Responses of body fat mobilization to isoproterenol or epinephrine challenge in adult cows: influence of energy level, breed, and body fatness

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ABSTRACT: The sustainability of livestock production systems facing climatic or economic changes is linked in part to the potential of the female ruminants to adapt to feeding constraints through metabolic and hormonal regulation, notably responses of body fat mobilization, depending on adipose tissue (AT) lipolysis. Our hypothesis was that these responses could change according to genotype (breed) and body fatness. Six fat, nonpregnant, nonlactating Charolais cows, six fat Holstein cows, and six lean Holstein cows were used in a 2 × 2 crossover design with two treatments (underfeeding or overfeeding, at 62% [low] or 128% [high] of maintenance energy requirements [MER], respectively) and two periods. Isoproterenol (ISO, a nonselective β -adrenergic agonist) or epinephrine (EPI, a β - and α 2-adrenergic agonist) was injected (6 nmol/kg of lean mass). Blood samples were collected regularly from -20 to 75 min after the injection and then were analyzed for NEFA, glycerol, glucose, and L-lactate. Underfeeding greatly increased ($P < 0.001$) basal plasma NEFA concentrations (+467%, +264%, and +600% for fat Charolais, fat

Holstein, and lean Holstein cows, respectively). For each drug, underfed cows had higher NEFA or glycerol responses to adrenergic challenges than overfed cows. Fat Charolais cows had higher basal plasma NEFA ($P < 0.05$) concentrations (+64.9%) than fat Holstein cows. The plasma NEFA or glycerol response at 5 min ($P < 0.05$) was higher for fat Charolais than for fat Holstein cows, whatever the injected drug. Basal plasma lactate concentration and lactate response to ISO or EPI were higher ($P < 0.05$) for fat Charolais cows than for fat Holstein cows. Fat Holstein cows had higher ($P < 0.01$) basal glycerol (+18.4%) than lean Holstein cows. This increase could be linked to the increased AT mass. ISO increased more lipolytic responses in fat than in lean Holstein cows, whereas EPI increased more these responses in lean than in fat Holstein cows (drug × fatness interaction), suggesting an increased antilipolytic effect due to α 2-AR stimulation in fat cows. Breed had a significant effect on basal and stimulated fat mobilization as well as lactate concentrations, suggesting that the Charolais breed could be more sensitive to stress.

Key words: body fatness, breed, cattle, epinephrine, lipomobilization, underfeeding

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INTRODUCTION

The ability of ruminants to cope with periods of food shortage results from a long evolutionary process in natural conditions where food availability seasonally fluctuates (Chilliard et al., 1998a).

As climate change increases, ruminant production systems will experience more frequent periods of feed restriction (FAO, 2006). In such contexts, the sustainability of livestock production systems depends on the efficiency of physiological (metabolic and hormonal) regulations that are involved in coping responses (Blanc et al., 2006).

The metabolic processes involved in fat mobilization depend firstly on adipose tissue (AT) lipolysis. Catecholamines (norepinephrine and

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epinephrine, **EPI**) are among the most effective regulators of AT lipolysis, which is stimulated through the β -adrenoceptors (**AR**; β 1-, β 2-, and β 3-AR) and inhibited through α 2-AR (Lafontan, 2012). The adrenergic regulation of AT lipolysis can be studied in vivo by measuring plasma NEFA and glycerol responses to adrenergic challenges through isoproterenol (**ISO**, a nonselective β -adrenergic agonist, β -AA) or EPI injection (Chilliard et al., 1998b).

Previous studies (Ferlay et al., 1996; Ferlay and Chilliard, 1999) documented the plasma NEFA responses to a β -AA or EPI challenge in underfed or overfed cows, but the effect of body fatness has not been evaluated in bovine. Thus, the first objective of this study was to analyze the effects of ISO or EPI injection on fat mobilization in interaction with body fatness to better understand the distinct roles of β - and α 2-components in the lipolytic response to undernutrition.

Few studies reported the effect of breed (Brown et al., 2012) or different genotypes (Xing et al., 1991; Fulkerson et al., 2008) on the adaptation of body fat mobilization to undernutrition. Therefore, the second objective was to assess the adaptation of the lipolytic response to undernutrition in two different bovine breeds: Holstein vs. Charolais.

MATERIALS AND METHODS

Experimental Design

All procedures on animals were in accordance with the guidelines for animal research of the French

Ministry of Agriculture and all other applicable national and European guidelines and regulations for experimentation with animals (see http://www2.vet-lyon.fr/ens/expa/acc_regl.html for details).

