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Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period

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Abstract

During the early postpartum period dairy cows mobilize fat and muscle to support lactation. This is associated with alterations in blood metabolite and hormone profiles which in turn influence milk yield and fertility. This study developed models to determine how metabolic traits, milk yield and body condition score were inter-related at different times in the periparturient period and to compare these relationships in primiparous (PP, $n = 188$) and multiparous (MP, $n = 312$) cows. Data from four previous studies which included information on blood metabolic parameters, parity, milk yield, body condition score and diet were collated into a single dataset. Coefficients of polynomial equations were calculated for each trait between −1 week pre-calving and week +7 postpartum using residual maximum likelihood modelling. The completed dataset was used in a multiple correlation model to determine how the best fit curves were related to each other over time. PP cows had higher concentrations of insulin-like growth factor-I and lower β-hydroxybutyrate concentrations throughout, higher leptin concentrations pre-partum and both the peak in non-esterified fatty acids and the nadir in urea concentration occurred earlier after calving. These differences were associated with significantly lower milk production. Leptin concentrations fell at calving and were related to body condition score.

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Insulin was negatively correlated with yield in MP cows only. In MP cows the relationship between insulin-like growth factor-I and yield switched from negative to positive between weeks +4 and +7. Both β-hydroxybutyrate and urea were positively related to yield in PP cows. In contrast, in MP cows β-hydroxybutyrate was negatively correlated with yield and urea was strongly related to body condition score but not yield. These results suggest that there are differences in the control of tissue mobilization between PP and MP cows which may promote nutrient partitioning into growth as well as milk during the first lactation.

Keywords: Dairy cow; Parity; IGF-I; Insulin; Milk yield; Body condition score

1. Introduction

The Holstein breed has been selected to produce high yields through a greater propensity for losing body condition to support milk production in early lactation and to target nutrients preferentially to the mammary gland. The late dry period coincides with the last phase of fetal growth, so the nutrient requirements for the gravid uterus increase, and within 4 days of calving a further dramatic rise in the demand for glucose, amino acids and fatty acids for milk synthesis occurs [1–3]. Non-esterified fatty acids (NEFA) released from lipid stores are either taken up by the udder to provide milk triglycerides or are oxidized in the liver as an alternative energy source. The plasma NEFA concentration is therefore an index of lipid mobilization, with a rise in NEFA pre-partum suggestive of an energy deficit at this time [4]. Following uptake by the liver, NEFA can be: (1) oxidized to carbon dioxide to provide energy; (2) partially oxidized to produce ketone bodies or acetate which are transported for use elsewhere in the body or (3) esterified to triglycerides or phospholipids [5]. β-Hydroxybutyrate (BHB) is the predominant form of ketone body in blood and its concentration is as an index of fatty acid oxidation. Blood urea in both late pregnant and early lactating ruminants may rise following mobilisation of amino acids stored in skeletal muscle [3] or in circumstances where dietary protein supply exceeds energy availability [6,7].

A number of metabolic hormone concentrations also change over this critical peripartum period. Genetic selection for milk production has been associated with a decline in circulating insulin levels in dairy cows [8,9] and insulin concentrations tend to fall in early lactation [10]. In addition adipose and muscle become insulin resistant in late gestation, but develop an increased sensitivity to lipolytic agents [3]. Together these changes increase lipolysis, reduce peripheral glucose uptake and increase glucose supply for milk lactose production. Circulating insulin-like growth factor-I (IGF-I) is released from the liver in response to growth hormone (GH) coupling to GH receptors [11]. IGF-I is believed to be the main mediator of GH on milk production [12] regulating milk synthesis by the mammary gland [13,14]. Liver GH receptors are, however, down regulated in the periparturient period [15] and in early lactation the relationship between IGF-I concentrations and yield is negative [16]. Plasma leptin concentrations in late pregnancy are strongly correlated to body condition score (BCS) [17] and also fall around parturition [18,19].

Dairy heifers are generally calved for the first time at about 24 months of age as this maximises economic benefit [20]. Animals are not however physically mature at this stage [21]. Cows approaching their first calving are therefore in a differing metabolic state to that experienced by multiparous cows as they require nutrients for their own continued growth in addition to that of their developing calf.

During early lactation most dairy cows enter a period of negative energy balance (NEB), due to their inability to consume sufficient feed to meet the metabolic demands outlined above [1,22]. The length and depth of NEB vary according to genetic merit and hence milk yield of the cows [8], pre-calving body condition [23,24], feed intake [25,26] and diet [27,28]. Previous work has established antagonistic links between energy balance and yield with fertility [29,30].

Relatively few previous studies have compared periparturient metabolic changes in first lactation (primiparous, PP) and more mature cows (multiparous, MP) and these have had limited numbers of animals in each age group (range $n = 21$–64) [8,31–33]. Available evidence indicates that parity can influence the pattern of changes in metabolic hormones and metabolites following calving but published data are inconsistent. NEFA was higher in the immediate periparturient period in PP cows [31,33] whereas NEFA tended to be higher in the first month of lactation in MP than PP cows [32]. BHB and IGF-I were reportedly either higher [31,33] or lower [32,33] in PP than MP cows, respectively. At least 30% of cows in the average UK herd will be in their first lactation. Understanding the basis for such metabolic responses may assist us in determining how they affect subsequent milk production and fertility. The aims of the present study were: (1) to use a modelling approach to determine how metabolic traits, milk yield and body condition score were inter-related at different times in the periparturient period and (2) to compare these relationships in PP and MP cows.

2. Materials and methods

2.1. Cows included in study and sample collection

Data from four previous studies conducted between 1997 and 2003 to investigate the relationships between metabolic parameters and fertility in Holstein Friesian dairy cows were collated into a single dataset (see Table 1). Each study included information on blood metabolic parameters, cow parity, diet, milk progesterone profile and fertility. This provided data from a total of 500 lactations: 38 of the cows were used in 2 consecutive years. All other animals were included once only. The fertility data are reported in a separate paper and are not considered further here. In each of these lactations, blood metabolites were measured at 1–2 weeks pre-calving and at weeks 2–3, 4–5 and 7–8 postpartum.

With two exceptions (groups which were milked three times daily for the first 4 months of lactation; see Table 1) all other groups of cows were milked twice daily. All cows were on total mixed rations (see below). New feed was provided after the morning milking and blood samples were collected approximately 2 h later. Blood was taken from the coccygeal vein into 9 ml heparinized vacutainer tubes. Samples were kept on ice, centrifuged immediately at $1600 \times g$ at 4 °C, aliquotted and stored at −20 °C. The metabolic hormones measured

Table 1
Summary information on dietary groups and milk yields for all cows included in the analysis

Farm	Diet group	Study	Year	*n*	Yield (kg/126 days)
Primiparous cows					
1	1	3	1999–2000	11	3000 ± 108
1	2	3	1999–2000	47	3265 ± 59
1	3	3	1999–2000	18	3477 ± 138
2	4[a]	3	1999–2000	38	3621 ± 89
2	5	3	1999–2000	44	3439 ± 90
3[b]	6	3	1999–2000	3	3756 ± 250
3[b]	7	3	1999–2000	3	3470 ± 21
4[b]	8	3	1999–2000	16	3867 ± 108
5[b]	9	3	1999–2000	8	4343 ± 365
Total PP				188	
Multiparous cows					
2	10	1	1997–1998	30	4128 ± 118
2	11[a]	2	1998–1999	28	5461 ± 123
2	12	2	1998–1999	20	4053 ± 140
2	13	3	1999–2000	26	5024 ± 205
2	14	3	1999–2000	15	5489 ± 198
2	15	3	1999–2000	15	5272 ± 179
2	16	4	2001–2002	49	4947 ± 86
3[b]	6	3	1999–2000	25	5212 ± 118
3[b]	7	3	1999–2000	18	4965 ± 198
4[b]	8	3	1999–2000	30	5116 ± 122
5[b]	9	3	1999–2000	22	5013 ± 134
6[b]	17	3	1999–2000	34	3904 ± 108
Total MP				312	

