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DAIRY COWS AND NUTRIENT PARTITIONING

Responses of North American and New Zealand strains of Holstein-Friesian dairy cattle to homeostatic challenges during early and mid lactation

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28 **Abstract**

29 This study investigated the physiological basis of differences in nutrient partitioning
30 between the North American (NA) and New Zealand (NZ) strains of Holstein Friesian
31 cattle by determining the responses to homeostatic challenges at two stages of lactation.
32 Glucose tolerance tests, epinephrine challenges, and insulin challenges were carried out
33 on consecutive days commencing on day 32 ± 0.48 (mean \pm s.e.m) of lactation (T1) and
34 again commencing on day 137 ± 2.44 of lactation (T2). The insulin and non-esterified
35 fatty acid (NEFA) responses to glucose infusion did not differ between the strains. The
36 NZ strain had a greater clearance rate (CR) of glucose (2.04 vs. 1.66 % / min) and tended
37 to have a shorter (34.4 vs. 41.1 min) glucose half-life ($t_{1/2}$) at T2 when infused with
38 glucose. The NA cows had a greater glucose response to epinephrine infusion across T1
39 and T2, and tended to have a greater insulin response to epinephrine infusion. Plasma
40 NEFA concentration declined to similar nadir concentrations for both strains at T1 in
41 response to insulin, though from a higher basal concentration in NA cows, resulting in a
42 greater (-2.29 vs. -1.38) NEFA area under the response curve (AUC) for NA cows.
43 Glucose response to insulin varied with time, tending to be greater for NA at T1, but
44 tending to be lower for NA at T2. The results indicated that NA cows had a greater
45 glycogenolytic response to epinephrine, but both strains had similar lipolytic responses.
46 The results also imply that higher basal circulating NEFA concentrations in the NA
47 strain in early lactation were not due to diminished adipose tissue responsiveness to
48 insulin. There were indications that glucose clearance rate was greater in NZ cows in
49 mid-lactation, and may form the basis of increased body tissue accretion during mid- to
50 late-lactation in this strain.

51 **Keywords:** Dairy cows, nutrient partitioning, genetic selection; homeostasis.

52

53 **Introduction**

54 The onset of lactation in the dairy cow represents a large and abrupt rise in nutrient
55 demand. The increased glucose requirements of the lactating mammary gland require
56 marked adjustments in nutrient partitioning and the metabolism of non-mammary tissues.
57 Hepatic gluconeogenesis is increased to meet mammary demands during early lactation,
58 while glucose utilization by adipose tissue and muscle is reduced. These responses are
59 mediated by reduced circulating insulin concentrations, and through a series of
60 coordinated adaptations – orchestrated by increased circulating growth hormone
61 concentrations – to reduce peripheral tissue sensitivity and responsiveness to insulin (Bell
62 and Bauman, 1997). In addition, adipose tissue responsiveness to lipolytic stimuli is
63 markedly increased in early lactation to facilitate body fat mobilization in support of
64 lactation (Bauman, 2000). Catecholamines are important signals to promote mobilization
65 of fuel stores to meet short-term energy needs. Epinephrine is a neurotransmitter in the
66 sympathetic nervous system with powerful lipolytic action, and induces maximum rates
67 of lipolysis in adipose tissue (Sechen *et al.*, 1990).

68

69 The administration of bovine somatotropin (bST) to lactating dairy cows alters tissue
70 responsiveness to homeostatic signals to increase nutrient partitioning towards milk
71 production (Etherton and Bauman, 1998). Alterations in responsiveness to homeostatic
72 signals may occur through changes in receptor number, binding kinetics or intracellular
73 expression of the signal (e.g. amplification, enzyme activation; Bauman and Elliot, 1983).

74 Genetic selection for increased milk yield has been associated with an increased ratio of
75 somatotropin to insulin (Bonczek *et al.*, 1988), though relative differences are reduced
76 when high producing cows are placed on a higher plane of nutrition (Hart, 1983).
77 Previous investigations have shown an association between genetic merit and changes in
78 insulin secretion in response to a glucose challenge in juvenile dairy bulls (Mackenzie *et*
79 *al.*, 1988), while Kolver *et al.* (2001) reported a greater lipolytic response to an
80 epinephrine challenge for cows of greater milk yield potential. The greater propensity for
81 body reserve mobilization that accompanies genetic selection for milk production may
82 thus result from changes in the set-points for physiological responses to homeostatic
83 signals.

84

85 The Moorepark strain comparison study evaluated the milk production, body condition
86 score (BCS), bodyweight and fertility characteristics of the North American (NA) and
87 New Zealand (NZ) strains of Holstein Friesian (HF) cattle managed under a range of
88 pasture feeding systems (Horan *et al.*, 2005). The NA strain had been selected for
89 increased milk yield in a confinement environment, whereas the NZ strain had been
90 selected for milk solids production, feed efficiency and survival in a pasture based system
91 with limited concentrate input. Among the principal findings of the study were greater
92 milk production, lower BCS, and inferior fertility performance for the NA strain across
93 the feeding systems. A notable feature of the Moorepark strain comparison study was the
94 greater milk production response to additional concentrate supplementation for NA cows
95 compared to NZ cows, indicating a greater capacity to partition additional ingested
96 nutrients to milk production for NA cows (Horan *et al.*, 2005). It also reported that

97 increasing concentrate supplementation in pasture-based systems is ineffective as a
98 strategy to reduce the extent of BCS loss for NA cows in early lactation. The current
99 study was carried out to test the hypothesis that differences in nutrient partitioning
100 between the NZ and NA strains are the result of altered tissue responsiveness to
101 homeostatic signals.

102

103 **Materials and Methods**

104 *Animals and experimental design*

105 Two groups of 10 spring-calving, multiparous Holstein-Friesian cows were selected from
106 the NA and NZ groups of the Moorepark strain comparison study. The origins and
107 establishment of the experimental groups from which the cows were selected have been
108 previously described by Horan *et al.* (2005). The NA strain was developed by mating the
109 top 50% of cows in Moorepark (based on pedigree index for milk production) with 5
110 North American Holstein sires, selected as the highest available in Ireland for pedigree
111 index for milk production. The NZ strain were imported as embryos from New Zealand
112 and implanted into Holstein heifers. These NZ embryos were generated by mating high
113 genetic merit New Zealand HF cows with 5 high genetic merit (based on Breeding
114 Worth, the New Zealand genetic evaluation system) New Zealand HF sires. The
115 experimental animals used in the current study were selected as representative of the NA
116 and NZ treatment groups involved in the Moorepark strain comparison study (Horan *et*
117 *al.*, 2005). The genetic merit for milk production of the experimental groups is outlined in
118 Table 1.

119

120 *Insert Table 1 Here*

121

122 *Animal management and sampling*

123 Cows were housed in a free-stall barn commencing three weeks prior to expected calving
124 date, and trained to use the Griffith Elder feeding system (Griffith Elder Ltd, Bury St
125 Edmunds, Suffolk, UK). Forage mangers were mounted on electronic load cells and
126 concentrates were dispensed through automatic feeders to facilitate measurement of DMI
127 and calculation of energy balance. Cows had ad libitum access to forage, which was fed
128 once daily and offered to allow for feed refusals of at least 5%. Refusals were removed
129 daily.

