasticity of residuals. When transformation was needed, least squares means and SEM were estimated from untransformed values, whereas *P*-values reflect statistical analysis of transformed data.

The significance level for treatment effects on IVGTT and IC measurements (CR, T½, $T_{basal}$, and AUC) was predefined at $P \leq 0.10$ and a trend toward significance at $0.10 < P < 0.15$. In experiment 2, contrasts were WTR vs. fat, and LIN vs. TAL. To reduce risk of type I error, the significance level was decreased to $P \leq 0.05$ for all other measurements and trends toward significance were considered at $0.05 < P \leq 0.10$. In experiment 1, treatment differences at individual time points were assessed using the SLICE option when treatment by time interaction was significant.

## RESULTS

### *Effect of Treatments on Serum FA Profile*

There were significant treatment effects on serum FA profile on d 5 of experiments. In experiment 1, infusion of LIN significantly increased the percentage of C18:2 and C18:3, and decreased the percentage of C14:0, C16:0, C16:1, and C18:1 (Table 2). In experiment 2, LIN increased the percentage of C18:3 and decreased C16:0 and C18:1 (LIN vs. TAL; Table 3). Infusion of fat altered percentage of C16:0 and C18:3 (WTR vs. fat; Table 3), reflecting the effect of LIN on FA profile. There was no sequence effect in experiment 1 or carryover effects from previous treatments during experiment 2.

### *Experiment 1*

***Glucose and Insulin in Daily Blood Samples Taken Before 0700 h Infusions.*** There was a time effect ($P = 0.01$) on plasma glucose concentration. Treatment and treatment by time effects were not significant. Glucose concentration was 65.1, 63.4, 64.4, 66.9, 65.5, and 64.6 ± 1.3 mg/dL from d 0 to d 5. Insulin concentration was determined in samples collected on d 0 (19.6 μIU/mL) and d 5 (23.4 ± 3.2 μIU/mL). There was a trend ($P = 0.10$) for a time effect on serum insulin.

**Table 3.** Serum fatty acid profile (% of total fatty acids) in experiment 2

| | Treatments | | | | *P*-value | |
|---|---|---|---|---|---|---|
| Fatty acid | Water | Linseed oil | Tallow | SEM | Water vs. Fat | Linseed vs. Tallow |
| C14:0 | 1.24 | 1.04 | 1.20 | 0.16 | 0.48 | 0.40 |
| C16:0 | 14.74 | 11.09 | 14.58 | 0.78 | 0.03 | 0.004 |
| C16:1 | 1.25 | 1.38 | 1.05 | 0.54 | 0.96 | 0.67 |
| C17:0 | 1.99 | 2.04 | 1.81 | 0.29 | 0.80 | 0.46 |
| C18:0 | 16.53 | 14.31 | 15.98 | 1.61 | 0.48 | 0.47 |
| C18:1 | 15.04 | 9.81 | 18.77 | 0.94 | 0.45 | <0.001 |
| C18:2 | 26.27 | 30.59 | 28.66 | 1.72 | 0.12 | 0.42 |
| C18:3 | 4.60 | 16.99 | 4.99 | 0.77 | <0.001 | <0.001 |
| Other | 18.36 | 12.76 | 12.98 | 2.53 | 0.10 | 0.95 |

**Table 4.** Experiment 1: Effect of abomasal infusion of linseed oil or tallow on glucose clearance measurements and serum insulin response after i.v. glucose tolerance test (IVGTT; 0.25 g/kg of BW of glucose i.v.)[1]

| | Treatments | | | |
|---|---|---|---|---|
| Item[2] | Linseed | Tallow | SEM | *P*-value |
| Glucose | | | | |
| Basal (mg/dL) | 65.6 | 62.8 | 1.9 | 0.01 |
| Peak (mg/dL) | 258 | 267 | 6.3 | 0.32 |
| $CR_{60}$ (%/min) | 2.46 | 2.27 | 0.16 | 0.42 |
| $T_{1/2}$ (min) | 28.9 | 30.9 | 2.2 | 0.64 |
| $T_{basal}$ (min) | 59.3 | 61.5 | 4.2 | 0.45 |
| $AUC_{60}$ [(mg/dL) × 60 min] | 3,778 | 3,897 | 277 | 0.45 |
| $AUC_{180}$ [(mg/dL) × 180 min] | 4,044 | 4,013 | 394 | 0.89 |
| Insulin | | | | |
| Basal (μIU/mL) | 28.7 | 28.1 | 3.1 | 0.81 |
| Peak (μIU/mL) | 272 | 369 | 49.2 | <0.001 |
| $AUC_{60}$ [(μIU/mL) × 60 min] | 8,663 | 11,729 | 1,655 | 0.002 |
| $AUC_{180}$ [(μIU/mL) × 180 min] | 9,810 | 12,589 | 1,863 | 0.04 |

[1]Cows were fed at a rate to meet maintenance requirements and treatments (0.54 g/d per kg of BW) were administered for 122 h before IVGTT.

[2]Basal = average concentration at 15 and 5 min before IVGTT; Peak = maximal concentration (estimated with PROC NLIN for glucose); $CR_{60}$ = clearance rate during first 60 min of IVGTT; $T_{1/2}$ = time to reach ½ concentration; $T_{basal}$ = time to reach basal concentration; $AUC_{60}$ and $AUC_{180}$ = area under the curve during first 60 and 180 min of IVGTT.

Glucose concentrations in samples collected before 0700 h infusions on d 5 were 65.6 and 63.6 ± 1.6 mg/dL ($P$ = 0.24), and insulin concentrations were 24.2 and 22.6 ± 3.6 μIU/mL ($P$ = 0.61) for LIN and TAL, respectively.

***Response to Glucose Tolerance Test.*** Basal glucose concentration (average concentration 5 and 15 min before glucose infusion) was greater for LIN than for TAL ($P$ < 0.01; Table 4) despite the absence of treatment differences on samples collected 2 h earlier, before 0700 h infusions. There were no treatment or treatment by time effects on glucose concentration during IVGTT (Figure 2, panel A; Table 4). Insulin response to glucose infusion was greater for TAL than LIN, leading to significantly greater insulin peak, $AUC_{60}$, and $AUC_{180}$ (Table 4). However, significance was not achieved ($P$ = 0.14) when insulin concentration was analyzed as repeated measures (Figure 2, panel B). This was probably due to differences in variance across time points, which were difficult to estimate due to limited degrees of freedom within each time. Treatment differences were significant ($P$ = 0.05) when statistical analysis was performed using insulin concentration during the first 60 min of IVGTT only, which is in agreement with the greater insulin peak observed with TAL (Table 4).