[a] Milked three times daily for the first 4 months of lactation. All other groups were milked twice daily.
[b] Milk yields were estimated from monthly milk recording records. All other groups used weekly recording.

were: insulin-like growth factor-I, insulin and leptin. The metabolites measured were: β-hydroxybutyrate, non-esterified fatty acids and urea. At each time point the BCS was also assessed using a 0–5 scale (lean to obese) with 0.5 intervals. In most of the groups parlour measurements of yield were automatically recorded at each milking for each animal, although measurements from some farms were based on monthly milk recording visits (see Table 1). All work was conducted under the Animals (Scientific Procedures) Act 1986.

2.2. *Dietary treatments*

All cows were fed a total mixed ration ad libitum. The characteristics of the diets fed to different groups are summarised in Table 2. Metabolizable energy (ME) values were in the range 11.0–12.4 MJ/kg dry matter (DM) and the crude protein content varied from 133 to 228 g/kg DM. Inclusion of dietary group in the model was used to account for management differences between the different groups of cows.

Table 2
Dietary information for all the nutritional groups included in the analyses

Group	Primiparous cow nutritional groups								
	1	3	2	4	5	6	7	8	9
Diet fed (kg/day DMI)									
Grass silage	5.4	5.0	5.0		2.5	3.1	3.1	4.2	7.2
Maize silage	5.1	6.3	6.3	6.7	7.4	5.0	5.0	5.3	7.2
Barley straw				0.3				0.4	
Lucerne				0.6					
Concentrates				5.5	7.8			6.4	2.6
Parlour cake						4.0	3.3	5.0	4.2
Wheat	3.4	1.5	1.7						
Rapeseed	2.2	1.3	2.3	0.8					
Regumaize 44				0.8	1.1				
Brewers grain						1.8	1.7		
Molasses				0.6				0.8	
Sugar beet pulp	2.7	1.8	1.3	0.6				1.3	
Linseed		1.8			0.7				
Soya	1.6	1.8	4.0			3.5	3.5		
Potatoes						1.0	1.0		
Bread						1.8	1.8		
Megalac							0.3		
Total DMI (kg/day)	20.4	19.5	20.2	15.9	19.5	20.2	19.7	23.4	21.2
CP (g/kg DM)	172	173	187	188	228	182	182	204	138
ME (MJ/kg DM)	118	123	122	119	119	121	124	124	110
CP:ME (g CP/MJ)	14.6	14.1	15.3	15.8	19.2	15.0	14.7	16.5	12.6

Group	Multiparous cow nutritional groups								
	6–9	10	11	12	13	14	15	16	17
Diet fed kg/day DMI									
Grass silage	As for PP cow groups	2.9	1.4	2.9				1.7	3.6
Maize silage		8.9	8.8	8.7	9.6	9.7	9.6	7.0	9.5
Barley straw			0.5		0.3	0.3	0.3		
Lucerne			3.2		0.9	0.9	0.9	1.2	
Hay								0.4	
Concentrates			8.7	8.1	7.9	8.0	7.9		4.3
Parlour cake									1.1
Wheat		4.2						2.9	
Rapeseed		2.1	1.1		1.1	1.2	1.1	1.9	
Regumaize 44					1.1	1.2	1.1	0.9	0.9
Molasses		0.8		1.0	0.9				0.7
Sugar beet pulp					0.9			0.9	
Ground maize							1.8	2.1	
Sopraline						1.8			
Soya		1.2						1.1	
Potatoes			1.1						
Megalac		0.2							
Fishmeal		0.3						0.3	
Total DMI (kg/day)		20.6	22.8	20.7	22.8	23.1	22.8	20.4	20.1
CP (g/kg)		198	178	172	188	184	189	165	133
ME (MJ/kg DM)		122	120	120	119	120	120	124	117
CP:ME (g CP/MJ)		16.2	14.8	14.5	15.8	15.4	15.8	13.3	11.4

All groups also included an appropriate mineral mix in their rations. DMI, dry matter intake; CP, crude protein; ME, metabolizable energy.

Table 3
Information on the actual number of samples obtained for measurement of milk yield, BCS, metabolites and metabolic hormones in primiparous (PP) and multiparous (MP) cows

	PP (*n*)	MP (*n*)	Comment
Total starting population	188	312	Some pre-calving samples were missed
Milk yield	93–97	159–202	Milk yields were estimated from monthly milk recording records for groups 6–9 and 17. All other groups used weekly recording
BCS	102–167	235–285	
IGF-I	180–184	258–291	
Insulin	178–184	262–291	
Leptin	143–167	105–246	Leptin results were not available for groups 10–12 and 16
BHB	157–184	228–246	BHB results were not available for groups 11 and 12
NEFA	140–162	175–199	NEFA results were not available for groups 11, 12 and 16
Urea	169–183	199–216	Urea results were not available for groups 10–12

Both the actual and modelled data are plotted in Figs. 1–3. Samples were collected pre-calving at week −1 and at +2, +4 and +7 weeks postpartum.

2.3. Measurement of blood traits

The IGF-I concentration in plasma was analyzed by radioimmunoassay after ethanol–acetone–acetic acid extraction of IGF binding proteins [34]. The inter- and intra-assay coefficients of variation were 11 and 7%, respectively. Plasma insulin was measured by bovine ELISA plate kits (DRG Diagnostics, Immuno Diagnostic Systems Ltd., Tyne and Wear, UK). The assay sensitivity was 0.20 ng/ml. The inter- and intra-assay coefficients of variation were both 9%. Leptin was measured by radioimmunoassay according to the method of Blache et al. [35]. This assay utilizes recombinant bovine leptin for the label with the primary antibody also raised against rb-leptin. Its validation has been described previously [35]. Inter- and intra-assay coefficients of variation were 13 and 8% and the sensitivity was 0.2 ng/ml. Plasma BHB, NEFA and urea concentrations were measured on an OPERA (OPerationally Enhanced Random Access) analyzer (Bayer, Newbury, Berks, UK) using kinetic enzymatic kits (Randox Laboratories Ltd. Co. Antrim, UK, NEFA test kit, BHB RANBUT D-3-hydroxybutyrate test kit, urea test kit). The ranges were BHB 0.1–1 mmol/l, NEFA 0.1–2 mmol/l and urea 0.1–7.5 mmol/l. Information on the actual number of samples measured at each time point is summarised in Table 3.