Details of the design of the experiment have been reported previously (Delavaud et al., 2002). Briefly, all cows were nonpregnant, nonlactating adults. Six fat Charolais cows (group 1; BW 773 [SD 77] kg, BCS [5-point scale] 3.6 [SD 0.3], 6 yr old [SD 1.9]), six fat Holstein cows (group 2; BW 714 [SD 48] kg, BCS 3.8 [SD 0.3], 6 yr old [SD 1.5]), and six lean Holstein cows (group 3; BW 537 [SD 57] kg, BCS 1.3 [SD 0.1], 4 yr old [SD 0.2]) were used in a 2 x 2 crossover design with two treatments (underfeeding or overfeeding, at 62% [low] or 128% [high] of maintenance energy requirements [MER], respectively) and two periods. The animals were divided into three blocks (each block consisted of two animals of each group, $n = 6$; Figure 1).

For each block, the pre-experimental and the experimental periods lasted 15 and 21 days, respectively. Pre-experimental period of the different blocks started with an interval of 1 wk from the previous blocks (Figure 1). During the pre-experimental periods, the animals were fed at 107% (medium) of MER. The diets were calculated from BW at the beginning of the experiment. The cumulative energy supply covered approximately 100% of the maintenance requirement during the whole experiment (pre-experimental and experimental periods). The animals were housed in tie stalls under a 12-h light regimen. They were treated with an intravaginal sponge containing progesterone (PRID, 2 mm,

Group 1 was constituted of fat Charolais cows (cow A, cow B, cow C, cow D, cow E and cow F).

Group 2 was constituted of fat Holstein cows (cow G, cow H, cow I, cow J, cow K and cow L).

Group 3 was constituted of lean Holstein cows (cow M, cow N, cow O, cow P, cow Q and cow R).

Figure 1. Summary of the experimental design. The arrows indicate the timing relative to measurements and samplings. Energy levels are low, medium, and high and correspond to 62%, 107%, and 128% of maintenance energy requirements, respectively.

Sanofi, Libourne, France) to prevent any oestrous cycles during the experiment.

The low diet consisted of 55% grassland hay, 24.6% pelleted barley, and 20.4% soybean meal on dry matter basis. The medium and high diets were 55.2% and 55.3% hay, 44.8% and 44.7% pelleted barley, respectively. Water was provided *ad libitum*. Each animal received 100 g of a mineral-vitamin premix daily. The total mixed diet was offered at 1400 h. Feed refusals were measured daily during the experimental periods. The total tract digestibility of OM, CP, crude fiber (CF), NDF, and ADF was determined by total collection of feces from each cow for 5 consecutive days (from days 17 to 21 of each experimental period). Samples of feedstuffs and refused feed were collected daily during the experimental period. The feed samples obtained were pooled to provide one sample per period, and the sample was stored at -20°C . The feed DM concentration was determined after drying at 103°C for 24 h. Samples of all feedstuffs (hay, barley, and soybean meal) were lyophilized, sieved through a 1-mm screen, and analyzed for OM, CP, and CF using standard procedures (AOAC, 1997), and NDF and ADF analyses were conducted according to Van Soest et al. (1991). The NE intake was estimated according to INRA (2007). The energy requirement for maintenance was assumed to be $0.32 \text{ MJ NE/kg of BW}^{0.75}$. The balance of NE was calculated by subtracting the maintenance requirement from intake. All rations were calculated (INRA, 2007) so that protein intake was similar and covered 130% of the requirements of protein digestible in the intestine (PDI; assumed to be $3.25 \text{ g of PDI/kg of BW}^{0.75}$).

Injections of ISO and EPI

ISO (Isuprel, Sanofi Winthrop Industrie, France) or EPI (Adréline, Laboratoire Aguettant, France) was injected as a bolus of 6 nmol/kg of lean mass (LM) via the jugular vein on day 18 or 19 and on day 20 or 21 of each experimental period (Figure 1). The sequences of the two daily injections were randomized across periods and groups, with one drug per day. The amount of LM per animal was calculated according to the equation: $\text{LM} = \text{empty BW} - \text{body lipids}$, where the empty BW was estimated as the BW of the animals minus 120 kg (mean gut content in dry, lean, or fat Holstein cows, Chilliard et al., 1983), and the amount of body lipids was calculated according to a ratio of 28 or 30 kg of body lipids per point of BCS for Holstein or Charolais cows, respectively (Rémond et al., 1988; Petit and Agabriel, 1993).

During the experimental period, one catheter was implanted into the jugular vein on the evening before the day of the injection. The catheter was kept patent by flushing with 2 mL of sterile heparinized (1%) saline. Injections were made at 10.00 h each day. From the contralateral vein, blood samples (10 mL) were obtained at -20 , -10 , -5 , 5 , 10 , 15 , 20 , 30 , 45 , 60 , and 75 min after the injection. Blood samples were collected into tubes containing EDTA (2.1 mg/mL) and centrifuged at $3,000 \times g$ for 20 min at 4°C . Plasma samples were frozen at -20°C until they were analyzed for NEFA, glycerol, glucose, and L-lactate using a multi-analyzer (Elan, Merck-Clevenot, Nogent-sur-Marne, France).