Basal plasma NEFA (average concentration at 5 and 15 min before glucose infusion) was 113 and 106 ± 6 μEq/L for LIN and TAL, respectively, and did not differ ($P$ = 0.41). Plasma NEFA concentration was greater for LIN than TAL (108 vs. 88 ± 4 μEq/L; $P$ < 0.001) after glucose infusion for IVGTT; there were no sequence ($P$ = 0.94) or treatment by time effects ($P$ = 0.68).

***Response to Insulin Challenge.*** There was a time effect ($P$ < 0.001) but no treatment ($P$ > 0.40) or treatment by time interaction ($P$ > 0.12) on glucose and insulin concentration after IC. Accordingly, there were no treatment effects on glucose and insulin clearance measurements after IC (Table 5). Basal NEFA concentration immediately before IC was greater ($P$ < 0.001) for LIN than for TAL (151 vs. 89 ± 14 μEq/L). There were treatment ($P$ < 0.001) and time ($P$ < 0.01) effects on plasma NEFA after administration of insulin for IC, but no treatment by time interaction ($P$ = 0.81). Plasma NEFA was 133 and 83 μEq/L for LIN and TAL during IC sampling, respectively.

### *Experiment 2*

***Response to Glucose Tolerance Test.*** There was a time effect ($P$ < 0.001), but no effect of fat supplementation (WTR vs. fat) or fat source (LIN vs. TAL) on plasma NEFA, glucose, and serum insulin concentrations in daily blood samples collected before 0700 h infusions (Table 6).

Basal glucose concentration (average concentration 5 and 15 min before glucose infusion) tended to be greater ($P$ = 0.09) for LIN than for TAL (61.0 vs. 58.1 ± 1.3 mg/dL; Table 7), despite the absence of differences in samples collected before 0700 h infusions (Table 6). As in experiment 1, there were no treatment or treatment by time effects on glucose concentration during IVGTT (Figure 3, panel A; Table 7).

There were no treatment effects on basal insulin concentration before IVGTT. Fat source (LIN vs. TAL) did not affect insulin response to glucose infusion for IVGTT (Figure 3, panel B; Table 7), despite numerically lower $AUC_{180}$ when cows received LIN. Water treat-

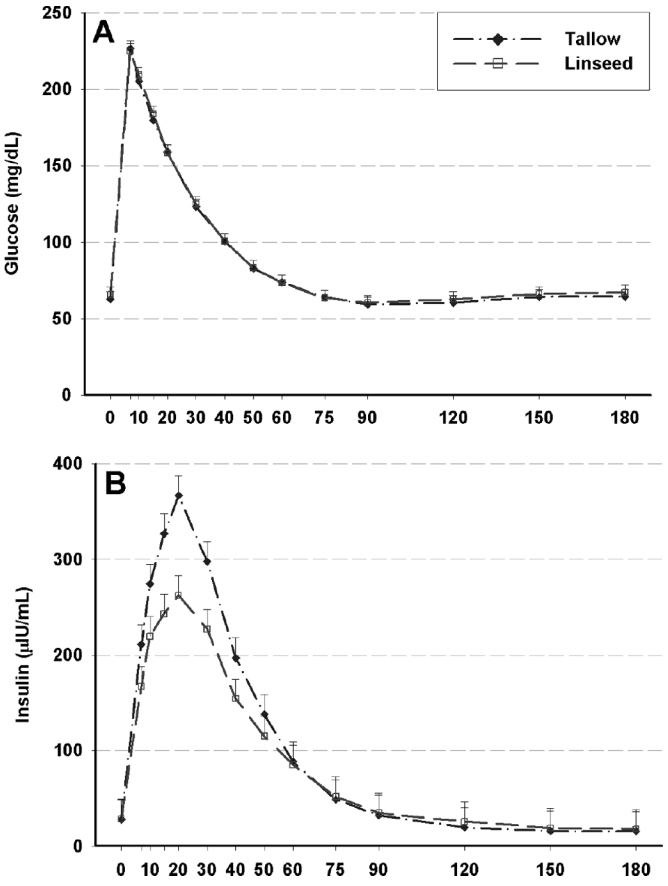


**Figure 2.** Experiment 1 (fed state): A) Effect of abomasal infusion of linseed oil or tallow (0.54 g/d per kg of BW) on plasma glucose concentration after i.v. glucose tolerance test (IVGTT; 0.25 g/kg of BW of glucose i.v.). Cows were fed at a rate to meet maintenance requirements and treatments were administered for 122 h before IVGTT. Fixed effects in the statistical model: sequence ($P = 0.13$), treatment ($P = 0.45$), time ($P < 0.001$), and treatment × time ($P = 0.94$). B) Effect of treatments on serum insulin concentration after IVGTT. Fixed effects in the statistical model: sequence ($P = 0.92$), treatment ($P = 0.14$), time ($P < 0.001$), treatment × time ($P = 0.24$), and BCS covariate ($P = 0.09$). *P*-values reflect statistical analysis with log-transformed data.

ment led to greater ($P = 0.04$) insulin $AUC_{180}$ when compared with infusion of both fat sources (WTR vs. fat; Table 7).

Basal NEFA concentration tended to be greater ($P = 0.09$) for LIN than for TAL (612 vs. 508 ± 42 μEq/L) despite no differences on plasma NEFA before infusion of treatments at 0700 h. During IVGTT, NEFA $CR_{60}$ and absolute value of $AUC_{180}$ were greater and $T_{1/2}$ was shorter for LIN than TAL, respectively (Figure 4; Table 7).

