Fatty Acid Metabolism in Liver of Dairy Cows Fed Supplemental Fat and Nicotinic Acid During an Entire Lactation¹

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ABSTRACT

Liver biopsies from 38 multiparous Holstein cows were used to determine rates of peroxisomal β -oxidation and total β -oxidation of palmitate in liver homogenates and contents of total lipid, triglyceride, and glycogen during the lactation cycle. Cows were assigned to one of four diets from wk 4 through wk 42 of lactation: control, control plus nicotinic acid (12 g/d), supplemental fat, or supplemental fat plus nicotinic acid. Liver biopsies were obtained at wk 3 (covariate), 6, 12, 24, and 42 of lactation. Neither supplemental fat nor nicotinic acid affected palmitate oxidation in liver homogenates or liver composition. Peroxisomal β -oxidation capacity and the ratio of peroxisomal to total β -oxidation decreased from wk 3 to 12 and then increased at wk 42. Contents of total lipid and triglyceride decreased, and content of glycogen increased, from wk 3 to 12. Total oxidation capacity in liver homogenates was correlated negatively with total lipid and triglyceride in liver, yields of milk and solids-corrected milk (SCM), and plasma nonesterified fatty acids (NEFA), and was correlated positively with liver glycogen, dry matter intake (DMI), energy balance, and plasma glucose. Peroxisomal β -oxidation was correlated negatively with yields of milk and SCM. The ratio of peroxisomal to total β -oxidation was correlated positively with liver total lipid, liver TG, and plasma NEFA and negatively with DMI and energy balance. When only data from wk 3 postpartum were considered, both total and peroxisomal β -oxidation were correlated negatively with hepatic concentrations of total lipid and TG. Peroxisomal β -oxidation in liver of dairy cows is not affected by feeding supplemental fat or nicotinic acid during wk 4 to 42 of lactation but may be a part of the hepatic adaptations to negative energy balance.

(**Key words:** peroxisomes, fatty acid metabolism, fat, liver)

Abbreviation key: LCFA = long-chain fatty acids, NA = nicotinic acid, PPAR α = peroxisome proliferator-activated receptor- α TG = triglyceride.

INTRODUCTION

Use of dietary fats has become a common practice to improve the energy status of high-producing dairy cows. Although most dietary long-chain fatty acids (LCFA) bypass the liver, mechanisms exist whereby dietary LCFA may be taken up by the liver and impact its metabolic processes (Grum et al., 1996a; Drackley, 1999). Skaar et al. (1989) fed diets supplemented with fat (5% DM basis) and nicotinic acid (NA) at 12 g/d from 17 d prepartum to 15 wk postpartum. They reported that fat and NA supplementation tended to increase hepatic concentrations of total lipid and triglyceride (TG). Fat supplemented to cows past peak lactation increased total metabolism of palmitate in liver slices and tended to increase palmitate oxidation (Grum et al., 1996a). When fat was supplemented to cows throughout the dry period, concentrations of total lipid and TG in liver were greatly decreased at d 1 and 21 after calving compared with cows fed control or high-grain diets during the dry period (Grum et al., 1996b). Lower hepatic concentrations of lipid and TG were accompanied by decreased capacity for esterification of palmitate in liver slices and increased peroxisomal β -oxidation in liver homogenates (Grum et al., 1996b). We demonstrated previously that hepatic peroxisomal β -oxidation of LCFA represents approximately 50% of the total capacity for first-cycle β -oxidation in the liver of dairy cows (Grum et al., 1994). Peroxisomal β -oxidation is inducible in rats by feeding high-fat diets (Ishii et al., 1980; Neat et al., 1980, 1981).

Although the largest potential impact of dietary fat on hepatic metabolism may be expected to occur during the periparturient period (Grum et al., 1996b; Drackley, 1999), the impact of dietary fat on the liver during mid- to late lactation and on the subsequent lactation also is of interest. In a short-term experiment, Grum et al. (1996a) found that supplemental fat did not affect he-

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patic TG concentration, despite increases in capacity for total metabolism of palmitate by liver slices. Vazquez-Añon et al. (1997) reported that supplementation of fat during mid- to late lactation did not affect hepatic TG content in mid- to late lactation but did tend to increase hepatic TG in the next lactation.