The plasma NEFA, glycerol, glucose, and L-lactate concentrations were determined using enzymatic kits (C-test Wako, Unipath, Dardilly, France; Sigma, reference 37-A, Saint-Quentin Fallavier, France; Merck-Clevenot, reference 12193, Nogent-sur-Marne, France; and BioMérieux, reference 61192, Marcy-l'Etoile, France, respectively), according to the methods described by Ferlay and Chilliard (1999).

Calculations and Statistical Analyses

The overall changes in blood metabolites during the 60-min postinjection period were estimated from calculation of the area under the response curve (AUC) and above the baseline (summation of the area between successive pairs of concentration-time coordinates). The baseline concentration was concentration at -5 min relative to the injection.

Data were analyzed by the GLM procedure of SAS (2003). For data obtained after the drug injection, the following model was used:

$$Y = \mu + \text{Block}_i + \text{Group}_j + \text{Group} * \text{Cow}(\text{Block})_{ijk} \\ + \text{Period}(\text{Block})_{il} + E_m + \text{Drug}_n + \text{Group} * E_{jm} \\ + \text{Group} * \text{Drug}_{jn} + E * \text{Drug}_{mn} \\ + \text{Group} * E * \text{Drug}_{jmn} + e_{ijklmn},$$

where the fixed effects were the overall mean μ , the group effect (2 df), the energy level effect (E , 1 df), the drug effect (1 df), and their interactions. The random effects were the block effect (2 df), the interaction between group and cow nested within the block effect (13 df), the period nested within the block effect (3 df), and the residual error. The following interactions were tested: drug x fatness, drug x breed, drug x fatness x energy level, and drug x

Table 1. Ingredient and selected nutrient intakes, and digestibilities of total mixed rations, energy balance, and characteristics of animals

Group	Fat Charolais		Fat Holstein		Lean Holstein		SEM	Effects ^a		
								Contrast	Contrast	
								Charolais vs. Holstein	Fat vs. lean	
Energy level	Low	High	Low	High	Low	High		Energy level		
<i>n</i>	6	6	6	6	6	6				
Ingredient intake, kg DM/d										
Hay	2.29	4.98	2.15	4.67	1.74	3.77	0.123	0.001	NS	0.001
Soybean meal	0.86	0.00	0.78	0.00	0.65	0.00	0.051	0.001	0.01	0.001
Barley	1.03	4.01	0.96	3.78	0.78	3.04	0.128	0.001	NS	0.001
Mineral and vitamin premix ^b	0.10	0.10	0.10	0.10	0.10	0.10	0.00	NS	NS	NS
Total	4.28	9.09	4.09	8.55	3.27	6.91	0.166	0.001	NS	0.001
Selected nutrient intake										
OM, kg/d	3.91	8.46	3.64	7.95	2.98	6.41	0.005	0.001	0.10	0.001
PDI ^c , g/d	614	717	567	673	476	542	1.57	0.001	0.10	0.001
Crude fiber, kg/d	0.86	1.83	0.80	1.72	0.65	1.38	0.036	0.001	NS	0.001
Ether extract ^d , g/d	76	164	71	154	58	123	3.3	0.001	NS	0.001
Digestibility, %										
OM	72.5	71.9	72.2	73.3	69.4	72.8	0.59	NS	NS	NS
Crude protein	69.6	59.9	71.4	62.5	66.7	62.5	0.60	0.001	0.10	0.10
Crude fiber	68.4	62.3	66.9	65.6	61.3	64.2	0.72	NS	NS	NS
NDF	63.6	58.1	64.2	61.9	60.6	60.2	0.76	0.05	NS	NS
ADF	57.7	53.6	59.2	58.7	54.9	56.3	0.93	NS	NS	NS
Energy intake, MJ NE/d	26.0	55.5	24.2	52.1	20.2	42.0	1.22	0.001	NS	0.001
Energy balance ^b , MJ NE/d	−16.6	+12.0	−15.2	+11.4	−11.3	+9.1	1.07	0.001	NS	0.05
BW, kg	767.8	787.7	689.8	722.8	506.7	543.2	1.05	NS	0.10	0.001
Body condition score	3.5	3.6	3.7	3.7	1.0	1.3	0.15	NS	NS	0.001

^a NS, not significant ($P > 0.10$).

^b Minerals, %: P, 14; Ca, 16; Mg, 3; Na, 4.7. Trace elements, mg/kg, Zn, 4000; Mn, 3000; Cu, 800; I, 60; Co, 20; Se, 5. Vitamins, mg/kg: retinyl acetate, 86; cholecalciferol, 2; *RRR*- α tocopheryl acetate, 67. ^{††} Balance = intake-maintenance requirements.

^c PDI, Protein digestible in the intestine (INRA, 1989).