***Response to Insulin Challenge.*** There was a time effect ($P < 0.001$), but no treatment ($P = 0.81$) or treatment by time ($P = 0.99$) effects on glucose concentration after insulin injection for IC (WTR vs. fat infusion: $P = 0.80$; LIN vs. TAL: $P = 0.56$), leading to no differences in glucose measurements during IC (Table 8). As for plasma glucose, there was a time effect ($P < 0.001$), but no treatment ($P = 0.31$) or treatment by time ($P = 0.98$) effects on serum insulin concentration during IC (WTR vs. fat infusion: $P = 0.17$; LIN vs. TAL: $P = 0.51$). Yet, insulin clearance was greater ($P < 0.10$) and $T_{1/2}$ ($P < 0.10$) and $T_{basal}$ ($P < 0.05$) was shorter for TAL than for LIN (Table 8). Fat infusion led to lower insulin $AUC_{30}$ and $AUC_{120}$ than WTR (Table 8). Basal NEFA concentration did not differ statistically across treatments, despite being 100 μEq/L numerically greater for LIN than TAL (Table 8). There were no treatment or treatment by time effects on plasma NEFA (Figure 5, panel A) or on NEFA change (Figure 5, panel B) after IC.

## DISCUSSION

Basal metabolite concentrations immediately before IVGTT did not always agree with concentrations collected approximately 2 h earlier, before 0700 h infusions on d 5. In experiment 1, basal glucose concentration immediately before IVGTT was significantly greater for LIN than for TAL (Table 4). In experiment 2, basal glucose and basal NEFA before IVGTT tended to be greater for LIN than TAL (Table 7). However, there were no treatment differences in samples collected 2 h before IVGTT in either experiment. The reason for these temporal differences is not known.

Infusion of LIN had an insulin-sensitizing effect in experiment 1 because lower insulin concentration led to the same clearance of glucose as TAL (Figure 2, Table 4). The magnitude of glucose-stimulated insulin secretion during IVGTT was decreased when cows were feed-restricted for 50 h in experiment 2 (Figure 3, panel B; Table 7), and differences between LIN and TAL on insulin response to IVGTT did not reach statistical significance. Smaller responses of insulin to IVGTT have been found during periods of energy deficit, such as in early lactation compared with the dry period (Hayirli et al., 2001; Holtenius et al., 2003). Feed restriction could also have changed the metabolic fate of C18:3 toward oxidation in experiment 2, thereby limiting its physiologic effects.

A WTR treatment was included in experiment 2 to assess whether fat supplementation could cause insulin resistance in feed-restricted nonlactating Holstein cows. There is limited evidence linking dietary fat with development of whole-body insulin resistance in lactating dairy cows (Palmquist and Moser, 1981; Chilliard and Ottou, 1995; Gaynor et al., 1996). For instance, clearance of glucose during IVGTT was impaired and there was a tendency for a lower antilipolytic effect of insulin during IC when lactating cows were infused

**Table 5.** Experiment 1: Effect of abomasal infusion of linseed oil or tallow on glucose and insulin clearance measurements after insulin challenge (IC; 0.10 IU/kg of BW of insulin i.v.)[1]

| | Treatment | | | |
|---|---|---|---|---|
| Item[2] | Linseed | Tallow | SEM | *P*-value |
| Glucose | | | | |
| Basal (mg/dL) | 68.7 | 66.2 | 1.9 | 0.13 |
| $CR_{30}$ (%/min) | 2.03 | 1.85 | 0.18 | 0.30 |
| $T_{1/2}$ (min) | 36.0 | 38.3 | 3.1 | 0.55 |
| $AUC_{30}$ [(mg/dL) × 30 min] | –487 | –426 | 35 | 0.23 |
| Insulin | | | | |
| Basal (μIU/mL) | 26.2 | 26.9 | 2.8 | 0.70 |
| Peak (μIU/mL) | 1,298.4 | 1,570.2 | 146.0 | 0.24 |
| $CR_{30}$ (%/min) | 3.86 | 4.39 | 0.54 | 0.27 |
| $T_{1/2}$ (min) | 18.6 | 16.9 | 1.8 | 0.33 |
| $T_{basal}$ (min) | 103.4 | 97.2 | 10.1 | 0.24 |
| $AUC_{30}$ [(μIU/mL) × 30 min] | 11,342 | 12,262 | 1,011 | 0.11 |

[1]Cows were fed at a rate to meet maintenance requirements and treatments (0.54 g/d per kg of BW) were administered for 134 h before IC.

[2]Basal = average concentration at 15 and 5 min before IC; $CR_{30}$ = clearance rate during first 30 min of IC; $T_{1/2}$ = time to reach ½ concentration; $T_{basal}$ = time to reach basal concentration; $AUC_{30}$ = area under the curve during first 30 min of IC; Peak = maximal insulin concentration (estimated with PROC NLIN).

with 640 g of TG/d into the duodenum, which was possibly due to establishment of insulin resistance in muscle and adipose tissue (Chilliard and Ottou, 1995). Lactating cows receiving abomasal infusions of 750 g of TG/d had greater insulin concentration after IVGTT but achieved the same glucose concentration as uninfused cows (Gaynor et al., 1996). We have previously shown that induction of hyperlipidemia by intravenous infusion of tallow-derived emulsion at a rate of 794 g of TG over 11 h causes insulin resistance in nonlactating, nongestating Holstein cows (Pires et al., 2007b). However, we found no evidence of fat-induced insulin resistance in experiment 2 (WTR vs. fat), probably because lower amounts of fat (409 ± 22 g/d; mean ± SD) were used.

In experiment 1, cows receiving LIN required lower concentrations of insulin than TAL to achieve the same clearance of glucose during IVGTT (Figure 2; Table 4). These IVGTT results resemble those from studies with nonruminants involving supplementation with fish oil, a source rich in n-3 long-chain FA. For instance, partial substitution of dietary fat for fish oil (6 g/d) led to lower insulin concentrations and unchanged clearance of glucose during oral glucose tolerance tests in healthy hu-