130 Glucose tolerance tests, epinephrine challenges, and insulin challenges were carried out at
131 two time periods for each cow. The first series of challenges were carried out on
132 consecutive days commencing on day 32 ± 0.48 (mean \pm s.e.m) of lactation (T1), and the
133 second series of challenges were carried out on consecutive days commencing on day 137
134 ± 2.44 of lactation (T2). Cows were moved to individual tie-stalls during the period when
135 homeostatic challenges were taking place, and were milked in the stall at 0730 h and
136 1530 h. Feed was removed at least 1 hour prior to the administration of each challenge.
137 The diet offered in the week preceding and during T1 consisted of ad libitum grass silage
138 (*L. perenne* spp) plus 8kg/d (as fed) of concentrate. The diet offered in the week
139 preceding and during T2, consisted of ad libitum freshly cut grass (*L. Perenne* spp.) plus
140 4kg/d (as fed) of concentrate. The daily concentrate allowance was fed in equal amounts
141 at each milking, and consisted of barley 220g/kg; rapeseed meal 210g/kg; beet pulp
142 200g/kg; maize gluten 170g/kg; soybean meal 140g/kg; vegetable oil 30g/kg, and mineral

143 mix 30g/kg. The chemical analyses of the forages and concentrate used are detailed in
144 Table 2.

145

146 *Insert Table 2 here*

147

148 Mean daily energy balance (EB) for the week preceding administration of homeostatic
149 challenges was estimated as the difference between energy intake and the sum of energy
150 requirements for maintenance and milk production. The French Net Energy system was
151 used, where one UFL is the NE content of 1 kg of air-dry standard barley for milk
152 production (Jarrige, 1989). Solids corrected milk (SCM) yield was calculated using the
153 equation of Tyrell and Reid (1965). On day 1 (Monday) of each time period, i.e. the day
154 before the first homeostatic challenge, cows were weighed to facilitate calculation of
155 dosage rates, and indwelling jugular catheters were fitted to facilitate collection of blood
156 samples and allow intravenous infusion of glucose, epinephrine and insulin. Body
157 condition score (Lowman et al, 1976) was also recorded for each animal on day 1 of each
158 time period.

159

160 *Administration of homeostatic challenges*

161 The glucose tolerance test was carried out on day 2 of each time period. Cows were
162 infused with glucose (50% wt/vol dextrose solution; Baxter Healthcare Ltd., Norfolk,
163 England) at a rate of 1.5 g glucose/kg of $BW^{0.75}$ via the jugular catheter at 0900 h and
164 immediately flushed with 10 mL saline. Blood samples were collected at -45, -40, -30,
165 -20, -10, -5, and 0 min relative to the start of infusion, and 2.5, 5, 7.5, 10, 15, 20, 30, 45,

166 60, 120, 150, and 180 min relative to completion of infusion. Mean infusion times (\pm
167 s.e.m) for NA cows were 495 s \pm 45 s and 400 s \pm 17 s at T1 and T2, respectively. Mean
168 infusion times for NZ cows were 401 s \pm 18 s and 363 s \pm 19 s at T1 and T2, respectively.

169

170 The epinephrine challenge was carried out on day 3 of each experimental week.
171 Epinephrine acid tartrate (1 mg/ml solution; Phoenix Pharma Ltd., Gloucester, England)
172 was infused at a rate of 1.4 μ g/kg BW via the jugular catheter at 0900 h and immediately
173 flushed with 10 mL of sterile saline. Blood samples were collected at -45, -40, -30, -20,
174 -10, -5, 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 120, 125, and 130 min relative to
175 epinephrine administration. The epinephrine dose chosen was previously reported to
176 result in a maximum lipolytic response (Sechen *et al.*, 1990)

177

178 The insulin challenge was carried out on day 4 of each experimental week. Bovine
179 pancreatic insulin (I-5500, lot 064K1582, 28.7 USP units/mg; Sigma, Dublin, Ireland)
180 was dissolved in a sterile solution of 0.01 M HCl to generate a 2 mg/ml solution, and this
181 was diluted with sterile saline to a final concentration of 1 mg/ml. The insulin solution
182 was prepared on the evening prior to the insulin challenges, and stored overnight at 4 °C.
183 Insulin was infused at a rate of 1.0 μ g/kg BW via the jugular catheter at 0900 h, and
184 immediately flushed with 10 mL of sterile saline. The blood sampling schedule for the
185 insulin challenge was identical to that of the epinephrine challenge

186

187 All blood samples collected during each challenge were decanted into tubes containing
188 100 international units of heparin, centrifuged at $2000 \times g$ for 15 mins at 4 °C, and the
189 plasma was harvested and stored at -20 °C until analysis.

190

191 *Laboratory procedures and sample analysis*

192 The DM, NDF, crude fiber and CP of the forage and concentrate samples were analyzed
193 as described by McNamara et al. (2003). Determination of in-vitro dry matter
194 digestibility (DMD) was carried out by near-infrared spectroscopy using a NIRsystems
195 6500 spectrophotometer (Perstorp Analytical Incorporated, Silver Springs, Maryland,
196 USA). Silage pH was measured on the juice pressed from the silage using a glass
197 electrode and a pH meter (Radiometer pHM2 standard pH meter-radiometer,
198 Copenhagen). The organic matter digestibility of grass was determined as described by
199 Morgan et al. (1994).

200 Blood plasma was analysed for glucose and NEFA concentrations by enzymatic
201 colorimetry, using an ABX Mira Autoanalyser (ABX Mira, Cedex 4, France) and
202 appropriate kits (NEFA kit supplied by Wako Chemicals, GmbH, Nissanstraße 2, D-
203 41468 Neuss Germany; glucose kit supplied by ABX Montpellier, Cedex 4, France).

204 Plasma insulin was assayed using a solid-phase fluoroimmunoassay (AutoDELFIA,
205 Perkin Elmer Life and Analytical Sciences, Turku, Finland; Perkin Elmer kit no. 312439,
206 supplied by Unitech BD Ltd, Dublin, Ireland). The inter- and intra-assay coefficients of
207 variation were 8.72% and 5.71% respectively. The minimum detectable concentration of
208 the assay was 1.52 $\mu\text{IU}/\text{mL}$.

209

210 *Data handling and statistical analysis*

211 Metabolite and hormone responses to each homeostatic challenge were calculated as area
212 under the response curve (AUC), corrected for differences in baseline value. Area under
213 the curve was calculated using the EXPAND procedure in SAS (SAS Institute, 1991).
214 Baseline values for each analyte were determined by calculating the overall mean
215 concentration of samples collected prior to administration and samples collected from
216 120 min post challenge. When measuring the AUC response to the hormone/metabolite
217 administered (i.e. glucose AUC in response to the glucose tolerance test, and insulin
218 AUC in response to the insulin challenge), calculations were from the time of
219 administration until the time of return to baseline concentration for the individual animal.
220 In all other instances, the AUC was calculated from 0 min until the average time that
221 maximal response had occurred to minimise the effect of counter-regulatory mechanisms.
222 Maximal responses occurred at 20 mins after administration of epinephrine for both
223 glucose and NEFA. For the insulin and glucose challenges, maximum NEFA responses
224 occurred at 30 mins after infusion.

