Gain and loss of subcutaneous and abdominal fat depot mass from late pregnancy to 100 days in milk in German Holsteins

Lena Ruda1,*,†, Claudia Raschka1,*, Korinna Huber2,†, Reka Tienken3, Ulrich Meyer3, Sven Dänicke3 and Jürgen Rehage1

1Clinic for Cattle, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany; 2Department of Physiology, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany and 3Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Germany

Abstract

This research paper addresses the hypothesis that in times of negative energy balance around parturition in dairy cattle, lipids stored in adipocytes are mobilised in a more intensive manner out of the abdominal depots than out of the subcutaneous adipose tissues. Furthermore, the impact of niacin supplementation and energy density of the ration on adipose tissue mass gain and loss was assessed. Absolute masses of subcutaneous (SCAT), retroperitoneal (RPAT), omental (OMAT), mesenteral (MAT) and abdominal adipose tissue as a whole (AAT) were estimated by ultrasonography at −42, 3, 21 and 100 DIM. Absolute and relative daily gain during dry period (−42 to 3 DIM) and loss in fresh cow period (3 to 21 DIM) and early lactation period (22 to 100 DIM) were calculated. Feeding regime neither by niacin nor by energy density exerted any effect on adipose tissue masses. The AAT was always bigger than SCAT, but RPAT, OMAT and MAT did not differ amongst each other. All depot masses showed similar patterns with an increase during dry period and a decrease after calving. In fresh cow period AAT absolutely and relatively lost more mass than SCAT. This confirms that AAT is more intensively mobilised than SCAT during that time span. Further absolute daily gain during dry period was strongly negatively correlated with absolute daily loss during fresh cow period. This underlines the impact of individual body condition on adipose mobilisation in periparturient dairy cows. According to these results, it has to be taken into account that the largest amount of fat mobilised in the fresh cow period originates from AAT. This might impact the pattern of adipose derived metabolites and metabolic effectors interacting in physiological and deregulated adaptation to negative energy balance.

Pathologically exceeding mobilisation of non-esterified fatty acids (NEFA) from adipose tissue in the periparturient period is still of major concern regarding dairy health, welfare and productivity (McArt et al., 2013). The necessity to mobilise metabolisable fuels out of body stores is caused by the disparity between dry matter intake (DMI) and energy expenditure via lactation referred to as negative energy balance (NEB). Besides NEB itself the individual cow’s condition is an important factor influencing the extent of lipomobilisation (Vanholder et al., 2015). Body condition score (BCS) and DMI in turn are largely influenced by energetic feeding level and parity (Janovick and Drackley, 2010; Janovick et al., 2011). In rodents and humans there are remarkable disparities in functional properties of subcutaneous and visceral fat depots (Yang and Smith, 2007) leading to dissimilarity in gain and loss and a varying strong impact on the development of metabolic disease (Bays, 2014). In dairy cows, functional differences between adipose tissue depots (Locher et al., 2011; Saremi et al., 2014) as well as asynchronicity of mobilisation and accumulation have been shown (von Soosten et al., 2011; Drackley et al., 2014; Kabara et al., 2014). Changes in BCS of the periparturient dairy cow and its possible impact on health and performance have been intensively studied in the last decades (Roche et al., 2009). However, comparatively little is known about the gain and loss of depot mass of different adipose tissues between late pregnancy and the first 100 days in milk (DIM).

Niacin is a potent antilipolytic agent applied in human and veterinary medicine. It is antagonistic to β-adrenergic activation of lipolysis and thereby reduces NEFA release from adipose tissue (Karpe and Fryan, 2004). In periparturient dairy cows β-adrenergic activation is one important pathway triggering lipolysis (Jaster and Wegner, 1981; Locher et al., 2011). Consequently, niacin has been successfully used in dairy cattle as an antilipolytic agent to lower plasma NEFA (Pires and Grummer, 2007). The present study aimed to estimate the mass of the respective adipose tissue depots at crucial time points in the period from drying off to 100 DIM and to describe possible impacts of parity, niacin supplementation and energy density in the ration. Further, it was intended to compare the gain and loss of depot mass in this period between the subcutaneous depot and the abdominal depot as a whole. By
comparison of the abdominal subunits retroperitoneal, mesenteric and omental adipose tissue amongst each other, it should be clarified, whether these tissues act similarly or divergently concerning gain and loss of depot mass during this time span. In a last step, the individual mass gain of a certain depot during dry period should be linked to its individual loss until 100 DIM.

