Lipid metabolic differences in cows producing small or large milk fat globules: Fatty acid origin and degree of saturation

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ABSTRACT

This study compared cows that consistently produce milk with small (volume-weighted mean diameter of 2.92–3.83 μm , with an average diameter of 3.29 μm) or large (volume-weighted mean diameter of 4.58–5.67 μm , with an average diameter of 4.92 μm) milk fat globule (MFG) size distributions in terms of the fatty acid (FA) composition of the MFG core. Selected cows fell into the respective size group over at least 3 independent measurements, including an observation period before the experiment. Further selection criteria were similar milk production traits between cows (milk yield, fat yield, fat/protein ratio) and established lactation (>50 d in milk). However, the selected groups differed in parity (parity 1–3 and 3–5 in the small and large MFG groups, respectively), and the small MFG group was an average of 25 d in milk later in their lactation period. All cows were under the same nutritional management and environmental conditions. Here, we show that cows with the small or large MFG phenotype differed in their lipid metabolism in terms of the FA composition of the MFG core. Our results indicate that cows with the small MFG phenotype produced milk with higher concentrations of unsaturated FA despite being fed the same diet. We suggest that this characteristic of the small MFG phenotype is the result of increased uptake of long-chain FA from the blood circulation. A relationship between the degree of unsaturation and MFG size was also identified in preliminary studies across other species—namely, camels, sheep, and goats. These findings show the potential for on-farm selection of cows (and potentially other dairy species) based on MFG size to produce milk with improved nutrient composition. This could lead to purpose-specific separation of milk based on MFG size and FA profile, both known to alter the technological properties of milk.

Key words: milk fat globule size, mammalian milk, fatty acid profile

INTRODUCTION

Milk fat exists as milk fat globules (MFG) in the milk of mammalian species. Each globule originates within the mammary epithelial cell (MEC) as a cytoplasmic lipid droplet (LD) that is enveloped upon secretion with a portion of the plasma membrane of the MEC. The result is the formation of the MFG, a lipid core surrounded by a trilayer membrane. The LD comprises a neutral lipid core containing mainly triacylglycerols (TAG) surrounded by a monolayer of phospholipids and proteins. Biogenesis of LD starts either within or in close proximity to the endoplasmic reticulum (Heid and Keenan, 2005; Robenek et al., 2009). Upon release from the endoplasmic reticulum, the LD are transported toward the apical side of the cell and can grow in size by fusion of LD (Masedunskas et al., 2017) or through local TAG and phospholipid synthesis on the surface of the LD (Krahmer et al., 2011; Wilfling et al., 2013). However, the latter process might not be relevant in MEC because, to date, the full set of enzymes required for on-surface TAG synthesis have not been detected in proteomic analyses of the LD envelope (Mather et al., 2019). Lipid droplet fusion, on the other hand, has been demonstrated in the context of mammary LD growth in vivo and in vitro studies and is believed to depend on the polar lipid and protein composition of the LD envelope (Argov-Argaman, 2019; Mather et al., 2019). After reaching the apical plasma membrane, LD are secreted by a budding process, which involves oxytocin-stimulated contraction of the myoepithelial cells surrounding the MEC layer (Masedunskas et al., 2017).

Differences in fatty acid (FA) composition between small and large MFG have been established. For example, milk with smaller MFG tends to contain less stearic acid (C18:0) and fewer de novo synthesized FA (<C16) but higher proportions of MUFA and PUFA (Couvreur and Hurtaud, 2017). Moreover, camel milk,

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which exhibits the smallest MFG size distribution among the main dairy species (El-Zeini, 2006), is high in UFA and contains very little short-chain FA (El-Agamy, 2017). Short-chain FA, on the other hand, are more abundant in goat milk, which is also among the species with a smaller average MFG size compared with cow milk (Park et al., 2007). The FA incorporated into milk fat in the MEC are obtained from de novo synthesis in the mammary gland (chain length of C4 to C16) or derived from the blood as preformed FA after hydrolysis of TAG from the diet or adipose tissue (chain length of C16 or higher; Palmquist, 2006). The balance between the 2 pathways depends on the physiological state of the animal (Palmquist, 2006). Therefore, the FA composition of milk provides an insight into the origin of FA utilized for milk fat production and therefore the lipid metabolism of the individual cow.

Here, we studied the differences between cows that consistently produce milk with small or large MFG size distributions. To identify these animals, the whole herd was tested over 7 sampling days across all seasons (Walter et al., 2019). Furthermore, many of the

selected animals were sampled additional times over the year before this study, resulting in 3 to 9 independent measurements per selected cow (Figure 1). The 2 groups were compared in terms of the FA composition of the MFG core to gain insight into the physiological differences between animals that consistently produce small or large MFG but are fed the same diet and are in similar stages of lactation. We hypothesized that differences in the lipid metabolism and specifically the FA origin could be the reason for the observed differences in MFG size between individual cows of the same breed. In this experiment we focused on the FA composition of the LD core because it contains the vast majority of FA in milk, and we wanted to exclude the MFG membrane (MFGM) from the current analysis to minimize the effect of increased membrane material per unit of fat in the small MFG phenotype. The MFGM comprises polar lipids with higher proportions of UFA (Sánchez-Juanes et al., 2009), and the higher proportion of MFGM material in the cows with small MFG could therefore have affected the interpretation of our results. A secondary objective was to compare milks from cows with small or large MFG size distribution with milks from sheep, goats, and camels. Milks from these species generally differ from bovine milk in MFG size distribution as well as FA composition (Park et al., 2007). We therefore compared the differences in FA compositions of the MFG core from differing mammalian species to see whether these differences are universal.

MATERIALS AND METHODS

Animals and Milk Sampling

Cows. The cows selected for this study were part of the University of Melbourne Dookie Dairy in northern Victoria, Australia (36°25'31.3" S, 145°42'36.6" E). The herd consisted of approximately 145 Holstein-Friesian cows with a year-round calving pattern and were milked using an automatic milking system (Lely Astronaut; Lely, Maassluis, the Netherlands). The cows were pasture fed all year and were fed concentrate (cereal grain-based pellets) during milking. When pasture was insufficient to meet the herd's nutritional requirements, the cows' diet was supplemented with silage and hay. The 12 cows used in this study were selected based on their average MFG size, with 6 cows falling into the group with small MFG (SMFG) and 6 falling into the group with large MFG (LMFG). The experiment was performed on 2 cohorts in separate seasons, with the first 6 cows (3/group) sampled in autumn and the second cohort in winter. Moreover, sampling of each cohort was repeated approximately 2 wk apart to obtain biological replicates for each cow. The selected