**Table 6.** Experiment 2: Effect of abomasal infusion of water, linseed oil, or tallow, and feed restriction on blood metabolites and insulin in Holstein cows

| | | | Treatment | | | | *P*-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Metabolite | Day of infusion | Hour of feed restriction | Water | Linseed | Tallow | SEM | Day | Treatment × Day | Water vs. Fat | Linseed vs. Tallow |
| NEFA (μEq/L) | 0 | | 106 | 126 | 123 | 17 | <0.001 | 0.70 | 0.16 | 0.87 |
| | 1 | | 130 | 137 | 153 | | | | | |
| | 2 | | 114 | 133 | 130 | | | | | |
| | 3 | 0 | 106 | 135 | 123 | | | | | |
| | 4 | 24 | 322 | 258 | 316 | | | | | |
| | 5 | 48 | 520 | 496 | 473 | | | | | |
| Glucose (mg/dL) | 0 | | 61.3 | 61.7 | 60.2 | 0.7 | <0.01 | 0.24 | 0.82 | 0.37 |
| | 1 | | 62.2 | 62.0 | 61.1 | | | | | |
| | 2 | | 59.3 | 62.2 | 62.1 | | | | | |
| | 3 | 0 | 64.8 | 63.1 | 60.9 | | | | | |
| | 4 | 24 | 63.1 | 63.6 | 64.1 | | | | | |
| | 5 | 48 | 59.2 | 61.0 | 59.9 | | | | | |
| Insulin (μIU/mL) | 0 | | 18.7 | 18.6 | 16.6 | 1.5 | <0.001 | 0.51 | 0.20 | 0.66 |
| | 1 | | 20.0 | 20.7 | 20.6 | | | | | |
| | 2 | | 20.1 | 19.9 | 17.0 | | | | | |
| | 3 | 0 | 25.3 | 23.6 | 20.6 | | | | | |
| | 4 | 24 | 21.9 | 16.4 | 16.3 | | | | | |
| | 5 | 48 | 13.9 | 12.4 | 14.2 | | | | | |

**Table 7.** Experiment 2: Effect of abomasal infusion of water, linseed oil, or tallow on glucose and NEFA clearance measurements and serum insulin response after i.v. glucose tolerance test (IVGTT; 0.25 g/kg of BW of glucose i.v.)[1]

| | Treatment | | | | *P*-value | |
|---|---|---|---|---|---|---|
| Item[2] | Water | Linseed | Tallow | SEM | Water vs. Fat | Linseed vs. Tallow |
| Glucose | | | | | | |
| Basal (mg/dL) | 57.5 | 61.0 | 58.1 | 1.3 | 0.14 | 0.09 |
| Peak (mg/dL) | 245.7 | 252.5 | 245.8 | 9.9 | 0.63 | 0.42 |
| $CR_{60}$ (%/min) | 1.46 | 1.51 | 1.49 | 0.13 | 0.66 | 0.82 |
| $T_{1/2}$ (min) | 48.5 | 48.6 | 47.7 | 4.9 | 0.90 | 0.79 |
| $T_{basal}$ (min) | 101.1 | 97.4 | 98.6 | 6.9 | 0.51 | 0.83 |
| $AUC_{60}$ [(mg/dL) × 60 min] | 5,608 | 5,392 | 5,521 | 162 | 0.39 | 0.52 |
| $AUC_{180}$ [(mg/dL) × 60 min] | 7,816 | 7,986 | 7,759 | 492 | 0.89 | 0.64 |
| Insulin | | | | | | |
| Basal (μIU/mL) | 12.3 | 11.8 | 12.5 | 1.3 | 0.82 | 0.51 |
| Peak (μIU/mL) | 232.5 | 209.6 | 233.0 | 42.0 | 0.46 | 0.20 |
| $AUC_{60}$ [(μIU/mL) × 60 min] | 8,800 | 8,178 | 8,705 | 1,642 | 0.39 | 0.25 |
| $AUC_{180}$ [(μIU/mL) × 180 min] | 13,028 | 11,601 | 12,455 | 2,105 | 0.04 | 0.19 |
| NEFA | | | | | | |
| Basal (μEq/L) | 548 | 612 | 508 | 44 | 0.81 | 0.09 |
| $CR_{60}$ (%/min) | 2.64 | 2.76 | 2.45 | 0.13 | 0.76 | 0.06 |
| $T_{1/2}$ (min) | 26.8 | 25.3 | 28.6 | 1.3 | 0.90 | 0.04 |
| $AUC_{60}$ [(μEq/L) × 60 min] | –12,876 | –14,280 | –12,286 | 1,377 | 0.81 | 0.32 |
| $AUC_{180}$ [(μEq/L) × 180 min] | –52,187 | –64,150 | –46,402 | 5,871 | 0.65 | 0.04 |

[1]Treatments (0.54 g/d per kg of BW) were administered for 122 h and cows were feed restricted for 50 h before IVGTT.

[2]Basal = average concentration at 15 and 5 min before IVGTT; Peak = maximal concentration (estimated with PROC NLIN for glucose); $CR_{60}$ = clearance rate during first 60 min of IVGTT; $T_{1/2}$ = time to reach ½ concentration; $T_{basal}$ = time to reach basal concentration; $AUC_{60}$ and $AUC_{180}$ = area under the curve during first 60 and 180 min of IVGTT.

mans (Delarue et al., 1996) and after induction of insulin resistance with dexamethasone (Delarue et al., 2006). Accordingly, poultry fed diets containing fish oil presented lower insulin concentration and unaltered clearance of glucose during IVGTT when compared with animals fed tallow or safflower oil (Newman et al., 2005).

Research focusing specifically on the effects of n-3 C18:3 on insulin resistance is scarce but there is evidence supporting an insulin-sensitizing effect in rodents. Partial replacement of dietary fat (mixture of tallow and safflower oil) with linseed oil (to provide 6% of dietary FA as n-3 C18:3) prevented the development of insulin resistance by a high-fat diet in rats, and increased muscle n-3 long-chain FA (C22:5 and C22:6) content (Storlien et al., 1991). Partial substitution of safflower oil (a source rich in C18:2) with linseed oil did not prevent insulin resistance, and did not change muscle content of n-3 long-chain FA (C22:5 and C22:6; Storlien et al., 1991). In contrast, partial replacement of safflower oil with fish oil prevented insulin resistance and increased muscle long-chain n-3 FA (C22:5 and C22:6; Storlien et al., 1991). The authors suggested a role for muscle long-chain n-3 FA in insulin resistance, and proposed that the ability for linseed oil to increase the responses to insulin may depend on C18:2 content in diet due to competition between C18:2 and C18:3 for elongation and desaturation enzymes.

Treatments did not affect glucose clearance after IC in either experiment 1 or 2 (Tables 5 and 8). Insulin concentration during IC increases above physiological concentrations and rapidly declines. The resulting clearance of plasma glucose can be interpreted as a maximal response to insulin. However, the increase in insulin concentration might not have been sustained long enough to allow the detection of potential treatment differences. In contrast to our results, feeding insulin-resistant ob/ob mice with a diet rich in n-3 C18:3 enhanced the response to intraperitoneal IC compared with a diet rich in C18:1. Feeding a diet rich in n-3 C18:3 led to increased n-3 FA (C18:3, C20:5, and C22:5) content in muscle phospholipids (Mustad et al., 2006). Again, the authors have implicated possible specific changes in membrane FA composition as a causal factor of increased response to insulin with C18:3 (Mustad et al., 2006).