2.4. Statistical analysis and model development

Primiparous (PP, lactation number = 1) and multiparous cows (MP, lactation number >1, range 2–8) were considered separately throughout. Using the ASREML software package [36] (residual maximum likelihood), coefficients of polynomial equations were calculated for each trait measured between −1 week pre-calving and week +7 postpartum, with dietary group included as a fixed factor in the model. As there were repeated observations on each

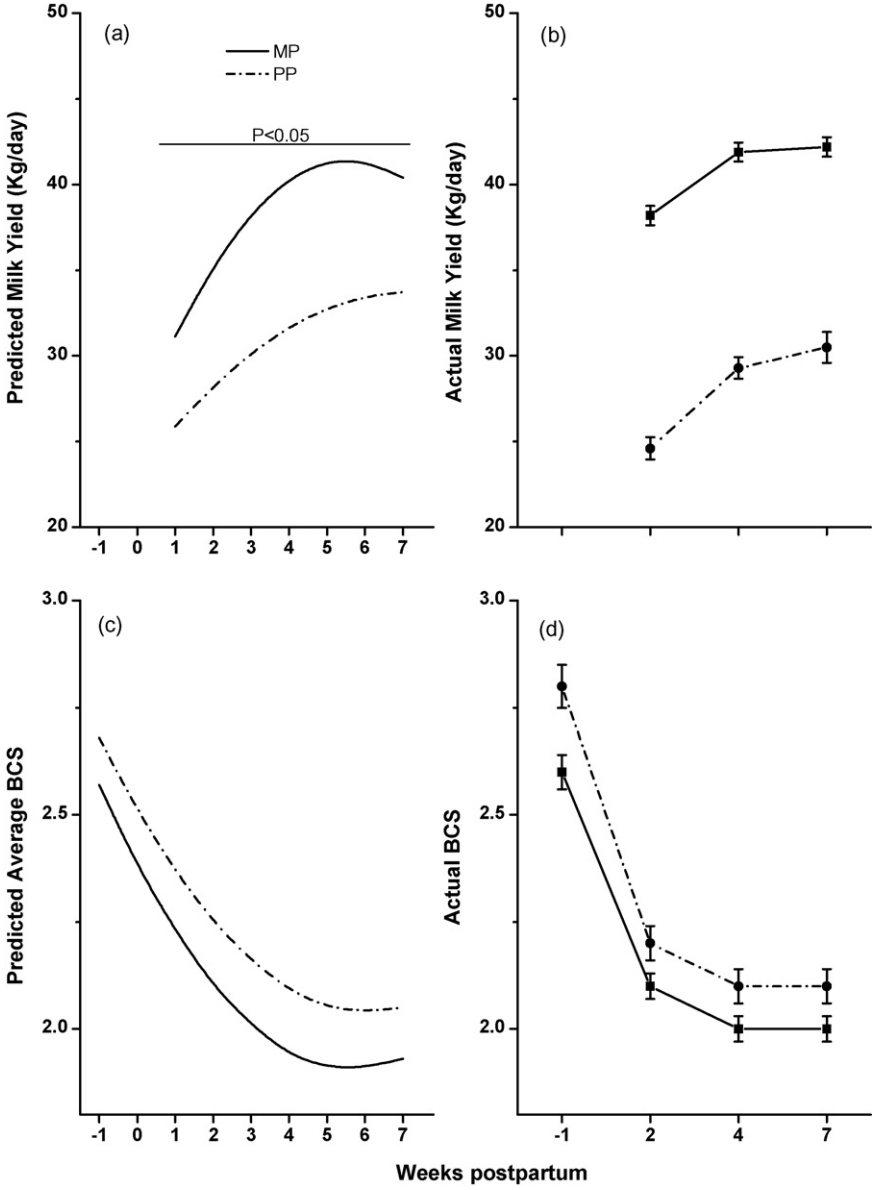

Fig. 1. Graphs showing the predicted LSM phenotypic values over time in comparison with the actual values obtained (mean ± S.E.M.) for (a and b) daily milk yield and (c and d) body condition score (BCS) for primiparous (PP) and multiparous (MP) cows. The number of animals sampled is summarized in Table 3. The SED values from the models were 1.24 for milk yield and 0.043 for BCS, respectively. Statistical analysis was only performed on the modelled data, which included dietary group as a fixed factor. Milk yield was significantly different throughout the study between MP and PP cows ($P < 0.05$).

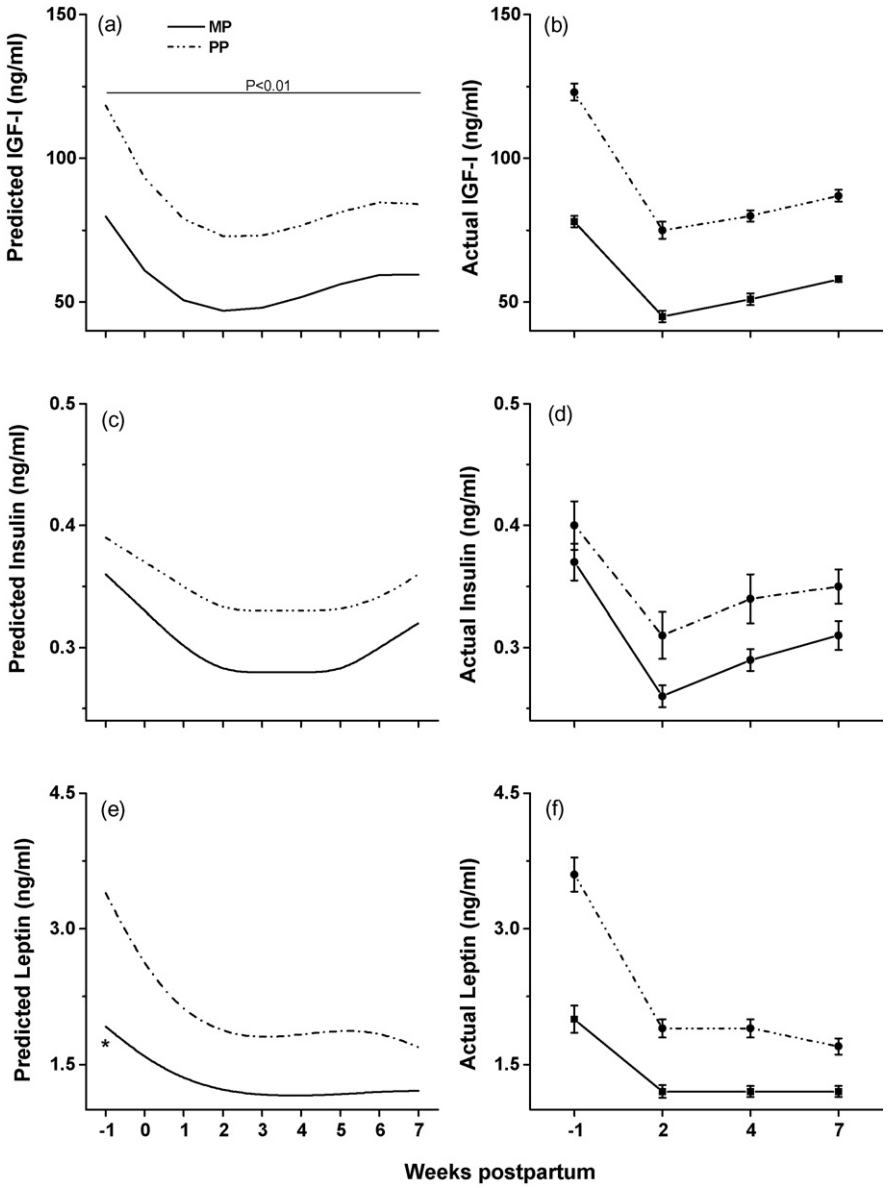

Fig. 2. Graphs showing the predicted LSM phenotypic hormone concentrations over time in comparison with the actual values obtained (mean ± S.E.M.) for (a and b) IGF-I, (c and d) insulin and (e and f) leptin for primiparous (PP) and multiparous (MP) cows. The number of animals sampled is summarized in Table 3. The SED values from the models were 2.89 for IGF-I, 0.017 for insulin and 0.15 for leptin, respectively. Statistical analysis was only performed on the modelled data, which included dietary group as a fixed factor. IGF-I was significantly different throughout the study between MP and PP cows ($P < 0.01$). Leptin was significantly different before calving only ($^{*}P < 0.01$).