Supplementation of NA has been used in an attempt to modulate the rate of body lipid mobilization because of the reported antilipolytic effects of NA in adipose tissue (Fronk and Schultz, 1979; Jaster et al., 1983). Supplemental NA also has increased milk protein content (Horner et al., 1986). Because NA may impact provision of NEFA to the liver by affecting lipolysis and because NA is a precursor to NAD, which is a cofactor in both mitochondrial and peroxisomal β -oxidation of NEFA in liver, NA might affect hepatic capacity for β -oxidation of NEFA. In previous studies (Skaar et al., 1989; Minor et al., 1998), NA tended (nonsignificantly) to increase liver TG concentrations during the early postpartal period.

The objectives of this experiment were 1) to determine how capacity for total and peroxisomal β -oxidation of palmitate changes over an entire lactation and 2) to determine the effects of supplemental fat and NA on hepatic total and peroxisomal β -oxidation of palmitate and on hepatic concentrations of total lipid, TG, and glycogen. The experiment was part of a larger study designed to determine production responses to long-term supplementation of fat and NA in dairy cows (Drackley et al., 1998).

MATERIALS AND METHODS

Experimental Design and Diets

Samples used in this study were obtained from cows in an experiment reported previously (Drackley et al., 1998). All procedures were conducted under protocols approved by the University of Illinois Laboratory Animal Care Advisory Committee. Briefly, 48 multiparous Holstein cows were fed a control diet for the first 3 wk postpartum and then were assigned to one of four dietary treatments in a 2×2 factorial arrangement: 1) control, 2) control plus NA (12 g/d; Lonza Inc., Fair Lawn, NJ), 3) supplemental fat (whole raw soybeans plus Qual-Fat Dairy Blend; National By-Products Inc., Mason City, IL), and 4) supplemental fat plus NA (12 g/d). Diet composition and analysis were reported previously (Drackley et al., 1998). Diets were based on alfalfa silage, corn silage, ground shelled corn, soybean meal, and soybean hulls. Dietary energy density was decreased from wk 26 through the remainder of lactation by removing the whole raw soybeans and a portion of the liquid fat and by increasing forage (Drackley et al., 1998). Cows were housed in tie stalls and were fed

twice daily for ad libitum intake; daily DMI and milk yields were recorded throughout lactation. Sampling and analysis of feeds and milk were described previously (Drackley et al., 1998). The control and fat-supplemented diets fed during wk 4 to 25 contained 2.75 and 6.04% total LCFA (DM basis); control and fat-supplemented diets fed during wk 26 to 43 contained 2.53 and 3.84% total LCFA (DM basis). Energy balance was calculated from results of DMI, dietary composition, BW, milk production, and milk composition, as described previously (Drackley et al., 1998) using principles defined by NRC (1989).

Sampling and Analysis of Liver and Blood

Samples of liver (approximately 3 to 5 g) were obtained via puncture biopsy under local anesthesia (Drackley et al., 1991) at the end of wk 3, 6, 12, 24, and 42 of lactation. A portion of the liver was frozen immediately in liquid N_2 until further analyses, and the remaining portion was placed immediately in ice-cold phosphate-buffered (0.015 M) saline (0.9% NaCl) at pH 7.4 and transported to the laboratory for in vitro metabolic assays.

Capacities for total and peroxisomal first-cycle β -oxidation of [$1-^{14}C$]palmitate (American Radiolabeled Chemicals, Inc., St. Louis, MO) were measured in liver homogenates using the procedures of Veerkamp and van Moerkerk (1986) modified as described previously (Grum et al., 1994, 1996a). Briefly, 0.5 g of liver was homogenized in a 10-ml homogenization buffer (pH 7.4). β -oxidation was measured in the absence and presence of antimycin A and rotenone (Sigma Chemical Co., St. Louis, MO) as inhibitors of mitochondrial β -oxidation. Radiolabel incorporated into CO_2 and acid-soluble products was measured by liquid scintillation spectroscopy. Capacity for total first-cycle β -oxidation of palmitate was that measured in the absence of mitochondrial inhibitors, whereas peroxisomal β -oxidation was that measured in the presence of the inhibitors. Triplicate flasks of each tissue treatment (blank, total β -oxidation, peroxisomal β -oxidation) were incubated for 30 min at 37°C.