Materials and methods

The study comprised 47 healthy German Holsteins, between 1st and 4th lactation (heifers: n = 18, cows: n = 29), housed in a free stall housing system at the Institute of Animal Nutrition, Friedrich-Löffler-Institut, Braunschweig, Germany. The trial started 42 d prior to the expected calving date (~42 DIM) and finished at 100 DIM. All procedures concerning treatment, handling and sampling were carried out in accordance with the German Animal Welfare Act (permit number: 33.9-42502-04-11/0444) pertaining to the protection of experimental animals and approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES). The feeding regime is described in detail by Tienken et al. (2015). In brief it was intended to stimulate lipid mobilisation postpartum by varying the percentage of concentrate in the ration during dry period and during lactation. Further niacin was supplemented to study a possible antilipolytic effect of this substance. More details are given in the online Supplementary File (see S1.1). Ultrasonography of subcutaneous and retroperitoneal fat layers and body size measurements were performed at ~42 DIM and 3, 21 and 100 DIM. For detailed description of the procedure see Raschka et al. (2016).

Data processing and statistical analysis

The estimated depot mass (eDM, kg) of subcutaneous adipose tissue (SCAT), abdominal adipose tissue as a whole (AAT) and its subunits retroperitoneal adipose tissue (RPAT), omental adipose tissue (OMAT) and mesenteric adipose tissue (MAT) was calculated using the equations described in Raschka et al. (2016). This method uses a multiple linear regression approach to estimate actual depot mass on the basis of ultrasonographic measurements of certain subcutaneous and retroperitoneal fat layers and selected body size measures. Root mean square error (RMSE) of the obtained equations is 3.4 kg for SCAT, 6.1 kg for AAT, 1.7 kg for RPAT, 3.2 kg for OMAT and 1.6 kg for MAT. The coefficients of determination ($R^2$) of correlations between predicted and actual weight are 0.85 for SCAT, 0.98 for AAT, 0.93 for RPAT, 0.84 for OMAT and 0.93 for MAT. Since the actual dry period differed between individuals, averages of daily gain (adG) and loss (adL) were calculated for dry period (~42 DIM to 3 DIM), fresh cow period (3 to 21 DIM) and early lactation period (21 and 100 DIM). Relative (rel) gain (%/day) and loss within dry period, fresh cow period or early lactation period respectively were calculated setting the eDM at the preceding time point as 100%. This was supposed to provide an indicator of the intrinsic activity of a certain depot during the respective time span. Thereby comparability between all depots measured should be facilitated. For more details of the calculations see section S2.1 in the Supplementary File.

Feeding regime neither by energy density nor by niacin supplementation exerted any effect on the variables of interest. Further, these variables were not influenced by parity. No interactions between these factors were found. For detailed description of the statistical model and results see S2.2. and tables S1 and S2a in the Supplementary File. Thus, the PROC MIXED of SAS 9.3 was used for the creation of a model with the factors time, parity and depot as fixed factors and cow as random factor. The model was initially run for SCAT and AAT and in a second step separately for the abdominal subunits RPAT, OMAT and MAT. To compare adG during dry period between depots, a paired t-test for SCAT and AAT and a one-way ANOVA with repeated measures for RPAT, OMAT and MAT were performed. The adL in fresh cow period and early lactation period were analysed by a two way ANOVA with repeated measures with the factors time and depot for SCAT and AAT and for RPAT, OMAT and MAT separately. Pearson’s correlations of adG during dry period with adL in fresh cow period and adL in early lactation period as well as of adL in fresh cow period with adL in early lactation period were calculated for each depot separately. Results were considered significant with $P < 0.05$.

Results

The effect of time ($P < 0.001$) was reflected by an increase of adipose tissue mass during dry period and a decrease during fresh cow period and early lactation period in each depot. As a main effect of the factor depot, AAT could be shown to be bigger than SCAT ($P < 0.001$). Further there was an interaction ($P < 0.001$) between depot and time concerning SCAT and AAT (Table 1). The kind of interaction becomes obvious by the differences in adG and adL as depicted below (Figs. 1 and 2). The abdominal subunits did not differ between each other. However, the interaction between time and depot was also significant for RPAT, OMAT and MAT (Table 2). Parity again had no effect on the parameters of interest.

In comparison to AAT, adG during dry period was significantly lower in SCAT ($P = 0.001$, Fig. 1a) whereas rel adG did not differ between these two depots ($P = 0.1987$, Fig. 1c). Concerning the abdominal subunits, adG and rel adG during dry period did not differ (Fig. 1b and d).