Figure 1. Consistency in volume weighted mean diameter $D_{4,3}$ of milk fat globules (MFG) from selected cows compared with the whole herd. Mean symbols (black triangles) and individual values (gray circles) are shown for the 12 selected cows individually, where error bars represent 95% CI. Boxplots are shown for the SMFG and LMFG group compared with the data collected for the whole herd including the pre-lactation period. Asterisks denote outliers 1.5 times the interquartile range above or below the upper or lower quartile, respectively. Each selected cow was measured a minimum of 3 and a maximum of 9 times. SMFG = cows naturally producing small MFG; LMFG = cows naturally producing large MFG.

cows received the same diet during the time of this study, and the concentrate consumption was recorded for each individual cow (Table 1). Another selection criterion was DIM, which was between 184 and 230 DIM for the first cohort of cows and between 57 and 135 for the second cohort of cows. This selection was made based on previous measurements of the whole herd (Walter et al., 2019) throughout the year before this study. Pretrial measurements to confirm MFG size were performed on 20-mL aliquots collected during afternoon milking (1300 to 1800 h) on 7 occasions, where measurements were performed on 50 to 100 animals. Additional measurements were taken for selected animals during morning milking when milk was collected for other experimental purposes. Particle size analysis was performed the day after milk collection following transport to the laboratory and storage on ice. Raw milk samples (3.5 L/animal) were taken during morning milking (0800 to 1000 h) and transported to the laboratory on ice. The fat and protein concentrations were determined using a LactoScope FTIR 20 (Delta Instruments, Melbourne, VIC, Australia) for each cow milk sample.

Camels, Goats, and Sheep. The camels selected for this study were part of the Camel Milk Co. in Kyabram, Victoria, Australia (36°21'19.7" S, 144°59'53.4" E). The goats and sheep used in this study were part of the Meredith Dairy in Meredith, Victoria, Australia (37°50'06.9" S, 143°57'51.4" E). Six camels, 6 goats, and 6 sheep were randomly selected and sampled once. Camels were wild Australian camels, goats were a mixed breed (~70% Saanen, 20% British Alpine, and 10% Toggenburg), and the selected sheep included 1 Awassi, 3 East Friesian, and 2 unknown mixed breeds. Raw milk samples (500 mL/animal) were taken during morning milking for camels and during afternoon milking for goats and sheep and were transported to the laboratory on ice. Extraction of the MFG core was performed on the following 2 d. Fat and protein concentrations in camel, sheep, and goat milk were determined by Dairy Technical Services, North Melbourne, Victoria, Australia.

MFG Size Analysis

Dynamic light scattering with a Mastersizer 2000 particle analyzer (Malvern Instruments, Malvern, UK) was used to measure MFG size distributions of the milk samples according to the procedure described in Logan et al. (2014). Before the analysis, milk was diluted 1:1 with 35 mM EDTA (pH 7.0) to dissociate casein micelles. The obscuration rate was kept between 12 and 15%, and refractive indices for milk fat and water were estimated as 1.46 and 1.33, respectively.

Table 1. Milk fat globule (MFG) size, milk composition, and animal production data for cows¹ producing milk with small (SMFG) or large (LMFG) average MFG and across several species

Item	SMFG cows (n = 6)			LMFG cows (n = 6)			P-value ²			Camels (n = 6)			Goats (n = 6)			Sheep (n = 6)		
	Mean	95% CI		Mean	95% CI		Mean		95% CI		Mean		95% CI		Mean		95% CI	
MFG D _{3,2} (μm) ³	2.89	2.69–3.10		4.04	3.74–4.33		0.003		0.003		2.76		2.62–2.90		3.26		2.98–3.53	
MFG D _{4,3} (μm) ⁴	3.29	2.96–3.62		4.92	4.58–5.25		0.003		0.003		3.33		3.00–3.66		4.00		3.50–4.50	
DIM	168.3	117.0–219.7		143.3	92.0–194.7		—		—		188.7		19.0–396.4		96.2		90.0–102.3	
Parity	1.8	1.1–2.6		3.8	3.1–4.6		0.028		0.028		1.3		0.8–1.9		3.0		1.9–4.2	
Concentrate intake (kg)	8.6	6.2–11.1		8.4	5.9–10.9		0.775		0.775		—		—		—		—	
Milkings	2.8	2.4–3.2		2.8	2.4–3.2		0.854		0.854		2.0		2.0		2.0		2.0	
Milk yield (kg)	27.84	19.41–36.27		29.25	20.82–37.68		0.854		0.854		4.87		2.58–7.15		4.95		3.71–6.19	
Fat content (%)	3.40	2.56–4.25		3.77	2.93–4.62		0.729		0.729		2.15		1.26–3.04		4.54		3.60–5.48	
Fat yield (kg/d)	0.92	0.76–1.07		1.01	0.86–1.16		0.729		0.729		0.10		0.05–0.15		0.22		0.16–0.29	
Protein content (%)	2.96	2.76–3.16		3.00	2.81–3.20		0.729		0.729		2.51		2.02–3.01		2.92		2.70–3.15	
Fat/protein ratio	1.15	0.92–1.37		1.24	1.01–1.47		0.729		0.729		0.85		0.59–1.12		1.57		1.21–1.92	

¹Six cows were sampled in biological replicates on 2 separate days; 3 were sampled in autumn and 3 were sampled in winter.

²P-values were calculated using a linear model with group (SMFG or LMFG cows), DIM, and sampling season as fixed effects. P-values were adjusted for multiple comparisons using the Benjamini and Hochberg (1995) procedure. Other species are shown for comparison purposes only.

³D_{3,2} = surface-weighted mean diameter.

⁴D_{4,3} = volume-weighted mean diameter.

Volume-weighted mean diameter and surface-weighted mean diameter were calculated by the software, and each sample was measured in duplicate.

Extraction of the MFG Core

Cows. Cow milk samples (3.2 L) were first separated into cream and skim milk, and the cream fraction was further separated into MFGM and MFG core fractions. Whole raw milk was centrifuged in 1-L centrifuge bottles (Nalgene, Sigma-Aldrich, Castle Hill, NSW, Australia) at $2,500 \times g$ for 30 min at 4°C, and the cream fractions were kept at 4°C overnight. On the next day, the cream was washed thrice in 1 L of double-distilled water using gentle agitation with a spoon for 5 to 10 min followed by centrifugation ($2,500 \times g$, 30 min, 4°C). The washed cream was crystallized overnight at 4°C. On the final day, the washed cream was churned, and the buttermilk and butter phases were separated by filtration through a double layer of cheesecloth. The butter fraction was then heated at 70°C for 30 min and left to solidify at 4°C to separate the milk fat from the butter serum. The butter serum was separated from the solid milk fat. The remaining milk fat after separation from the butter serum represents the MFG core. The washed MFG core fraction was kept frozen at -80°C for FA analysis.