Samples of frozen liver were analyzed for concentrations of total lipid by gravimetric methods after chloroform-methanol extraction (Drackley et al., 1991), TG by a colorimetric procedure (Foster and Dunn, 1973), and glycogen by a colorimetric procedure (Lo et al., 1970). Plasma obtained from the coccygeal vein before the a.m. feeding was analyzed for concentrations of glucose using glucose oxidase (kit number 315, Sigma Chemical Co.), NEFA using a coupled enzymatic-colorimetric procedure (Drackley et al., 1991), and BHBA using an enzymatic procedure (Cant et al., 1993).

Table 1. Least-squares means for production and blood variables by week postpartum.¹

Variable	wk 6	wk 12	wk 24	wk 42	SE
DMI, ² kg	21.2	24.3	23.6	19.4	0.4
Milk, kg	40.2	38.4	34.0	19.8	0.7
SCM, ³ kg	36.6	34.8	31.1	20.1	0.6
Energy balance, ² Mcal/d	0.20	6.67	7.94	7.68	0.64
Glucose, mg/dl	68.0	69.6	68.5	70.7	0.8
NEFA, ^{4,5} µeq/L	203	112	82	70	9
BHBA, ⁵ mg/dl	6.3	4.4	4.3	4.6	0.4

¹Effect of week was significant ($P < 0.05$) for all variables.²Fat $\times$ week postpartum, $P < 0.02$.³Higher for NA, $P < 0.05$.⁴Higher for fat, $P < 0.01$.⁵Fat $\times$ NA $\times$ week, $P < 0.01$.

Statistical Analysis

A total of 44 cows completed the production experiment (Drackley et al., 1998); 40 of those cows had been selected a priori for liver measurements. Liver data were available for 38 cows (10 control, 10 control + NA, 9 supplemental fat, and 9 supplemental fat + NA); liver could not be obtained from two cows. Data for liver measurements, blood metabolites, and selected production data (DMI, milk yield, SCM yield, and energy balance) for the week preceding each liver biopsy were subjected to repeated-measures ANOVA for a continuous design, using the MIXED procedure of SAS (1996). The effects of fat supplementation, addition of NA, and the interaction of fat and NA were tested using cow as the subject, with covariance structures fit using the autoregressive procedure (SAS, 1996). Cow was considered a random factor. The effects of time postpartum and the interactions of dietary treatments with time were tested using the residual error. The respective measurements made during wk 3 (before dietary treatments were initiated) were included in the model as continuous variables. Least-squares means are reported throughout. Pearson correlations among variables were computed using the entire dataset, including the wk-3 (covariate) data, and also for the wk-3 data only. Statistical significance was set at $P < 0.05$; comparisons with $P < 0.15$ are discussed as trends.

RESULTS

Results for effects of diets on production variables and metabolites in blood were presented earlier (Drackley et al., 1998). Variables were re-analyzed here for only the weeks in which liver samples were taken so that liver data could be compared directly with production and blood variables from the same cows. In general, results followed the patterns reported earlier for the full data set. Means by week across dietary treatments are shown in Table 1. The interaction of the main effect of

Figure 1. Least squares means for the main effect of fat supplementation, demonstrating the interactions of fat $\times$ week ($P < 0.05$) for cows fed control diets (◇) or diets supplemented with fat (◆). Panel a: DMI. Largest SE of the least-squares means = 0.6 kg/d. Significant effects from the model: week ($P < 0.0001$), fat $\times$ week ($P < 0.02$), fat $\times$ NA $\times$ week ($P < 0.13$). Panel b: calculated energy balance. Largest SE of the least squares means = 0.9 Mcal/d. Significant effects from the model: week ($P < 0.0001$), fat $\times$ week ($P < 0.0001$), fat $\times$ NA $\times$ week ($P < 0.09$).