^d Ether extract values from ingredients were estimated from INRA (2007).

breed x energy level. For data obtained before the drug injection, the same model was used without the drug effect. The orthogonal contrasts for all variables were 1) fat Charolais vs. fat Holstein and 2) fat Holstein vs. lean Holstein. The significance level was predefined at $P \leq 0.05$, and trends toward significance were considered at $0.05 < P \leq 0.10$.

RESULTS

Animals and Diets

Energy level did not modify the BW or BCS of the animals (Table 1). Fat Charolais cows tended

to have higher BW ($P < 0.10$) than fat Holstein cows. Fat Holstein cows had higher BW and BCS ($P < 0.001$) than lean Holstein cows. Total dry matter, CF, and ether extract intakes were 3.75 kg/d, 0.77 kg/d, 68 g/d, 8.08 kg/d, 1.64 kg/d, and 147 g/d for under and overfed cows, respectively (Table 1). Total dry matter intake was higher ($P < 0.001$) for fat than for lean Holstein cows (6.32 vs. 4.99 kg/d, respectively). Intakes of CF and ether extract were higher ($P < 0.001$) for fat than for lean cows (1.26 and 1.02 kg/d of CF intake, and 113 and 91 g/d of ether extract intake for fat and lean cows, respectively). Underfeeding tended to increase ($P < 0.10$) the NDF digestibility (62.8% vs. 60.1% for under

and overfed cows, respectively). Energy intake was higher ($P < 0.001$) for fat than for lean Holstein cows. By experimental design, the NE balance of the underfed cows was negative, while that of the overfed cows was positive ($P < 0.001$).

Effects of energy level, breed, and body fatness on basal plasma metabolite concentrations of animals

Underfeeding highly increased ($P < 0.001$) basal plasma NEFA concentrations (+467%, +264%, and +600% for fat Charolais, fat Holstein, and lean Holstein cows, respectively, Table 2). The basal glycerol concentrations were on average slightly higher ($P < 0.01$) for under- than for overfed cows (+28.5%, +10.0%, and +26.9% for fat Charolais, fat Holstein, and lean Holstein cows, respectively). Fat Charolais cows had higher basal plasma NEFA concentrations ($P < 0.05$) than fat Holstein cows. Fat Holstein cows had higher ($P < 0.01$) basal glycerol concentrations than lean Holstein cows.

Basal plasma lactate concentrations were higher ($P < 0.05$) in fat Charolais cows than in fat Holstein cows (0.74 vs. 0.55 mM, respectively, Table 3). Fat Holstein cows had higher ($P < 0.05$) glycemia than lean Holstein cows (Table 3).

Effects of energy level, breed, and body fatness on the responses of plasma metabolite concentrations to ISO or EPI injection

For each drug, underfed cows tended to have higher NEFA response at 5 min ($P < 0.10$) than overfed cows (Table 2 and Figure 2). Underfed cows had higher NEFA ($P < 0.001$) or glycerol ($P < 0.01$) response at 10 min, maximal NEFA ($P < 0.001$) or glycerol ($P < 0.05$) response, and NEFA or glycerol AUC ($P < 0.001$) than overfed cows. The increases due to underfeeding in NEFA response at 10 min ($P < 0.001$), maximal response ($P < 0.001$), and AUC (+25.5 and +4.13 mM/min for under- compared with overfed cows for ISO and EPI injection, respectively, $P < 0.001$) were higher with ISO than with EPI injection (energy level $\times$ drug interaction). The same increases were observed for glycerol response at 10 min ($P < 0.01$), maximal response ($P < 0.05$), and AUC (+1.78 and +0.42 mM/min for under- compared with overfed cows for ISO and EPI injection, respectively, energy level $\times$ drug interaction, $P < 0.001$).

For each drug, the plasma NEFA or glycerol response at 5 min ($P < 0.05$) was higher for fat Charolais than for fat Holstein cows. For ISO, plasma glycerol response at 10 min and maximal response tended to be higher ($P < 0.10$) for fat than for lean

Holstein cows, and AUC was higher ($P < 0.05$). ISO increased more glycerol responses at 5 min ($P < 0.05$) and 10 min ($P < 0.01$), maximal response ($P < 0.01$), and AUC in fat than in lean Holstein cows (2.9 and 0.69 mM/min for ISO and EPI in fat Holstein cows, 1.46 and 0.88 mM/min for ISO and EPI in lean Holstein cows, respectively, $P < 0.001$), whereas EPI increased more these responses in lean than in fat Holstein cows (drug $\times$ fatness interaction).

The BCS was related to the maximal plasma NEFA response to ISO or EPI in overfed cows ($r = 0.74$ and 0.67 , $n = 18$, $P < 0.001$ and 0.01 , respectively), whereas these relationships were not significant in underfed cows.