225

226 The glucose response to the insulin challenge was expressed as the fractional rate of
227 glucose clearance (FCR). This was calculated as the slope of the natural logarithm of
228 glucose concentration over the initial declining phase (0 to 20 min) plotted versus time.

229

230 The half life ($t_{1/2}$) and clearance rate (CR) of glucose and insulin were calculated using
231 the NLIN procedure in SAS (SAS Institute, 1991). Data from the first 60 min following
232 infusion of each challenge were fitted to the following equation:

233 $f(t) = b * e^{(c * t)}$

234 where

235 t = time,

236 b = parameter for starting concentration,

237 c = parameter for rate of decay.

238

239 Clearance rate is the slope of this exponential function. Therefore, CR and $t^{1/2}$ of glucose
240 and insulin were calculated as follows:

241 $CR, \%/min = 100 * (\ln [t_a] - \ln [t_b]) / (t_b - t_a)$

242 $t^{1/2} = \ln(2) / CR$

243 where

244 $[t_a]$ = concentration of metabolite or hormone at time a (t_a)

245 $[t_b]$ = concentration of metabolite or hormone at time b (t_b)

246

247 Data from T1 and T2 were analyzed as repeated measures using the MIXED procedure of
248 SAS (SAS Institute, 1991). Treatment, time and a treatment by time interaction term were
249 included in the models as fixed effects; cow was treated as a random variable nested
250 within treatment, and an autoregressive covariance structure was used. The P-values
251 presented in Tables 4-6 represent the main effects of strain, time, and the interaction
252 between strain and time. Pair-wise comparisons of strain effects within time period and
253 time effects within strain were adjusted using the Tukey-Kramer test; where reported in
254 the text of the results section, these comparisons are described using adjusted P-values.

255

256 **Results**

257 *Milk production, EB, BCS and bodyweight*

258 The milk yield, DMI and EB data of the strains during the periods of administration of
259 homeostatic challenges are presented in Table 3. The NA strain had higher milk yield at
260 T1 ($P = 0.02$) compared to the NZ strain. Solids-corrected milk yield did not differ ($P =$
261 0.37) between the strains at T1 due to a higher milk fat content for NZ cows ($P = 0.02$).
262 Mean daily EB ($P = 0.92$) was similar for the strains at T1, and differences in DMI were
263 not significant ($P = 0.13$). The NA cows had a greater milk yield ($P = 0.01$) and lower
264 milk fat concentration ($P = 0.04$) compared to the NZ strain at T2. Solids-corrected milk
265 yield ($P = 0.04$) and DMI ($P = 0.03$) were greater for NA compared to NZ cows at T2.
266 However, DMI as a percentage of body weight ($P = 0.91$) and daily EB ($P = 0.16$) did not
267 differ between the strains during this time period. The NA cows were heavier ($P < 0.05$)
268 during both experimental periods. There was no difference in BCS between the strains at
269 T1 ($P = 0.46$) or at T2 ($P = 0.13$). The milk production, postpartum EB, and metabolic
270 profiles of the animals used in the current study have been previously reported (Patton *et*
271 *al.*, 2008).

272

273 ***Insert Table 3 Here***

274

275 *Glucose tolerance test*

276 Intravenous infusion of glucose resulted in an acute increase in plasma insulin
277 concentration, and a reduction in plasma NEFA concentrations (Table 4; Figure 1). The

278 NA and NZ strains had similar glucose AUC (P = 0.73), insulin AUC (P = 0.32), NEFA
279 AUC (P = 0.85), glucose CR (P = 0.37), and glucose t_{1/2} (P = 0.93) at T1.

280

281 *Insert Table 4 Here*

282 *Insert Figure 1 Here*

283

284 The CR of glucose was greater for NZ compared to NA cows at T2 (P = 0.03), while t_{1/2}
285 for glucose tended to be greater for NA cows at that time (P = 0.07). There were no
286 differences between the strains at T2 in insulin AUC (P = 0.89), glucose AUC (P = 0.17)
287 or NEFA AUC (P = 0.63). A significant effect of time was observed, where the insulin
288 response to glucose infusion was greater at T2 versus T1 (P < 0.01).

289

290 *Epinephrine Challenge*

291 Plasma concentrations of glucose were acutely elevated by intravenous infusion of
292 epinephrine at T1 and T2 (Table 5; Figure 2). The NA strain tended to have a greater
293 glucose response to epinephrine at T1 (P = 0.10) and T2 (P = 0.14), as measured by AUC,
294 resulting in an overall greater response (P = 0.04) for NA cows across the two time
295 periods.

296

297 *Insert Table 5 Here*

298 *Insert Figure 2 Here*

299

300 Epinephrine infusion also resulted in an acute increase in circulating concentrations of
301 NEFA, which returned to baseline concentrations by 45 min post infusion. The NEFA
302 response to epinephrine, measured as AUC, was similar for both strains at T1 ($P = 0.25$)
303 and T2 ($P = 0.73$). The NA cows tended to have a greater ($P = 0.07$) insulin response to
304 epinephrine infusion across the 2 time periods.

305

306 *Insulin Challenge*

307 The NA cows had a greater NEFA AUC compared to NZ cows at T1 ($P = 0.02$), whereas
308 the strains had a similar ($P = 0.51$) AUC at T2 (Figure 3; Table 6). The NEFA AUC in
309 response to the insulin challenge was greater in both strains at T1 versus T2 (Time effect,
310 $P < 0.01$). The baseline plasma NEFA concentration, measured as the mean concentration
311 from -45 min to 0 min relative to infusion of insulin, was greater at T1 compared to T2
312 (0.25 vs 0.11 mmol/L; $P < 0.01$).

313

314 *Insert Table 6 Here*

315 *Insert Figure 3 Here*

316

317 The FCR of glucose in response to the insulin challenge was similar for both strains at T1
318 ($P = 0.77$) and T2 ($P = 0.45$) (Table 6, Figure 4). Glucose response to the insulin
319 challenge was also measured as AUC. The NA cows tended to have a greater ($P = 0.11$)
320 glucose response area at T1, whereas NZ cows tended to have a greater ($P = 0.13$)
321 response area at T2 (Table 6). This resulted in a significant strain by time interaction for
322 glucose AUC in response to the insulin challenge ($P = 0.04$).The baseline value for

323 glucose was greater for NA cows compared to NZ cows at T1 (2.94 vs. 2.58 Mmol/L; P =
324 0.04), whereas both strains had similar baseline plasma glucose concentrations at T2
325 (3.38 vs. 3.55 Mmol/L; P = 0.30), resulting in a significant strain by time interaction for
326 baseline glucose concentrations during the insulin challenges (P = 0.04).