Camels, Goats, and Sheep. Extraction of the MFG core fractions from whole milk differed from the process used for cow samples because churning was not feasible due to smaller sample volume and physical properties of camel, goat, and sheep milk. Separation of cream from skim milk was performed using four 230-mL centrifuge tubes at $3,500 \times g$ for 30 min at 4°C. Washing was performed by mixing the cream fraction with double-distilled water followed by centrifugation ($3,500 \times g$, 30 min, 4°C). Goat and sheep milk samples were washed thrice, whereas camel milk was washed only twice to reduce losses due to the small MFG size. The washed cream was kept at 4°C overnight. The cream was mixed with a small volume of double-distilled H₂O in a 230-mL centrifuge tube, shaken by hand for 1 min, and sonicated for 5 min in a sonication water bath at room temperature. Last, the tubes were placed in a water bath at 70°C for 30 min, followed by 15 min of centrifugation at $1,500 \times g$ at 4°C. The milk fat layer was separated from the aqueous phase and kept at -80°C.

FA Analysis

The FA composition of milk fats was determined as mentioned in Shen et al. (2001) with some modifications. Briefly, FAME of milk fat were prepared by

mixing 10 to 20 mg of fat in a glass vial with 0.3 mL of hexane and 0.3 mL of 2 M potassium hydroxide in methanol for 5 min at room temperature. After cooling the mixture, the hexane phase was transferred to a GC vial and was diluted 4 times with hexane. The FAME were analyzed using an Agilent 7890A GC (Agilent Technologies Australia, Mulgrave, VIC, Australia) with a 30-m BPX70 column (0.25-mm inner diameter, 0.25- μ m film thickness; Trajan Scientific and Medical, Ringwood, VIC, Australia). The column temperature was set for 3 min at 40°C and then was increased at a rate of 20°C/min to 160°C, which was held for 16 min. The column temperature was further increased to 240°C at a rate of 20°C/min and held for 3 min. Helium was used as a carrier gas. The injector temperature was programmed at 240°C with 11.691 psi pressure and 55.58 mL/min flow of helium. The samples were injected in the split mode with a ratio of 50:1 and with a split flow of 51.549 mL/min. The flame ionization detector temperature was 280°C with a flow of 40 mL/min hydrogen gas and 400 mL/min air. The FAME peaks were identified on the basis of retention time of FAME standards (GLC-607 and GLC-674 commercially available from Nu-Chek Prep Inc. USA and the milk fat reference CRM164 from CSIRO, Agriculture and Food, Werribee, Australia). Standard CRM 164 has a FA composition that was determined by gas-liquid chromatography and certified by the Commission of the European Communities (Brussels, Belgium). Peak areas of GLC 607 were used to determine the response factors for individual FAME peaks in the GC and were applied to correct the area percentages of the milk FAME peaks.

Statistical Analysis

Statistical analysis was performed using R (R Core Team, 2018). Duplicate measurements of individual cows were averaged, and a linear model was fitted with group (SMFG or LMFG cows), DIM, and sampling season as fixed effects. The model was fitted using the “lm” function in combination with the “tidy” function from the broom package (Robinson and Hayes, 2018). The *P*-values were adjusted for multiple testing using the procedure by Benjamini and Hochberg (1995), which was applied individually per data set—namely, the milk production data analysis (Table 1), the grouped FA analysis (Table 2), and the individual FA analysis (Table 3). Statistical significance was assumed for *P*-values <0.05, and *P*-values <0.10 were defined as trends toward statistical significance. Means and 95% confidence intervals were calculated. Graphs were produced using the “ggplot2” package (Wickham, 2016).

Table 2. Fatty acid (FA) origin and degree of unsaturation in the milk fat globule (MFG) core from cows¹ producing milk with small (SMFG) or large (LMFG) average MFG size and across several species

Item ²	SMFG cows (n = 6)			LMFG cows (n = 6)			P-value ³	Camels (n = 6)			Goats (n = 6)			Sheep (n = 6)		
	LSM	95% CI		LSM	95% CI			Mean	95% CI		Mean	95% CI		Mean	95% CI	
Preformed FA ($\geq$ C18)	39.31	37.29–41.33		34.75	32.73–36.77		0.024	49.08	46.32–51.84		43.90	40.77–47.03		39.49	31.97–47.01	
De novo FA (C4 to C14)	23.16	20.85–25.46		24.79	22.49–27.10		0.387	15.24	13.1–17.38		26.77	25.16–28.38		31.34	25.39–37.29	
SCFA (C4 to C8)	2.25	2.03–2.47		2.39	2.17–2.62		0.387	0.11	0.08–0.14		4.05	3.72–4.39		4.07	3.15–4.99	
MCFA (C9 to C14)	19.13	16.97–21.30		20.47	18.30–22.63		0.387	12.80	10.91–14.69		21.33	19.79–22.87		25.07	19.69–30.45	
C16 group (C16:0, C16:1 <i>cis</i> -9)	37.54	34.94–40.13		40.46	37.86–43.05		0.267	35.68	32.17–39.19		29.34	27.46–31.22		29.17	26.62–31.72	
UFA	32.94	31.56–34.33		27.67	26.29–29.06		0.003	42.52	39.08–45.96		31.78	29.18–34.38		32.29	27.81–36.77	
SFA	67.06	65.67–68.44		72.33	70.94–73.71		0.003	57.48	54.04–60.92		68.22	65.62–70.82		67.71	63.23–72.19	
MUFA	28.23	26.92–29.53		23.98	22.68–25.29		0.005	38.84	35.42–42.26		28.51	26.16–30.86		27.52	23.50–31.54	
PUFA	4.72	4.28–5.16		3.69	3.25–4.13		0.024	3.68	3.24–4.12		3.27	2.88–3.66		4.77	3.71–5.82	
SCDI14	10.50	8.91–12.09		9.48	7.88–11.07		0.387	10.79	10.03–11.55		3.26	2.99–3.53		4.29	3.05–5.54	
SCDI16	4.21	3.42–5.00		3.63	2.84–4.43		0.387	19.74	17.69–21.79		1.86	1.50–2.22		4.08	2.77–5.38	
SCDI18	69.21	64.26–74.16		64.23	59.28–69.18		0.298	62.62	54.8–70.44		66.47	62.17–70.77		70.85	63.88–77.82	

¹Six cows were sampled in biological replicates on 2 separate days.²SCFA = short-chain FA; MCFA = medium-chain FA; SCDI = stearoyl-CoA desaturase index. $SCDI14 = (C14:1 \text{ cis-9}) / (C14:0 + C14:1 \text{ cis-9}) \times 100$. $SCDI16 = (C16:1 \text{ cis-9}) / (C16:0 + C16:1 \text{ cis-9}) \times 100$. $SCDI18 = (C18:1 \text{ cis-9}) / (C18:0 + C18:1 \text{ cis-9}) \times 100$.³P-values were calculated using a linear model with group (SMFG or LMFG cows), DIM, and sampling season as fixed effects. P-values were adjusted for multiple comparisons using the Benjamini and Hochberg (1995) procedure. Other species are shown for comparison purposes only.