The adL ($P < 0.0001$) and rel adL ($P = 0.0237$) during fresh cow period were significantly lower in SCAT than in AAT but were not different in early lactation period ($P > 0.05$; Fig 2a and c). Intra-depot adL was higher in fresh cow period than in early lactation period (SCAT: $P = 0.0001$; AAT: $P < 0.0001$; Figure 2a).

Table 1. Effects of depot (SCAT vs. AAT) and time (~42 to 100 DIM) on estimated adipose depot mass (kg) in German Holsteins (LSmeans ± SE)

<table>
<thead>
<tr>
<th>Item</th>
<th>Depot</th>
<th>x</th>
<th>Time</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>~42 DIM</td>
<td>3 DIM</td>
<td>21 DIM</td>
<td>100 DIM</td>
<td>~42 DIM</td>
</tr>
<tr>
<td>eDM</td>
<td>15.8 ± 1.4</td>
<td>19.3 ± 1.8</td>
<td>14.5 ± 1.5</td>
<td>10.9 ± 1.5</td>
</tr>
</tbody>
</table>

eDM, estimated depot mass; SCAT, subcutaneous adipose tissue; AAT, abdominal adipose tissue.
In OMAT adL in fresh cow period was lower than in MAT (P = 0.0045) whereas RPAT ranged in between and was not different from the other depots. This difference was levelled out in early lactation period (P > 0.05; Fig. 2b). Neither in fresh cow period nor in early lactation period did rel adL differ between abdominal subunits (Fig. 2d). Intra-depot adL in fresh cow period was higher than in early lactation period for RPAT (P < 0.0001), OMAT (P = 0.0011) and MAT (P < 0.0001; Fig. 2b).

Correlations between adG during dry period and adL in fresh cow period intra-depot were consistently significant (P < 0.001) and ranged from $r = -0.58$ for SCAT to $r = -0.49$ for AAT. The adG in dry cow period was significantly correlated with adL in early lactation period in OMAT (P < 0.05; $r = -0.34$). The adLs in fresh cow period and early lactation period were not correlated in any of the depots. Details are given in Table S3 in the Supplementary File.

**Discussion**

All adipose depots showed the quantitative changes usually expected in periparturient dairy cows with an increase during dry period when energy balance is positive and a decrease in fresh cow period and early lactation period driven by NEB.

Body composition measurements reported an average quantity of 42 to 54 kg of mobilised fat during NEB in dairy cows. Whereas Andrew *et al.* (1994) reported 42.9 kg within the first 62.9 DIM, performing a direct chemical body composition analysis at slaughter, animals lost 54 kg between 14 d antepartum and 35 DIM in a study of Komaragiri and Erdman (1997) that was using the D$_2$O dilution technique. Converted to a daily basis, animals in these studies lost about 0.6 and 1.1 kg of total body fat respectively. Animals in the present study lost about 1.1 kg per day in fresh cow period until 21 DIM (Fig. 2). Thus, assessment of body fat depots by ultrasonography was in similar range with direct chemical body composition measurements or D$_2$O dilution technique.

As shown in the companion study by Tienken *et al.* (2015) multiparous cows showed higher DMI and a more positive energy balance during the dry period than primiparous cows (P < 0.001). This effect was more pronounced in animals receiving a high percentage of concentrate during this time span (P < 0.005). In lactating animals only parity influenced DMI and energy balance; primiparous cows showed a lower DMI but a more positive energy balance than multiparous cows. Despite these differences no parity or concentrate effects on BCS and the estimated mass of adipose tissue stored in the different depots could be found.
in the present study. However, body weight was influenced by parity (Tienken et al., 2015). This indicates that in the setting of the present study the differences in body weight of about 12% that according to Tienken et al. (2015) are driven by parity are mostly smaller than the individual variations in the amount of fat stored in a certain AT depot of a single animal. The reasons for the absence of an effect of niacin supplementation in the present study are also explicitly discussed by Tienken et al. (2015). Even with comparatively high amounts of 24 g niacin per day, only nicotinamide but not niacin could be detected in the serum of the animals in the present study (data not shown). The antilipolytic effect of dietary niacin in bovine adipose tissue is exerted by niacin and not by nicotinamide (Kenéz et al., 2014). Thus the absence of niacin in the bloodstream of the experimental animals is considered the main reason for the lack of effects of a dietary niacin supplementation in the present study.

