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Genetic ancestry modifies fatty acid concentrations in different adipose tissue depots and milk fat

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The objective of this study was to determine the effect of cow genetic strain on fatty acid (FA) profiles in adipose tissue and milk. Adipose samples from two subcutaneous (shoulder and tail-head) and three visceral (kidney channel, mesenteric and omental) depots were obtained post mortem from New Zealand (NZ; $n=8$) and North American (NA; $n=8$) Holstein–Friesian cows. At the time of slaughter cows were in similar body condition (NZ: 4·0 ± 0·03, NA: 4·0 ± 0·02; ± SD) and stage of lactation (NZ: 90 ± 11·2 d; NA: 83 ± 4·3 d; ± SD). Milk was collected during the a.m. milking prior to slaughter and milk fat was extracted. Adipose and milk fat FA were quantified using gas chromatography. NZ cows had a lower proportion of saturated FA in shoulder, tail-head and omental adipose tissue and a greater proportion of mono-unsaturated FA and an elevated Δ9-desaturase index in shoulder and tail-head adipose tissue. The proportions of individual FA differed between adipose depots, with proportions of de-novo FA greater in subcutaneous compared with visceral adipose depots. Milk from NZ cows contained greater concentrations of short chain FA (C8 : 0–12 : 0) and CLA, and less *cis*-9 18 : 1 than milk from NA cows. Regression analysis identified moderate associations between milk FA and shoulder adipose tissue FA for 18 : 2 ($R^2=0·24$), 18 : 3 $n-3$ ($R^2=0·39$), and polyunsaturated fatty acids ($R^2=0·38$). Results from this study support the hypothesis that genetic strain dictates FA profiles in adipose tissue and milk and may alter the metabolic status of the various adipose depots differently. The data further support the premise that genetic strain affects the metabolic status of the various adipose depots differently. Elucidating the mechanisms that regulate the different adipose depots in the NZ and NA strains will increase our understanding of tissue mobilization and replenishment.

Keywords: Genetic strain, fatty acid, adipose, milk, dairy cow.

Differing selection priorities among dairying nations have led to the development of divergent genetic strains of Holstein–Friesian (HF) dairy cows (Harris & Kolver, 2001). Studies comparing North American (NA) and New Zealand (NZ) HF strains report an effect of genetic strain on energy partitioning and milk production. Under similar management systems, milk yields, milk composition and body condition (BC) profiles differed between for NA and NZ cows (Horan et al. 2005; Roche et al. 2006; McCarthy et al. 2007; Macdonald et al. 2008; Wales et al. 2009). These data support an effect of genetic strain on adipose tissue metabolism, especially in early lactation. Consistent with this hypothesis, genetic selection has altered insulin signalling, the level of insulin resistance (Chagas et al. 2009), hepatic gluconeogenesis (White et al. 2012) and functional components of the somatotropic axis (Lucy et al. 2009; McCarthy et al. 2009; Meier et al. 2010; Grala et al. 2011). When considered together, these data provide evidence that the mechanisms and pathways controlling nutrient partitioning differs between NZ and NA HF cows. A better understanding of the physiology of individual adipose depots from differing genetic strains may provide new insights into the preferential mobilization and replenishment of these depots.

A key component of nutrient partitioning is the mobilization of adipose tissue in response to the onset of lactation (Bauman & Currie, 1980; McNamara & Hillers, 1986). This is evident in the reported difference in BC among NZ and NA cows after calving (Roche et al. 2006; Macdonald et al. 2008). Mobilization and accumulation of fat in different depots occurs in a coordinated manner with intermuscular and subcutaneous adipose depots mobilized first, followed by the internal and visceral depots (Butler-Hogg et al. 1985;

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Pethick & Dunshea, 1996). In turn, differences in adipocyte size, fatty acids composition, and gene expression are evident among the different adipose depots (Hood & Allen, 1973; Hausman et al. 2009; Dodson et al. 2010). Based on the BC loss and gain that occurs with NZ and NA cows, it appears that genetic selection has altered adipose tissue metabolism (mobilization and replenishment). Thus, it was hypothesized that genetic selection of dairy cows has resulted in differences in the composition and metabolic status of adipose tissues. The objective of the current study was to evaluate FA profiles in samples from two sub-cutaneous (SC) and three visceral (VC) adipose depots collected from NZ and NA cows. A secondary objective was to investigate associations among milk and tissue FA to evaluate whether relationships exist and if so, evaluate any such associations.