Table 4
Best fit equation of each phenotypic trait for primiparous cows[a] ($n = 188$)

Trait
Milk yield = $14.93 + 0.4276\text{DIM} - 0.0043\text{DIM}^2$
BCS = $2.444 - 0.0221\text{DIM} + 0.00026\text{DIM}^2$
IGF-I = $120.3 - 0.2765\text{DIM} + 0.111\text{DIM}^2 - 0.0012\text{DIM}^3$
Insulin = $0.5476 - 0.0033\text{DIM} + 0.000066\text{DIM}^2$
Leptin = $2.905 - 0.0924\text{DIM} + 0.0035\text{DIM}^2 - 0.00004\text{DIM}^3$
BHB = $0.6057 + 0.0066\text{DIM} - 0.00009\text{DIM}^2$
NEFA = $0.2706 + 0.0026\text{DIM} - 0.00013\text{DIM}^2$
Urea = $3.842 - 0.0180\text{DIM} + 0.00065\text{DIM}^2$

DIM, days in milk. Milk yield was estimated from weekly or monthly milk records for the first 7–8 weeks of lactation. All other traits were based on records collected −1 week pre-calving and at approximately +2, +4 and +7 weeks postpartum.

[a] Dietary group was included in each model to account for differences in diet and management between farms.

cow, cow was included in all models as a random effect and cow deviations from the overall curve were allowed to vary linearly. Graphs for each trait were drawn using each equation, using the optimal statistical model that best fitted the data and was parsimonious. All equations were then used to predict missing values in the original dataset. The new dataset allowed changes for all metabolites, between each sample time point, to be determined. The completed dataset was used in a multiple correlation model to estimate the covariance between curves to determine how these were related to each other over time. This was done independently for: (a) the actual values at each time point and (b) the change in values between time points.

3. Results

3.1. Comparison according to parity

The best fit equation for the curves for each of these phenotypic traits are given in Table 4 (PP cows) and Table 5 (MP cows). The best fitting model was the model for milk yield (MY),

Table 5
Best fit equation of each of each phenotypic trait for multiparous cows[a] ($n = 312$)

Trait
Milk yield = $14.93 + 2.921 + 0.806\text{DIM} - 0.0105\text{DIM}^2$
BCS = $0.6057 + 0.1611 + 0.0209\text{DIM} - 0.000027\text{DIM}^2$
IGF-I = $120.3 - 32.32 - 2.032\text{DIM} + 0.0875\text{DIM}^2 - 0.00095\text{DIM}^3$
Insulin = $0.5475 - 0.0414 - 0.0042\text{DIM} + 0.000083\text{DIM}^2$
Leptin = $2.905 - 0.2995 - 0.0408\text{DIM} + 0.0012\text{DIM}^2 - 0.000016\text{DIM}^3$
BHB = $2.444 - 0.1272 - 0.0245\text{DIM} + 0.00031\text{DIM}^2$
NEFA = $0.2706 - 0.0864 + 0.013\text{DIM} - 0.00029\text{DIM}^2$
Urea = $3.842 + 0.3138 - 0.0144\text{DIM} + 0.00027\text{DIM}^2$

DIM, days in milk. Milk yield was estimated from weekly or monthly milk records for the first 7–8 weeks of lactation. All other traits were based on records collected −1 week pre-calving and at approximately +2, +4 and +7 weeks postpartum.

[a] Dietary group was included in each model to account for differences in diet and management between farms.

with approximately 94% of the variation in yield being accounted for, whereas the model for BHB only accounted for 33% of the variance (equivalent to a correlation between actual values and predicted values of 0.58). For the other traits the proportion of the variance accounted for by the model varied from 51% (NEFA) to 86% (BCS). Least square mean (LSM) phenotypic values for each trait over the 8-week period from −1 week pre-calving until week +7 of lactation are presented in Figs. 1 (MY and BCS), 2 (IGF-I, insulin and leptin) and 3 (NEFA, BHB and urea). These are compared with the actual values obtained for each trait at each of the four sampling time points, which indicate that there was good concordance between the two sets of graphs.

Best fit milk yield curves in both age groups were quadratic, with MP cows producing significantly more milk throughout the study period ($P < 0.05$). BCS loss over the first 7 weeks of lactation also followed a quadratic form. BCS values tended to be slightly higher pre-partum in the PP cows and a similar differential was maintained throughout the study period, indicating a similar rate of BCS loss which had stabilized by the end of the study period. There was, however, no significant difference between the BCS of PP and MP cows ($P > 0.05$ at all time points).

The change in IGF-I and leptin concentrations over time were best described by cubic polynomial equations. Values had started to decline pre-calving, reaching a minimum between 2 and 3 weeks postpartum. IGF-I concentrations then recovered slightly, whereas leptin concentrations remained low. Throughout the time period of the study IGF-I values were significantly higher in PP than MP cows ($P < 0.001$), but although leptin levels were also significantly higher in PP cows pre-calving ($P < 0.01$), no significant difference was detected from calving onwards. Insulin concentrations were described by a quadratic polynomial equation, again falling over parturition and reaching a nadir at 2–3 weeks postpartum. In all cases the LSM values were consistently, but not significantly, higher in PP than MP cows.

Best fit curves for BHB, NEFA and urea all followed a quadratic form. BHB LSM values increased over the study period, peaking at +5 weeks in PP cows and stabilizing by +7 weeks in MP cows. LSM values started much lower in PP cows and never reached those measured in MP cows despite a steeper initial rise. BHB values pre-calving and for 2 weeks after calving differed significantly between PP and MP cows ($P < 0.05$), but thereafter no significant difference was detected. The change in LSM values for NEFA followed a different pattern over time according to parity, although from calving onwards this difference was not statistically significant. PP cows calved with higher NEFA values which showed little further increase postpartum and started to fall from 2 weeks after calving. MP cows showed a steep rise and fall with values peaking at +3 weeks postpartum. The LSM values for urea did not differ significantly between PP and MP cows although in PP cows the nadir which occurred at +2 weeks was followed by a steeper rise.