RESULTS AND DISCUSSION

MFG Size Distribution

Milk was collected from cows shown to consistently produce milk of either small or large MFG size distributions, selected through an observation period before this study (Walter et al., 2019). According to the pre-trial measurements, cows were classified into 2 study groups: SMFG and LMFG. Figure 1 shows the MFG size distributions of the selected cows during this study and the pre-trial period. This shows the remarkable consistency in average MFG size of individual cows, which in some cows was seen over 2 consecutive lactation cycles (Figure 1).

The average MFG size, milk composition, and animal production data of both study groups are shown in Table 1 compared with the same data, where known, for milk collected from camel, goat, and sheep species. The milk from cows in the SMFG and LMFG groups varied significantly in volume-weighted mean diameter MFG size ($P = 0.003$; Table 1), with an average diameter of 3.29 and 4.92 μm , respectively. This confirmed that pre-trial selection indeed led to 2 study groups with significantly different MFG size distributions. Moreover, all cows selected for this study were in established lactation (>50 DIM), received the same diet, and did not differ in milk production traits (milk yield, fat and protein content, and fat/protein ratio; Table 1). This was important because it is known that factors such as fat/protein ratio can influence MFG size (Couvreur and Hurtaud, 2017). We hypothesized that there may be 2 distinct phenotypes of cows consistently producing milk with small and large MFG within our herd, thereby allowing for on-farm selection. To test this hypothesis, it was necessary to limit the effect of environmental conditions and milk production traits to isolate physiological differences between the SMFG and LMFG groups. Although milk production parameters were similar between the studied groups (Table 1), the selected cows differed slightly in DIM (168 in SMFG and 143 in LMFG) and parity (1–3 in SMFG and 3–5 in LMFG), the former being known to affect MFG size (Altenhofer et al., 2015). However, we do not believe this to contribute considerably to the differences observed between the studied groups because we studied the effect of DIM and parity on MFG size in a previous study using the same herd as in the present study (Walter et al., 2019). Based on this analysis, we estimate that an additional 25 DIM, observed here for the SMFG group compared with the LMFG group, would lead to a 0.05- μm decrease in MFG size. Furthermore, an increase in MFG size of 0.16 μm was observed between parity 2 and 3 (Walter et al., 2019), which

Table 3. Fatty acid composition (% of total FAME) in the small (SMFG) and large (LMFG) milk fat globule phenotype in cows and across species

Fatty acid	SMFG cows			LMFG cows			P-value ¹	Camels			Goats			Sheep		
	LSM	95% CI		LSM	95% CI			Mean	95% CI		Mean	95% CI		Mean	95% CI	
C4:0	0.71	0.62-0.80		0.74	0.65-0.83		0.856	0.00	0.00-0.00		1.00	0.84-1.17		1.17	0.90-1.45	
C5:0	0.00	0.00-0.00		0.00	0.00-0.01		0.668	0.00	0.00-0.00		0.00	0.00-0.01		0.02	0.00-0.03	
C6:0	0.78	0.70-0.86		0.83	0.75-0.91		0.615	0.05	0.03-0.06		1.24	1.12-1.36		1.20	0.91-1.49	
C7:0	0.01	0.00-0.01		0.01	0.01-0.02		0.310	0.00	0.00-0.00		0.02	0.01-0.03		0.05	0.02-0.08	
C8:0	0.75	0.66-0.84		0.80	0.71-0.90		0.659	0.07	0.04-0.09		1.79	1.68-1.89		1.63	1.16-2.10	
C9:0	0.02	0.01-0.03		0.03	0.02-0.04		0.310	0.00	0.00-0.00		0.05	0.02-0.09		0.12	0.04-0.20	
C10:0	2.31	1.89-2.73		2.56	2.14-2.98		0.659	0.11	0.07-0.14		7.11	6.54-7.68		6.78	4.72-8.85	
C10:1n-1	0.22	0.19-0.24		0.21	0.19-0.24		0.917	0.02	0.01-0.02		0.16	0.11-0.20		0.23	0.15-0.31	
C11:0	0.04	0.02-0.07		0.07	0.04-0.10		0.310	0.01	0.01-0.01		0.09	0.01-0.16		0.23	0.05-0.40	
C12:0	3.23	2.61-3.85		3.53	2.91-4.15		0.731	0.77	0.58-0.95		3.29	2.64-3.94		4.78	3.17-6.39	
C12:1n-9	0.08	0.07-0.10		0.08	0.06-0.09		0.917	0.02	0.02-0.03		0.05	0.03-0.07		0.08	0.05-0.11	
C13:0	0.07	0.04-0.11		0.11	0.08-0.15		0.310	0.08	0.06-0.09		0.09	0.05-0.13		0.22	0.08-0.35	
C13:0 isomer	0.09	0.07-0.11		0.09	0.07-0.11		0.954	0.02	0.01-0.02		0.05	0.03-0.08		0.10	0.05-0.15	
C14:0	11.63	10.50-12.77		12.39	11.26-13.53		0.629	10.32	8.79-11.85		10.02	9.31-10.73		11.92	10.29-13.55	
C14:0 isomer	0.09	0.07-0.10		0.09	0.07-0.11		0.917	0.22	0.18-0.26		0.08	0.07-0.10		0.07	0.04-0.11	
C14:1 <i>cis</i> -9	1.36	1.15-1.56		1.29	1.09-1.50		0.856	1.24	1.09-1.40		0.34	0.29-0.38		0.53	0.36-0.71	
C15:0	1.12	0.87-1.37		1.32	1.07-1.57		0.579	1.38	1.22-1.54		0.93	0.69-1.17		1.58	1.14-2.03	
C15:0 ante isomer	0.56	0.45-0.66		0.54	0.44-0.65		0.917	0.88	0.74-1.01		0.43	0.33-0.52		0.58	0.48-0.68	
C15:0 isomer	0.10	0.09-0.11		0.07	0.06-0.08		0.020	0.08	0.05-0.11		0.03	0.02-0.04		0.04	0.03-0.05	
C16:0	35.65	33.22-38.08		38.69	36.27-41.12		0.310	27.74	25.64-29.84		28.36	26.43-30.29		27.48	25.22-29.74	
C16:0 isomer	0.09	0.08-0.11		0.09	0.08-0.11		0.930	0.23	0.14-0.32		0.06	0.05-0.08		0.12	0.05-0.19	
C16:1 <i>cis</i> -7	0.22	0.21-0.23		0.20	0.19-0.21		0.310	0.77	0.61-0.92		0.38	0.33-0.42		0.37	0.31-0.43	
C16:1 <i>cis</i> -9	1.57	1.22-1.93		1.47	1.11-1.82		0.856	6.94	5.53-8.35		0.54	0.45-0.62		1.19	0.74-1.65	
C17:0	0.63	0.56-0.70		0.64	0.57-0.71		0.917	0.74	0.63-0.85		0.56	0.5-0.62		0.64	0.52-0.77	
C17:1 <i>cis</i> -9	0.31	0.26-0.35		0.27	0.22-0.31		0.504	0.71	0.61-0.80		0.29	0.23-0.35		0.37	0.26-0.49	
C18:0	9.10	7.27-10.93		9.59	7.76-11.42		0.868	14.46	10.99-17.93		12.69	10.31-15.07		8.71	4.75-12.67	
C18:1 <i>cis</i> -9	20.54	19.52-21.55		16.81	15.79-17.83		0.020	24.00	21.70-26.30		25.04	23.07-27.01		19.92	15.45-24.39	
C18:1 isomers	3.84	3.46-4.22		3.57	3.19-3.95		0.615	4.85	4.38-5.33		1.66	1.3-2.01		4.71	2.26-7.17	
C18:2 <i>trans</i> -11, <i>cis</i> -15	0.46	0.43-0.49		0.40	0.37-0.43		0.201	0.31	0.24-0.37		0.24	0.19-0.29		0.41	0.22-0.61	
C18:2 <i>trans</i> -9, <i>trans</i> -12	0.40	0.34-0.45		0.31	0.25-0.36		0.310	0.25	0.22-0.28		0.22	0.15-0.28		0.49	0.32-0.66	
C18:2	2.23	1.78-2.69		1.65	1.19-2.10		0.310	1.69	1.41-1.97		2.32	2.15-2.50		2.92	2.33-3.51	
C18:3	0.71	0.62-0.80		0.59	0.50-0.68		0.310	0.68	0.53-0.83		0.30	0.26-0.35		0.42	0.32-0.51	
CLA C18:2 <i>cis</i> -9, <i>trans</i> -11	0.92	0.78-1.05		0.74	0.61-0.87		0.310	0.76	0.59-0.93		0.19	0.10-0.27		0.53	0.27-0.78	
C20:0	0.08	0.04-0.13		0.10	0.06-0.15		0.786	0.35	0.22-0.48		0.32	0.30-0.33		0.26	0.13-0.39	
C20:1n-9	0.10	0.09-0.11		0.08	0.07-0.09		0.310	0.30	0.19-0.40		0.07	0.06-0.08		0.11	0.07-0.15	