Materials and methods

Animals and their management

A full description of the genetic strains of HF cows in this experiment has been reported previously (Macdonald et al. 2008). Cows were managed in a single herd and grazed ryegrass/white clover pastures in an intensive rotation manner. Grazing cows were allocated a pasture allowance of >40 kg of dry matter (DM)/cow per day (measured to ground level). Grazing residuals were used to ensure adequate pasture allowance: post grazing residuals of greater than 1800 kg DM/ha were targeted. Briefly, the NZ cows contained an average of ⩾ 76% NZ genetics ($n=8$) and the NA cows ⩾ 91% NA genetics ($n=8$). Cows in this study were part of a larger study investigating endometrial FA (Meier et al. 2009) with eight cows on day 17 of the oestrous cycle (NZ $n=4$, NA $n=4$) and the balance on day 17 of pregnancy (NZ $n=4$, NA $n=4$).

Individual milk yields were recorded at each milking using in-line milk meters (Tru-Test Ltd, Auckland, NZ) and milk composition was determined fortnightly by Fourier-transform infrared spectroscopy (FT120, Foss Electric, Hillerød, Denmark) from composite samples (p.m. and a.m.). An aliquot of the milk sample collected at the a.m. milking prior to slaughter was stored at −70 °C for fat extraction and subsequent FA analysis. BC was assessed fortnightly using a scale of 1–10 (where 1 is emaciated and 10 obese; Roche et al. 2004). Table 1 summarizes cow parameters and milk production and composition for the NZ and NA cows in this study. The Ruakura Animal Ethics Committee (Hamilton, NZ) approved the procedures undertaken during this study.

Cows were slaughtered at a commercial abattoir (AgResearch Abattoir, Hamilton, NZ). Adipose tissue samples (approximately 0·5–1·0 g) were collected from five depots (front-shoulder, tail-head, kidney channel (kidney), mesenteric, and omental) within 45 min after slaughter. Following inspection, the samples were cut to size before being transferred to cryo-vials. Samples were frozen in liquid nitrogen and subsequently stored at −70 °C until analysis.

Table 1. Phenotypic description of the dairy cows used, including ancestry, lactation number, days in milk (DIM), milk yield and milk composition. Cows were of North American (NA; $n=8$) and New Zealand (NZ; $n=8$) origin. Data represent the means and the ranges

Variable	NZ	Range	NA	Range
Cows, *n*	8	—	8	—
% NA genetics	22	11–31	93	88–93
Lactation, *n*	3·5	2–6	3·9	2–5
Days in milk†	90	81–117	83	75–90
Milk‡				
Yield, kg/d	28·0	22·1–36·5	28·2	22·0–35·1
Protein yield, kg/d	1·0	0·78–1·16	1·0	0·77–1·16
Fat yield, kg/d	1·1	0·86–1·13	1·1	0·63–1·43
Protein, %	3·6	3·12–4·27	3·4	3·11–3·60
Fat, %	4·1	3·23–4·94	3·8	2·42–4·63
Milk at slaughter§				
Yield, kg/d	27·6	22·0–31·5	30·0	25·0–33·5
Protein yield, kg/d	1·0	0·81–1·46	1·0	0·88–1·13
Fat yield, kg/d	1·2	0·92–1·46	1·1	0·57–1·40
Protein, %	3·6	3·13–4·19	3·5	3·32–3·59
Fat, %	4·2	3·04–4·63	3·9	2·29–5·05

†DIM on the day of slaughter
‡Milk yield and composition when 78 ± 1·4 DIM (range: 65 to 85 DIM)
§Milk yield and composition during the 24 h before slaughter; NZ $n=8$, NA $n=6$

Fat extraction and fatty acid methylation

Adipose tissue. A combined fat extraction and trans-methylation method was used to extract and generate FA methyl esters (FAME). Samples were firstly freeze dried, then methylation reagents added (5 ml of 2% H_2SO_4 in methanol : toluene 1 : 1 v/v), along with an internal standard (23 : 0 methyl ester). Samples were heated to 80 °C for 2 h under N_2. After cooling to room temperature, 5 ml of saturated NaCl were added and the sample mixed, and then centrifuged at 2500 ***g*** for 10 min. The toluene fractions containing the FAME were removed, the sample washed with an additional 2 ml of toluene, and the two toluene fractions combined for FAME analysis.

Milk. Milk fat was extracted from milk samples using a modified Röse–Gottlieb gravimetric method (IDF, 1996). FAME were prepared from the milk fat sample using a cold base catalysation method (IUPAC, 1987). Briefly, 30 mg of melted fat sample was dissolved in 2 ml heptane and 20 µl 2 m-potassium hydroxide (in methanol) was added. The mixture was vortexed for 30 s and allowed to stand for 5 min before being neutralized by adding 25 µl 2 m-HCl. The sample was again vortexed at 3000 rpm for 2 min, and the layer containing the milk FAME removed for analysis.