dietary fat and week was significant ($P < 0.02$) for DMI (Figure 1); means were greater for cows fed fat-supplemented diets at wk 6 but lower for cows fed fat-supplemented diets at wk 24 and 42. Milk yield was not affected by diet, although the difference between cows fed control and NA (32.3 vs. 33.9 kg/d, respectively) approached significance ($P < 0.11$). Yield of SCM was increased ($P < 0.05$) by NA (31.5 vs. 29.8 kg/d for NA and control, respectively). A weak statistical tendency ($P < 0.12$) for an interaction of fat $\times$ week was detected for SCM (36.2, 34.3, 31.4, and 18.9 kg/d for control and 37.1, 35.4, 30.8, and 21.2 kg/d for fat-supplemented diets at wk 6, 12, 24, and 42, respectively). The interaction of fat $\times$ week was significant for calculated energy balance (Figure 1), demonstrating that energy balance was greater for cows fed fat-supplemented diets at wk 6, 12, and 24 but was greater for cows fed control diets at wk 42. Similar to results for the complete data set (Drackley et al., 1998), a tendency ($P < 0.09$) was de-

Figure 2. Least squares means for concentration of NEFA in plasma from cows fed a control diet (○), the control diet plus 12 g/d of nicotinic acid [(NA, □)], a diet supplemented with fat (●), or the diet supplemented with fat plus 12 g/d of NA (■). Largest SE of the least squares means = 19.1 µeq/L. Significant effects from the model: fat ($P < 0.006$), fat $\times$ NA ($P < 0.09$), week ($P < 0.0001$), fat $\times$ NA $\times$ week ($P < 0.0001$).

tested for the three-way interaction of fat $\times$ NA $\times$ week for energy balance; cows fed the fat-supplemented diet without NA had greater energy balance during early lactation, whereas cows fed the control diet with NA remained in lower energy balance for a greater length of time than did cows fed the other diets.

The concentration of glucose in plasma was not affected by diets. Plasma NEFA were higher for cows fed the fat-supplemented diet than for cows fed the control diet, and the three-way interaction of fat $\times$ NA $\times$ week was significant (Figure 2), similar to data from the full data set reported earlier (Drackley et al., 1998). The concentration of NEFA was lower for cows fed the fat-supplemented diet plus NA than for cows fed the fat-supplemented diet without NA in early lactation, but cows fed the fat-supplemented diet plus NA had the greater NEFA concentration later in lactation. In contrast, NEFA for cows fed the control diet plus NA were greater than for unsupplemented cows at wk 6 but then were lower than unsupplemented cows at the other weeks. The three-way interaction of fat $\times$ NA $\times$ week also was significant for BHBA in plasma (data not shown) and followed the same pattern as NEFA, although mean concentrations of BHBA were less than 8 mg/dl for all groups at all time points.

Rates of total and peroxisomal β -oxidation and the ratio of peroxisomal to total β -oxidation were not affected by diets (Figure 3). Accumulation of ^{14}C in acid-soluble products was the primary fate of oxidation of $[1-^{14}\text{C}]$ palmitate; negligible amounts of $^{14}\text{CO}_2$ were pro-

Figure 3. Least squares means for in vitro metabolism of palmitate by liver homogenates from cows fed a control diet (○), the control diet plus 12 g/d of nicotinic acid [(NA, □)], a diet supplemented with fat (●), or the diet supplemented with fat plus 12 g/d of NA (■). Panel a: total first-cycle β -oxidation. Largest SE of the least squares means = 0.78 µmol/(h $\times$ g wet weight). Significant effects from the model: week ($P < 0.02$). Panel b: peroxisomal first-cycle β -oxidation. Largest SE of the least squares means = 0.59 µmol/(h $\times$ g wet weight). Significant effects from the model: week ($P < 0.0001$). Panel c: ratio of peroxisomal to total β -oxidation. Largest SE of the least squares means = 0.04. Significant effects from the model: week ($P < 0.01$), fat $\times$ week ($P < 0.12$).

duced in the presence of antimycin A and rotenone. The rate of total β -oxidation differed ($P < 0.02$) by week postpartum, with the greatest rates measured at wk 42 (Figure 3A). Although the fat $\times$ week interaction did not reach statistical significance ($P < 0.19$), means for cows fed the fat-supplemented diets were slightly lower

than those for control cows at wk 12 but higher than controls at wk 42. The rate of peroxisomal β -oxidation differed ($P < 0.0001$) by week postpartum, with means decreasing from wk 3 to 12 and then increasing at wk 24 and 42 (Figure 3B). As a result of these changes, the ratio of peroxisomal to total oxidation (Figure 3C) also was affected by week postpartum ($P < 0.02$); ratios were highest at wk 3 and then decreased by wk 12 before increasing again at wk 42. The weak tendency ($P < 0.12$) for interaction between fat and week postpartum indicates that the ratio tended to be lower for control cows at wk 12, but higher for controls at wk 24 and 42.