The greater insulin concentration observed during IVGTT for cows receiving TAL in experiment 1 (Figure 2, panel B; Table 4) could have resulted from increased insulin secretion, impaired insulin clearance, or a combination of both. Because clearance of exogenous insulin after IC did not differ across treatments (Table 5), the greater insulin response to IVGTT observed with TAL compared with LIN in experiment 1 was probably due to modifications in insulin secretion. In contrast, clearance of insulin after IC was greater for TAL than

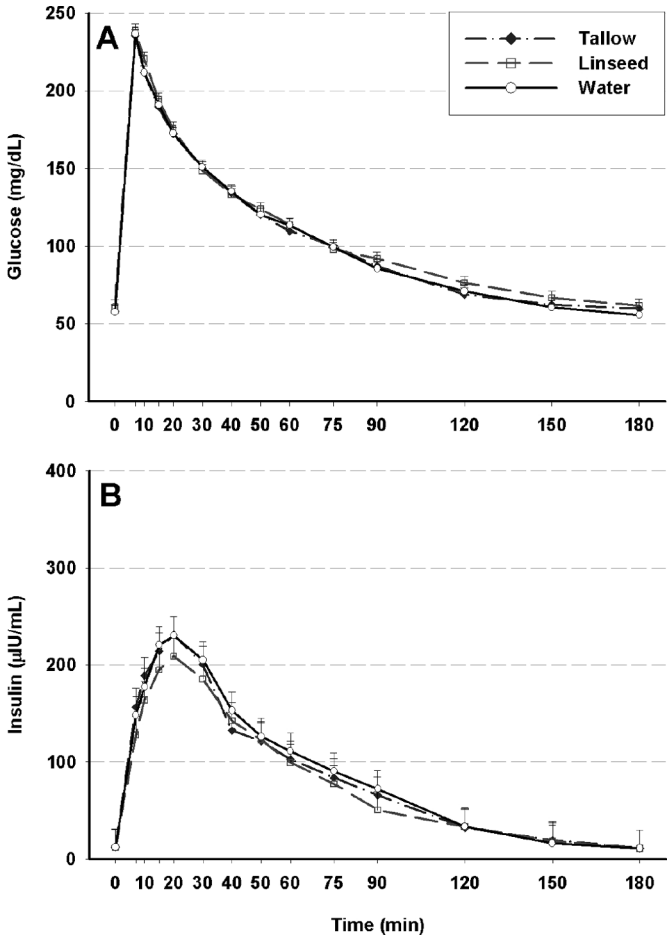


**Figure 3.** Experiment 2 (feed restriction): A) Effect of abomasal infusion of water, linseed oil, or tallow (0.54 g/d per kg of BW) on plasma glucose concentration after i.v. glucose tolerance test (IVGTT; 0.25 g/kg of BW of glucose i.v.). Treatments were administered for 122 h and cows were feed restricted for 50 h before IVGTT. Fixed effects in the statistical model: treatment ($P = 0.40$), time ($P < 0.001$), and treatment × time ($P = 0.95$). Contrasts: water vs. fat infusion ($P = 0.53$); linseed oil vs. tallow ($P = 0.24$). B) Effect of treatments on serum insulin concentration after IVGTT. Fixed effects in the statistical model: treatment ($P = 0.51$), time ($P < 0.001$), and treatment × time ($P = 0.97$). Contrasts: water vs. fat infusion ($P = 0.50$); linseed oil vs. tallow ($P = 0.65$). *P*-values reflect statistical analysis with log-transformed data.

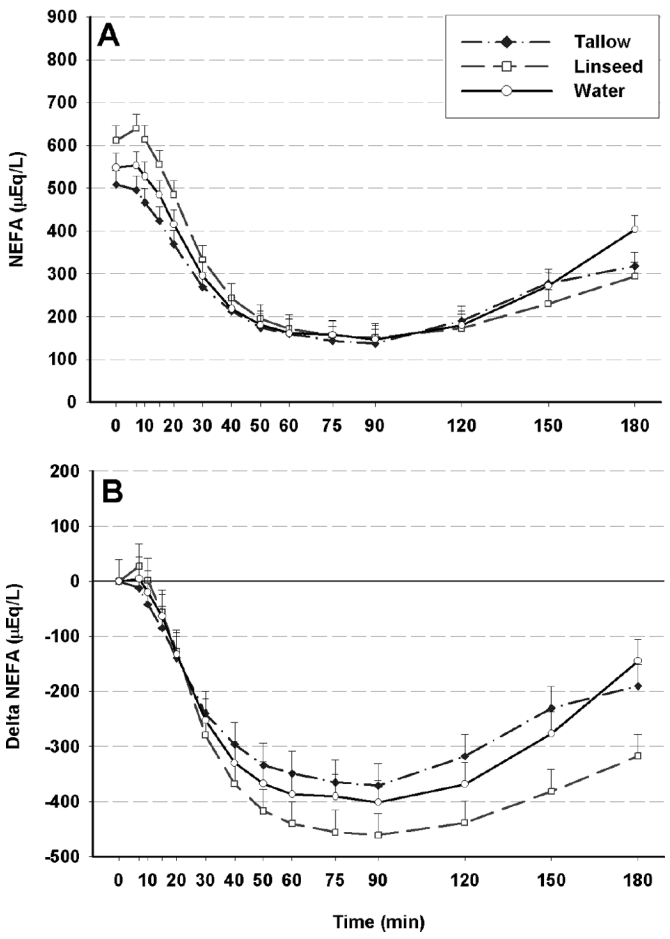


**Figure 4.** Experiment 2 (feed restriction): A) Effect of abomasal infusion of water, linseed oil or tallow (0.54 g/d per kg of BW) on plasma NEFA concentration after i.v. glucose tolerance test (IVGTT; 0.25 g/kg of BW of glucose i.v.). Treatments were administered for 122 h and cows were feed restricted for 50 h before IVGTT. Fixed effects in the statistical model: treatment ($P = 0.48$), time ($P < 0.001$), and treatment × time ($P = 0.92$). Contrasts: water vs. fat infusion ($P = 0.85$); linseed oil vs. tallow ($P = 0.23$). *P*-values reflect statistical analysis with log-transformed data. B) Effect of treatments on plasma NEFA concentration change (delta NEFA) after IVGTT. Fixed effects in the statistical model: treatment ($P = 0.34$), time ($P < 0.001$), and treatment × time ($P = 0.73$). Contrasts: water vs. fat infusion ($P = 0.64$); linseed oil vs. tallow ($P = 0.17$).