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Fatty acid analysis

FAME were quantified by gas chromatography (MacGibbon, 1988) using a HP 6890 equipped with a flame ionization detector (Agilent Technologies, Santa Clara CA, USA) on a RTX 2330 column (40 m length × 0·18 mm i.d. and 0·1 μM film thickness; Restek Corporation, Bellefonte PA, USA). A 1-μl sample was injected using an injection ratio of 150 : 1. The injector temperature was 260 °C. The initial oven temperature was 75 °C for 2 min; it was then increased to 220 °C at a rate of 15°/min, held for 1·5 min and then increased to 260 °C at 30°/min, and held for 30 s. The column was set to the constant flow mode, with H_2 at 47 cm/s average linear velocity. The injector and detector temperatures were 260 °C. The same FAME standards were used for both adipose tissue and milk FA identification. These were obtained from Matreya Inc. (Pleasant Gap PA, USA): a CLA isomer mix from Sigma Chemicals (St Louis MO, USA), Nu Check Prep (Eylsian MN, USA), and Supelco® 37 Component FAME Mix (Supelco, Sigma Aldrich, St Louis MO, USA). Adipose and milk FA are expressed as the percentage of total FA (% of total FA).

Statistical analyses

Statistical analyses were undertaken using GenStat Release 11 statistical package (Payne et al. 2008). All data are presented as estimated means ± SED unless otherwise stated. Differences in BC were analysed by repeated measures analysis using a compound symmetry covariance structure to model within-cow measurements through time. This structure was chosen as the fit was improved somewhat compared with other covariant structures. Fixed effects included were day of measurement (day group), strain, reproductive status (pregnant or not pregnant) and their interactions, and a constant. Random effects in the model included cow and cow-by-day group interactions.

Differences in individual or grouped FA within adipose tissue were analysed with mixed models fitted using restricted maximum likelihood (REML). The model included adipose tissue, strain, and their interactions as fixed effects, and cow within strain as a random effect. Milk FA (individual and grouped FA) were analysed using a model that included strain as a fixed effect, and cow within strain as a random effect. Parity was not included in the model, as all cows were multiparous. Within strain, adipose tissue FA were regressed against milk FA. Milk FA were grouped based on their origin, de novo (< 16 : 0 included: 4 : 0, 6 : 0, 8 : 0, 10 : 0, 12 : 0, 14 : 0 and 14 : 1), mixed origin (16 : 0 and 16 : 1) and preformed [> 16 : 0 included: 17 : 0, 18 : 0, *trans*-6–8 18 : 1, *trans*-11 18 : 1 *cis*-9 18 : 1, *cis*-11 18 : 1, 18 : 2,*cis*-9, *trans* − 11 18 : 2 (CLA) and 18 : 3 *n* − 3]. Adipose FA origin groups were de novo (< 16 : 0 included: 14 : 0, 14 : 1 and 15 : 0), mixed origin (16 : 0 and 16 : 1) and preformed [> 16 : 0 included: 17 : 0, 18 : 0, *trans*-11 18 : 1, *cis*-9 18 : 1, *cis*-11 18 : 1, 18 : 2, *trans*-11 18 : 2 (CLA) and 18 : 3 *n* − 3]. FA were also grouped based on the degree of saturation for both adipose tissue and milk.

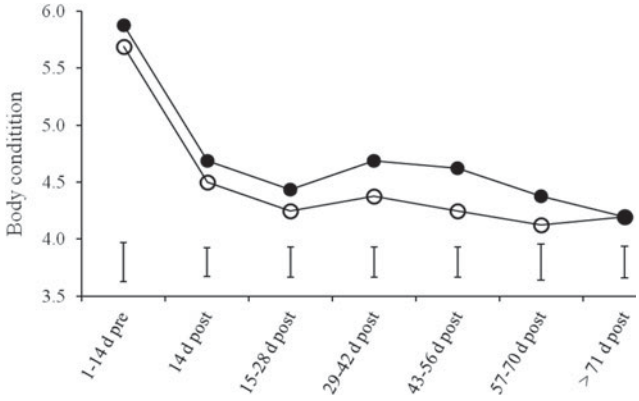

Fig. 1. Pre- and post-calving body condition score (scale 1–10) for the North American (○, *n* = 8) and New Zealand (●, *n* = 8) Holstein Friesian cows. Standard errors represent the SED within strain. Statistical significance: Strain (*P* = 0·35), Day group (*P* < 0·01), Strain × day group (*P* = 0·98).