Changes in liver composition are depicted in Figure 4. For the concentrations of total lipid (Figure 4A) and TG (Figure 4B) in liver, the three-way interaction of fat $\times$ NA $\times$ week was significant ($P < 0.01$). The three-way interactions evidently arose mainly from the differences in response to NA at wk 6. Concentrations of total lipid and TG decreased for all treatments from wk 3 to 12 as expected. The concentration of glycogen (Figure 4C) tended to be lower ($P < 0.09$) for cows supplemented with NA, although differences were small (5.2 vs. 4.7% of wet weight for control and NA, respectively). Hepatic glycogen was lowest for all treatments during early lactation and increased throughout the remainder of lactation ($P < 0.0001$).

To identify associations between hepatic lipid metabolism and other variables, we computed correlation coefficients for the entire dataset, including the wk-3 covariate samples (Table 2). Total and peroxisomal β -oxidation rates were highly correlated ($r = 0.748$). Total β -oxidation was correlated negatively with hepatic concentrations of total lipid ($r = -0.362$) and TG ($r = -0.324$) and positively (albeit weakly) with hepatic glycogen content ($r = 0.148$). In contrast, peroxisomal β -oxidation was not correlated with liver composition. Modest positive correlations existed between total β -oxidation capacity and DMI ($r = 0.209$) and energy balance ($r = 0.307$), but total β -oxidation was correlated negatively with yields of milk ($r = -0.179$) and SCM ($r = -0.204$). The rate of peroxisomal β -oxidation was correlated negatively with milk ($r = -0.260$) and SCM ($r = -0.230$) but was not correlated with DMI or energy balance. The ratio of peroxisomal to total β -oxidation was correlated negatively with DMI ($r = -0.324$) and energy balance ($r = -0.300$) but was uncorrelated with yields of milk or SCM. Weak correlations also existed between total oxidation, but not peroxisomal oxidation, and plasma glucose ($r = 0.234$) and NEFA ($r = -0.222$).

The concentrations of total lipid and TG in liver were highly related ($r = 0.983$). Both hepatic total lipid and TG were related negatively to hepatic glycogen ($r = -0.61$ for both), DMI ($r = -0.62$ for both), energy balance ($r = -0.73$ for both), and plasma glucose ($r = -0.455$ for

Figure 4. Least squares means for composition of liver from cows fed a control diet ($\circ$), the control diet plus 12 g/d of nicotinic acid (NA, $\square$), a diet supplemented with fat ($\bullet$), or the diet supplemented with fat plus 12 g/d of NA ($\blacksquare$). Panel a: total lipid in liver tissue (% of wet weight). Largest SE of the least squares means = 0.8%. Significant effects from the model: week ($P < 0.0001$), fat $\times$ NA $\times$ week ($P < 0.01$). Panel b: triglyceride (TG) in liver tissue (% of wet weight). Largest SE of the least squares means = 0.7%. Significant effects from the model: week ($P < 0.0001$), fat $\times$ NA $\times$ week ($P < 0.003$). Panel c: glycogen in liver tissue (% of wet weight). Largest SE of the least squares means = 0.4%. Significant effects from the model: NA ($P < 0.09$), week ($P < 0.0001$).

total lipid and $r = -0.438$ for TG). Both total lipid and TG in liver were positively correlated with SCM yield ($r = 0.25$ for both), plasma NEFA ($r = 0.77$ for both), and plasma BHBA ($r = 0.428$ for total lipid and $r = 0.436$ for TG). Hepatic glycogen content was correlated

Table 2. Pearson correlation coefficients among selected variables for entire dataset.¹