As shown in Table 1, the AAT depot as a whole was considerably bigger than the SCAT depot whereas the masses of the abdominal depots RPAT, OMAT and MAT did not differ amongst each other. Data of the present study are in accordance with previous studies of Raschka et al. (2016) and von Soosten et al. (2011) that determined quantity of adipose tissue depots by carcass analysis in periparturient dairy cattle. However, in the study by von Soosten et al. (2011) AAT is three to four times bigger than SCAT, whereas in the present study AAT was only two times bigger than SCAT. This difference might be due to the fact that von Soosten et al. (2011) used primiparous cows whereas the present study comprised primi- and multiparous cows. Further von Soosten et al. (2011) describe also RPAT, OMAT and MAT masses but don’t give statistical analysis of differences between these depots. Nevertheless the mass of OMAT seemed to be numerically bigger than RPAT and MAT in that study. These differences might also be due to differences in the study protocols concerning the definition of the respective depots. In a study on nonpregnant and nonlactating cows, Drackley et al. (2014) found numerically similar amounts of mesenteric and omental adipose tissue but that study did not assess subcutaneous adipose tissue.

Higher adG during dry period in AAT compared with SCAT in combination with a similar rel adG during dry period becomes explicable by the higher amount of fat stored in AAT at −42 DIM (Table 1). If both depots grow by the same percentage per day, the absolute value of adG is necessarily bigger in AAT compared to SCAT. For the moment these data contradict a constitutionally higher adipogenic activity in AAT, at least during the dry period. Even though Weber et al. (2016a, 2016b) in companion studies found higher mRNA expression of insulin dependent antilipolytic factors like hydroxycarboxylic receptor (HCAR) 1 and 2 in RPAT than in SCAT at −42 DIM, this did not lead to a relatively higher actual accretion of fat in AAT during dry period.

Several studies reveal discrepancies between changes in subcutaneous fat layers as represented by BCS, back fat thickness, fat depth or changes in body weight and biochemical markers of lipid mobilisation (NEFA and BHB). Therefore, it is often hypothesised that changes in internal or visceral adipose depots would be of greater importance in the context of lipomobilisation in dairy cows (Pires et al., 2013) but unfortunately this is not reflected by common methods for the assessment of body condition. Not only the absolute weight of AAT depot (Table 1) but also adL in fresh cow period (Fig. 2a) was always bigger than in SCAT in our study. This supports the assumption that in times of NEB in fresh cow period quantitatively more fat is mobilised
out of AAT than out of SCAT. Further, a higher intrinsic lipolytic activity is hypothesised for selected abdominal adipose tissues in the dairy cow (Locher et al., 2011; von Soosten et al., 2011; Kabara et al., 2014). According to the present study this hypothesis could be confirmed for fresh cow period, because the relative adL in fresh cow period in AAT was also bigger than in SCAT (Fig. 2c). This higher intrinsic lipolytic activity seemed to be a property of AAT as a whole, for the abdominal subunits RPAT, OMAT and MAT despite higher adL during fresh cow period in MAT (Fig. 2b) did not differ in rel adL (Fig. 2d). Further, these differences between AAT and SCAT seemed to be diminished across lactation, for neither adL nor rel adL differed between depots during early lactation period (Fig. 2a,b). On the ultrastructural level this might become explicable by changes in morphology and functionality accompanying the depletion of fat cells during lipomobilisation. In dairy cattle, adipocyte size is considered to reflect, independently of its anatomic location, the actual ‘fill’ of a certain adipose depot (Locher et al., 2015; De Koster et al., 2016). In a companion study Kenéz et al. (2015) found bigger fat cells in RPAT than in SCAT at 3 DIM and a more pronounced cell size decrease in RPAT postpartum. Recently it could be further shown in dairy cattle for SCAT and OMAT that bigger fat cells have a higher basal lipolytic activity and are more reactive to lipolytic stimuli (De Koster et al. 2016). Assuming that OMAT and MAT underwent the same ultrastructural changes as RPAT, the higher adL and rel adL in AAT might be due to bigger fat cells with higher lipolytic activity in this depot. This was further supported by the fact that in the present study differences disappeared after 21 DIM when cell size is not different between depots anymore (Kenéz et al., 2015). Furthermore, the mentioned companion studies of Weber et al. (2016a, 2016b) again confirm higher lipolytic potential in RPAT compared to SCAT due to differences in mRNA and protein expression of several effectors of lipolytic pathways. In the present study, MAT showed the biggest adL during fresh cow period. In studies on non-pregnant non-lactating cows, a comparatively low lipogenic potential for MAT is postulated (Ji et al., 2014). Another study found this thesis confirmed by smaller adipocytes in MAT compared to OMAT and RPAT at the end of dry period (Depreester et al., 2018). Following these argumentations,
higher adL during fresh cow period in MAT might point to a comparatively higher propensity of this depot to lose fat mass stored during dry period. In most of the studies on visceral adipose tissue in the bovine species, fat samples were harvested in a slaughter process, reflecting a single time point for each individual. Due to technical reasons, only for RPAT repeated biopsy sampling in vivo has been performed (Locher et al., 2011). A cross sectional study on different adipose tissues in dairy cattle by von Soosten et al. (2011) compares actual mass of the respective depots in heifers slaughtered at 1, 42 and 105 DIM. In this study significant changes over time are found only for RPAT, but not for OMAT and MAT. These considerable differences in study design and protocol limit comparability of the mentioned studies with the present data.

