BLOOD GLUCOSE AND INSULIN RESPONSES DURING THE GLUCOSE TOLERANCE TEST IN RELATION TO DAIRY COW BODY CONDITION AND MILK YIELD

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Abstract. For dairy cows, insulin sensitivity plays a key role in *post partum* lipid mobilization. In addition to genetic factors, body condition at calving affects the development of *post partum* insulin resistance. The aim of this study was to examine glucose and insulin responses at the time of the glucose tolerance test (GTT) in relation to Body Condition Score (BCS) and milk yield in Estonian Holstein (EH, n=16) and Estonian Red (ER, n=15) cows. The GTT was carried out at 31±1.9 days *post partum*. Blood samples were collected at -15, -5, 5, 10, 20, 30, 40, 50 and 60 min relative to infusion of 0.15 g/kg BW glucose, and were analyzed for glucose, insulin and non-esterified fatty acids (NEFA). Energy-Corrected Milk (ECM) yield, at the time of the GTT, was calculated as the mean ECM of the day before and the day after the GTT. Spearman correlations for BCS measurements (BCS at calving and at the time of the GTT, and BCS loss) and milk production traits (ECM yield and milk fat content) with basal concentration of metabolites, maximum increase and area under the curve (AUC) of glucose and insulin, clearance rate (CR) of glucose, and Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) were calculated. Correlations between BCS and GTT characteristics were different between the breeds. For EH cows, BCS at calving was correlated positively with basal NEFA; BCS at the time of the GTT with maximum increase and AUC of insulin, and with basal NEFA. In ER cows BCS at calving was correlated positively with maximum increase and AUC of glucose and negatively with CR of glucose. BCS loss was negatively correlated with RQUICKI. Milk fat content was correlated positively with basal NEFA in EH cows; ECM yield and milk fat content with basal NEFA in ER cows. Observed glucose and insulin responses in the breeds examined indicate greater impairment of insulin function of thin cows amongst EH, and of over-conditioned cows amongst ER, compared to cows with moderate BCS at calving. In addition, the results indicate an association between milk production and adipose tissue mobilization.

Keywords*:* glucose tolerance test, glucose response, insulin response, area under the curve, clearance rate, RQUICKI, body condition score, NEFA, milk yield.

KRAUJO GLIUKOZĖS IR INSULINO REAKCIJŲ PRIKLAUSOMYBĖ NUO PIENINIŲ KARVIŲ KŪNO RIEBALŲ KIEKIO IR PIENINGUMO, NUSTATYTA GLIUKOZĖS TOLERANCIJOS TESTU