Underfeeding tended to decrease the plasma glucose AUC ($P < 0.10$; 30.1 vs. 23.0 mM/min for over- and underfed cows, respectively, Figure 3). EPI increased ($P < 0.001$) the plasma glucose AUC compared with ISO (46.6 vs. 11.5 mM/min for EPI and ISO, respectively). Underfeeding decreased the plasma lactate AUC for ISO, whereas it was unchanged for EPI (38.7 and 51.8 mM/min for ISO in under- and overfed cows, and 44.4 and 44.9 mM/min for EPI in under- and overfed cows, respectively, energy level $\times$ drug interaction, $P < 0.05$). The plasma lactate AUC for ISO or EPI was higher ($P < 0.001$) for fat Charolais cows than for fat Holstein cows (58.7 vs. 34.1 mM/min, respectively, Figure 3). Fat Holstein cows tended to have lower ($P < 0.10$) plasma glucose AUC than lean Holstein cows (23.0 vs. 26.3 mM/min, respectively), whatever the drug. Plasma glucose AUC tended to be higher ($P < 0.10$) for fat than for lean Holstein cows.

DISCUSSION

Few experiments have examined the effects of body fatness on basal and stimulated body fat mobilization using different drugs in cows, and none have studied simultaneously the influence of body fatness and breed. Novel aspects of this study concern particularly the energy level $\times$ drug and fatness $\times$ drug interactions, allowing to evaluate the respective part of β - and α 2-components in the lipolytic response in cows.

Effect of Energy Level on the Basal Metabolite Concentrations and Responses of Plasma Metabolite Concentrations to ISO or EPI Injection

Underfeeding markedly enhanced basal plasma NEFA and glycerol concentrations in our study, in agreement with previous studies (Blum et al., 1982; Xing et al., 1991; Chilliard et al., 1998b).

Table 2. Effects of injection of ISO or EPI on plasma nonesterified fatty acid and glycerol concentrations in underfed or overfed fat Charolais, fat or lean Holstein nonpregnant adult cows

Group	Fat Charolais						Fat Holstein						Lean Holstein						Effects ^a			
	ISO		EPI		Low		High		Low		High		Low		High		Low		High		EPI	
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
Energy level																						
NEFA																						
Basal, mM	0.403	0.109	0.539	0.057	0.280	0.065	0.248	0.080	0.181	0.033	0.211	0.023	0.121	0.001	-	-	0.05	NS	NS	-	-	-
Response at 5 min, mM	0.560	0.451	0.180	0.164	0.369	0.371	0.066	0.081	0.528	0.344	0.126	0.077	0.038	NS	0.001	0.10	0.05	NS	NS	NS	NS	NS
Response at 10 min, mM	1.081	0.629	0.276	0.160	0.960	0.662	0.156	0.135	1.171	0.406	0.250	0.069	0.050	0.001	0.001	0.001	NS	NS	NS	NS	NS	0.05
Maximal response, mM	1.245	0.629	0.358	0.176	1.142	0.662	0.251	0.136	1.253	0.406	0.272	0.084	0.059	0.001	0.001	0.001	NS	NS	NS	NS	NS	0.10
Glycerol																						
Basal, µM	39.6	41.9	56.1	32.5	41.3	30.4	44.2	47.4	37.8	34.1	39.3	26.7	4.1	0.01	-	-	0.05	0.01	-	-	-	-
Response at 5 min, µM	135.3	113.0	65.9	37.1	103.5	91.5	35.7	24.0	74.5	64.0	41.7	18.9	7.9	0.05	0.001	NS	0.05	NS	NS	0.05	NS	NS
Response at 10 min, µM	158.9	83.4	79.1	29.8	174.1	69.6	37.0	19.3	100.8	47.7	49.8	9.3	12.1	0.001	0.001	0.01	NS	0.10	0.01	0.01	0.05	0.05
Maximal response, µM	162.8	113.0	79.9	39.8	175.6	93.4	43.3	26.6	103.6	64.0	53.6	21.5	10.7	0.01	0.001	0.05	NS	0.10	0.01	0.01	0.10	0.10

^a NS, not significant. For all variables, drug x breed interaction, and drug x breed x energy level interaction were not significant.^b Iso, isoproterenol; EPI, epinephrine.

Table 3. Effects of energy level, breed, and fatness on basal plasma glucose and lactate concentrations in underfed or overfed, fat Charolais, fat or lean Holstein nonpregnant adult cows

Group	Fat Charolais		Fat Holstein		Lean Holstein		SEM	Effects ^a		
Energy level	Low	High	Low	High	Low	High		Energy level	Contrast Charolais vs. Holstein	Contrast fat vs. lean
Glucose, mM	3.75	3.81	3.79	3.81	3.44	3.61	0.04	NS	NS	0.05
Lactate, mM	0.72	0.76	0.48	0.63	0.46	0.53	0.03	NS	0.05	NS

^a NS, not significant.