Based on our results we can summarise, that higher rel adL of AAT during fresh cow period might be partly explicable by differences in cellularity, mRNA and protein expression in RPAT whereas the underlying processes in OMAT and MAT in the context of lipomobilisation in fresh cow period remain unclear so far. In humans and rodents, visceral adipose tissue depots are postulated as the instant resource for maintenance of energy homeostasis in times of energy surplus or deprivation (Romao et al. 2013; Fried et al. 2015). Higher deposition during dry period combined with higher actual and relative loss during fresh cow period pointed to a similar function for AAT in dairy cattle, too. This is supported by the fact that dairy breeds accumulate relatively more abdominal fat than beef breeds (Wright and Russel, 1984). Metabolites like NEFA and glycerol mobilised out of the abdominal adipose depots are directly drained to the liver (Montague and O’Rahilly, 2000), thereby providing an immediate energy flux to meet the energy demands of this organ. The energy demands of the liver in the periparturient cow are particularly high in dairy breeds, not only because the liver synthesises large amounts of glucose that is needed for lactose synthesis but also to accomplish its tasks concerning periparturient protein turnover and acute phase reactions (Bertoni et al., 2008; Bertoni and Trevisi, 2013).

Correlations between adG during dry period and average daily loss during fresh cow period were significant in all depots and ranged between \( r = -0.49 \) and \( r = -0.58 \). Therefore, 24–34% of the total variation in the amount of fat mobilised in the fresh cow period may be attributable to the amount of fat stored during the dry period. It would appear that the more an individual accumulates fat in the dry period, the more it mobilises in fresh cow period. Since the size of bovine adipocytes increases with the increase of adipose mass in a certain depot (at least in the short term), it can be assumed that individuals in the present study that gained more fat in dry period had bigger fat cells at the beginning of fresh cow period. Bigger fat cells have a higher basal lipolytic activity and are more reactive to lipolytic stimuli (De Koster et al., 2016).

Bigger fat cells might be the reason for the bigger adL during fresh cow period in individuals with higher adG during dry period. With the start of lactation, this relationship was lost in the largest part of the depots and only AAT showed a relationship of adG during dry period and adL in early lactation period. In addition adL during fresh cow period and adL in early lactation period were not correlated in any depot. This possibly indicates that higher lipolytic activity in adipose tissue in fatter cows was a transient and reversible effect that disappeared, when adipose tissue amount and thereby fat cell size decreased.

When assessing a dairy cow’s condition, it should be taken into account, that the largest part of adipose tissue is stored in AAT and that this depot besides its mere quantity further appears to have a higher intrinsic lipolytic activity. Thus it can be assumed that a remarkable part of adipose derived metabolites and metabolic effectors interacting in physiological and deregulated adaptation to NEB origins from AAT. To clarify the impact of the abdominal subunits RPAT, OMAT and MAT, longitudinal studies linking biopsy sampling with adipose tissue weight over time would be necessary. Strong correlations between gain during dry period and loss in fresh cow period point to the special importance of the individual’s body condition on adipose tissue mobilisation.

In conclusion, we have shown that the largest amount of fat mobilised in the fresh cow period origins from abdominal stores. This might impact the pattern of adipose derived metabolites and metabolic effectors interacting in physiological and deregulated adaptation to negative energy balance.

**Supplementary material.** The supplementary material for this article can be found at [https://doi.org/10.1017/S0022029919000542](https://doi.org/10.1017/S0022029919000542)

**Acknowledgements.** The authors thank the German Research Foundation (DFG, Bonn, Germany) for financial support. Further the technical assistance of Lea Fieguth and Anja Schacht and the cowworkers of the Experimental Station of the Institute of Animal Nutrition Friedrich-Loeffler-Institut Braunschweig, Germany is gratefully acknowledged.

**References**


Bays H (2014) Central obesity as a clinical marker of adiposopathy; increased visceral adiposity as a surrogate marker for global fat dysfunction. *Current Opinion in Endocrinology, Diabetes and Obesity* 21, 345–351.