¹P-values were calculated using a linear model with group (SMFG or LMFG cows), DIM, and sampling season as fixed effects. P-values were adjusted for multiple comparisons using the Benjamini and Hochberg (1995) procedure. Other species are shown for comparison purposes only.

in the current study could contribute to the difference between the SMFG group (parity 1–3, average = 1.8) and the LMFG group (parity 3–5, average = 3.8). However, these results suggest that the difference in average MFG diameter between the groups in the current study (1.63 μm) is considerably greater than the combined effect of the differences in DIM and parity (0.21 μm). Nonetheless, DIM was included in the statistical model as a fixed effect to control for its known effect on MFG size. Parity, on the other hand, was significantly different between the studied groups ($P = 0.028$; Table 1) and could have contributed to some of the observed differences in FA origin and saturation. This is a limitation of our study because different parities are related to metabolic changes mainly in the transition period but also later in lactation. For example, increased milk yields in cows in later parities (4 and higher) can lead to a higher loss of body condition during early lactation (Lee and Kim, 2006). Furthermore, in these cows, the recovery from the loss of body condition experienced during early lactation can be delayed and can take up to 4 to 5 mo into the lactation cycle (Lee and Kim, 2006). Therefore, although the effect of parity on MFG size remains equivocal in the literature (Martini et al., 2004; Fleming et al., 2017), we cannot separate the observed differences in our study from the potential effect of differences in the lipid metabolism of cows in later parities (here the LMFG group) and earlier parities (the SMFG group).

Figure 2 shows the variation in MFG size distribution between the selected SMFG and LMFG groups compared with the MFG size distribution of several other mammalian species. Interestingly, the average MFG size distribution for camel milk was similar to that of the SMFG cow group measured in the current study. Moreover, the variation in MFG size between the 6 randomly selected camels was smaller than that in the other species (Table 1), indicating that camel milk may naturally contain MFG of a narrower size range and smaller average MFG size, as previously suggested (El-Agamy, 2017). For sheep and goat milk, however, we observed considerable variation in the average MFG size between the 6 randomly selected animals (Table 1), similar to the range generally found in cow milk (Logan et al., 2014).

MFG Core FA Composition Differs Between SMFG and LMFG Cows

We found clear differences in the FA composition of the MFG core obtained from SMFG and LMFG cows even though these animals were under the same nutritional management and produced milk of similar composition. The MFG core, comprising predominantly

but not exclusively nonpolar lipids, contained a higher proportion of total UFA (33.1% of total FAME) in the SMFG group compared with the MFG core from LMFG cows (27.5%; Table 2; Figure 3). This was due to higher concentrations of MUFA and PUFA in the MFG core from SMFG cows (28.4 and 4.8%, respectively) compared with LMFG cows (23.9 and 3.6%, respectively; Figure 3). Our results are in accordance with a study by Couvreur et al. (2007) but in conflict with another (Mesilati-Stahy and Argov-Argaman, 2014). This could at least in part be due to the difference in study design: Mesilati-Stahy and Argov-Argaman (2014) used microfiltration to separate milk into groups with different MFG sizes, whereas in our study and that of Couvreur et al. (2007) the milk of small and large MFG groups was sourced from cows with naturally occurring differences in MFG size. Comparison of these results suggests that although FA compositions of MFG in a certain size group can vary, the relationship between the overall FA composition in milk and its MFG size is also specific to the individual animal. These results supported our hypothesis that MFG size could reflect individual differences in the cow's lipid metabolism.