327

328 ***Insert Figure 4 here***

329

330 The half-life of insulin following insulin administration did not differ between strains at
331 T1 (P = 0.52) or at T2 (P = 0.15). The CR of insulin was similar for both strains at both
332 T1 (P = 0.66) and T2 (P = 0.22). The CR of insulin tended to be greater (P = 0.08) in both
333 strains at T1 compared to T2.

334

335 **Discussion**

336 The NA and NZ strains of Holstein Friesian cattle differ considerably in their nutrient
337 partitioning and BCS profiles when managed in a pasture-based feeding system. Previous
338 reports have indicated that these two strains have a comparable rate of BCS loss during
339 early lactation; however NZ cows maintain a higher BCS throughout lactation and have a
340 greater rate of BCS gain post-nadir (Horan *et al.*, 2005; McCarthy *et al.*, 2007), indicating
341 that NA cows maintain preferential partitioning of nutrients to milk production for a
342 greater duration *post partum*. In the current study, a series of metabolic challenges were
343 carried out to investigate if strain differences in nutrient partitioning were associated with
344 alterations in tissue responsiveness to homeostatic stimuli. The challenges were carried
345 out during wk 4-5 of lactation, and again during wk 19-20 of lactation, to determine

346 whether the responses of the strains to the challenges varied according to stage of
347 lactation and EB status.

348

349 Calculated EB was more negative for both strains at T1 compared to T2 as expected,
350 though differences in EB between the strains were minor during both time periods. Daily
351 milk yield was greater for NA cows at both time periods, whereas solids-corrected milk
352 (SCM) production was similar for both strains at T1 and greater for the NA strain at T2.
353 The NZ cows had higher milk fat concentration, consistent with previous reports from
354 strain comparison studies (Horan *et al.*, 2005; Kolver *et al.*, 2002). Differences in BCS
355 profiles between the strains were less pronounced in the current study than previously
356 documented (Harris and Kolver, 2001; Horan *et al.*, 2005). In the current study, both
357 strains had comparable BCS loss during early lactation (T1), and had similar BCS in mid
358 lactation (T2); however the NZ cows accumulated significantly more body reserves from
359 T2 to the end of lactation (Patton *et al.*, 2008).

360

361 Intravenous infusion of glucose resulted in an acute increase in plasma insulin
362 concentrations. The insulin response was similar for both strains at each time period,
363 though both strains had a greater insulin response at T2 compared to T1. This observation
364 is likely a reflection of superior energy balance and reduced mammary glucose demand at
365 T2 compared to T1. In support of this, pancreatic insulin secretion in response to glucose
366 and propionate infusions is greater in non-lactating cows than lactating cows (Lomax *et*
367 *al.*, 1979). Similarly, Sano *et al.* (1993) used a hyperglycemic clamp to demonstrate that
368 the increase in circulating insulin concentrations in response to glucose infusion is

369 reduced in lactating compared to non-lactating cows. Staufenbiel *et al.* (1992) concluded
370 that flow of metabolites to the mammary gland in early lactation was supported by both
371 reduced pancreatic response to insulinotropic stimuli, and decreased responsiveness of
372 peripheral cells to insulin. In any case, there was no evidence of differences in insulin
373 response to an insulinotropic stimulus between the strains in the present study. Chagas *et*
374 *al.* (2003) showed that New Zealand Friesian cows had a greater insulin response to a
375 glucose challenge than North American Holstein cows when fed a TMR diet, but the
376 opposite occurred on a pasture-only diet. Strain differences in the insulin response to a
377 glucose challenge appear therefore to be dependent on the basal diet (i.e., environment
378 effect).

379

380 It is well documented that peripheral tissue responses to insulin are attenuated during
381 early lactation. These tissue-specific adaptations are collectively described as '*insulin*
382 *resistance*', and include reduced stimulation of lipogenesis in adipose tissue and whole-
383 body oxidation of glucose (Bauman, 2000). The net effect is to increase the availability of
384 glucose in support of mammary glucose requirements. Insulin resistance occurs whenever
385 normal concentrations of insulin produce a less than normal biologic response. This may
386 be due to a decrease in sensitivity to insulin (i.e. a shift in the dose-response curve to the
387 right), a decrease in maximal response to insulin, or a combination of both (Kahn, 1978).
388 Glucose response to the glucose challenge was measured as area under the response curve
389 (AUC), half-life ($t_{1/2}$) and clearance rate (CR). While no strain differences were apparent
390 for measures of glucose response at T1, it was found that glucose had a greater CR and
391 shorter $t_{1/2}$ in NZ cows at T2. This indicates greater insulin responsiveness in the NZ cows

392 compared to NA cows in mid-lactation, as insulin resistance is associated with slower
393 CR, longer $t_{1/2}$, and a greater AUC for glucose at similar insulin concentrations (Mertz,
394 1993). Greater insulin responsiveness in the NZ cows in mid-lactation is consistent with
395 accumulation of more body reserves from mid-lactation to the end of lactation (Patton *et*
396 *al.*, 2008).

397

398 Insulin regulates adipose tissue metabolism by suppressing lipid mobilization and
399 increasing rates of reesterification (Brockman and Laarveld, 1986). Sechen *et al.* (1989)
400 reported that bST-treated cows entered negative EB and had increased basal NEFA
401 concentrations compared to control cows, presumably as part of the coordinated
402 responses necessary to support greater milk production. During glucose and insulin
403 challenges, bST-treated cows had greater decreases in plasma NEFA, indicating that bST-
404 treated cows were more sensitive to the antilipolytic effects of insulin (Sechen *et al.*,
405 1989). Intravenous infusion of glucose resulted in an acute increase in plasma insulin and
406 a decline in plasma NEFA concentration in the current study, the magnitude of which did
407 not vary between strains or time periods. In contrast to the glucose challenge, the NEFA
408 response to insulin administration varied with both genetic strain and time period. Basal
409 concentrations of NEFA were greater at T1 compared to T2, explaining the greater NEFA
410 AUC responses at T1 compared to T2. The higher basal NEFA concentration at T1
411 compared to T2 is in agreement with observed differences in EB between the time
412 periods. This is also consistent with the reduced NEFA response to the insulin challenge
413 at T2, where basal NEFA concentrations were lower. Following insulin administration at
414 T1, NEFA was reduced to a similar concentration at 30 min post infusion in both strains,

415 indicating that the higher basal NEFA concentration in the NA strain was not due to
416 diminished adipose tissue responsiveness to the antilipolytic actions of insulin.