Adipose tissue saturation groups included: saturated FA (SFA; 14 : 0 + 16 : 0 + 17 : 0 + 18 : 0), mono-unsaturated FA (MUFA; 14 : 1 + 16 : 1 + *trans*-11 18 : 1 + *cis*-9 18 : 1 + *cis*-11 18 : 1) and polyunsaturated FA (PUFA; 18 : 2 + CLA + 18 : 3). For milk the saturation groups were: SFA (4 : 0 + 6 : 0 + 8 : 0 + 10 : 0 + 12 : 0 + 14 : 0 + 16 : 0 + 17 : 0 + 18 : 0), MUFA (14 : 1 + 16 : 1 + *trans* − 6–8 18 : 1 + *trans*-11 18 : 1 + *cis*-9 18 : 1 + *cis*-11 18 : 1) and PUFA [18 : 2 + *cis*-9, *trans*-11 18 : 2 (CLA) + 18 : 3*n* − 3]. The Δ9-desaturase indices were calculated using the ratios of 14 : 1 to 14 : 0, 16 : 1 to 16 : 0, *cis*-9 18 : 1/18 : 0, CLA/*trans*-11 18 : 1 and overall desaturase index (14 : 1 + C16 : 1 + *cis*-9 18 : 1 + CLA)/(14 : 0 + 16 : 0 + 18 : 0 + *trans*-11 18 : 1) + (14 : 1 + 16 : 1 + *cis*-9 18 : 1 + CLA) (Kay et al. 2005; Meier et al. 2009).

Results

BC data for both genetic strains are summarized in Fig. 1. There was no effect of strain on BC (*P* = 0·35), with the average BC immediately prior to slaughter being 4·1 and 4·1 (max SED 1·4) for the NZ and NA cows, respectively.

Significant (*P* < 0·05) strain by adipose depot interactions were identified for SFA, MUFA and Δ9-desaturase indices (Table 2). Concentrations of SFA were greater (*P* < 0·05) in omental, shoulder and tail-head adipose tissue from the NA cows. Concentrations of MUFA and Δ9-desaturase indices were greater (*P* < 0·05) in the shoulder and tail-head adipose tissue of NZ compared with NA cows (Table 2).

The main effects of strain and adipose depot on individual FA and groups based on origin (de novo, mixed and preformed) are summarized in Table 3. Fatty acid composition of shoulder and tail-head adipose tissue was different, with greater (*P* < 0·05) proportions of 17 : 0, 18 : 1 and pre-formed FA in tail-head compared with shoulder adipose. Variations in the FA profiles among the internal adipose depots were also evident. Omental adipose contained greater (*P* < 0·05) proportions of 16 : 0 and

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Table 2. Proportions of fatty acid groups (% of total) in five adipose tissue depots (kidney, mesenteric, omental, shoulder and tail-head) in samples obtained from North American (NA) and New Zealand (NZ) Holstein-Friesian cows. Data represent the mean with variance represented as the SED

		Adipose tissue						*P* values		
Fatty acid groups	Strain†	Kidney	Mesenteric	Omental	Shoulder	Tail-head	SED	Strain	AT depot	S × AT‡
Saturation										
SFA§	NA	$58{\cdot}04^{b}$	$58{\cdot}23^{b}$	$62{\cdot}96^{c,y}$	$50{\cdot}22^{a,y}$	$48{\cdot}32^{a,y}$	1·544	<0·05	<0·001	0·002
	NZ	$58{\cdot}00^{b}$	$58{\cdot}63^{b}$	$59{\cdot}29^{b,x}$	$43{\cdot}87^{a,x}$	$42{\cdot}71^{a,x}$				
MUFA¶	NA	$33{\cdot}11^{b}$	$32{\cdot}07^{b}$	$28{\cdot}66^{a}$	$39{\cdot}20^{c,x}$	$42{\cdot}53^{d,x}$	1·532	<0·05	<0·001	<0·05
	NZ	$33{\cdot}65^{a}$	$32{\cdot}30^{a}$	$31{\cdot}66^{a}$	$43{\cdot}78^{b,y}$	$47{\cdot}53^{c,y}$				
PUFA††	NA	2·61	2·70	2·58	2·76	2·68	0·203	0·36	<0·05	0·39
	NZ	$2{\cdot}54^{a}$	$2{\cdot}74^{a,b}$	$2{\cdot}74^{a,b}$	$3{\cdot}12^{b}$	$2{\cdot}99^{a,b}$				
Δ9-desaturase indices										
14 : 1/14 : 0	NA	$0{\cdot}08^{a}$	$0{\cdot}07^{a}$	$0{\cdot}08^{a}$	$0{\cdot}17^{b,x}$	$0{\cdot}19^{b,x}$	0·027	<0·001	<0·05	0·08
	NZ	$0{\cdot}08^{a}$	$0{\cdot}07^{a}$	$0{\cdot}10^{a}$	$0{\cdot}24^{b,y}$	$0{\cdot}27^{b,y}$				
16 : 1/16 : 0	NA	$0{\cdot}06^{a}$	$0{\cdot}05^{a}$	$0{\cdot}04^{a}$	$0{\cdot}11^{b}$	$0{\cdot}12^{b,x}$	0·014	<0·001	0·15	0·05
	NZ	$0{\cdot}06^{a}$	$0{\cdot}05^{a}$	$0{\cdot}05^{a}$	$0{\cdot}14^{b}$	$0{\cdot}16^{b,y}$				
cis-9 18 : 1/18 : 0	NA	$0{\cdot}85^{a}$	$0{\cdot}75^{a}$	$0{\cdot}63^{a}$	$1{\cdot}69^{b,x}$	$1{\cdot}76^{b,x}$	0·210	<0·001	<0·05	<0·01
	NZ[a]	$0{\cdot}86^{a}$	$0{\cdot}87^{a}$	$0{\cdot}80^{a}$	$2{\cdot}32^{b,y}$	$2{\cdot}68^{b,y}$				
CLA/*trans* − 11 18 : 1	NA	$0{\cdot}11^{a}$	$0{\cdot}09^{a}$	$0{\cdot}08^{a}$	$0{\cdot}41^{c,y}$	$0{\cdot}22^{b}$	0·034	<0·001	0·57	<0·01
	NZ	$0{\cdot}10^{a}$	$0{\cdot}10^{a}$	$0{\cdot}09^{a}$	$0{\cdot}28^{b,x}$	$0{\cdot}27^{b}$				
Δ9-desaturase index‡‡	NA	$0{\cdot}32^{b}$	$0{\cdot}30^{a}$	$0{\cdot}26^{a}$	$0{\cdot}42^{c,x}$	$0{\cdot}43^{c,x}$	0·019	0·10	<0·001	0·02
	NZ	$0{\cdot}32^{a}$	$0{\cdot}30^{a}$	$0{\cdot}29^{a}$	$0{\cdot}47^{b,y}$	$0{\cdot}49^{b,y}$				