3.2. Correlations between traits at individual time points

The completed dataset was then used in a multiple correlation model to estimate the covariance between curves to determine how the values at each of the four sampling time points were related to each other. The significant correlations between traits at each time are summarised in Table 6 (PP cows) and Table 7 (MP cows) and are illustrated diagram-

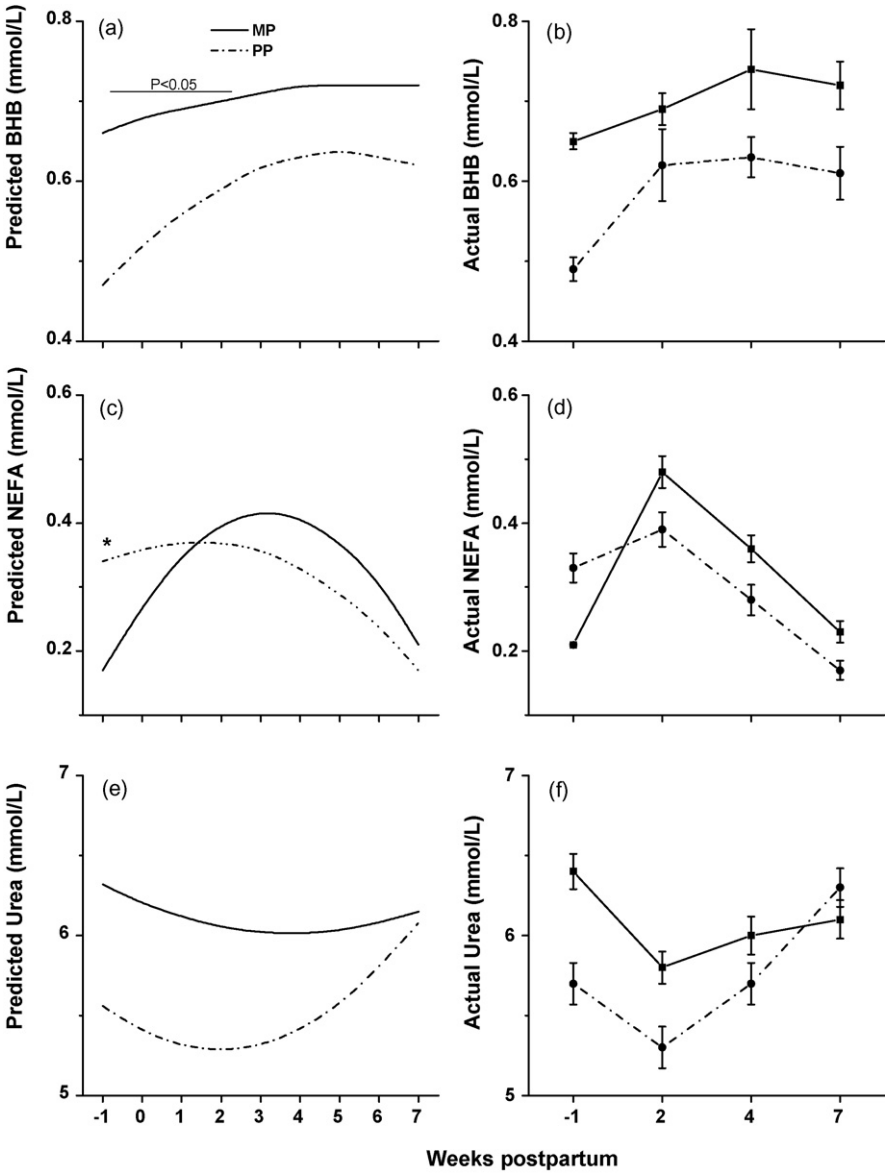

Fig. 3. Graphs showing the predicted LSM phenotypic metabolite concentrations over time in comparison with the actual values obtained (mean ± S.E.M.) for (a and b) BHB, (c and d) NEFAs and (e and f) urea for primiparous (PP) and multiparous (MP) cows. The number of animals sampled is summarized in Table 3. The SED values from the models were 0.032 for BHB, 0.023 for NEFAs and 0.12 for urea, respectively. Statistical analysis was only performed on the modelled data, which included dietary group as a fixed factor. BHB was significantly different from weeks −1 to +2 between MP and PP cows ($P < 0.05$). NEFAs were significantly different before calving only ($^{*}P < 0.001$).

Table 6
Correlations between the traits recorded at each of four time points in relation to calving for primiparous cows

	BCS	IGF	INSUL	LEPT	BHB	NEFA	Urea
Week −1							
BCS		ns	ns	ns	ns	ns	ns
IGF-I			0.31^{***}	ns	-0.30^{***}	-0.13^{*}	-0.14^{*}
INSUL				ns	ns	-0.16^{**}	ns
Leptin					ns	ns	-0.12^{*}
BHB						0.26^{***}	0.20^{***}
NEFA							ns
Week +2							
MY*	ns	ns	ns	ns	ns	ns	ns
BCS		0.13^{***}	ns	0.21^{***}	ns	ns	ns
IGF-I			0.30^{***}	ns	-0.33^{***}	-0.38^{***}	ns
INSUL				ns	ns	-0.17^{**}	ns
Leptin					ns	-0.13^{*}	ns
BHB						0.36^{***}	ns
NEFA							ns
Week +4							
MY*	ns	ns	ns	ns	0.19^{**}	ns	0.12^{*}
BCS		0.20^{***}	ns	ns	ns	ns	ns
IGF-I			0.34^{***}	ns	-0.24^{***}	-0.29^{***}	0.27^{***}
INSUL				ns	ns	ns	0.12^{*}
Leptin					ns	ns	ns
BHB						0.61^{***}	ns
NEFA							-0.23^{***}
Week +7							
MY*	ns	ns	ns	ns	ns	ns	0.14^{*}
BCS		ns	ns	0.16^{**}	ns	-0.13^{*}	ns
IGF-I			0.26^{***}	ns	-0.15^{*}	ns	ns
INSUL				0.13^{*}	ns	-0.20^{**}	ns
Leptin					ns	ns	ns
BHB						ns	ns
NEFA							ns

Milk yield (MY) was only included in the model for the times after calving. Significant correlations are indicated: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$; ns, not significant.

matically in Fig. 4. In PP cows, MY was positively related to BHB in week +4 and to urea in weeks +4 to +7 but showed no relationships with other metabolic traits. In MP cows MY was strongly negatively related to insulin at all postpartum time points. It also showed a negative relationship with IGF-I at week +4 which switched to positive at week +7. At week +7 there was an additional relationship of MY with BHB (−ve) and NEFA (+ve).

In PP cows, BCS was related to the IGF-I (+ve) and leptin (+ve) concentration after calving, but showed no significant correlation with any of the metabolic trait measurements made before calving. In MP cows, the relationship between BCS and urea was consistently negative throughout the study period, whereas a positive relationship with IGF-I and insulin was present from week +4. BCS and leptin in MP cows were positively related before and at week +7 after calving, but not during the early part of lactation.

Table 7
Correlations between the traits recorded at each of four time points in relation to calving for multiparous cows

	BCS	IGF	INSUL	LEPT	BHB	NEFA	Urea
Week −1							
BCS	ns	ns	ns	0.19^{**}	ns	ns	-0.12^{*}
IGF-I			0.21^{***}	ns	ns	-0.22^{***}	ns
INSUL				ns	0.15^{*}	-0.15^{*}	ns
Leptin					ns	-0.14^{*}	-0.15^{*}
BHB						ns	0.13^{*}
NEFA							ns
Week +2							
MY	ns	ns	-0.22^{***}	ns	ns	ns	ns
BCS		ns	ns	ns	ns	ns	-0.18^{**}
IGF-I			0.15^{*}	0.19^{**}	-0.19^{**}	-0.15^{*}	ns
INSUL				ns	0.24^{***}	ns	ns
Leptin					ns	ns	ns
BHB						0.28^{***}	ns
NEFA							ns
Week +4							
MY	ns	-0.13^{*}	-0.17^{**}	ns	ns	ns	ns
BCS		0.27^{***}	0.17^{**}	ns	ns	ns	-0.14^{*}
IGF-I			0.28^{***}	ns	-0.19^{***}	-0.22^{***}	ns
INSUL				ns	ns	ns	ns
Leptin					ns	ns	ns
BHB						0.32^{***}	ns
NEFA							ns
Week +7							
MY	ns	0.15^{*}	-0.22^{***}	ns	-0.16^{**}	0.15^{*}	ns
BCS		0.31^{***}	ns	0.16^{**}	ns	ns	-0.22^{***}
IGF-I			0.15^{*}	ns	-0.16^{**}	ns	ns
INSUL				ns	ns	ns	ns
Leptin					ns	ns	ns
BHB						ns	ns
NEFA							ns

Milk yield (MY) was only included in the model for the times after calving. Significant correlations are indicated: $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$; ns, not significant.