417

418 There were significant interactions between strain and time period for glucose AUC in
419 response to the insulin challenge, and also for basal glucose concentration during the
420 insulin challenge. The strain with the greater basal glucose concentration had the greater
421 AUC in response to insulin at both time periods, indicating that the magnitude of the
422 response to insulin was dependent on basal concentration. The fractional clearance rate
423 (FCR) of glucose measures the response of insulin-sensitive tissues. No differences were
424 observed in FCR between the strains at either stage of lactation in this study. Mammary
425 tissue utilizes 60-90% of circulating glucose during lactation, and mammary tissue is
426 insulin-insensitive (Bell and Bauman, 1997). Potential strain differences in glucose
427 utilization by insulin-sensitive tissues only affect a small proportion of the total
428 circulating glucose pool, and consequently are difficult to detect. Differences in FCR may
429 be more apparent in later lactation, when cows are in a more positive EB and have a
430 relatively larger proportion of glucose available for tissue accretion (Sechen *et al.*, 1990).
431 In the current study, the mean daily EB of the NA and NZ strains were similar; both were
432 in mild NEB at T1 and both were close to neutral EB at T2.

433

434 Norepinephrine is a neurotransmitter that stimulates lipolysis and NEFA release in
435 adipose tissue of ruminants and other species (Himms-Hagen, 1972), and this effect is
436 simulated by epinephrine administration. The effect of epinephrine treatment on adipose
437 tissue mobilization may be determined from the plasma NEFA response profile.

438 Epinephrine binds to both the β - and α 2- adrenergic receptors on adipocytes. The elevated
439 concentrations of plasma NEFA after an epinephrine challenge represents mobilization of
440 fatty acids, the net effect of β -adrenergic induced lipolysis minus fatty acid
441 reesterification. Sechen *et al* (1989) reported a 2.2-fold increase in NEFA response to
442 epinephrine when lactating Holstein cows were treated with bST, demonstrating that
443 adipose tissue mobilization in response to lipolytic stimuli was enhanced by bST
444 treatment. In the current study, NEFA response (i.e. mobilization) to the epinephrine
445 challenges was not affected by strain or stage of lactation. Similarly, Kolver *et al.* (2001)
446 reported no difference in plasma NEFA response to an epinephrine challenge between
447 North American HF and New Zealand HF cows, either in a TMR or pasture-feeding
448 scenario. Their study also reported a trend towards a strain by diet interaction for glycerol
449 response to epinephrine, with pasture-fed North American cows and TMR-fed NZ cows
450 having a greater response. These groups had lower EB than their strain counterparts on
451 opposite diets, which suggested that the degree of lipolytic response to epinephrine, but
452 not NEFA mobilization, was influenced by differences in energy status. The EB of the
453 NA and NZ strains was similar within experimental period in the present study. The
454 NEFA AUC of the strains did not differ between time periods however, indicating that
455 energy status did not influence the NEFA mobilization response to epinephrine.

456

457 Epinephrine stimulated an acute increase in circulating glucose concentrations,
458 presumably reflecting increased hepatic glycogenolysis and reduced glycogenesis in both
459 strains. The glucose response to epinephrine infusion was greater in NA cows compared
460 to NZ cows in the current study. In contrast, Kolver *et al.* (2001) found no difference in

461 glucose response to epinephrine between the North American HF and New Zealand HF
462 strains; however cows fed a total mixed ration (TMR) had a greater response to
463 epinephrine than pasture-fed cows. Sechen *et al.* (1989) also observed an acute increase
464 in plasma glucose after administration of epinephrine, but no difference in response due
465 to bST treatment. As in the current study, Sechen *et al.* (1989) observed that plasma
466 insulin concentration was acutely elevated in response to epinephrine. This insulin
467 response counter-regulates the effects of epinephrine on plasma glucose concentration;
468 the degree of insulin response is determined by the magnitude of glucose increase.

469

470 **Conclusions**

471 The NA and NZ strains of Holstein Friesian cows exhibited some different responses to
472 acute metabolic challenges that varied with stage of lactation. The greater glucose
473 response to epinephrine of the NA strain indicates enhanced hepatic glycogenolysis
474 and/or reduced glycogenesis. The NA cows also had a greater reduction in plasma NEFA
475 in response to insulin compared to NZ cows in early lactation, due to a higher basal
476 NEFA concentration for NA cows at that time. The NZ cows had a greater glucose
477 clearance rate and shorter half-life than the NA cows in mid-lactation when infused with
478 glucose, indicating that the NA cows may have a greater degree of insulin resistance at
479 that stage of lactation. It is plausible that this plays a key role in the continued preferential
480 partitioning of nutrients to the mammary gland at the expense of body reserve repletion
481 during mid- to late-lactation in the NA cows, whereas NZ cows accumulate body reserves
482 during this period on grass-based diets.

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600 **Table 1** *Genetic merit of the North American and New Zealand Holstein Friesian cows*
 601 *based on predicted differences¹ and standard deviations (SD) for milk production,*
 602 *calving interval and survival*

Trait	Strain ²	
	NA	NZ
Milk (kg)	+ 210 (117)	+ 1 (157)
Fat (kg)	+ 6.2 (3.5)	+ 6.5 (5.0)
Protein (kg)	+ 7.4 (4.4)	+ 3.7 (4.0)
Fat (g/kg)	+ 0.10 (1.4)	+ 1.13 (0.62)
Protein (g/kg)	+ 0.40 (0.32)	+ 0.75 (0.43)
Calving interval (days)	+ 0.99 (1.98)	- 2.86 (1.53)
Survival (%)	+ 0.04 (0.29)	+ 1.14 (0.48)

603 ¹ All predicted differences obtained from the February 2004 international evaluations of the INTERBULL
 604 Animal Centre (Uppsala, Sweden).

605 ² NA = North American Holstein Friesian; NZ = New Zealand Holstein Friesian

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620 **Table 2** *Chemical composition of forages and concentrate*¹

	Grass Silage	Grass	Concentrate
Dry Matter (DM), g/kg	273 ± 53	172 ± 23	871 ± 32
Crude Protein, g/kg DM	117 ± 10	155 ± 31	186 ± 71
NDF, g/kg DM	589 ± 27	390 ± 23	256 ± 20
ADF, g/kg DM	368 ± 23	-	-
Ash, g/kg DM	58 ± 8	79 ± 8	91 ± 3
Starch (g/kg DM)	-	-	182 ± 15
Dry Matter Digestibility ² , g/kg DM	697 ± 40	-	-
Organic Matter Digestibility, g/kg DM	630 ± 33	813 ± 17	-
pH	4.11 ± 0.36	-	-
Net Energy ^{3,4,5} , UFL ⁶ /kg DM	0.79 ³	1.02 ⁴	1.14 ⁵
Net Energy ⁷ , Mcal/kg DM	1.34	1.73	1.96

621 ¹ Values reported are mean ± standard deviation622 ² Estimated using near infrared spectroscopy623 ³ The net energy value of silage was related to its *in-vitro* DMD concentration (O'Mara *et al.*, 1997)624 ⁴ The net energy value of grass was determined according to Jarrige (1989)625 ⁵ The net energy of concentrate was calculated from the net energy values for ingredients (Jarrige 1989)626 ⁶ Unité Fourragère Lait, net energy for lactation equivalent of 1 kg standard air-dry barley (Jarrige, 1989)627 ⁷ Estimated based on 1 UFL = 1.7 Mcal/kg (Vermorel, 1989)