	Peroxisomal oxidation	P:T ²	Liver total lipid	Liver TG ³	Liver glycogen	DMI	Milk	SCM	Energy balance	Plasma glucose	Plasma NEFA	Plasma BHBA
Total oxidation	0.748 (<0.001)	-0.148 (<0.05)	-0.362 (<0.001)	-0.324 (<0.001)	0.148 (<0.05)	0.209 (0.005)	-0.179 (0.02)	-0.204 (0.007)	0.307 (<0.001)	0.234 (0.002)	-0.222 (0.003)	NS ⁴
Peroxisomal oxidation		0.529 (<0.001)	NS	NS	NS	NS	-0.260 (<0.001)	-0.230 (0.002)	NS	NS	NS	NS
P:T			0.265 (<0.001)	0.273 (<0.001)	NS	-0.324 (<0.001)	NS	NS	-0.300 (<0.001)	NS	0.185 (0.02)	NS
Liver total lipid				0.983 (<0.001)	-0.611 (<0.001)	-0.621 (<0.001)	NS	0.254 (<0.001)	-0.727 (<0.001)	-0.455 (<0.001)	0.770 (<0.001)	0.428 (<0.001)
Liver TG					-0.610 (<0.001)	-0.616 (<0.001)	NS	0.254 (<0.001)	-0.726 (<0.001)	-0.438 (<0.001)	0.774 (<0.001)	0.436 (<0.001)
Liver glycogen						0.342 (<0.001)	-0.387 (<0.001)	-0.465 (<0.001)	0.589 (<0.001)	0.364 (<0.001)	-0.657 (<0.001)	-0.286 (<0.001)

¹Number in parentheses below each correlation coefficient (r) is probability that absol. val.: $|r| > 0$.

²Ratio of peroxisomal to total β -oxidation.

³Triglyceride.

⁴ $P > 0.05$.

positively with DMI ($r = 0.342$), energy balance ($r = 0.589$), and plasma glucose ($r = 0.364$) and negatively with milk yield ($r = -0.387$), SCM yield ($r = -0.465$), plasma NEFA ($r = -0.657$), and plasma BHBA ($r = -0.286$).

Because of the dynamic changes in hepatic LCFA metabolism during the periparturient period, and the larger range of hepatic total lipid and TG contents in the pretreatment liver samples obtained at wk-3 postpartum, we also computed correlation coefficients among variables for wk-3 data only (Table 3). Correlations between total β -oxidation in liver homogenates and peroxisomal β -oxidation ($r = 0.842$), the ratio of peroxisomal to total β -oxidation ($r = -0.367$), liver total lipid ($r = -0.517$), and liver TG ($r = -0.468$) were all larger than corresponding correlations for the full dataset. In contrast to the full dataset, peroxisomal β -oxidation in liver homogenates was not correlated significantly with the ratio of peroxisomal to total β -oxidation but was correlated negatively with concentrations of total lipid ($r = -0.450$) and TG ($r = -0.403$) in liver. Relationships among other variables at wk 3 generally were similar to those for the full dataset.

DISCUSSION

The primary objectives of this experiment were to determine how hepatic peroxisomal and total β -oxida-

tion and liver composition change throughout an entire lactation in dairy cows, and to determine if these variables are affected by long-term dietary supplementation of fat, NA, or both. Neither supplemental fat nor NA significantly affected hepatic capacities for total or peroxisomal β -oxidation. Previously, we showed that total and peroxisomal β -oxidation in liver homogenates were increased at d 1 postpartum, decreased at d 21, and then increased again by d 60 postpartum (Grum et al., 1996b). Furthermore, peroxisomal β -oxidation was greater during the periparturient period for cows that were fed a fat-supplemented diet throughout the dry period. These changes were accompanied by a lack of increase in hepatic total lipid and TG concentrations at 1 d postpartum, compared with marked increases in control cows and cows fed a high-grain diet that was isocaloric to the high-fat diet (Grum et al., 1996b). We interpret the results of the present experiment to indicate that increased dietary fat per se is not sufficient to cause increases in peroxisomal β -oxidation per unit of liver mass in dairy cows.