† Strain: NA = North American ($n = 8$), NZ = New Zealand ($n = 8$)
‡ S × AT = Strain by adipose tissue interactions
§ SFA = Saturated fatty acids (14 : 0 + 16 : 0 + 17 : 0 + 18 : 0)
¶ MUFA = monounsaturated fatty acids (14 : 1 + 16 : 1 + *trans* − 11 18 : 1 + *cis*-9 18 : 1 + *cis*-11 18 : 1)
†† PUFA = polyunsaturated fatty acids (18 : 2 + CLA + 18 : 3)
‡‡ Δ9-desaturase index (14 : 1 + 16 : 1 + *cis*-9 18 : 1 + CLA)/(14 : 0 + 16 : 0 + 18 : 0 + *trans* − 11 18 : 1) + (14 : 1 + 16 : 1 + *cis*-9 18 : 1 + CLA)
[a,b,c,d] Within each fatty acid category, means without common superscripts differ ($P < 0{\cdot}05$) between depots
[x,y] Within each fatty acid category, means without common superscripts differ between NA and NZ HF cows ($P < 0{\cdot}05$)

de-novo FA whereas kidney adipose contained lower ($P < 0{\cdot}05$) proportions of 15 : 0, 17 : 0 and 18 : 0. There was a strain by depot interaction ($P < 0{\cdot}05$) detected for palmitic acid (16 : 0); such that concentrations differed between depots in NA cows but were similar for all depots in NZ cows (Table 3).

De-novo FA were most abundant in shoulder adipose and intermediate in tail-head, omental and kidney adipose, which were not different from each other, and were in lowest concentrations in mesenteric adipose. Higher concentrations of mixed-origin FA were evident in shoulder relative to tail-head adipose tissue, which in turn contained greater concentrations than the visceral adipose tissue depots (omental, mesenteric and kidney). Preformed FA had the reverse pattern to mixed-origin FA, with visceral adipose tissue containing the greatest concentrations, tail-head being intermediate, and shoulder adipose tissue containing the least concentrations of preformed FA.

Proportions of 8 : 0, 10 : 0, 12 : 0, *trans*-6–8 18 : 1, CLA and total de-novo FA were greater ($P < 0{\cdot}05$) in milk from NZ cows whereas milk from NA cows contained proportionately more 4 : 0, and *cis*-9 18 : 1 (Table 4).

Few associations ($P < 0{\cdot}05$) were observed among milk and adipose fatty acids. Associations ($P < 0{\cdot}05$) among milk and shoulder adipose were evident for 18 : 2 ($R^2 = 0{\cdot}24$), 18 : 3 ($R^2 = 0{\cdot}39$) and total PUFA ($R^2 = 0{\cdot}38$). Furthermore there were associations between FA in mesenteric adipose and milk (18 : 3, $R^2 = 0{\cdot}39$) and FA in omental adipose and milk (18 : 2 $R^2 = 0{\cdot}16$, 18 : 3 $R^2 = 0{\cdot}29$).