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Santrauka. Pieninių karvių jautrumas insulinui atlieka pagrindinį vaidmenį lipidų mobilizacijoje po veršiavimosi (*post partum). Post partum* atsparumas insulinui priklauso ne tik nuo genetinių veiksnių, bet ir nuo karvės kūno riebalų kiekio (BCS) veršiavimosi metu. Šio darbo tikslas – gliukozės tolerancijos testu (GTT) ištirti, kaip karvių kūno riebalų kiekis ir pieningumas veikia gliukozės ir insulino reakcijas. Tyrimas atliktas su Estijos holšteinais (EH, n=16) ir Estijos žalmargėmis (ER, n=15). GTT tolerancijos testas atliktas praėjus 31±1,9 dienos po veršiavimosi. Kraujo mėginiai paimti -15, -5, 5, 10, 20, 30, 40, 50 ir 60 min. po 0,15 g/kg BW gliukozės injekcijos. Mėginiuose nustatytas gliukozės, insulino ir neesterifikuotų sočiųjų rūgščių (NEFA) kiekis. Gliukozės tolerancijos testo metu pilnos energinės vertės pieno kiekis (ECM) apskaičiuotas kaip ECM kiekio vidurkis dieną prieš ir dieną po GTT. Buvo apskaičiuota: Spearmano koreliacija tarp BCS parametrų (BCS veršiavimosi metu, GTT metu ir BCS pablogėjimas), koreliacija tarp pieno savybių (ECSM kiekis ir pieno riebalų koncentracija) ir bazinių metabolitų koncentracijos, gliukozės ir didžiausia insulino koncentracija bei testo tikslumo laipsnis (AUC), gliukozės pašalinimo greitis (CR) ir kiekybinis insulino jautrumo testo indeksas (RQUICKI). Koreliacija tarp skirtingų veislių karvių BCS ir GTT charakteristikų skyrėsi. Estijos holšteinų BCS koreliacija su bazinėmis neesterifikuotomis rūgštimis (NEFA) veršiavimosi metu ir GTT metu, esant aukščiausioms insulino ir AUC vertėms, buvo teigiama. Veršiavimosi metu Estijos žalmargių karvių BCS teigiamai koreliavo su aukščiausiomis gliukozės AUC vertėmis ir neigiamai su gliukozės pašalinimo greičiu (CR). BCS praradimas neigiamai koreliavo su RQUICKI. Teigiama koreliacija tarp pieno riebalų ir NEFA nustatyta Estijos holšteino veislės karvėms. Estijos žalmargėms nustatyta teigiama koreliacija tarp ECM, pieno riebalų ir NEFA. Pastebėtos gliukozės ir insulino reakcijos tirtų veislių karvėse parodė, kad, lyginant su normalaus svorio karvėmis, veršiavimosi metu insulino funkcija labiau sutrinka per mažo svorio Estijos holšteinams ir per didelio svorio Estijos žalmargėms. Be to, tyrimų rezultatai rodo, kad egzistuoja ryšys tarp pieningumo ir kūno riebalų kiekio.

Raktažodžiai: gliukozės tolerancijos testas, gliukozės reakcija, insulino reakcija, šalinimo kreivė, RQUICKI, kūno riebalai, NEFA, pieningumas.

Introduction. Recent increases in milk production per cow in many countries are considered to be a result of selection of cows capable of partitioning proportionally more energy into milk, and less into body reserves (Agnew & Yan 2000, Yan et al. 2006). In addition, selection for higher milk yield has been associated with an increase in cow dry matter intakes (Korver 1988, Sæther et al. 2010) and intensified mobilization of body reserves (Theilgaard et al. 2002, Beerda et al. 2007). However, milk production potential appears to be mainly related to intensified lipid mobilisation, and rather less importantly to feed intakes (van Arendonk et al. 1991, Veerkamp et al. 2000). This has caused deeper nadirs and prolonged periods of negative energy balance (Veerkamp et al. 2003), accompanied by increased incidence of *post partum* metabolic diseases (Goff 2006), reduced fertility (Lucy 2001) and poor welfare (Nielsen 1999).