In the earlier experiment (Grum et al., 1996b), cows fed the fat-supplemented diet consumed less dietary NE_L than cows fed the other diets. Starvation causes an increase of peroxisomal β -oxidation per unit of liver mass in rodents (Mannaerts et al., 1979; Veerkamp and van Moerkerk, 1986). However, depriving nonlactating cows of all feed for 7 d did not increase hepatic peroxi-

Table 3. Pearson correlation coefficients among selected variables for measurements at wk 3 postpartum (pretreatment period) only.¹

	Peroxisomal oxidation	P:T ²	Liver total lipid	Liver TG ³	Liver glycogen	DMI	Milk	SCM	Energy balance	Plasma glucose	Plasma NEFA	Plasma BHBA
Total oxidation	0.842 (<0.001)	-0.367 (<0.03)	-0.517 (<0.001)	-0.468 (<0.004)	NS	NS	NS	NS	NS	NS	NS	NS
Peroxisomal oxidation		NS	-0.450 (<0.006)	-0.403 (<0.01)	NS	NS	NS	NS	NS	NS	NS	NS
P:T			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Liver total lipid				0.988 (<0.001)	-0.560 (<0.001)	-0.659 (<0.001)	NS	NS	-0.682 (<0.001)	-0.456 (<0.005)	0.616 (<0.001)	NS
Liver TG					-0.564 (<0.001)	-0.645 (<0.001)	NS	NS	-0.690 (<0.001)	-0.435 (<0.007)	0.651 (<0.001)	NS
Liver glycogen						0.353 (<0.03)	NS	NS	0.412 (<0.01)	0.358 (<0.03)	-0.472 (<0.003)	NS

¹Number in parentheses below each correlation coefficient (r) is probability that abso. val.: $|r| > 0$.

²Ratio of peroxisomal to total β -oxidation.

³Triglyceride.

⁴ $P > 0.05$.

somal β -oxidation (Grum et al., 1994). Consequently, we speculate that some combination of increased LCFA from dietary or endogenous sources and the hormonal milieu characteristic of the periparturient period is required to elicit increases in capacity for peroxisomal and total β -oxidation in bovine liver as observed earlier (Grum et al., 1996b).

The weak tendency ($P < 0.12$) for an interaction of fat $\times$ week postpartum for the ratio of peroxisomal to total β -oxidation (Figure 3C) resulted from the trend for cows fed fat-supplemented diets to have a slightly higher ratio at wk 12 but a lower ratio at wk 24 and 42. The changes in this ratio, however, appeared to be a result of changes in capacity for total β -oxidation (Figure 3A) rather than changes in peroxisomal β -oxidation capacity (Figure 3B). This result is opposite to what we observed previously when a fat-supplemented diet was fed to cows throughout the dry period (Grum et al., 1996b); in that experiment, total β -oxidation capacity and the peroxisomal-to-total ratio tended to increase as a result of significantly greater peroxisomal β -oxidation in response to the fat-supplemented diet. Results also differ from research with rodents; for example, in rats, hepatic peroxisomal β -oxidation was increased 150% by feeding high-fat diets (Neat et al., 1980, 1981). Neat et al. (1980) fed a control diet (5% soybean oil) or diets containing 15% fat supplied as either marine oil or soybean oil. By d 17 of feeding, hepatic peroxisomal β -oxidation was greater in rats fed

diets containing either 15% marine oil or 15% soybean oil than for controls. The amount of supplemental fat fed to cows in our experiment may not have been high enough to elicit an increase of peroxisomal β -oxidation, as reported for rats. Alternately, the profile of LCFA reaching the intestine as a result of ruminal biohydrogenation may not have provided the appropriate LCFA to cause induction of peroxisomal β -oxidation.

In rodents, increases of hepatic peroxisomal β -oxidation result from increased transcription of the enzymes secondary to activation and binding of a specific lipid-activated nuclear receptor called peroxisome proliferator-activated receptor- α (PPAR α). When activated by binding of a lipid ligand, PPAR α binds to response elements in the promoter region of target genes to activate their transcription (see Desvergne and Wahli, 1999, for review). A variety of LCFA can activate PPAR α , but polyunsaturated LCFA are much more potent activators than are saturated LCFA (Kliwer et al., 1997). Because the dietary LCFA provided in our experiment were a combination of unprotected fat sources that would be extensively hydrogenated in the rumen, the additional LCFA absorbed from the intestine from the fat-supplemented diet would be mostly saturated LCFA. Consequently, this profile of absorbed LCFA may not have been effective in increasing transcription of the genes encoding enzymes for peroxisomal and mitochondrial β -oxidation.