Figure 2. Volume weighted milk fat globule (MFG) size distributions of the selected SMFG^a and LMFG^b cows compared with camels^a, sheep^c, and goats^c. Different superscript letters indicate statistical significance ($P < 0.05$) between the studied species and SMFG and LMFG cows, determined through a pairwise comparison *t*-test adjusted for multiple comparisons using the Benjamini and Hochberg (1995) procedure. SMFG = cows naturally producing small milk fat globules; LMFG = cows naturally producing large milk fat globules.

Figure 3. Degree of unsaturation (A) and origin of fatty acids (B) in the small and large milk fat globule phenotypes. Dot plots show individual data points and predicted means as thick bars and numbers. Error bars represent the 95% CI. *P*-values were calculated using a linear model with group (SMFG or LMFG cows), DIM, and sampling season as fixed effects. *P*-values were adjusted for multiple comparisons using the Benjamini and Hochberg (1995) procedure. De novo = fatty acids derived from de novo synthesis (C4 to C14), C16 group = fatty acids with a chain length of 16 (C16:0 and C16:1), Preformed = fatty acids ($\geq$ C18). SMFG = cows naturally producing small milk fat globules; LMFG = cows naturally producing large milk fat globules.

The content of UFA can be modified through the cow's diet (Lopez et al., 2008); however, most UFA are first converted to *trans* FA isomers, and, if biohydrogenation of the double bonds is complete, they are ultimately converted to the SFA stearic acid in the rumen (Bauman et al., 2011). Hence, the majority of UFA found in milk are produced from the desaturation of SFA by an enzyme called Δ^9 -desaturase or stearoyl-CoA desaturase (SCD), and this process takes place in mammary and adipose tissue. The SCD activity increases with ongoing lactation (Soyeurt et al., 2008) and may change between seasons, where it increases with higher proportions of pasture in the diet (Lock and Garnsworthy, 2003). Furthermore, SCD activity can vary between individual animals and breeds (Lock and Garnsworthy, 2003; Soyeurt et al., 2008). Indices for SCD (SCDI), expressing each product of SCD as a proportion of the sum of product and precursor, can be used as indicators for SCD activity (Garnsworthy et al., 2010). Couvreur et al. (2007) reported higher C18:1/C18:0 ratio in SMFG cows, and the use of SCDI for breeding selection programs has already been suggested (Garnsworthy et al., 2010). In the current study, cows in the SMFG group exhibited a higher SCDI for FA with a chain length of 14 carbons (SCDI14; $P = 0.387$; Table 2), 16 carbons (SCDI16; $P = 0.387$; Table 2), and 18 carbons (SCDI18; $P = 0.298$). However, this was not statistically significant.

Others have related the K232A polymorphism on the diacylglycerol acyltransferase 1 (*DGAT1*) gene to changes in milk FA composition as well as MFG size (Schennink et al., 2007; Argov-Argaman et al., 2013). These studies reported changes in MFG size and FA composition due to genotypic differences on the *DGAT1* gene that are comparable with the differences observed between the SMFG and LMFG groups in our study, with cows of the 232K genotype producing milk with less UFA, more C16:0 (Schennink et al., 2007), and larger MFG (Argov-Argaman et al., 2013). However, genotypic information on the selected cows was not available in our study and remains speculative at this stage.

Possible Lipid Metabolic Differences Between the SMFG and LMFG Phenotypes

To gain insight into possible lipid metabolic differences between SMFG and LMFG cows, we further looked at the origin of the FA in their MFG core. Figure 3 shows the concentrations of de novo synthesized and preformed FA in the studied groups. The results indicate that the proportion of preformed FA taken up from the blood was higher in SMFG cows than in LMFG cows ($P = 0.024$), whereas the proportion of de novo FA was similar between the groups. Based on this analysis, we suggest 2 possible explanations

for the observed differences between the SMFG and LMFG phenotypes: (1) long-chain SFA in the SMFG group were utilized to produce polar lipids needed for MFGM production and were therefore reduced in the MFG core, or (2) the observed changes could indicate increased lipid mobilization from adipose tissue in the SMFG group.

Regarding the first suggested mechanism, Mesilati-Stahy et al. (2012) showed that preformed long-chain SFA are preferentially incorporated into the MFGM fraction (polar lipids) rather than the core (TAG) and suggested that this could supply the increased membrane material needed for the formation of smaller MFG. Although we did not determine the FA composition of the MFGM, the increased proportion of blood-derived FA in the MFG core of the SMFG group was due to increased relative proportions of UFA, not long-chain SFA (Table 3). This could imply that long-chain SFA are channeled toward polar lipid synthesis and could partly explain their reduced proportion in the MFG core of the SMFG group. Moreover, the study by Mesilati-Stahy et al. (2012) suggested that MUFA were preferentially partitioned into the MFG core in cows with higher plasma insulin concentrations, whereas cows with lower insulin levels preferentially partitioned MUFA into the MFGM compartment, suggesting a potential hormonal regulation of this process.

The second possible mechanism is based on the effect of negative energy balance on the milk FA profile. Cows in negative energy balance (i.e., in the early stages of lactation or during feed restriction) exhibit a FA composition similar to the FA profile of the MFG core from SMFG cows in our study, with more oleic acid (C18:1 *cis*-9), less palmitic acid (C16:0), and less de novo synthesized FA (Gross et al., 2011). Moreover, oleic acid has been suggested as a potential indicator for subclinical ketosis (Van Haelst et al., 2008). This could indicate increased lipid mobilization from adipose tissue in SMFG cows. However, whereas dietary FA are derived from TAG packaged in lipoprotein particles and require hydrolysis by lipoprotein lipase, FA from lipid mobilization reach the mammary gland in the form of nonesterified FA. In vitro evidence suggests that higher nonesterified FA supply to the mammary gland increases LD size (Mesilati-Stahy and Argov-Argaman, 2018), which would be in conflict with the hypothesis that SMFG cows obtain preformed long-chain FA from lipid mobilization. Although we did not measure nonesterified FA in the current study to draw a conclusion either way, the concentrate intake, BW, milk yield, and fat and protein measurements provided to us by the milking system suggest that the cows were probably not in negative energy balance and indicate that the first mechanism is more likely to have resulted in the

observed differences in the FA profiles of the SMFG and LMFG groups. However, as previously noted, the effect of the observed differences in parity between the study groups and its possible effect on body condition recovery could have contributed to some of the observed differences.