In the dairy cow insulin and tissue sensitivity to insulin play a key role in *post partum* lipid mobilization. At the beginning of lactation blood insulin concentration decreases compared to the *pre partum* level (Blum et al. 1973, Jaakson et al. 2007), insulin response to glucose infusion reduces (Opsomer et al. 1999), and adipose tissue becomes resistant to the lipogenic effect of insulin (Debras et al. 1989). Insulin resistance is defined as a condition in which a normal concentration of insulin produces less than the normal biological response (Kahn 1978) or, a greater amount of insulin is required to evoke a normal response (Berson & Yalow 1970). Hayirli (2006) proposed a broader definition: insulin resistance is a state that comprises insulin deficiency, decreased pancreatic glucose-induced insulin production and a reduced effect of insulin on the target tissues. Moderate insulin resistance is common in dairy cows at the beginning of lactation. It enhances non-esterified fatty acid (NEFA) release from adipose tissue to cover energy requirements and supports milk production under negative energy balance conditions. At the same time, elevated NEFA levels in turn play an important role in the development of insulin resistance (Pires et al. 2007, Kokkonen et al. 2009). Insulin resistance is related to several factors: it has been shown that within one breed cows with higher genetic merit and higher milk production potential experience more pronounced *post partum* insulin resistance (Hammon et al. 2007, Chagas et al. 2009); in addition, a higher extent of insulin resistance has been observed in dairy cows compared to beef cows (Shingu et al. 2002), and in high producing breeds compared to breeds with lower milk production potential (Jaakson et al. 2010). In addition to genetic factors, Body Condition Score (BCS) plays a role in the development of *post partum* insulin resistance: cows with moderate BCS experience milder insulin resistance compared to those with either a suboptimal BCS (Oikawa & Oetzel 2006) or a high BCS (Holtenius et al 2003). Nevertheless, the aetiology of insulin resistance in thin and obese cows seems to be different. Although accompanied by lipid mobilization in both cases, the development of *post partum* insulin resistance in thin cows is mainly related to previous malnutrition and reduced pancreatic insulin secretion (Hayirli 2006), while in obese cows insulin resistance develops secondarily as a consequence of *pre partum* hyperinsulinaemia (Flores-Riveros et al. 1993) and the long-term appetite-depressing effect of hyperleptinaemia (Ingvartsen & Boisclair 2001).

It has been demonstrated previously, using the glucose tolerance test (GTT), that a less pronounced glucose-induced increase in blood insulin concentration was accompanied by higher blood glucose concentrations in Estonian Holstein (EH) compared to Estonian Red (ER) cows (Jaakson et al. 2010). In the present study it was hypothesized that glucose-induced insulin and glucose responses are related to cows' BCS and milk production level. Therefore, the aim of this study was to examine blood glucose and insulin responses in relation to BCS and milk yield in EH and ER cows, using the GTT.

Materials and Methods. The field study was carried out on two dairy farms under similar management (Farms A and B) during the indoor period on clinically healthy 2^{nd} to 5^{th} parity EH (n=16; six cows from farm A and 10 from farm B) and ER (n=15; nine cows from farm A and six from farm B) cows. Cows' bodyweights (BW) were 674±23 kg for EH and 583±22 kg for ER. On both farms cows were housed in tie-stall barns and offered a total mixed ration (TMR) *ad libitum* twice a day. Samples of TMR ingredients were collected once a week throughout the whole experimental period (March and April 2005 on farm A and February 2008 on farm B) and pooled. TMR feed values were calculated on the basis of the chemical composition of the ingredients, according to AOAC (2005) methods, on the pooled samples. TMR offered to cows provided 11.4 MJ metabolizable energy (ME) and 96.6 g metabolizable protein (MP) per kg dry matter (DM) on farm A, and 12.0 MJ ME and 105.5 g MP per kg DM on farm B (Table 1). Approximate DM intake (DMI) was about 22 kg/d per cow on both farms, and this was evaluated as follows. On both farms, during the whole experimental period, the amount of TMR offered was weighed and, on the basis of visual daily assessment of feed refusal, mean weekly DMIs of the feeding groups (15 to 150 days in milk) including experimental cows during the glucose tolerance test (GTT), were estimated. Using the procedure described by Edmonson et al. (1989), the cows BCs were scored weekly from the week before expected calving to week of the GTT, and BCS loss from calving to the time of the GTT was calculated. Cows were milked thrice a day on both farms. Mean 305-day energy corrected milk (ECM) yield from the previous lactation in the experimental groups was 8,999±319 kg for EH and 8,253±287 kg for ER cows. ECM yields at the time of the experiment, calculated as the mean ECM of the day before and the day after the GTT, as described by Saunja et al. (1991), were 36.8±2.6 kg/d (EH) and 38.0±2.5 kg/d (ER); mean milk constituents, determined by infrared spectrometry (MilkoScan 4300, Foss Electric A/S), were 4.56±0.25 % and 4.66±0.25 % for fat, and 3.11±0.11 % and 3.64±0.11 % for protein in EH and ER respectively.