IGF-I and insulin showed a significant positive correlation at all time points in both parity groups. In PP cows IGF-I was also negatively related to BHB and NEFA before and after calving, whereas the relationship with urea switched from negative pre-partum to positive in week +4. In MP cows IGF-I showed negative relationships to BHB after calving and to NEFA pre- and postpartum. In addition to the positive relationship with IGF-I, insulin in PP cows was negatively related to NEFA before and after calving. It was weakly positively related to urea in week +4 but showed no significant correlations with BHB. In MP cows a negative relationship between insulin and NEFA was only present before calving, whereas insulin was unrelated to urea but was positively related to BHB at weeks −1 and +2. In addition to the relationships with BCS described above, leptin values in PP cows were correlated with urea (−ve week −1), NEFA (−ve week +2) and insulin (+ve week +7). In

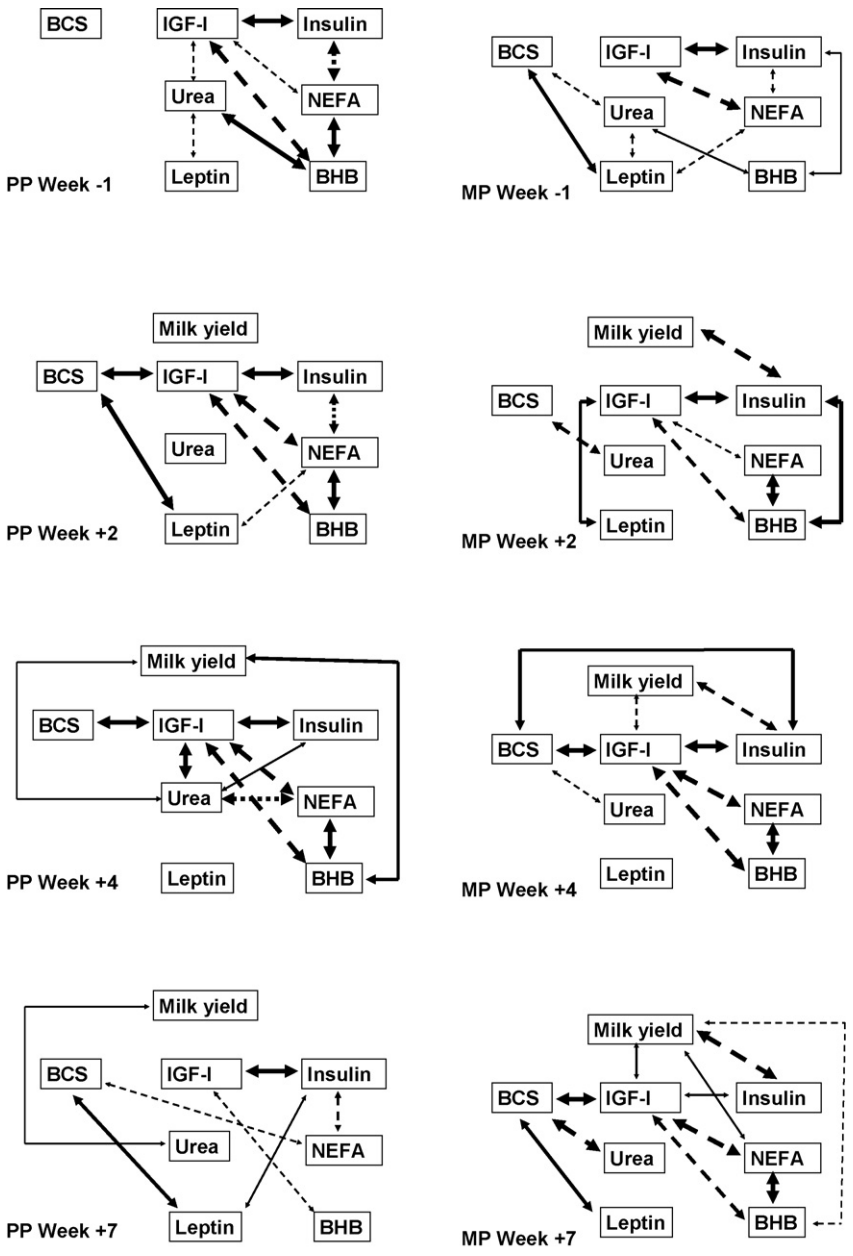

Fig. 4. Summary diagram showing how the correlations between phenotypic traits recorded at each of four time points change in relation to calving and how these relationships differ for primiparous (PP) and multiparous (MP) cows. The times were −1 week pre-partum and +2, +4 and +7 weeks postpartum. Solid arrows represent positive and dotted lines negative correlations. The thickness of each arrow indicates the degree of significance, increasing from $P < 0.05$ to $P < 0.01$ and $P < 0.001$. See Tables 6 and 7 for the actual P-values.

MP cows there were similarly negative relationships of leptin with NEFA and urea (both week −1) and in addition a positive correlation with IGF-I was present in week +2.

There were consistent significant correlations between BHB with NEFA (+ve, as mentioned above) and BHB was also related to IGF-I (−ve) in both parity groups and at several time points. In both parity groups BHB was also positively related to urea before calving. In PP cows MY in week +4 was positively correlated with BHB, in contrast to the negative correlation between BHB with MY in week +7 in MP cows. For NEFA, in addition to the consistent correlations with BHB (+ve) and IGF-I (−ve) already described, there was also a negative correlation with insulin before calving in both parity groups which persisted throughout the postpartum period in PP cows. A negative relationship with leptin was present around calving in both parity groups. NEFA was also negatively related to urea in week +4 in PP cows and positively to MY in week +7 in MP cows.

Summarizing the correlations for urea, these showed a completely different set of relationships according to parity. In PP cows there were significant negative correlations with leptin (week −1) and NEFA (week +4) and positive correlations with BHB (week −1) and insulin (week +4), whereas the relationship with IGF-I switched from negative to positive over this period. Urea was positively related to MY in weeks +4 to +7. In MP cows the

Table 8
Correlations between the changes in traits over time for primiparous cows

	BCS	IGF	INSUL	LEPT	BHB	NEFA	Urea
Weeks −1 to +2							
BCS		ns	ns	ns	ns	ns	ns
IGF-I			0.21^{***}	0.14^{*}	-0.34^{***}	-0.28^{***}	ns
INSUL				ns	-0.14^{*}	ns	ns
Leptin					-0.24^{***}	ns	ns
BHB						0.31^{***}	ns
NEFA							ns
Weeks +2 to +4							
MY	ns	ns	ns	ns	ns	ns	0.13^{*}
BCS		ns	ns	ns	ns	ns	0.17^{**}
IGF-I			ns	ns	-0.21^{***}	-0.33^{***}	0.27^{***}
INSUL				ns	ns	ns	ns
Leptin					-0.21^{***}	ns	ns
BHB						0.35^{***}	ns
NEFA							-0.22^{***}
Weeks +4 to +7							
MY	ns	ns	ns	ns	ns	ns	ns
BCS		ns	ns	ns	ns	ns	ns
IGF-I			0.26^{***}	ns	-0.28^{***}	-0.21^{***}	0.25^{***}
INSUL					ns	-0.17^{**}	ns
Leptin						ns	ns
BHB						0.37^{***}	ns
NEFA							-0.15^{*}

Milk yield (MY) was only included in the model for the times after calving. Significant correlations are indicated: $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$; ns, not significant.

predominant relationship was a negative one with BCS throughout the study period. There was also a relationship with leptin (−ve) and BHB (+ve) before calving.