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629 **Table 3** Milk production, dry matter intake (DMI), bodyweight and energy balance (EB)
 630 during periods of homeostatic challenges

Variable	NA ¹	NZ ¹	S.E.D ²	P-Value
<i>T1</i> ³				
Milk yield (kg/d)	35.3	30.5	1.40	0.02
Milk fat (g/kg)	45.4	56.0	2.88	0.02
Milk protein (g/kg)	30.4	29.6	0.60	0.41
Solids corrected milk (kg/d)	35.0	33.4	1.24	0.37
Dry matter intake (kg/d)	17.6	15.8	0.88	0.13
DMI (% body weight)	2.99	2.94	0.23	0.80
Energy balance (UFL ⁴ /d)	-4.72	-4.61	0.87	0.92
Bodyweight (kg)	596	540	19	<0.01
BCS	2.80	2.92	0.16	0.46
<i>T2</i> ³				
Milk yield (kg/d)	26.1	22.3	0.93	0.01
Milk fat (g/kg)	41.2	46.0	1.85	0.02
Milk protein (g/kg)	32.4	33.3	0.50	0.24
Solids corrected milk (kg/d)	24.9	22.4	1.14	0.04
Dry matter intake (kg/d)	17.2	15.8	0.64	0.03
DMI (% body weight)	2.93	2.94	0.15	0.91
Energy balance (UFL/d)	0.32	0.38	0.72	0.91
Bodyweight (kg)	593	538	24	0.03
BCS	2.46	2.68	0.14	0.13

631 ¹NA = North American Holstein Friesian; NZ = New Zealand Holstein Friesian

632 ²S.E.D = standard error of difference

633 ³T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk

634 ⁴Unité Fourragère Lait, net energy for lactation equivalent of 1 kg standard air-dry barley (Jarrige, 1989)

635 **Table 4** *Effect of cow strain on responses to intravenous glucose tolerance tests in early*
 636 *and mid-lactation*

	T1 ¹		T2 ¹		<i>P</i> -values			
	NA ²	NZ ²	NA	NZ	S.E.D ³	S ⁴	T ⁴	S x T
Glucose AUC ⁵	254	262	258	227	22.1	0.52	0.27	0.18
Insulin AUC	1617 ^A	2195 ^A	3289 ^B	3368 ^B	412	0.42	<0.01	0.55
NEFA AUC	-4.62	-4.88	-3.17	-2.45	1.52	0.81	0.11	0.67
t ½ glucose ⁶	36.9	36.6	41.1	34.4	3.59	0.19	0.71	0.22
CR glucose ⁷	1.78	1.93	1.66 ^a	2.04 ^b	0.17	0.02	0.96	0.38

637 ¹T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk

638 ² NA = North American Holstein Friesian; NZ= New Zealand Holstein Friesian

639 ³ SED = Standard error of difference

640 ⁴ S = Strain, T = time period

641 ⁵ AUC = Area under the response curve. Expressed in units of Mmol*min/L for glucose and NEFA and

642 μIU*min/mL for insulin

643 ⁶ t ½ = glucose half-life (min)

644 ⁷ CR = clearance rate (%/min)

645 ^{ABab} Means having different upper case superscripts differ significantly within strain across time period (P <

646 0.05). Means having different lower case superscripts differ significantly within time period across strain

647 (P < 0.05)

648 **Table 5** *Effect of cow strain on responses to intravenous epinephrine challenges in early*
 649 *and mid-lactation*

	T1 ¹		T2 ¹		<i>P-values</i>			
	NA ²	NZ ²	NA	NZ	S.E.D ³	S ⁵	T ⁵	S x T
Insulin AUC ⁴	585	339	753	463	172	0.07	0.17	0.83
Glucose AUC	43.7 ^A	36.8 ^A	32.2 ^B	26.1 ^B	4.11	0.04	<0.01	0.88
NEFA AUC	6.11	5.03	5.14	5.44	0.93	0.56	0.65	0.27

650 ¹T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk

651 ² NA = North American Holstein Friesian; NZ= New Zealand Holstein Friesian

652 ³ SED = Standard error of difference

653 ⁴ AUC = Area under the response curve. Expressed in units of Mmol*min/L for glucose and NEFA and

654 μIU*min/mL for insulin

655 ⁵ S = Strain, T = time period

656 ^{ABab} Means having different upper case superscripts differ significantly within strain across time period (P <

657 0.05). Means having different lower case superscripts differ significantly within time period across strain

658 (P < 0.05)

659 **Table 6** Effect of cow strain on responses to intravenous insulin tolerance tests in early
 660 and mid-lactation

	T1 ¹		T2 ¹		P-values			
	NA ²	NZ ²	NA	NZ	S.E.D ³	S ⁴	T ⁴	S x T
NEFA AUC ⁵	-2.29 ^{Aa}	-1.38 ^{Ab}	-0.69 ^B	-0.42 ^B	0.40	0.01	<0.01	0.33
t ½ insulin ⁶	6.27	5.84	5.99	5.04	0.65	0.20	0.18	0.51
CR insulin ⁷	6.53	6.82	6.27	5.50	0.64	0.59	0.08	0.24
FCR glucose ⁸ , min	-0.019	-0.019	-0.017	-0.019	0.002	0.49	0.51	0.73
Glucose AUC	-17.0	-12.7 ^A	-17.7	-21.8 ^B	2.68	0.95	0.02	0.04