Table 1. **Ingredients and chemical composition of TMR in farms A and B**

	Farm A	Farm B
Ingredients (% DM)		
Grass silage	37.31	20.29
Corn silage	-	15.91
Hay	2.44	3.67
Barley meal	14.08	-
Corn meal	23.23	9.64
Wheat meal	-	9.62
Rape cake	18.92	25.87
Soyabean meal	2.58	7.56
Palm oil	-	2.24
Brewers grains	-	4.93
Mineral feed	0.36	0.55
Limestone	0.24	0.42
Salt	0.57	0.42
Soda	-	0.85
Chemical composition		
Dry matter (%)	53.4	56.2
In dry matter		
NDF (g/kg)	314.2	372.0
ADF (g/kg)	213.9	227.8
Crude protein (g/kg)	164.5	197.5
Crude fat (g/kg)	57.9	70.2
Ca (g/kg)	8.6	6.2
P (g/kg)	5.1	5.3
ME (MJ/kg)	11.4	12.0
MP (g/kg)	96.6	105.5

Cows were deprived of feed 60 min before and during the GTT, which was carried out 31±1.9 days *post partum*. Glucose (0.15 g/kg BW; 40%-solution; Inj. Glucosi 40%; Vetoquinol Biowet Sp. z o.o.) was infused and blood was sampled using a jugular vein catheter (12G, Lenght 80mm; Jørgen Kruuse A/S). Blood samples were collected into vacuum tubes with Li-heparin (BD Vacutainer Systems) at the following times: -15, -5, 5, 10, 20, 30, 40, 50 and 60 min relative to the start of infusion. Plasma was separated by centrifugation (5000xg, 15 min) and kept at -24°C until analysed spectrophotometrically (Helios β; Unicam Ltd.) for glucose (mmol/l) using test kits from Human Gesellschaft für Biochemica und Diagonostica GmbH, for NEFA (μmol/l) using test kits from Randox Laboratories Ltd., and radio-immunologically (Wallac 1470 Wizard Gamma Counter; Perkin Elmer Life and Analytical Sciences Inc.) for insulin concentration (μIU/ml) using ^{125}I radioimmunoassay test kits (Coat-A-Count Insulin) from Siemens Medical Solutions Diagnostics. The inter- and intra-assay coefficients of variation of the methods were below 6 %.

The following GTT characteristics were calculated: basal concentration (mean of pre-infusion samples) for all metabolites; maximum increase (MAX), calculated as the difference between the basal concentration and the highest concentration, and area under the curve (AUC), as described by Holtenius et al. (2003), for glucose and insulin; corrected maximum increase (MAX_c) and corrected AUC (AUC_c) for insulin according to Bossaert et al. (2008); clearance rate (CR) for glucose according to Pires et al. (2007) and the Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) by Rabasa-Lhoret et al. (2003). Relationships between GTT characteristics, BCS measurements (BCS at calving and at the time of the GTT, and BCS loss) and milk production traits (ECM-yield and fat content) were analyzed using Spearman Correlation Analysis. For comparison of the breeds' BCS at calving, BCS at the time of the GTT, BCS loss, ECM-yield, and milk fat and protein content at the time of the GTT, a two-factorial analysis of variance considering effects of breed and farm was used. The breed by farm interaction effect was omitted as it was not statistically significant (P>0.05) for any of the studied characteristics. Statistical analyses were performed using the SAS System (version 9.1.3). Values given in the text are least square means presented with standard errors. Significance has been declared as follows: significant (P≤0.05), tendency (P≤0.10). Correction for multiple testing was made by the Bonferroni-Holm method.