Figure 2. Effects of injection of ISO (6 nmol/kg of lean mass) or EPI (6 nmol/kg of lean mass) on plasma NEFA and glycerol concentrations in underfed (open symbols) or overfed (filled symbols) fat Charolais (filled or open ovals), and fat (filled or open squares) or lean (filled or open triangles) Holstein adult, nonpregnant cows. The plasma NEFA AUC to ISO were 36.99, 10.31, 39.46, 15.60, 33.04, and 6.96 mM.min in underfed and overfed fat Charolais, underfed and overfed fat, and underfed and overfed lean Holstein cows, respectively. The plasma NEFA AUC to EPI were 4.75, 3.48, 7.78, 2.96, 7.39, and 1.09 mM.min, respectively. The plasma glycerol AUC to ISO were 3.76, 1.53, 3.85, 1.95, 2.06, and 0.85 mM.min in underfed and overfed fat Charolais, underfed and overfed fat, and underfed and overfed lean Holstein cows. The plasma glycerol AUC to EPI were 1.31, 1.14, 0.98, 0.40, 1.13, and 0.63 mM.min in underfed and overfed fat Charolais, underfed and overfed fat, and underfed and overfed lean Holstein cows. Energy level ($P < 0.001$) and drug effects ($P < 0.001$), energy level $\times$ drug interaction ($P < 0.001$) were significant for plasma NEFA area under the response curve (AUC) and plasma glycerol AUC. The drug $\times$ fatness interaction ($P < 0.10$) tended to be significant for plasma NEFA AUC. The contrast “fat vs. lean” ($P < 0.05$), drug $\times$ fatness interaction ($P < 0.001$) were significant for plasma glycerol AUC.

Underfeeding increased the NEFA and glycerol responses to ISO, in agreement with previous studies in cattle (Blum et al., 1982; Chilliard et al., 1998b; Ferlay and Chilliard, 1999) and sheep (Chilliard et al., 2000).

ISO induced higher lipolytic effects than EPI, and this difference was more pronounced during

underfeeding. This result agrees with *in vivo* data obtained previously in cattle (Blum et al., 1982; Ferlay et al., 1996). In contrast, the dialysate glycerol response area to EPI, perfused into AT *in situ* by microdialysis method at 10^{-4} or 10^{-5} M, was similar to that to ISO in underfed ewes (Ferlay et al., 2000). Similarly, *in vitro* NEFA response

Figure 3. Effects of injection of ISO or EPI (6 nmol/kg of lean mass) on plasma glucose and lactate concentrations in underfed (open symbols) or overfed (filled symbols) fat Charolais (filled or open ovals), and fat (filled or open squares) or lean (filled or open triangles) Holstein non-pregnant adult cows. The plasma glucose AUC to ISO were 14.0, 14.7, 5.8, 15.5, 9.4, and 9.6 mM.min, and the plasma glucose AUC to EPI were 32.6, 48.0, 45.5, 56.0, 30.9, and 36.6 mM.min, in underfed and overfed fat Charolais, underfed and overfed fat, and underfed and overfed lean Holstein cows, respectively. The plasma lactate AUC to ISO were 50.2, 67.6, 37.2, 33.2, 28.8, and 54.7 mM.min, and the plasma lactate AUC to EPI were 58.8, 58.0, 26.5, 39.4, 47.9, and 37.4 mM.min in underfed and overfed fat Charolais, underfed and overfed fat, and underfed and overfed lean Holstein cows. Energy level ($P < 0.10$) and drug effects ($P < 0.001$), the contrast “fat vs. lean” ($P < 0.10$), the drug $\times$ fatness and drug $\times$ breed interactions were significant for plasma glucose area under the response curve (AUC). The contrast “Charolais vs. Holstein”, the energy level $\times$ drug ($P < 0.05$), drug $\times$ fatness $\times$ energy level, and drug $\times$ breed $\times$ energy level interactions ($P < 0.001$) were significant for plasma lactate AUC.

to EPI was equivalent to that to ISO in AT from well-fed ewes (Gilson et al., 1996). However, in AT from prepartum lactating cows, the in vitro lipolytic efficiencies of ISO and EPI were similar, whereas at lower doses, EPI tended to inhibit lipolysis because EPI has higher affinity than ISO to the antilipolytic α_2 -receptors (Smith and McNamara, 1989). Furthermore, in vitro AT lipolysis glycerol release stimulated by ISO was decreased by the α_2 -agonist clonidine from cows in early and mid-lactation, whereas there was no effect on NEFA release (Gagliostro and Chilliard, 1991).