Discussion

This study provides further evidence that genetic strain is a factor affecting adipose tissue (Horan et al. 2005; Roche et al. 2006; Macdonald et al. 2008). Genetic strain modified adipose tissue in shoulder, tail-head and omental adipose depots, but there was no effect on either mesenteric or kidney channel depots. These differences were evident under conditions where days post partum and BC were similar between the two groups of cows. Additionally, the FA profiles of milk fat differed between NZ and NA cows.

The discovery that FA profiles within the shoulder, tail-head and omental adipose depot differed between NZ and NA cows provides evidence that the metabolic status of these three adipose depots differ. During adipose deposition, SFA are desaturated to MUFA before being incorporated into triacylglycerides for storage in adipocytes (Etherton & Evock, 1986). Hence, during lipogenesis, the proportions of SFA decrease and MUFA increase. The rate-limiting enzyme in the desaturation of SFA is stearoyl-CoA desaturase (SCD;

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Table 3. Fatty acid proportions (% of total) in five adipose tissue depots (kidney, mesenteric, omental, shoulder and tail-head) obtained from North American (NA) and New Zealand (NZ) Holstein-Friesian cows. Data represent means with variance represented as SED

Fatty acids (% of total FA)	Strain†			Adipose tissue						*P* values‡		
	NA	NZ	SED	Kidney	Mesenteric	Omental	Shoulder	Tail-head	SED	Strain	AT	S × AT
14 : 0	2·76	2·83	0·159	2·84	2·61	2·87	2·91	2·73	0·123	0·68	0·12	0·12
14 : 1	0·32	0·43	0·053	$0·24^{a}$	$0·18^{a}$	$0·25^{a}$	$0·60^{b}$	$0·62^{b}$	0·056	0·08	<0·001	0·18
15 : 0	0·51	0·52	0·035	$0·46^{a}$	$0·52^{b,c}$	$0·55^{c}$	$0·53^{b,c}$	$0·51^{b}$	0·018	0·91	0·001	0·06
16 : 0	22·86	22·77	0·845	$22·32^{a,b}$	$21·67^{a}$	$23·04^{b}$	$24·41^{c}$	$22·65^{a,b}$	0·658	0·92	0·002	0·02
16 : 1	1·78	2·14	0·293	$1·34^{a}$	$1·02^{a}$	$0·98^{a}$	$3·22^{b}$	$3·26^{b}$	0·234	0·25	<0·001	0·23
17 : 0	1·32	1·37	0·075	$1·37^{c}$	$1·51^{d}$	$1·51^{d}$	$1·13^{a}$	$1·20^{b}$	0·029	0·49	<0·001	0·48
18 : 0	28·11	25·02	1·558	$31·03^{b}$	$32·12^{b,c}$	$33·16^{c}$	$18·08^{a}$	$18·43^{a}$	0·961	0·07	<0·001	0·07
trans − 11 18 : 1	4·21	4·59	0·561	$4·89^{c}$	$5·35^{c}$	$5·29^{c}$	$2·65^{a}$	$3·82^{b}$	0·250	0·51	<0·001	0·65
cis-9 18 : 1	28·12	29·91	1·257	$26·22^{b}$	$25·01^{b}$	$22·95^{a}$	$34·14^{c}$	$36·75^{d}$	0·907	0·18	<0·001	0·25
cis-11 18 : 1	0·77	0·80	0·063	$0·70^{a}$	$0·64^{a}$	$0·70^{a}$	$0·88^{b}$	$0·98^{b}$	0·078	0·65	<0·001	0·65
18 : 2	1·24	1·29	0·081	1·20	1·31	1·28	1·32	1·22	0·070	0·58	0·30	0·58
CLA§	0·59	0·66	0·059	$0·51^{a}$	$0·48^{a}$	$0·43^{a}$	$0·81^{b}$	$0·89^{b}$	0·050	0·23	<0·001	0·44
18 : 3 *n* − 3	0·84	0·88	0·064	$0·87^{b,c}$	$0·93^{c}$	$0·96^{c}$	$0·81^{a,b}$	$0·73^{a}$	0·056	0·59	0·001	0·10
Unidentified	6·65	6·89	0·517	$6·02^{a}$	$6·66^{a}$	$6·09^{a}$	$8·52^{b}$	$6·54^{a}$	0·518	0·65	<0·001	0·30
Origin groups												
De novo¶	3·59	3·77	0·201	$3·54^{a,b}$	$3·31^{a}$	$3·67^{b}$	$4·03^{c}$	$3·86^{b,c}$	0·150	0·40	<0·001	0·34
Mixed origin††	24·64	24·91	1·076	$23·66^{a}$	$22·69^{a}$	$24·02^{a}$	$27·63^{c}$	$25·90^{b}$	0·784	0·81	<0·001	0·10
Preformed‡‡	65·10	64·43	1·195	$66·78^{c}$	$67·35^{c}$	$66·27^{c}$	$59·83^{a}$	$63·62^{b}$	0·841	0·59	<0·001	0·28