Results and Discussion. Mean BCS was lower in EH compared to ER cows both at calving (3.27±0.12 and 3.50±0.12, P<0.05) and at the time of the GTT (2.71±0.11 and 3.02±0.11, P<0.05; Figure 1B), representing typical BCSs for these breeds on Estonian commercial farms. BCS decreased from calving to GTT in both breeds (P<0.05) there being no significant between-breed difference in the BCS loss (0.56±0.08 in EH and 0.48±0.08 in ER; Figure 1B). Differences in BCS at calving between EH and ER cows, housed and fed similarly before the experiment, could reflect differences in energy and nutrient utilization in the breeds. Indeed Holsteins, as high producers, appear to be less efficient at restoring fat stores before the next lactation cycle compared to Red breeds (Ling et al. 2006, Rodriguez-Martinez et al. 2008).

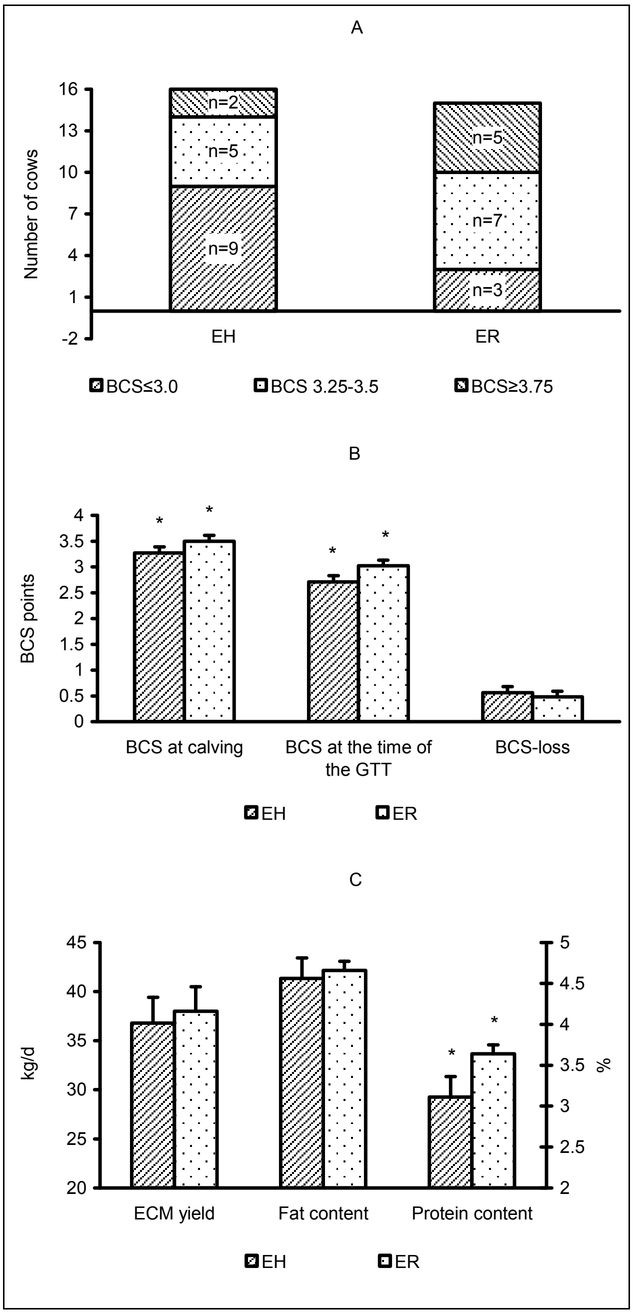

Fig. 1**. Distribution of Estonian Holstein** (EH) **and Estonian Red** (ER) **cows into: A, Body Condition Score** (BCS) **groups at calving; B, least square means, presented with standard errors, for breeds BCS measurements and C, milk production traits at the time of the glucose tolerance test** (GTT). **Asterisks above the error bars indicate significant differences** (P<0.05) **between the breeds**