In our study, in vivo, EPI, contrary to ISO, enhanced glycemia up to 4.9 mM. High plasma glucose concentrations (above 3.5 mM) can induce an antilipolytic effect (Brockman, 1984) and result in reduced plasma NEFA and glycerol responses. Thus, an in vivo antilipolytic effect of glucose could explain the difference in NEFA and glycerol

responses between EPI and ISO, contrary to in vitro or in situ situations without interaction with other metabolites or tissues. Another explanation could be that ISO has a higher affinity than EPI for β -AR, as demonstrated in human adipocytes (Lafontan, 2012). Alternatively, underfeeding could have decreased the number of α_2 -AR, which are coupled with antilipolytic responses (as shown in monogastric species, Lafontan and Berlan, 1995).

Underfeeding decreased plasma lactate AUC in response to ISO, whereas plasma lactate AUC in response to EPI was unchanged. Our data are in agreement with data from heifers underfed during 28 days and receiving an ISO injection (Ferlay and Chilliard, 1999) but in disagreement with data from steers fasted for 4 d (Blum et al., 1982), for which increase in plasma lactate AUC to EPI was observed, and with data from dairy cows fasted for 12 h (Fröli and Blum, 1988), for which fasting

reduced response of plasma lactate AUC to infusion of EPI. The differences in these respective responses could be due to the different duration of undernutrition or physiological status of animals among these studies.

Effect of Body Fatness on Basal Plasma Metabolite Concentrations and Responses of Plasma Metabolite Concentrations to ISO or EPI Injection

The lack of changes in basal plasma NEFA according to the level of body fatness in our study is surprising and is in disagreement with previous studies: Bines and Morant (1983) with lean or fat underfed or overfed nonlactating cows, and McCann et al. (1992) with lean, fat, or obese nonlactating sheep fed at maintenance or ad libitum, Chilliard et al. (1998b) with underfed or overfed dry and lactating cows, and Pires et al. (2013) with early lactating cows with low or high BCS. The absence of changes in basal and stimulated NEFA responses in this study could be explained partly by the difference of age between the two groups of Holstein cows, with fat Holstein cows being 2 yr older than lean cows. Aging is indeed associated with a decline in ability to mobilize NEFA during β -AR challenge in humans (Lafontan and Langin, 2009), which could have masked in part an increase due to body fatness in ruminants. Nevertheless, the higher basal plasma glycerol concentrations for fat compared with lean Holstein cows are in line with lipomobilization determined by plasma NEFA in these previous studies in cows and sheep.

The increased basal plasma glycerol concentrations in our fat animals could be due to their increased AT mass, as was suggested by Lafontan and Langin (2009) for obese human subjects. Furthermore, the hyperglycemia (observed in this study) and the hyperinsulinemia (Delavaud et al., 2002) in fat Holstein cows suggest that fat cows could present insulin resistance. The adipocytes enlarge in the fat animals, in concert with the development of hormonal refractoriness, and thus, insulin is less efficient at inducing antilipolytic action (Lafontan and Langin, 2009). However, the higher age of fat vs. lean Holstein cows could also have induced a lower sensitivity of adipocytes to the antilipolytic effect of insulin, as reported by Engfeld and Arner (1988) in adipocytes from old humans.

Plasma glycerol, but not NEFA, concentrations after injection of ISO were higher in fat than in lean Holstein cows. The response of plasma NEFA to an ISO challenge was positively related to BCS

in both dry and lactating cows (Chilliard et al., 1998b) and to the amount of body lipids in well-fed and overfed dry ewes (Chilliard et al., 2000) and in well-fed lactating ewes (Chilliard et al., 2000). However, the plasma NEFA response to ISO was high and not related to body lipids in underfed ewes (Chilliard et al., 2000), which is in agreement with the high glycerol and NEFA maximal responses from lean Holstein cows in this study when they were underfed, but not when they were overfed (Table 2 and significant drug $\times$ fatness $\times$ energy level interaction). This could reflect a priority for fat mobilization in underfed animals, whereas during overfeeding, AT basal mobilization (or turnover) is only observed in fat animals (Chilliard et al., 2000).

There was a linear relationship between adipocyte volume (measured by Delavaud et al., 2002, in the same animals, on average 73 and 523 pL for lean and fat Holstein cows, respectively) and plasma maximal NEFA response to ISO or EPI in overfed cows ($r = 0.66$ and 0.61 , $n = 18$, $P < 0.01$, respectively) but not in underfed cows. These results are in agreement with in vivo glycerol concentrations obtained by microdialysis in humans (Barbe et al., 1997), reporting a positive correlation between adipocyte diameter and the catecholamine-stimulated rate of lipolysis. Other studies have reported that in vitro lipolysis, basal or stimulated by β -AA or norepinephrine, was positively correlated with adipocyte volume in sheep (Vernon, 1996) and in lactating cows (Gagliostro and Chilliard, 1991). One explanation could be that adipocyte volume is positively related to β -AR number and cyclic AMP levels (Engfeldt and Arner, 1988; Reynisdottir et al., 1997). However, plasma NEFA (basal or response to ISO) is better correlated to energy balance than to adipocyte volume in lactating cows (Gagliostro and Chilliard, 1991).