661 ¹T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk

662 ² NA = North American Holstein Friesian; NZ= New Zealand Holstein Friesian

663 ³ SED= Standard error of difference

664 ⁴ S = Strain, T = time period

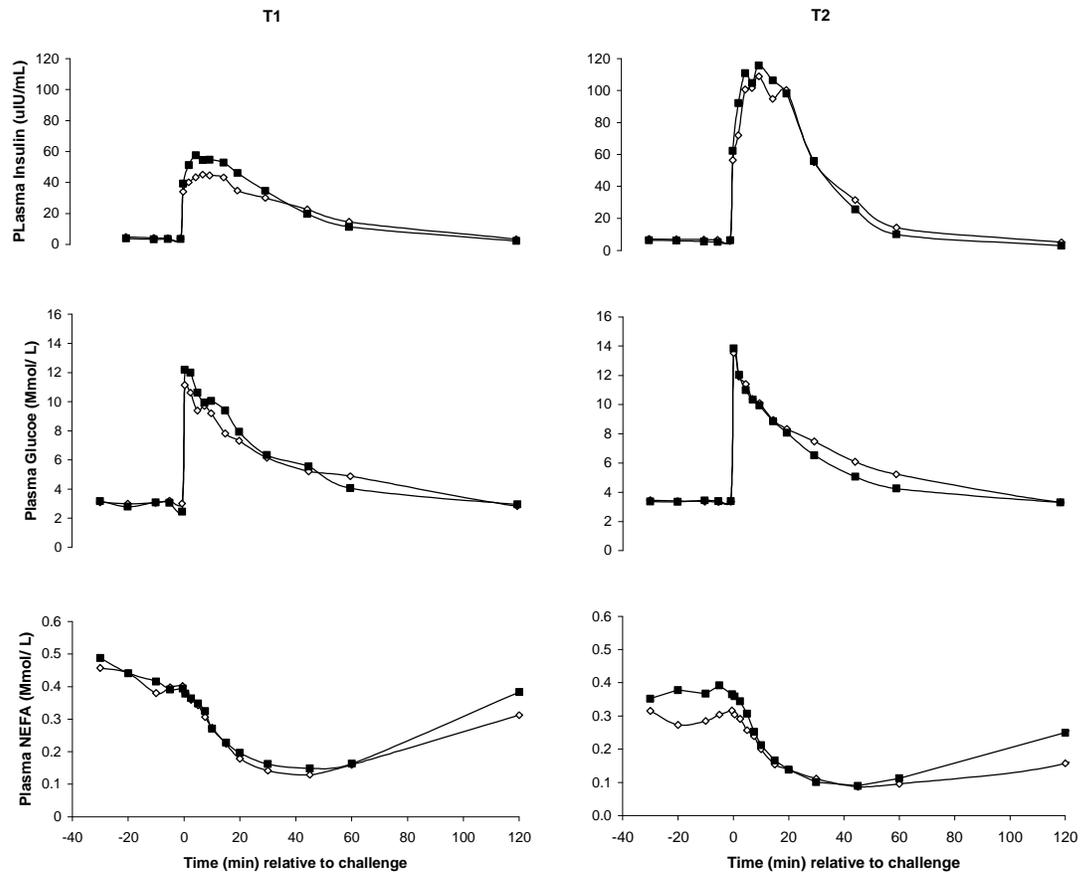
665 ⁵AUC= Area under the response curve. Expressed in units of Mmol*min/L

666 ⁶ t ½ = Insulin half-life (min)

667 ⁷ CR = clearance rate (%/min)

668 ⁸ FCR = Fractional clearance rate of glucose between 0 and 20 minutes after insulin administration. Values
 669 represent the slope of the natural logarithm of glucose concentrations (Mmol/L).

670 ^{ABab} Means having different upper case superscripts differ significantly within strain across time period (P <
 671 0.05). Means having different lower case superscripts differ significantly within time period across strain
 672 (P < 0.05).

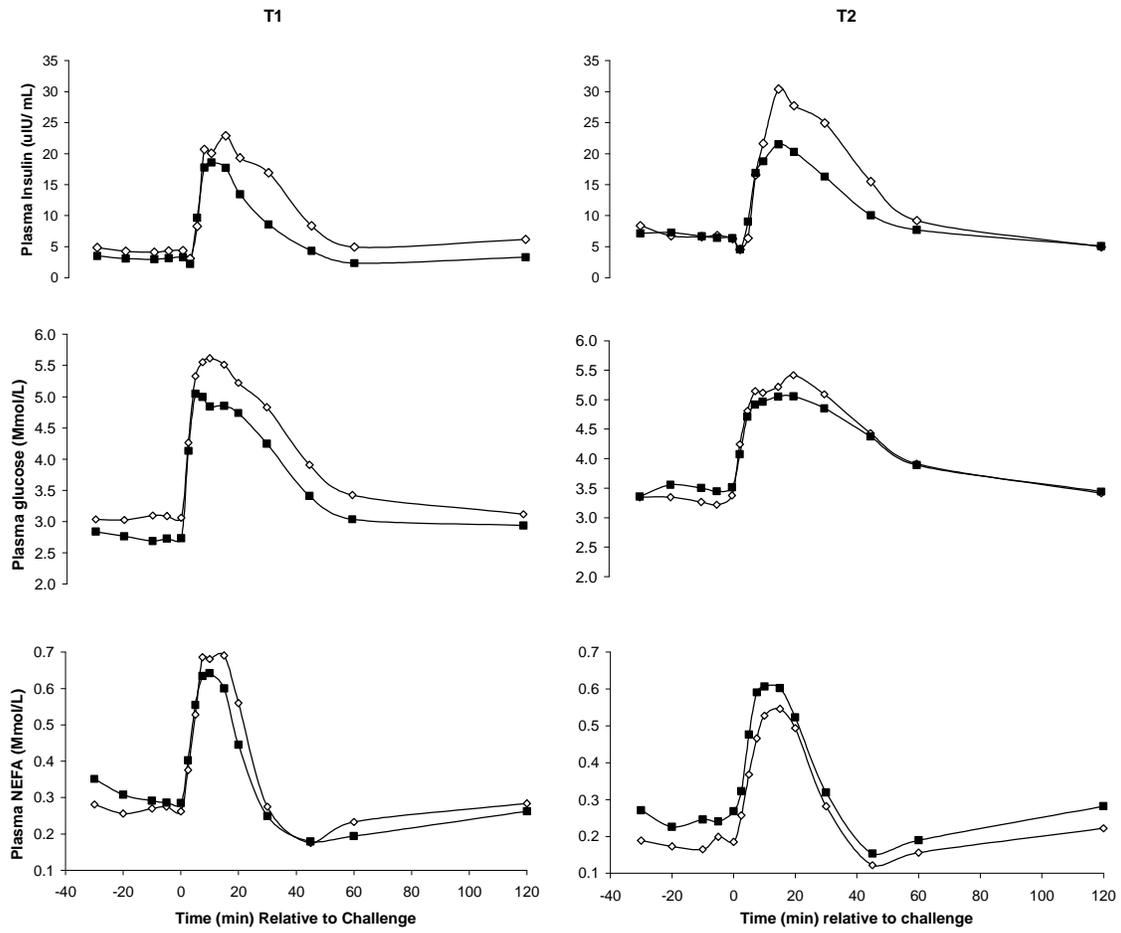


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Figure 1

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689 **Figure 1** Responses of the NA (\diamond) and NZ (\blacksquare) strains of Holstein-Friesian cattle to intravenous
690 glucose tolerance tests at 2 stages of lactation (T1 = 32 ± 0.48 (mean \pm s.e.m) days in milk; T2
691 = 137 ± 2.44 days in milk). Cows were infused with 1.5g glucose (50% wt/vol)/kg of BW^{0.75}
692 via a jugular catheter. Areas under the response curve and statistical analysis are outlined in
693 Table 4.