Mean values of GTT characteristics investigated and blood NEFA basal concentrations are presented in Table 2. There were no significant differences between the breeds, except NEFA basal level, which was higher (P<0.01) in EH (842±144 μmol/l) compared to ER (539±70 μmol/l) cows. Correlations of BCS measurements with GTT characteristics were different between breeds (Table 3). In the EH breed, representing mostly thin cows and cows with moderate BCS at calving (Fig. 1A), a higher BCS, both at calving and at the time of the GTT, was associated with a more pronounced insulin response: BCS at calving tended to correlate positively with the MAX_c of insulin and BCS at the time of the GTT correlated positively with MAX_c and the AUC_c of insulin (Table 3). The relationships between BCS measurements and GTT characteristics for insulin indicate greater impairment of insulin function in thinner cows compared to those with a higher BCS in EH. As noted by Kerestes (2009) impaired insulin function in thin cows is mainly related to reduced pancreatic insulin secretion as a consequence of previous malnutrition. Hove (1978) observed reduced glucose-induced insulin secretion in starved cows compared to controls. In addition, Hove (1978) also demonstrated that reduced insulin secretion was accompanied by decreased blood glucose clearance, another characteristic of impaired insulin function.

In this study the EH breed at calving represented mostly thin cows or cows with moderate BCS (Fig. 1A). BCS at calving was correlated positively with NEFA basal concentration (Table 3), which indicates greater NEFA release from the adipose tissue in EH cows with higher BCS compared to those with lower BCS. To explain less pronounced NEFA mobilization in EH cows with a lower BCS at calving it is suggested that they may not have had sufficient body reserves available to mobilize to meet requirements. This is supported by the relationship between BCS at calving and BCS loss found in this study: EH cows with a lower BCS at calving had smaller BCS losses in the *post partum* period (r=0.63; P<0.01).

In the ER breed, representing for the most part cows with moderate BCS and over-conditioned cows (Fig. 1A), BCS at calving was positively correlated with MAX and with the AUC of glucose, and negatively with the CR of glucose (Table 3) indicating reduced post-infusion glucose clearance in cows with higher BCS at calving in this breed. In addition, BCS loss was positively correlated with MAX of glucose, and tended to correlate negatively with CR of glucose in this breed (Table 3). Furthermore, the positive correlation between BCS at calving and BCS loss (r=0.61; P<0.05) indicates a more rapid lipid mobilization in over-conditioned ER cows. These results accord with a lower clearance rate of glucose during the GTT in cows that were over-conditioned at calving observed by Holtenius et al. (2003), indicating reduced glucose uptake by body tissues in these cows, compared to the thinner cows. One of the factors linking *pre partum* obesity with impaired *post partum* insulin resistance appears to be the long term appetite-depressive effect of *pre partum* hyperleptinaemia in over-conditioned cows (Ingvartsen & Boisclair 2001) leading to more pronounced depression of feed intake, followed by excessive lipid mobilization, rapid BCS loss and the development of an insulin resistant state. In addition, over-conditioning before calving is associated with the development of transient *pre partum* hyperinsulinaemia in cows (Holtenius et al. 2003), which could be another link between *pre partum* obesity and *post partum* insulin resistance. As has been demonstrated in non-ruminant species, hyperinsulinaemia is related to a reduced number of insulin receptors (Suagee et al. 2011) and insulin-sensitive glucose transporters in adipose tissue (Flores-Riveros et al. 1993, Brennan et al. 2004, Suagee et al. 2011). This has not been reported in cows; however, if existing it could contribute to *post partum* development of more pronounced insulin resistance, along with reduced glucose uptake by adipocytes and intensified NEFA release from adipose tissue in over-conditioned cows at calving, compared to those in a moderate condition.