FA Composition Across Species in Relation to MFG Size

The MFG core FA profiles from SMFG and LMFG cows compared with camels, goats, and sheep showed general differences between species (Table 3). For example, the MFG core from goat and sheep milk contained higher concentrations of C8:0 and C10:0, and milk fat extracted from camel milk contained very low amounts of short-chain FA (Table 3). Most of these differences have been described elsewhere and are not within the scope of our study. Here, we aimed to relate certain FA groups (e.g., UFA and de novo synthesized FA) to differences in the MFG size distributions observed between the camel, goat, and sheep milk compared with the milk from SMFG and LMFG cows. Concentrations of UFA seemed to be inversely related to MFG size (Figure 4). Figure 4 shows the concentrations of UFA across the studied species ordered according to increasing average MFG sizes from camel to LMFG cows. Due to the broad distribution of MFG size in goat and sheep milk, the data were analyzed further by grouping the results from all species into the categories small ($\leq 3.5 \mu\text{m}$), medium ($3.5\text{--}4.5 \mu\text{m}$), and large ($\geq 4.5 \mu\text{m}$; Figure 4) to reveal a clear relationship between MFG size and UFA across all species (Figure 4).

CONCLUSIONS

The analysis of the FA profile in cows producing milk with either small or large average MFG size distributions revealed a possible link between the origin of FA incorporated into LD, the desaturation activity of the individual animal, and its ultimate effect on MFG size. More specifically, cows with the SMFG phenotype incorporated more FA with a carbon number of 18 or higher into their LD and transformed more of these FA into UFA. In the current study, we defined the SMFG and LMFG phenotypes as cows within a single herd that consistently produced MFG of either small or large average size, were fed the same diet, and were similar in terms of their milk production traits. Based on this selection, we showed that the SMFG phenotype could be exploited to produce milk with higher nutritional value. However, further studies are needed to test this hypothesis and should focus on the possible links between the *DGAT1* gene polymorphisms and MFG size.

Figure 4. Concentrations of UFA in the milk fat globule core from the small and large milk fat globule (MFG) phenotypes as well as camel, sheep, and goat milk (A) and grouped into small ($\leq 3.5 \mu\text{m}$), medium (3.5 to $4.5 \mu\text{m}$), and large ($\geq 4.5 \mu\text{m}$) MFG (B). Dot plots show individual data points and predicted means as thick bars and numbers. Error bars represent the 95% CI. *P*-values were calculated using a linear model with group (SMFG or LMFG cows), DIM, and sampling season as fixed effects. *P*-values were adjusted for multiple comparisons using the Benjamini and Hochberg (1995) procedure. SMFG = cows naturally producing small milk fat globules; LMFG = cows naturally producing large milk fat globules.

Further studies should also look at the identification of the small and large MFG phenotypes in goats and sheep and investigate the reason why camels do not seem to show the same variation in MFG size seen in the main dairy species.

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REFERENCES

- Altenhofer, C., W. Holzmüller, F. Wolfertstetter, D. W. Ribeiro, U. Kulozik, M. W. Pfaffl, and E. Viturro. 2015. Temporal variation of milk fat globule diameter, fat and cholesterol content and milk epithelial cell gene expression in dairy cows. *Int. J. Dairy Technol.* 68:519–526. <https://doi.org/10.1111/1471-0307.12220>.
- Argov-Argaman, N. 2019. Symposium review: Milk fat globule size: Practical implications and metabolic regulation. *J. Dairy Sci.* 102:2783–2795. <https://doi.org/10.3168/jds.2018-15240>.
- Argov-Argaman, N., K. Mida, B.-C. Cohen, M. Visker, and K. Hettlinga. 2013. Milk fat content and DGAT1 genotype determine lipid composition of the milk fat globule membrane. *PLoS One* 8:e68707. <https://doi.org/10.1371/journal.pone.0068707>.
- Bauman, D. E., M. A. McGuire, and K. J. Harvatine. 2011. Mammary gland, milk biosynthesis and secretion. Pages 352–358 in *Encyclopedia of Dairy Sciences*. 2nd ed. J. W. Fuquay, ed. Academic Press, San Diego, CA.
- Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B Stat. Methodol.* 57:289–300.
- Couvreur, S., and C. Hurtaud. 2017. Relationships between milks differentiated on native milk fat globule characteristics and fat, protein and calcium compositions. *Animal* 11:507–518. <https://doi.org/10.1017/S1751731116001646>.
- Couvreur, S., C. Hurtaud, P. G. Marnet, P. Faverdin, and J. L. Peyraud. 2007. Composition of milk fat from cows selected for milk fat globule size and offered either fresh pasture or a corn silage-based diet. *J. Dairy Sci.* 90:392–403. [https://doi.org/10.3168/jds.S0022-0302\(07\)72640-1](https://doi.org/10.3168/jds.S0022-0302(07)72640-1).
- El-Agamy, E.-S. I. 2017. Camel milk. Pages 409–480 in *Handbook of Milk of Non-Bovine Mammals*. 2nd ed. Y. W. Park, G. F. W. Haenlein, and W. L. Wendorff, ed. Wiley-Blackwell, Hoboken, NJ.
- El-Zeini, H. M. 2006. Microstructure, rheological and geometrical properties of fat globules of milk from different animal species. *Pol. J. Food Nutr. Sci.* 56:147–153.
- Fleming, A., F. S. Schenkel, J. Chen, F. Malchiodi, R. A. Ali, B. Mallard, M. Sargolzaei, M. Corredig, and F. Miglior. 2017. Variation in fat globule size in bovine milk and its prediction using mid-infrared spectroscopy. *J. Dairy Sci.* 100:1640–1649. <https://doi.org/10.3168/jds.2016-11427>.
- Garnsworthy, P. C., S. Feng, A. L. Lock, and M. D. Royal. 2010. Short communication: Heritability of milk fatty acid composition and stearoyl-CoA desaturase indices in dairy cows. *J. Dairy Sci.* 93:1743–1748. <https://doi.org/10.3168/jds.2009-2695>.
- Gross, J., H. A. van Dorland, R. M. Bruckmaier, and F. J. Schwarz. 2011. Milk fatty acid profile related to energy balance in dairy