3.3. Correlations between changes in traits over time

The completed dataset was then used in a multiple correlation model to estimate how the change in values between time points for each trait were related to each other. The significant correlations are summarized in Table 8 (PP cows) and Table 9 (MP cows). During the increase in yield between weeks +2 and +4, yield was positively related to changes in urea in both PP and MP cows. Once peak milk yield had been reached in MP cows, yield was positively related to the changes in IGF-I and negatively to changes in BHB.

As both BCS and leptin decreased over calving and had not yet increased again by week +7, the overall differences between weeks −1 and +7 were also calculated for these traits. In both parity groups the overall fall in BCS between weeks −1 and +7 was positively correlated to the fall in leptin over the same period (correlation 0.12 for PP and 0.19 for MP, both $P < 0.05$; not shown in Tables 8 and 9). The fall in BCS between weeks +2 and +4 in PP cows was also positively related to urea. In MP cows the fall in BCS was negatively related to the change in urea over calving (weeks −1 to +2) but positively related to the

Table 9
Correlations between the changes in traits over time for multiparous cows

	BCS	IGF	INSUL	LEPT	BHB	NEFA	Urea
Weeks −1 to +2							
BCS		ns	ns	ns	-0.12^{*}	ns	-0.13^{*}
IGF-I			0.25^{***}	ns	ns	-0.21^{***}	-0.14^{*}
INSUL				ns	ns	ns	ns
Leptin					ns	ns	ns
BHB						0.13^{*}	ns
NEFA							ns
Weeks +2 to +4							
MY	ns	ns	ns	ns	-0.16^{**}		0.14^{*}
BCS		-0.13^{*}	ns	ns	ns	ns	ns
IGF-I				0.15^{*}	-0.15^{*}	-0.12^{*}	ns
INSUL					ns	-0.19^{**}	ns
Leptin						ns	ns
BHB						0.17^{**}	-0.24^{***}
NEFA							-0.15^{**}
Weeks +4 to +7							
MY	ns	0.12^{*}	ns	ns	-0.17^{**}	ns	ns
BCS		ns	-0.13^{*}	ns	0.13^{*}	ns	0.15^{*}
IGF-I			ns	ns	-0.19^{**}	-0.18^{**}	ns
INSUL				ns	ns	ns	ns
Leptin					ns	ns	ns
BHB						0.29^{***}	ns
NEFA							ns

Milk yield (MY) was only included in the model for the times after calving. Significant correlations are indicated: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$; ns, not significant.

change in urea later in lactation (weeks +4 to +7). There were also significant correlations for BCS with the change in IGF-I between weeks +2 and +4 (−ve), the change in insulin between weeks +4 and +7 (−ve) and the change in BHB between weeks +4 and +7 (+ve).

The change in IGF-I was positively related to the change in insulin in both PP and MP cows in weeks −1 to +2 over calving and again in PP cows between weeks +4 and +7. The change in IGF-I was consistently negatively correlated to changes in NEFA and BHB in both parity groups. The change in insulin was also negatively related to changes in BHB and NEFA in PP cows and to NEFA and BCS in MP cows. In addition to its relationship with BCS, the change in leptin over calving was positively related to the change in IGF-I in both parity groups and negatively to the change in BHB in PP cows only.

As lactation established in the PP cows, the change in urea was correlated to the increase in milk yield (+ve), the change in IGF-I concentration (+ve) and the change in NEFA (−ve). In the MP cows the fall in urea over calving was negatively related to BCS and IGF-I. As lactation established, the change in urea was related to the change in BHB (−ve), NEFA (−ve), milk yield (+ve) and BCS (+ve).

4. Discussion

The large dataset on metabolic changes in dairy cows during the periparturient period available for this study has enabled us to model how the inter-relationships between different metabolic traits alter over time, to study their relationship to milk yield and body condition, and to determine how these factors differed according to the parity of the cows. Inclusion of the nutrition group as a fixed factor in the model accounted for both management and dietary differences between herds. It should be noted that identifying the effects of particular dietary variations on metabolic parameters was not an aim of the present study, so this aspect is not considered further. Furthermore, all herds in the various studies were fed diets which were intended to meet the protein and energy requirements of the animals. The results obtained from the combined analyses showed co-ordinated changes in the circulating concentrations of IGF-I, insulin, NEFA and BHB over the calving period in both parity groups. However, both the actual concentrations and the pattern of change over time differed with parity.

Information showing in detail how metabolic hormone concentrations in high yielding dairy cows change as animal mature is currently sparse. Dairy heifers are generally calved for the first time at about 24 months as this has been shown to maximize economic benefit [20]. Coffey et al. [21] analyzed the growth trajectories of dairy heifers from birth until the end of their third lactation and found that cows continued to grow throughout the entire period, although growth rates slowed once animals reached about 450 days. At the start of their first lactation the competing demands of the mammary gland are thus superimposed on the requirements for growth. Both insulin and IGF-I have positive growth promoting effects [37,38] and IGF-I is the primary regulator of postnatal muscle hypertrophy, stimulating protein synthesis and inhibiting degradation [37].

Our data showed higher concentrations of IGF-I in PP than MP cows. We have confirmed in a subsequent experiment that this is an age rather than a lactation number effect as IGF-I concentrations declined between 2 and 3 years of age, regardless of whether cows calved for the first time as 2 or 3 year olds (D.C. Wathes, A. Swali, N. Bourne, unpublished

observations). Insulin followed a similar declining trend with age in our study but in this case the difference was not significant. In US Holsteins peak insulin was similarly lower in the second and third lactations in a control group of cows but not in a group selected for yield in which insulin levels were generally lower [8]. Like us, Vandehaar et al. [31] found higher concentrations of IGF-I pre-partum in PP cows. A study of Australian Holstein cows reported similar findings for IGF-I, but a trend for insulin to be lower in PP than MP cows [39], whereas in Uruguay PP Holsteins had lower concentrations of IGF-I but no differences in insulin [33]. These somewhat conflicting reports suggest that management and/or genetic differences between continents can have important effects on the relative maturity at which cows calve for the first time and their subsequent response to lactation. In the PP cows in our study there was no relationship between the insulin concentration and milk production, whereas in the MP cows these two parameters were always strongly negatively related ($P < 0.01$–0.001). This suggests that in PP cows insulin is less important in controlling the relative partitioning of nutrients between body tissue and milk synthesis, possibly due to the prevailing higher IGF-I concentrations. PP cows also have a lower MY so require less glucose for milk production. This might spare more glucose for uptake to other tissues, and Santos et al. [32] found higher glucose concentrations in the immediate peripartum period in PP cows. Although glucose was not measured in the present study, we have however in another study compared glucose concentrations in the same cows in their first and second lactations and found no differences in the period between −1 and +7 weeks postpartum (D.C. Wathes, A. Swali, N. Bourne, unpublished observations).