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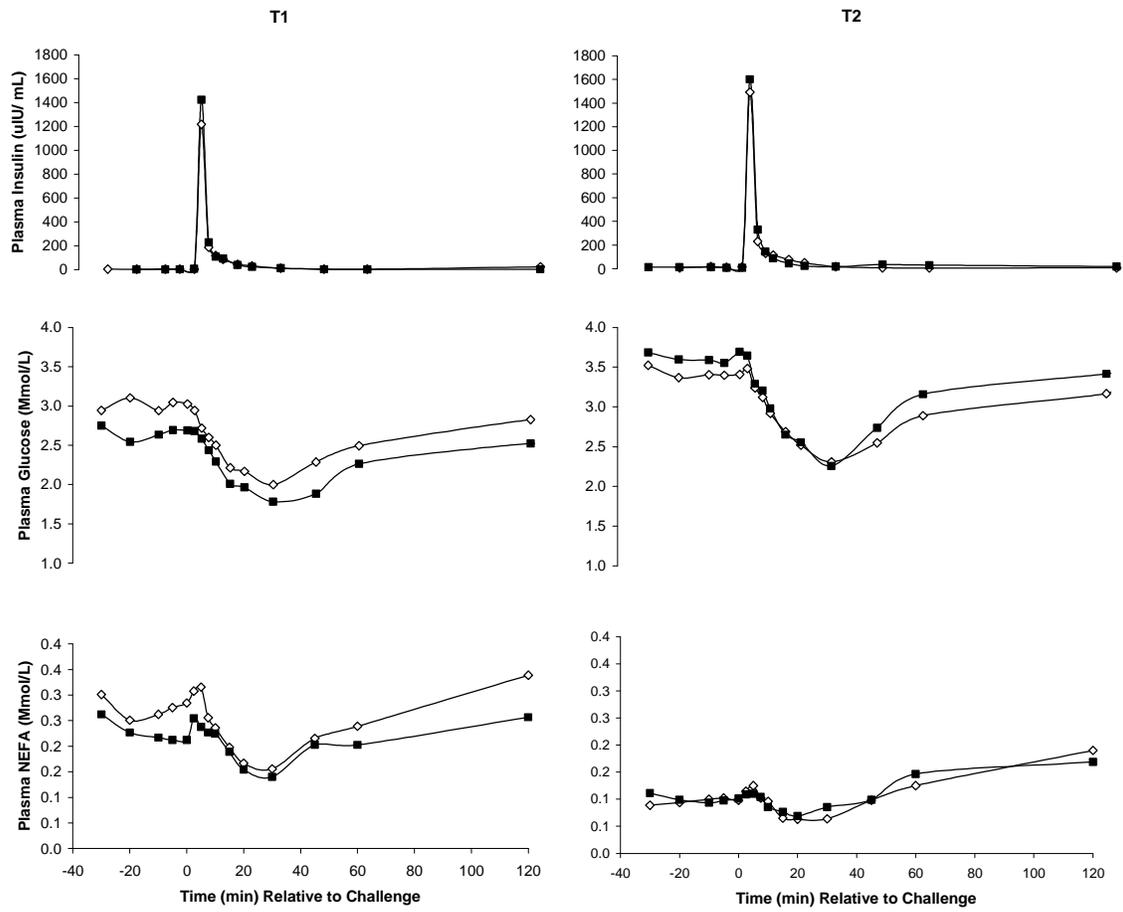
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Figure 2

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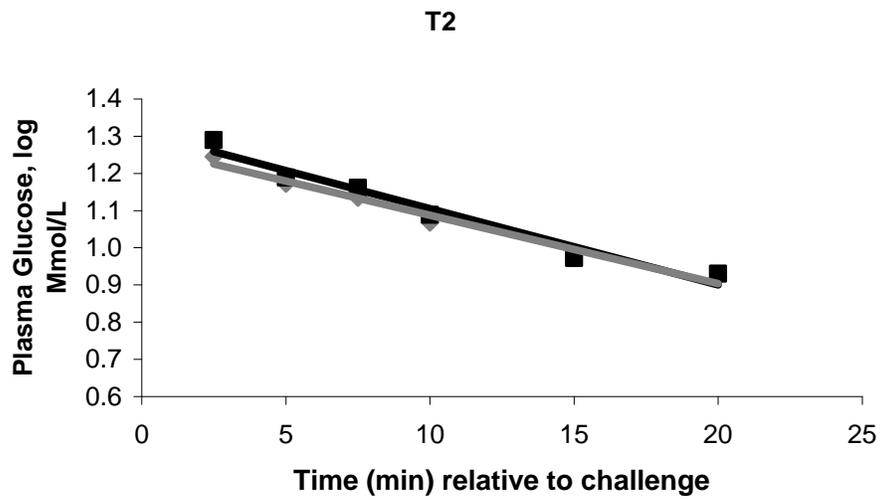
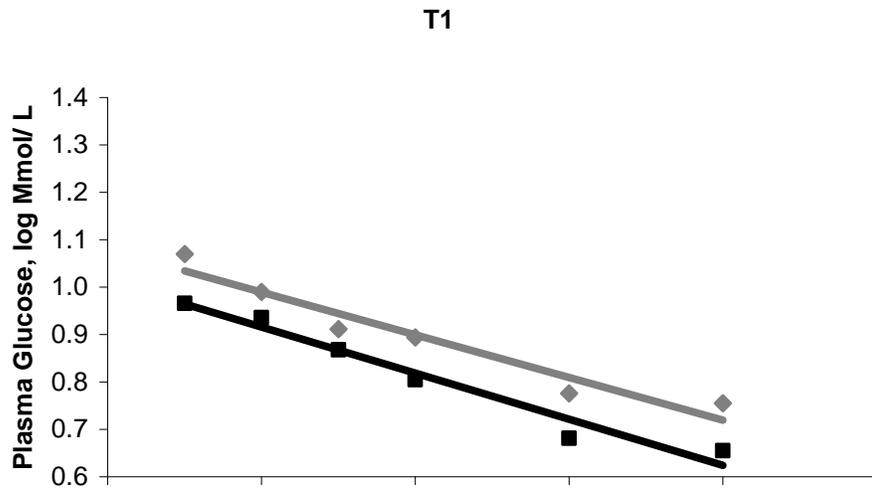
709 **Figure 2** Responses of the NA (\diamond) and NZ (\blacksquare) strains of Holstein-Friesian cattle to
710 intravenous epinephrine challenges at 2 stages of lactation (T1 = 32 ± 0.48 (mean \pm
711 s.e.m) days in milk; T2 = 137 ± 2.44 days in milk). Epinephrine acid tartrate ($1.4 \mu\text{g}/\text{kg}$
712 BW) was administered via a jugular catheter. Areas under the response curve and
713 statistical analysis are outlined in Table 5.



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Figure 3

728 **Figure 3** Responses of the NA (\diamond) and NZ (\blacksquare) strains of Holstein-Friesian cattle to
729 intravenous insulin challenges at 2 stages of lactation (T1 = 32 ± 0.48 (mean \pm s.e.m)
730 days in milk; T2 = 137 ± 2.44 days in milk). Cows were infused with $1.0 \mu\text{g}/\text{kg}$ BW of
731 bovine pancreatic insulin, administered via a jugular catheter. Areas under the response
732 curve and statistical analysis are outlined in Table 6.



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Figure 4

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745 **Figure 4** Fractional clearance rate (FCR) of glucose in response to intravenous insulin for
746 the NA (◆) and NZ (■) strains of Holstein-Friesian cattle at 2 stages of lactation (T1 = 32
747 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk). FCR was calculated
748 as the slope of the natural logarithm of glucose concentration over the initial declining
749 phase (0 to 20 min) plotted versus time. Results are detailed in Table 6. The standard
750 error of mean FCR was 0.002.

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