The lipolytic effect of EPI was lower in fat than in lean Holstein cows, whereas ISO induced a higher effect in fat than in lean Holstein cows. These opposite effects could be linked to the specific properties of each drug. The lower lipolytic action of EPI could be due to an increased antilipolytic response linked to α 2-AR stimulation or to an increase in α 2-AR number in fat cows. This fact has been observed in subcutaneous adipocytes of obese subjects (Lafontan and Langin, 2009). In addition, EPI has a higher affinity for α 2-AR than β -AR (Smith and McNamara, 1989; Lafontan, 2012), which could reinforce the antilipolytic effect of EPI in fat cows. The higher lipolytic effect of ISO in fat vs. lean cows could be due to an enhancement of β 2-AR number or lipolytic

response to β -AR stimulation in larger adipocytes. Nevertheless, this lipolytic action of ISO in fat cows is contradictory to the effects observed in obese humans, since body fatness decreased the ISO sensitivity in humans (Lafontan and Langin, 2009). To understand the effect of aging in lipomobilization in cows is difficult because of the lack of studies in ruminants involving this factor. In our study, it seems that aging had an impact on the α 2-AR component of the lipolytic response. Indeed, aging induced a loss of α 2-AR number in adipocytes from old humans (Engfeldt and Arner, 1988) and a drop of in vitro maximum lipolysis in response to EPI in old rats (Fain and Garcia-Sainz, 1983). Such effects could explain the lower effect of EPI in fat vs. lean cows. In contrast, aging decreased the expression of β 2-AR in adipocytes from humans (Lafontan and Langin, 2009), and this effect is in disagreement with the higher action of ISO in "old" fat cows observed in our study. Further investigation is needed to better understand the respective influences of body fatness and aging on body fat mobilization in cows.

Effect of Breed on Basal Metabolite Concentrations and Responses of Plasma Metabolite Concentrations to ISO or EPI Injection

Fat Charolais cows had higher basal plasma NEFA concentration than fat Holstein cows, which is surprising because adipocyte diameter and plasma leptin were lower (Delavaud et al., 2002).

The plasma NEFA or glycerol response at 5 min to ISO or EPI was greater for fat Charolais than for fat Holstein cows. Nevertheless, this effect did not persist since the response at 10 min, and the AUC were similar between the two groups. Simultaneously, the plasma lactate response AUC to drug was higher for Charolais than for Holstein cows. As plasma lactate may be antilipolytic in rodent or human adipocytes (Lafontan and Langin, 2009; Ahmed et al., 2010), this action could contribute to the nonpersistence of the NEFA or glycerol response. Moreover, the higher lactate concentrations in fat Charolais than in fat Holstein cows could be due to a putatively higher stress level, since it was shown that plasma lactate concentrations are higher in stressed animals (Lindsay, 1978). Putative stressful situations could also result in the increased plasma NEFA concentration for fat Charolais cows, as already observed by Earley et al. (2013) in beef bulls that were subjected to stress linked to transport. Enhancement of mobilization is due to an increase in central

nervous system drive and the secretion of stress hormones (Lafontan and Langin, 2009). Another explanation could be related to the difference in muscularity between the two breeds, which could make Charolais cows susceptible to higher plasma lactate release from the carbohydrate metabolism. Nevertheless, results contradictory to this hypothesis were obtained by McGilchrist et al. (2011), demonstrating that high-muscled Angus steers had lower basal plasma lactate than low-muscled Angus steers.

CONCLUSION

Underfeeding strongly increased basal plasma NEFA and glycerol concentrations in nonlactating adult cows due to body lipid mobilization, in agreement with increased NEFA and glycerol responses to ISO or EPI challenge, despite decreases in plasma leptin concentrations. Fat Holstein cows had higher basal glycerol, glucose, leptin, and insulin concentrations as well as glycerol response to ISO than lean Holstein cows. The higher body fat mobilization in fat cows could be due to the increased AT mass, adipocyte size, and leptinemia and/or insulin resistance. The lipolytic effect of EPI was lower in fat than in lean Holstein cows, whereas ISO induced a higher effect in fat than in lean Holstein cows, suggesting an increased antilipolytic effect due to α 2-AR stimulation in fat cows. Breed had a significant effect on basal plasma NEFA, glycerol, and lactate concentrations. Moreover, breed modified plasma NEFA, glycerol, and lactate response to adrenergic challenge, with higher values for fat Charolais cows despite lower adipocyte size and leptin, suggesting that this breed is more sensitive than Holstein to stress. This study shows that there are strong variations in basal and stimulated body fat mobilization, indicating different metabolic and hormonal adaptive responses to underfeeding, and according to breed and/or body fatness.

Conflict of interest statement. None of the authors have any conflict of interest to declare.

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