- cows. *J. Dairy Res.* 78:479–488. <https://doi.org/10.1017/S0022029911000550>.
- Heid, H. W., and T. W. Keenan. 2005. Intracellular origin and secretion of milk fat globules. *Eur. J. Cell Biol.* 84:245–258. <https://doi.org/10.1016/j.ejcb.2004.12.002>.
- Krahmer, N., Y. Guo, F. Wilfling, M. Hilger, S. Lingrell, K. Heger, H. W. Newman, M. Schmidt-Supprian, D. E. Vance, M. Mann, R. V. Farese Jr., and T. C. Walther. 2011. Phosphatidylcholine synthesis for lipid droplet expansion is mediated by localized activation of CTP:phosphocholine cytidyltransferase. *Cell Metab.* 14:504–515. <https://doi.org/10.1016/j.cmet.2011.07.013>.
- Lee, J.-Y., and I.-H. Kim. 2006. Advancing parity is associated with high milk production at the cost of body condition and increased periparturient disorders in dairy herds. *J. Vet. Sci.* 7:161–166. <https://doi.org/10.4142/jvs.2006.7.2.161>.
- Lock, A. L., and P. C. Garnsworthy. 2003. Seasonal variation in milk conjugated linoleic acid and $\Delta 9$ -desaturase activity in dairy cows. *Livest. Prod. Sci.* 79:47–59. [https://doi.org/10.1016/S0301-6226\(02\)00118-5](https://doi.org/10.1016/S0301-6226(02)00118-5).
- Logan, A., M. Auldist, J. Greenwood, and L. Day. 2014. Natural variation of bovine milk fat globule size within a herd. *J. Dairy Sci.* 97:4072–4082. <https://doi.org/10.3168/jds.2014-8010>.
- Lopez, C., V. Briard-Bion, O. Menard, F. Rousseau, P. Pradel, and J.-M. Besle. 2008. Phospholipid, sphingolipid, and fatty acid compositions of the milk fat globule membrane are modified by diet. *J. Agric. Food Chem.* 56:5226–5236. <https://doi.org/10.1021/jf7036104>.
- Martini, M., C. Scolozzi, F. Cecchi, and F. Abramo. 2004. Morphometric analysis of fat globules in ewe's milk and correlation with qualitative parameters. *Ital. J. Anim. Sci.* 3:55–60. <https://doi.org/10.4081/ijas.2004.55>.
- Masedunskas, A., Y. Chen, R. Stussman, R. Weigert, and I. H. Mather. 2017. Kinetics of milk lipid droplet transport, growth, and secretion revealed by intravital imaging: Lipid droplet release is intermittently stimulated by oxytocin. *Mol. Biol. Cell* 28:935–946. <https://doi.org/10.1091/mbc.E16-11-0776>.
- Mather, I. H., A. Masedunskas, Y. Chen, and R. Weigert. 2019. Symposium review: Intravital imaging of the lactating mammary gland in live mice reveals novel aspects of milk-lipid secretion. *J. Dairy Sci.* 102:2760–2782. <https://doi.org/10.3168/jds.2018-15459>.
- Mesilati-Stahy, R., and N. Argov-Argaman. 2014. The relationship between size and lipid composition of the bovine milk fat globule is modulated by lactation stage. *Food Chem.* 145:562–570. <https://doi.org/10.1016/j.foodchem.2013.08.077>.
- Mesilati-Stahy, R., and N. Argov-Argaman. 2018. Changes in lipid droplets morphometric features in mammary epithelial cells upon exposure to non-esterified free fatty acids compared with VLDL. *PLoS One* 13:e0209565. <https://doi.org/10.1371/journal.pone.0209565>.
- Mesilati-Stahy, R., H. Malka, and N. Argov-Argaman. 2012. Association of plasma insulin concentration to fatty acid distribution between milk fat and membrane synthesis. *J. Dairy Sci.* 95:1767–1775. <https://doi.org/10.3168/jds.2011-4583>.
- Palmquist, D. L. 2006. Milk fat: Origin of fatty acids and influence of nutritional factors thereon. Pages 43–92 in *Advanced Dairy Chemistry. Vol. 2: Lipids*. 3rd ed. P. F. Fox and P. L. H. McSweeney, ed. Springer International, New York, NY.
- Park, Y. W., M. Juárez, M. Ramos, and G. F. W. Haenlein. 2007. Physico-chemical characteristics of goat and sheep milk. *Small Rumin. Res.* 68:88–113. <https://doi.org/10.1016/j.smallrumres.2006.09.013>.
- R Core Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Robenek, H., I. Buers, O. Hofnagel, M. J. Robenek, D. Troyer, and N. J. Severs. 2009. Compartmentalization of proteins in lipid droplet biogenesis. *Biochim. Biophys. Acta* 1791:408–418. <https://doi.org/10.1016/j.bbalip.2008.12.001>.
- Robinson, D., and A. Hayes. 2018. broom: Convert statistical analysis objects into tidy tibbles. R package version 0.5.0. <https://CRAN.R-project.org/package=broom>.
- Sánchez-Juanes, F., J. M. Alonso, L. Zancada, and P. Hueso. 2009. Distribution and fatty acid content of phospholipids from bovine milk and bovine milk fat globule membranes. *Int. Dairy J.* 19:273–278. <https://doi.org/10.1016/j.idairyj.2008.11.006>.
- Schennink, A., W. M. Stoop, M. H. P. W. Visker, J. M. L. Heck, H. Bovenhuis, J. J. V. D. Poel, H. J. F. V. Valenberg, and J. A. M. V. Arendonk. 2007. DGAT1 underlies large genetic variation in milk-fat composition of dairy cows. *Anim. Genet.* 38:467–473. <https://doi.org/10.1111/j.1365-2052.2007.01635.x>.
- Shen, Z., A. Birkett, M. A. Augustin, S. Dungey, and C. Versteeg. 2001. Melting behavior of blends of milk fat with hydrogenated coconut and cottonseed oils. *J. Am. Oil Chem. Soc.* 78:387–394. <https://doi.org/10.1007/s11746-001-0273-4>.
- Soyeurt, H., F. Dehareng, P. Mayeres, C. Bertozzi, and N. Gengler. 2008. Variation of $\Delta 9$ -desaturase activity in dairy cattle. *J. Dairy Sci.* 91:3211–3224. <https://doi.org/10.3168/jds.2007-0518>.
- Van Haelst, Y. N. T., A. Beeckman, A. T. M. Van Kneegsel, and V. Fievez. 2008. Short communication: Elevated concentrations of oleic acid and long-chain fatty acids in milk fat of multiparous subclinical ketotic cows. *J. Dairy Sci.* 91:4683–4686. <https://doi.org/10.3168/jds.2008-1375>.
- Walter, L., S. Finch, B. Cullen, R. Fry, A. Logan, and B. J. Leury. 2019. The effect of physiological state, milk production traits and environmental conditions on milk fat globule size in cow's milk. *J. Dairy Res.* <https://doi.org/10.1017/S0022029919000748>.
- Wickham, H. 2016. *Ggplot2: Elegant Graphics for Data Analysis*. 2nd ed. Springer International, New York, NY.
- Wilfling, F., H. Wang, J. T. Haas, N. Krahmer, T. J. Gould, A. Uchida, J.-X. Cheng, M. Graham, R. Christiano, F. Fröhlich, X. Liu, K. K. Buhman, R. A. Coleman, J. Bewersdorf, R. V. Farese Jr., and T. C. Walther. 2013. Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocating from the ER to lipid droplets. *Dev. Cell* 24:384–399. <https://doi.org/10.1016/j.devcel.2013.01.013>.

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