			Adipose tissuedepot						*P* values		
	16 : 0	Strain†	Kidney	Mesenteric	Omental	Shoulder	Tail-head	SED	Strain	AT depot	S × AT
		NA	$22·54^{a}$	$20·38^{a,x}$	$23·03^{b}$	$25·47^{c}$	$22·87^{b}$	1·073	0·92	0·002	0·02
		NZ	22·10	$22·95^{y}$	23·06	23·55	22·42				

† Strain: NA = North American (n = 8), NZ = New Zealand (n = 8)
‡ S × AT = Strain by adipose tissue interactions
§ CLA = *cis*-9, *trans* − 11 18 : 2
¶ De novo fatty acids (< 16 : 0)
†† Mixed origin fatty acids (16 : 0 + 16 : 1)
‡‡ Preformed fatty acids (> 16 : 0)
^{a,b,c,d} Within each fatty acid category, means without common superscripts differ (P < 0·05)
^{x,y} Within each fatty acid category, means without common superscripts differ between NA and NZ HF cows (P < 0·05)

Table 4. Proportions of fatty acids (% of total) in milk from North American (NA) and New Zealand (NZ) Holstein–Friesian cows. Data represent means with variance presented as SED

Fatty acids (%)	NA†	NZ	SED	*P* values
4:0	4·07	3·65	0·134	<0·01
6:0	2·45	2·46	0·070	0·85
8:0	1·45	1·63	0·067	<0·05
10:0	3·09	3·71	0·190	<0·01
12:0	3·46	4·20	0·247	<0·01
14:0	10·80	11·41	0·401	0·16
14:1	0·86	0·89	0·146	0·87
16:0	24·64	23·34	1·073	0·25
16:1	0·88	1·05	0·106	0·13
17:0	0·65	0·59	0·060	0·34
18:0	10·56	10·11	0·671	0·51
trans − 6–8 18:1	0·44	0·50	0·020	<0·05
trans − 11 18:1	4·38	5·18	0·398	0·07
cis-9 18:1	16·62	14·92	0·591	<0·01
cis-11 18:1	0·59	0·59	0·028	0·89
18:2	1·53	1·33	0·140	0·18
cis-9, *trans*-11 18:2 (CLA)	1·71	1·97	0·100	<0·05
18:3 *n* − 3	0·85	0·97	0·079	0·16
Unidentified	10·94	11·47	0·534	0·34
Origin groups				
De novo‡	26·2	28·0	0·80	<0·05
Mixed origin§	25·5	24·4	1·08	0·32
Preformed¶	37·4	36·2	0·90	0·22
Saturation groups				
SFA††	61·2	61·1	0·99	0·95
MUFA‡‡	23·8	23·1	0·49	0·21
PUFA§§	4·1	4·3	0·25	0·49
Δ9 desaturase indices				
C14:1/C14:0	0·08	0·08	0·013	0·92
C16:1/C16:0	0·04	0·05	0·005	0·06
cis-9 C18:1/C18:0	1·62	1·48	0·129	0·31
CLA/*trans* − 11 C18:1	0·40	0·38	0·032	0·66
Δ9 desaturase index¶¶	0·29	0·27	0·010	0·29

† NA ($n=8$), NZ ($n=8$)
‡ De novo fatty acids (<16:0)
§ Mixed origin fatty acids (16:0+16:1)
¶ Preformed fatty acids (>16:0)
†† SFA = Saturated fatty acids (4:0+6:0+8:0+10:0+12:0+14:0+16:0+17:0+18:0)
‡‡ MUFA = monounsaturated fatty acids (14:1+16:1+*trans* − 68 18:1+*trans* − 11 18:1+*cis*-9 18:1+*cis*-11 18:1)
§§ PUFA = polyunsaturated fatty acids [18:2+*cis*-9, *trans*-11 18:2 (CLA)+18:3 *n* − 3]
¶¶ Δ9 desaturase index (14:1+16:1+*cis*-9 18:1+CLA)/(14:0+16:0+18:0+*trans* − 11 18:1)+(14:1+16:1+*cis*-18:1+CLA)

Miyazaki et al. 2004; Flowers & Ntambi, 2008). A 5–6% decline in adipose tissue MUFA concentrations was reported to be associated with a 40% decline in SCD activity (Yang et al. 1999). In the current study, the differences in MUFA (+5%) and SFA (−7%) in sub-cutaneous adipose tissues between NZ and NA cows could indicate a tissue-specific increase in SCD activity in the NZ strain. The data presented here provide preliminary support for genetic influence on adipose tissue function.