LIN in feed-restricted cows for unknown reasons (Table 8), despite the absence of treatment differences on insulin concentration over time.

Fatty acids can directly influence glucose-stimulated insulin secretion. For instance, saturated FA are insulinogenic compared with unsaturated fatty acids (Stein et al., 1997; Dobbins et al., 2002). These effects have been found both in vivo, by feeding rats with different fat sources, and in vitro, by perfusing rat pancreas with distinct FA (Stein et al., 1997; Dobbins et al., 2002).The latter method excludes the influence of extrapancreatic tissues on insulin secretion or clearance, and has confirmed that long-chain saturated FA (C16:0 and C18:0) are insulin secretagogues in rodents compared with unsaturated FA (C18:1, C18:2; Stein et al., 1997; Dobbins et al., 2002). Additionally, fat supplements with distinct fatty acid composition can alter secretion of gut peptides such as glucose-dependent insulinotropic peptide 1 and glucagon-like peptide 1 in Holstein cows, and these peptides may stimulate glucose-mediated insulin secretion (Relling and Reynolds, 2007). Potential treatment effects on plasma concentration of gut peptides under these experimental conditions deserve further investigation. Assessment of insulin resistance using tech-

**Table 8.** Experiment 2: Effect of abomasal infusions of water, linseed oil, or tallow on plasma glucose, NEFA, and serum insulin clearance measurements after insulin challenge (IC; 0.10 IU/kg of BW of insulin i.v.)[1]

| | Treatment | | | | *P*-value | |
|---|---|---|---|---|---|---|
| Item[2] | Water | Linseed | Tallow | SEM | Water vs. Fat | Linseed vs. Tallow |
| Glucose | | | | | | |
| Basal (mg/dL) | 58.9 | 60 | 59.8 | 1.5 | 0.76 | 0.58 |
| $CR_{30}$ | 1.12 | 1.08 | 1.03 | 0.08 | 0.32 | 0.31 |
| $T_{1/2}$ | 63.8 | 65.5 | 68.8 | 5.4 | 0.38 | 0.38 |
| $AUC_{30}$ [(mg/dL) × 30 min] | –253 | –251 | –238 | 21 | 0.67 | 0.93 |
| $AUC_{120}$ [(mg/dL) × 120 min] | –2,190 | –2,247 | –2,236 | 207 | 0.66 | 0.51 |
| Insulin | | | | | | |
| Basal (μIU/mL) | 10.2 | 8.7 | 9.6 | 1.4 | 0.21 | 0.31 |
| Peak (μIU/mL) | 1,964 | 1,776 | 1,976 | 139 | 0.47 | 0.17 |
| $CR_{30}$ | 3.84 | 3.77 | 4.18 | 0.16 | 0.41 | 0.08 |
| $T_{1/2}$ | 18.2 | 18.4 | 16.6 | 0.8 | 0.35 | 0.07 |
| $T_{basal}$ | 137.7 | 141.4 | 128.5 | 3.9 | 0.56 | 0.02 |
| $AUC_{30}$ [(μIU/mL) × 30 min] | 18,109 | 16,659 | 16,738 | 1,157 | 0.09 | 0.96 |
| $AUC_{120}$ [(μIU/mL) × 120 min] | 21,729 | 20,039 | 20,079 | 1,486 | 0.09 | 1.0 |
| NEFA | | | | | | |
| Basal (μEq/L) | 689 | 764 | 664 | 72 | 0.78 | 0.24 |
| $CR_{30}$ | 2.54 | 2.45 | 2.20 | 0.29 | 0.32 | 0.26 |
| $T_{1/2}$ | 28.4 | 29.1 | 34.0 | 3.8 | 0.36 | 0.18 |
| $AUC_{30}$ [(μEq/L) × 30 min] | –5,831 | –6,331 | –4,996 | 1,220 | 0.83 | 0.25 |
| $AUC_{120}$ [(μEq/L) × 120 min] | –23,285 | –26,862 | –17,892 | 11,188 | 0.98 | 0.25 |

[1]Treatments (0.54 g/d per kg of BW) were administered for 134 h and cows were feed restricted for 62 h before IC.

[2]Basal = average concentration at 15 and 5 min before IC; $CR_{30}$ = clearance rate during first 30 min of IC; $T_{1/2}$ = time to reach ½ concentration; $T_{basal}$ = time to reach basal concentration; $AUC_{30}$ and $AUC_{120}$ = area under the curve during first 30 and 120 min of IC; Peak = maximal insulin concentration (estimated with PROC NLIN).

niques that depend on endogenous insulin secretion, such as IVGTT used in these experiments, may incorporate direct and indirect effects of specific fatty acids on the pancreas. Nevertheless, the insulin sensitizing effects of n-3 C18:3 in peripheral tissues has been confirmed using hyperinsulinemic euglycemic clamp in rodents (Storlien et al., 1991) and using intraperitoneal IC (Mustad et al., 2006).

Plasma NEFA concentrations during experiment 1 were low, and reflect a postprandial state because cows were being fed and infused with fat sources. Feeding and infusion of treatments were performed 2 h before IVGTT and IC. Plasma NEFA concentration was greater ($P < 0.001$) for LIN than for TAL during IVGTT and IC. The reasons for the greater concentration of NEFA with LIN are not known and multiple factors could be involved. Plasma NEFA concentrations reflect the balance between NEFA production and clearance. Plasma NEFA originate from lipolysis of body reserves, and hydrolysis of dietary TG in intestinally synthesized chylomicrons and TG in very low density lipoprotein secreted by the liver. Lipolytic rates should have been low in experiment 1 because cows were in positive energy balance. We have assumed minimal differences in the digestibility of both fat sources because relatively modest amounts were infused every 4 h and feed intake was low (experiment 1) or limited to 1 kg of wheat middlings per day (experiment 2), which would lead to minimal flow of fat to the intestine. Therefore, we speculate that the digestibility of both lipid sources would be high. The uptake of TG from blood by peripheral tissues is a complex process, which includes hydrolysis of TG by lipoprotein lipase, release of free FA and binding to albumin, dissociation of FA from albumin, and translocation of FA across cytoplasmatic membrane, either through diffusion or protein-mediated transport, followed by intracellular association with FA binding proteins and acyl-CoA binding proteins (Doege and Stahl, 2006). Treatment effects on any of the previously enumerated processes could have affected plasma NEFA concentration, as observed during IVGTT and IC in experiment 1.