Table 2. **Least square means of glucose tolerance test** (GTT) **characteristics for blood glucose and insulin, and NEFA basal concentrations in Estonian Holstein** (EH) **and Estonian Red** (ER) **cows**

Characteristic	Breed	
	EH	ER
Glucose		
Basal concentration; mmol/l	5.10±0.30	4.25±0.21
Maximum increase relative to basal concentration (MAX of glucose); mmol/l	6.01±0.34	5.60±0.45
Area under the curve (AUC of glucose); mmol/l×min	128.31±10.23	111.02±11.29
Clearance rate (CR); %/min	1.47±0.11	1.47±0.10
Insulin		
Basal concentration; µIU/ml	3.7±0.7	1.8±0.5
Maximum increase relative to basal concentration (MAX of insulin); µIU/ml	60.0±15.4	53.6±11.5
Corrected maximum increase relative to basal concentration (MAX_c of insulin); µIU/ml	0.48±0.1	0.66±0.2
Area under the curve (AUC of insulin); µIU/ml×min	1428.2±398	1021.4±237
Corrected area under the curve (AUC_c of insulin); µIU/ml×min	11.3±2.8	11.9±3.5
NEFA		
Basal concentration; µmol/l*	842±144	539±70

*Significant (P<0.01) differences between the breeds.

Table 3. **Spearman correlations of glucose tolerance test** (GTT) **characteristics and blood non-esterified fatty acids** (NEFA) **basal concentration with body condition score** (BCS) **measurements** (BCS at calving, BCS at the time of the GTT, BCS loss)**, with ECM-yield and milk fat content in Estonian Holstein** (EH) **and Estonian Red** (ER) **cows**

Characteristic	BCS						ECM-yield		Milk fat content	
	At calving		At the time of GTT		Loss					
	EH	ER	EH	ER	EH	ER	EH	ER	EH	ER
Glucose										
Basal concentration; mmol/l	-0.04	0.06	0.15	0.14	-0.19	0.12	**-0.49**†	-0.41	-0.09	-0.21
Maximum increase relative to basal concentration (MAX of glucose); mmol/l	-0.06	**0.72****	0.24	0.41	-0.30	**0.52***	0.21	**0.48**†	0.06	0.13
Area under the curve (AUC of glucose); mmol/l×min	0.04	**0.58***	0.06	0.33	-0.11	0.39	0.04	0.21	0.14	-0.24
Clearance rate (CR); %/min	-0.08	**-0.58***	-0.24	-0.37	0.15	**-0.46**†	0.21	**-0.44**†	-0.13	-0.03
Insulin										
Basal concentration	0.09	-0.05	0.40	0.30	-0.23	-0.28	0.03	0.04	0.26	0.08
Corrected maximum increase relative to basal concentration (MAX_c of insulin); µIU/ml	**0.43**†	-0,36	**0.60***	0.03	0.07	**-0.47**†	0.11	-0.33	0.38	-0.01
Corrected area under the curve (AUC_c of insulin); µIU/ml×min	0.37	-0.32	**0.63****	0.04	-0.07	-0.39	0.03	-0.41	0.39	-0.03
NEFA										
Basal concentration; µmol/l	**0.74****	0.32	**0.52***	*0.29*	***0.49***†	0.31	0.08	**0.51***	**0.65****	**0.69****

X.XX** – P<0.01 (not significant after Bonferroni-Holm correction)
X.XX* – P<0.05 (not significant after Bonferroni-Holm correction)
X.XX† – P<0.1 (no tendency after Bonferroni-Holm correction)

The differences in relationships observed between BCS measurements and GTT characteristics in ER and EH cows in this study can be explained by differences in the BCSs of the cows of the two breeds around calving (Fig. 1A). It has been suggested that cows should calve at approximately BCS 3.5 (Samarütel et al. 2006, Roche et al. 2009). If grouped as follows: thin (BCS≤3.0), moderate BCS (3.25-3.5) and over conditioned (BCS≥3.75) at calving, only two cows amongst the EH were classified as over-conditioned, and three cows amongst the ER as thin (Fig. 1A). For EH the correlations found may reflect relationships between BCS measurements and GTT parameters mainly for cows with a moderate BCS at calving, in comparison to thin cows. For the ER cows these relationships were found mainly for comparisons between cows with moderate BCS and over-conditioned cows. These results, together with previous reports (Holtenius et al. 2003, Oikawa & Oetzel 2006), therefore suggest impaired insulin function in over-conditioned cows in ER, and in thin cows in EH, indicating the possibility of a non-linear relationship between BCS and insulin function.