There was a highly significant positive correlation between insulin and IGF-I in both parity groups and at all time points. Circulating IGF-I seems to be synthesized mainly in the liver where production is stimulated by the action of GH on GH receptors (GHR). The liver specific variant of the GHR (GHR1A) is down regulated at parturition in both PP and MP dairy cows but not in MP beef cows [40,41]. The concentration of circulating IGF-I decreased over calving in both parity groups in this study but, because the starting point was approximately double in the PP cows, the nadir value was also greater. Hepatic GHR abundance was lower on day +3 than on day +35 and had returned to late pregnancy values by day +56 [42], a time course which paralleled the change in circulating IGF-I reported here. This down regulation of liver GHR1A transcription around calving is thought to be due the energy balance deficit and concurrent hypoinsulinaemia. Treatment of dairy cows with insulin infusion for 96 h in early lactation (day +10) increased abundance of both hepatic GHR1A and IGF-1 mRNA and plasma IGF-I increased during the infusion period [43]. Exogenous GH (bST) is widely used in the dairy industry to promote milk production, an effect thought to be mediated via the enhanced IGF-I secretion promoting galactopoietic effects [11,12,14]. The MP cows in this study showed a negative relationship between IGF-I and milk yield in the first 4 weeks when the cows would have been in NEB. However, by week +7 the relationship had become positive. This time course fits well with the studies cited above showing the recovery in liver GHR expression over the same period.

Another consistent finding in both parity groups was the positive correlation between the NEFA and BHB concentrations from weeks −1 to +4. After this time the NEFA concentration was falling whereas BHB concentrations continued to rise until about week +6. In the early postpartum period NEFAs are esterified to triacylglycerols (TAGs) which accumulate in the liver, peaking in concentration there at 7–13 days after calving then gradually declin-

ing [5,22,24,27]. The period of high BHB with declining blood NEFA may thus reflect utilization of liver TAGs. In PP but not in MP cows the positive relationship between NEFA and BHB was established pre-calving and the NEFA values were already elevated at week −1. This suggests that the PP cows were in a worse energy status before calving [4] and agrees with the data of Meikle et al. [33] and Vandehaar et al. [31]. In both age groups there were significant negative correlations between IGF-I and NEFA from weeks −1 to +4, as also reported previously by Vandehaar et al. [31]. Rodent models have provided evidence that circulating fatty acid concentrations are sensed within the hypothalamus which can then modulate hepatic gluconeogenesis via signalling through the efferent hepatic branch of the vagus [44]. In the cow this is potentially another mechanism for central determination of the negative energy balance status.

At least 50% of all dairy cows are thought to go through a temporary period of subclinical ketosis in the first month of lactation [4]. The previously reported higher prevalence of hyperketonemia with increasing lactation number [45,46] is in accord with our study in which BHB concentrations were higher in MP than PP cows. The relationship between BHB and milk yield also differed according to parity. In PP cows at week +4 there was a significant positive correlation between BHB and milk yield, suggesting that in the less mature animals an increased ability for fatty acid oxidation made more nutrients available for milk production. In contrast, in the MP cows at +7 weeks a negative relationship was seen. In this parity group continued elevation of BHB may be indicative of underlying clinical/subclinical ketosis. Our data thus agree well with a previous study by Solbu [47] and may help to explain why previous reports are conflicting as to whether hyperketonemia is associated with higher (e.g. refs. [45,48]) or reduced (e.g. ref. [49]) milk yields.

Another important variable over the periparturient period is the extent to which individual animals mobilize adipose tissue to support lactation. In both PP and MP cows there was a positive relationship between IGF-I and BCS during the postpartum period. A positive feedback loop thus exists such that cows with higher IGF-I concentrations lost less condition and conversely better conditioned cows experience less negative feedback to down regulate hepatic IGF-I synthesis through mechanisms outlined above. By week +7 in PP cows BCS and NEFA were negatively related. In most animals the NEFA peak has decreased by this time so these data show that animals which continue to mobilise NEFA to support lactation for longer will not surprisingly lose more condition.

In agreement with previous studies, the leptin concentration decreased at calving and then remained low [18,19,33,50]. As reported by Meikle et al. [33] the decrease was steeper in PP than MP cows, and in addition circulating leptin was significantly higher in PP than MP cows before calving. This may relate to the tendency towards a slightly higher BCS in our PP cows. In PP cows leptin and BCS were significantly correlated at weeks +2 and +7, in MP cows leptin and BCS were positively related before calving and in both parity groups the change in BCS loss from weeks −1 to +7 was also highly correlated to the change in leptin over this same period. These data confirm the strong link between leptin and body condition [33], and Ehrhardt et al. [17] estimated that BCS in late pregnant cows explained 37% of the variation in plasma leptin. However, the leptin concentrations remained low postpartum even when the energy balance status had improved (current study, [18]). Pre-partum leptin concentrations are increased by treatments with glucose, insulin and lipid [50,51], whereas postpartum IGF-I was a stronger predictor of circulating leptin than insulin [52]. In our

study we also found a positive correlation with IGF-I at week +2 in the MP cows, and in addition pre-partum leptin was negatively related to urea in both age groups. The precise mechanisms causing the timing and extent of the periparturient leptin decline thus remain uncertain, although there is clearly a genetic component as the pattern varies with different leptin promoter mutations [53]. Possible roles for leptin during this period were reviewed by Ingvartsen and Boisclair [18] who suggested that postpartum hypoleptinemia may enhance voluntary feed intake and contribute to the peripheral insulin resistance which occurs in periparturient ruminants.

Many factors contribute to the actual blood urea level measured in both late pregnant and early lactating ruminants. In late gestation if either protein or energy supply are limiting to the demands for fetal growth, glucose availability for oxidation is supplemented by increased catabolism of amino acids at the expense of protein synthesis, thus increasing urea production [3]. In our study there was a positive correlation between the urea and BHB concentrations in both PP and MP cows before calving. Following parturition up-regulation of liver gluconeogenesis cannot meet the requirement for mammary glucose uptake, which can only be made up by mobilization of amino acids stored in skeletal muscle and other tissue proteins [3]. These underlying changes in the physiology of nutrient utilization are also influenced by dietary factors and their relationship to protein synthesis by rumen microorganisms [5–7,27,54]. If energy supply is limiting, the rate of ammonia production from dietary crude protein exceeds the ability of the rumen microbes to convert it into microbial protein, circulating ammonia concentrations will rise and this will be converted to urea by the liver [55]. Both the pattern of change in urea over time and its relationships with other metabolic indices differed between our PP and MP postpartum cows. In PP cows the urea concentration increased more steeply than in MP cows and was positively related to milk yield. This is probably indicative of animals with a greater supply/intake of rumen degradeable protein using this to enhance milk production. In the MP cows the predominant relationship, which was present at all time points, was a negative correlation of urea with BCS which was unrelated to milk yield. In this case, a likely explanation is that mature cows calving in poor condition have less adipose tissue available for milk production so may have to mobilise more protein to support gluconeogenesis.

In summary, comparison of the metabolic data from PP and MP cows showed that PP cows had consistently higher concentrations of IGF-I throughout the period from −1 to +7 weeks after calving and leptin was also significantly higher before calving in this age group. In PP cows the BHB concentration remained lower throughout the periparturient period and the increase in NEFA occurred earlier than in MP cows in relation to the time of calving. These differences were associated with significantly lower milk production in first calving cows. These results suggest that the differing endocrine background in the less mature animals may limit partitioning of nutrients into milk.

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