A major objective of experiment 2 was to assess the effect of fat source on the antilipolytic effects of insulin. Therefore, lipolysis was stimulated by feed restriction because the antilipolytic effects of insulin may only be evident in lipolytic states, when plasma NEFA is elevated (Sechen et al., 1990). Results suggest that LIN enhanced the antilipolytic effects of insulin in feed-restricted cows. The fact that treatment differences were found in clearance of plasma NEFA but not glucose agrees with the concept that insulin sensitivity is greater for antilipolytic effects in adipose tissue than for whole-body glucose utilization (Petterson et al., 1993, 1994). Therefore, improvement of insulin resistance by n-3 FA should first be observed in adipose tissue. However, the data presented herein should be interpreted with caution because for unknown reasons, plasma

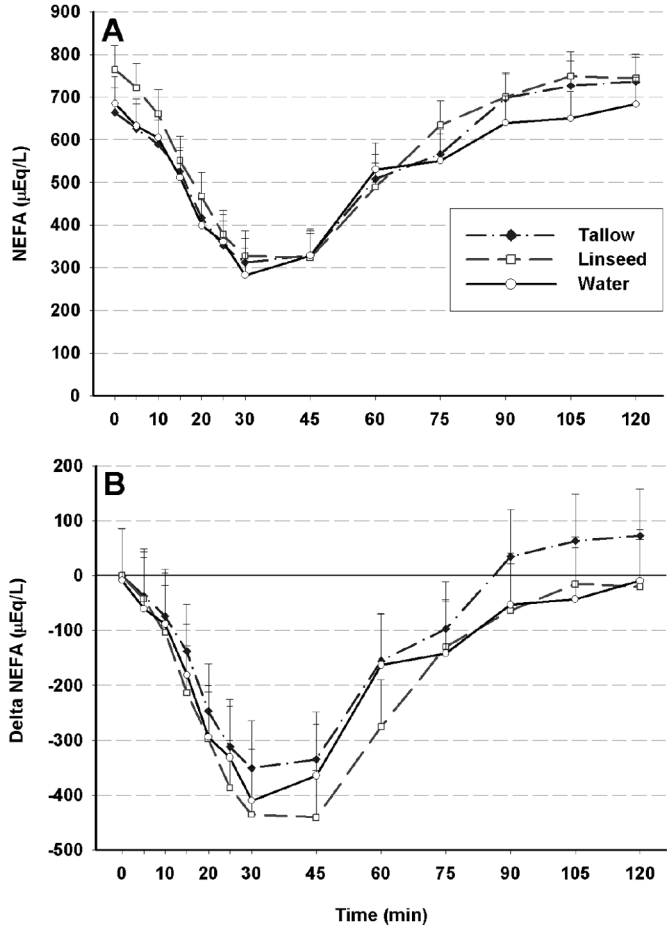


**Figure 5.** Experiment 2 (feed restriction): A) Effect of abomasal infusion of water, linseed oil or tallow (0.54 g/d per kg of BW) on plasma NEFA concentration after insulin challenge (IC; 0.1 IU/kg of BW of insulin i.v.). Treatments were administered for 134 h and cows were feed restricted for 62 h before IC. Fixed effects in the statistical model: treatment ($P = 0.67$), time ($P < 0.001$), and treatment × time ($P = 0.91$). Contrasts: water vs. fat infusion ($P = 0.52$); linseed oil vs. tallow ($P = 0.54$). *P*-values reflect statistical analysis with log-transformed data. B) Effect of treatments on plasma NEFA concentration change (delta NEFA) after IC. Fixed effects in the statistical model: treatment ($P = 0.72$), time ($P < 0.001$), and treatment × time ($P = 0.90$). Contrasts: water vs. fat infusion ($P = 0.88$); linseed oil vs. tallow ($P = 0.43$).

NEFA concentrations immediately before IVGTT and IC were approximately 100 μEq/L greater for LIN than for TAL (Tables 7 and 8; Figures 4 and 5). Factors such as potential differences in lipoprotein lipase affinity to TG from each fat source or different uptake and metabolism of FA may have been involved.

## CONCLUSIONS

The main objective was to assess the effects of abomasal infusion of linseed oil, a source rich in n-3 C18:3, on whole-body responses to insulin. Treatments were infused at rates within the range currently used for fat supplementation in dairy rations. Infusion of LIN had an insulin-sensitizing effect in fed cows (experiment 1) compared with TAL because lower insulin concentrations led to the same clearance of glucose as TAL. This effect was lost when cows were feed restricted for 50 h before IVGTT (experiment 2).

Lipolysis was stimulated by feed restriction (experiment 2) to assess whether treatments could modulate antilipolytic effects of insulin. Infusion of LIN may have enhanced the antilipolytic effect of insulin because clearance of plasma NEFA and magnitude of $AUC_{180}$ after IVGTT were greater for LIN than TAL. Yet, other factors could have been involved because, for unknown reasons, plasma NEFA concentration before IVGTT and IC were approximately 100 μEq/L greater for LIN than for TAL. The experiments presented herein were conducted using a dry-cow model and the effects of supplementation with n-3 C18:3 on responses to insulin need to be investigated in periparturient cows.

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## REFERENCES

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804–2819.

Bell, A. W., and D. E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. J. Mammary Gland Biol. Neoplasia 2:265–278.

Chilliard, Y., and J. F. Ottou. 1995. Duodenal infusion of oil in midlactation cows. 7. Interaction with niacin on responses to glucose, insulin, and beta-agonist challenges. J. Dairy Sci. 78:2452–2463.

Clarke, S. D. 2000. Polyunsaturated fatty acid regulation of gene transcription: A mechanism to improve energy balance and insulin resistance. Br. J. Nutr. 83(Suppl. 1):S59–S66.

Delarue, J., C. Couet, R. Cohen, J. F. Brechot, J. M. Antoine, and F. Lamisse. 1996. Effects of fish oil on metabolic responses to oral fructose and glucose loads in healthy humans. Am. J. Physiol. Endocrinol. Metab. 270:E353–E362.