The RQUICKI has been used as an index of reduced insulin sensitivity in humans (Rabasa-Lhoret 2003). Holtenius and Holtenius (2007) reported a relationship between over-conditioning at calving and lower *post partum* RQUICKI in Swedish Red and White breed cows, and proposed that RQUICKI could have potential as an insulin resistance measure in dairy cows. Bobowiec et al. (2011) described similar relationships in Holstein-Friesian cows. In the current study there was no significant correlation between BCS at calving and RQUICKI. However, in ER, representing more over-conditioned cows compared to EH (Fig. 1A), there was a significant negative correlation between BCS loss and RQUICKI ($R=-0.52$; $P<0.05$). As more pronounced BCS loss was correlated with a higher BCS at calving ($R=0.67$; $P<0.01$), the results for ER indirectly support the idea of a link between higher BCS and lower RQUICKI and, together with relationships between BCS measurements and GTT characteristics, indicate a greater degree of insulin resistance in over-conditioned cows in this breed. More importantly, in ER RQUICKI was negatively correlated with MAX of glucose ($R=-0.71$; $P<0.01$) and with AUC of glucose ($R=-0.67$; $P<0.01$), indicating that a lower RQUICKI is associated with reduced blood glucose clearance and therefore could be used as an insulin resistance marker. At the same time, results from a Hungarian experiment on Holstein cows, with BCSs comparable to the EH cows in this study, identified no relationships between RQUICKI and BCS on days 7 or 25 *post partum*, or with BCS loss (Kerestes et al. 2009). It might be possible that RQUICKI is not a reliable insulin resistance marker across the whole range of BCSs.

Mean ECM yields at the time of the GTT (36.8±2.6 kg/d in EH and 38.0±2.5 kg/d in ER) and milk fat contents (4.56±0.25 % in EH and 4.66±0.25 % in ER) were not different between breeds, although milk protein contents were lower in EH compared to ER (3.11±0.11 % and 3.64±0.11 %; $P<0.05$; Fig. 1C). Relationships between ECM-yield and milk fat content with GTT characteristics are presented in Table 3. In EH cows ECM-yield at the time of the GTT tended to be correlated negatively with blood glucose basal concentration, and milk fat content was correlated positively with blood NEFA basal level (Table 3), indicating that dairy cows preferentially partition circulating glucose and mobilized fatty acids into milk. In ER cows a higher ECM yield at the time of the GTT tended to be correlated with a higher MAX of glucose and with a slower CR of glucose (Table 3). This indicates delayed stabilization of blood glucose levels, probably due to impaired insulin signalling and reduced insulin-dependent uptake of glucose by tissues, therefore increasing the availability of glucose that can be taken up insulin-independently by the mammary gland for use in milk synthesis. This is in accordance with Hammon et al. (2007) who reported a relationship between higher milk yield at the time of the GTT and a lengthened glucose half-life. Bossaert et al. (2008) found that higher milk yield was related to a larger AUC of glucose, suggesting slower post-infusion stabilization of blood glucose levels in cows with a higher milk yield. In addition; in ER cows higher ECM-yields and higher milk fat contents were associated with higher blood NEFA basal concentrations, indicating relationships between impaired insulin signalling and greater release of NEFAs from adipose tissue, which in turn can be used in milk fat synthesis.

Conclusions

Relationships between BCS measurements and GTT characteristics were different between breeds. The results indicate greater impairment in insulin function in thin and over-conditioned cows compared to cows with a moderate BCS at calving. Relationships were found between milk production and adipose tissue mobilization.

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