The main effect of genetic strain on the FA profiles is evident in three of the five adipose depots investigated. The adipose depots that are the first to be mobilized and the first to be replenished are those affected most by genetic ancestry in the current study. A hierarchy of adipose mobilization and replenishment exists, with intramuscular depots being mobilized first, followed by subcutaneous and inter-muscular depots. The visceral depots (kidney, mesenteric and omental) are the last to be mobilized (Butler-Hogg et al. 1985; Pethick & Dunshea, 1996) with replenishment occurring in the reverse order. The differences found in the proportions of MUFA and SFA in the shoulder, tail-head and mesenteric adipose tissue support the premise that these tissues were undergoing modification in the experimental model investigated. The reduction in SFA and increase in MUFA in the mesenteric adipose tissue is suggestive of a gradient effect, where genetic strain altered the subcutaneous depots, partially altered the omental depot, and had no effect on the kidney and mesenteric adipose tissues. This pattern is very similar to the established hierarchy within which the adipose depots are mobilized or replenished and may reflect differences in the metabolic status or FA storage status of the different depots. It remains to be determined whether the degree of lipogenesis of the adipose depots from the two genetic strains are indeed different.

Genetic ancestry has been reported to modify metabolic function and signalling through early lactation with differences demonstrated in the degree of somatotropic axis uncoupling in NA compared with NZ cows (Lucy et al. 2009; McCarthy et al. 2009; Meier et al. 2010; Grala et al. 2011). Additionally, glucose clearance rates were slower in NA cows in early (Chagas et al. 2009) and mid-lactation (Patton et al. 2009) indicating lower peripherial tissue glucose update and greater insulin insensitivity in NA cows. The difference in FA profiles, particularly the SFA and MUFA concentrations of the subcutaneous and omental adipose tissues suggest that these tissues are in different metabolic status. Although an indirect measure of tissue status, these data provide further evidence that the adipose tissues of NA cows have lower rates of lipogenesis and hence more insulin-resistant compared with the NZ cows. Previous studies support the premise that the NA cows have a longer period of insulin insensitivity and uncoupling of the somatotrophic axis (Lucy et al. 2009; McCarthy et al. 2009; Grala et al. 2011). These phenomena result in an elevated growth hormone concentration, which in turn antagonizes insulin action, essentially blocking lipogenesis and promoting lipolysis and gluconeogenesis (Etherton et al. 1987; Beswick & Kennelly, 1998).

In the current study, the effect of genetic strain on subcutaneous FA profiles is evident although the BC of the cows was similar (condition score 4·1) at the time of tissue collection. The BC of the groups were not manipulated and hence represent the normal changes for the groups (Table 2). The NA strain has greater BC loss in early lactation, reaching their post calving nadir later (Horan et al. 2005; Macdonald et al. 2008). Whether adipose FA profiles reflect difference in

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BC or whether adipose FA profiles are a more sensitive measure of adipose function compared with BC is unclear. Future studies to evaluate the metabolic function of adipose tissue should benefit from examination of several adipose tissue depots (e.g. subcutaneous, visceral and/or intramuscular) at a range of BC, as information is likely to be specific for each depot.

The effect of genetic strain on the milk FA profiles is consistent with previous reports of the effects of genetic HF strain and breed on milk fatty acid composition. Both the current and previous work (Wales et al. 2009) support the premise that NZ cows contain greater concentrations of de-novo FA than milk from NA cows. Kay et al. (2005) also reported that cows with high genetic merit for high milk yield produced milk with lower Δ9-desaturase indices than cows with lower genetic merit. Fatty acid content of milk and milk fat also differ across breeds, with low to moderate heritability ($h^2 = 0{\cdot}05–0{\cdot}42$; Soyeurt et al. 2007; Soyeurt et al. 2008). Candidate genes controlling milk FA composition include diacylglycerol acyltransferase (DGAT1) and SCD-1 (Schennink et al. 2007, 2008). Altered milk FA associated with changes in milk FA composition may also be reflective of the changes in SCD activity within adipose tissues, as reflected in the SFA to MUFA ratios. Elucidating the mechanisms that influence milk fatty acid profiles among NZ and NA cows could further inform the genetic mechanisms controlling fatty acid synthesis within both the mammary gland and adipose tissues.

Few significant associations between milk and adipose tissue FA were evident in the current study. Previous studies reported an effect of calving BC on milk FA profiles, with milk from cows calving in better BC producing more long-chain FA, 18:0 and 18:1 (Thomson et al. 2002; Stockdale et al. 2005). This effect of BC at calving on milk FA in early lactation reflects the difference in adipose tissue utilization, with cows calving in low BC using less adipose reserves for milk production (Pedron et al. 1993). In the current study, the associations were tested at a later stage of lactation than those reported for BC at calving and milk FA. Whether the associations reported here between milk FA and shoulder adipose are markers for adipose status in mid lactation cannot be answered here but warrants further consideration.

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