Delarue, J., C. LeFoll, C. Corporeau, and D. Lucas. 2004. N-3 long chain polyunsaturated fatty acids: A nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity? Reprod. Nutr. Dev. 44:289–299.

Delarue, J., C. H. Li, R. Cohen, C. Corporeau, and B. Simon. 2006. Interaction of fish oil and a glucocorticoid on metabolic responses to an oral glucose load in healthy human subjects. Br. J. Nutr. 95:267–272.

Dobbins, R. L., L. S. Szczepaniak, J. Myhill, Y. Tamura, H. Uchino, A. Giacca, and J. D. McGarry. 2002. The composition of dietary fat directly influences glucose-stimulated insulin secretion in rats. Diabetes 51:1825–1833.

Doege, H., and A. Stahl. 2006. Protein-mediated fatty acid uptake: Novel insights from in vivo models. Physiology (Bethesda) 21:259–268.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497–509.

Frayn, K. N. 2002. Adipose tissue as a buffer for daily lipid flux. Diabetologia 45:1201–1210.

Gaynor, P. J., R. A. Erdman, B. B. Teter, A. V. Capuco, and D. R. Waldo. 1996. Glucose and norepinephrine challenges during the abomasal infusion of *cis* or *trans* octadecenoates in Holstein cows. J. Dairy Sci. 79:1590–1595.

Gingras, A.-A., P. J. White, P. Y. Chouinard, P. Julien, T. A. Davis, L. Dombrowski, Y. Couture, P. Dubreuil, A. Myre, K. Bergeron, A. Marette, and M. C. Thivierge. 2007. Long-chain omega-3 fatty acids regulate bovine whole-body protein metabolism by promoting muscle insulin signalling to the akt-mtor-s6k1 pathway and insulin sensitivity. J. Physiol. 579:269–284.

Gressley, T. F., S. M. Reynal, J. J. O. Colmenero, G. A. Broderick, and L. E. Armentano. 2006. Technical Note: Development of a tool to insert abomasal infusion lines into dairy cows. J. Dairy Sci. 89:3965–3967.

Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882–3896.

Hayirli, A., D. R. Bremmer, S. J. Bertics, M. T. Socha, and R. R. Grummer. 2001. Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. J. Dairy Sci. 84:1218–1230.

Holtenius, K., S. Agenas, C. Delavaud, and Y. Chilliard. 2003. Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. J. Dairy Sci. 86:883–891.

Lewis, G. F., A. Carpentier, K. Adeli, and A. Giacca. 2002. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr. Rev. 23:201–229.

Mashek, D. G., S. J. Bertics, and R. R. Grummer. 2002. Metabolic fate of long-chain unsaturated fatty acids and their effects on palmitic acid metabolism and gluconeogenesis in bovine hepatocytes. J. Dairy Sci. 85:2283–2289.

Mashek, D. G., S. J. Bertics, and R. R. Grummer. 2005. Effects of intravenous triacylglycerol emulsions on hepatic metabolism and blood metabolites in fasted dairy cows. J. Dairy Sci. 88:100–109.

Mashek, D. G., and R. R. Grummer. 2003. Effects of long chain fatty acids on lipid and glucose metabolism in monolayer cultures of bovine hepatocytes. J. Dairy Sci. 86:2390–2396.

Mustad, V. A., S. DeMichele, Y.-S. Huang, A. Mika, N. Lubbers, N. Berthiaume, J. Polakowski, and B. Zinker. 2006. Differential effects of n-3 polyunsaturated fatty acids on metabolic control and vascular reactivity in the type 2 diabetic ob/ob mouse. Metabolism 55:1365–1374.

Newman, R. E., W. L. Bryden, A. C. Kirby, L. H. Storlien, and J. A. Downing. 2005. Dietary n-3 and n-6 fatty acids alter avian glucose metabolism. Br. Poult. Sci. 46:104–113.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Palmquist, D. L., and T. C. Jenkins. 2003. Challenges with fats and fatty acid methods. J. Anim. Sci. 81:3250–3254.

Palmquist, D. L., and E. A. Moser. 1981. Dietary fat effects on blood insulin, glucose utilization, and milk protein content of lactating cows. J. Dairy Sci. 64:1664–1670.

Petterson, J. A., F. R. Dunshea, R. A. Ehrhardt, and A. W. Bell. 1993. Pregnancy and undernutrition alter glucose metabolic responses to insulin in sheep. J. Nutr. 123:1286–1295.

Petterson, J. A., F. R. Dunshea, R. A. Ehrhardt, and A. W. Bell. 1994. Pregnancy but not moderate undernutrition attenuates insulin suppression of fat mobilization in sheep. J. Nutr. 124:2431–2437.

Pires, J. A. A., J. B. Pescara, and R. R. Grummer. 2007a. Reduction of plasma NEFA concentration by nicotinic acid enhances the response to insulin in feed-restricted Holstein cows. J. Dairy Sci. 90:4635–4642.

Pires, J. A. A., A. H. Souza, and R. R. Grummer. 2007b. Induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in Holstein cows. J. Dairy Sci. 90:2735–2744.

Relling, A. E., and C. K. Reynolds. 2007. Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. J. Dairy Sci. 90:1506–1515.

Sechen, S. J., F. R. Dunshea, and D. E. Bauman. 1990. Somatotropin in lactating cows: Effect on response to epinephrine and insulin. Am. J. Physiol. Endocrinol. Metab. 258:E582–E588.

Stein, D. T., B. E. Stevenson, M. W. Chester, M. Basit, M. B. Daniels, S. D. Turley, and J. D. McGarry. 1997. The insulinotropic potency of fatty acids is influenced profoundly by their chain length and degree of saturation. J. Clin. Invest. 100:398–403.

Storlien, L. H., J. A. Higgins, T. C. Thomas, M. A. Brown, H. Q. Wang, X. F. Huang, and P. L. Else. 2000. Diet composition and insulin action in animal models. Br. J. Nutr. 83(Suppl. 1):S85–S90.

Storlien, L. H., A. B. Jenkins, D. J. Chisholm, W. S. Pascoe, S. Khouri, and E. W. Kraegen. 1991. Influence of dietary-fat composition on development of insulin resistance in rats - Relationship to muscle triglyceride and omega-3-fatty-acids in muscle phospholipid. Diabetes 40:280–289.