Although correlations cannot demonstrate cause and effect, the capacity for total β -oxidation was correlated negatively with liver total lipid and TG contents, plasma NEFA, and yields of milk and SCM (Table 2). Correlations were positive between total β -oxidation capacity and liver glycogen, DMI, energy balance, and plasma glucose. These correlations, although statistically significant, were relatively weak compared with those between liver lipid or TG and DMI, energy balance, and NEFA. Negative correlations between either total or peroxisomal β -oxidation and hepatic concentrations of total lipid and TG were much stronger when only wk-3 data were considered (Table 3), similar to our previous observations during the periparturient period (Grum et al., 1996b). Whether the negative associations between hepatic total or peroxisomal β -oxidation capacity and total lipid or TG contents indicate that increased β -oxidation capacity is protective against hepatic lipid accumulation or that hepatic lipid accumulation results in lower total β -oxidation capacity cannot be determined from our data. Peroxisomal β -oxidation capacity was not correlated significantly with any production or blood variables except for negative correlations with yields of milk and SCM in the full dataset (Table 2). We suggest that our data are consistent with an increased relative importance of peroxisomal β -oxidation during times of negative energy balance, as shown previously during the periparturient period (Grum et al., 1996b).

The trend for total and peroxisomal β -oxidation capacities to increase by wk 42 of lactation (Figure 3) is interesting, but we can offer no explanation for its cause or significance. Aiello et al. (1984) noted that ketogenic capacity of liver slices incubated with [1- 14 C]palmitate decreased from d 30 to d 60 to 90 of lactation, but then increased at d 180. Oxidation of [1- 14 C]palmitate to CO_2 was unchanged between d 30 and 60 but then increased at d 90 and 180 (Aiello et al., 1984).

Main effects of fat and NA were not significant for concentrations of total lipid and TG in liver tissue, but the three-way interaction of fat $\times$ NA $\times$ week postpartum was significant for both variables (Figure 4A and 4B). The interactions arose mainly from differences among diets at wk 6. The pattern among diets is generally similar to that for the concentration of NEFA in plasma (Figure 2). Increased NEFA concentration in plasma in response to negative energy balance is the major factor that drives TG accumulation in liver (Vazquez-Añon et al., 1994; Drackley, 1999). Although differences in energy balance among treatments were not significant in the smaller data set used here, a pattern among treatments inverse to those for liver TG and plasma NEFA was observed for the full dataset (Drackley et al., 1998), in that energy balance was lowest during early lactation for cows fed the control diet

supplemented with NA. Despite these transient effects in early lactation, supplemental fat did not affect the concentrations of total lipid or TG in liver during mid- to late lactation, which agrees with results of Vazquez-Añon et al. (1997). The effect of NA on the concentration of NEFA in plasma has been variable, including decreases (Dufva et al., 1983) or no change (Skaar et al., 1989; Minor et al., 1998;). Supplementation of NA beginning prepartum (Skaar et al., 1989; Minor et al., 1998) or postpartum (Minor et al., 1998) did not significantly affect liver TG concentrations during the periparturient period, although in both reports means were slightly higher for NA-supplemented cows.

The concentration of glycogen in liver tended ($P < 0.09$) to be lower for cows supplemented with NA. Because NA significantly increased production of SCM but did not affect DMI, the slightly lower overall mean for hepatic glycogen may reflect the greater demand for glucose for milk synthesis. Changes in hepatic composition with stage of lactation reflect the well-characterized increases of lipid and decreases of glycogen in early lactation, followed by decreases in lipid content and increases in glycogen content in later lactation (Skaar et al., 1989; Vazquez-Añon et al., 1994, 1997; Grum et al., 1996b). The strong correlations among contents of total lipid, TG, and glycogen in liver with DMI, energy balance, and plasma NEFA highlight the importance of these factors in determining the degree of lipid infiltration and glycogen depletion observed during the periparturient period and early lactation (Vazquez-Añon et al., 1994).

CONCLUSIONS

The rate of hepatic peroxisomal β -oxidation in lactating dairy cows did not change appreciably between wk 3 and wk 24 of lactation, but increased by wk 42 of lactation. Peroxisomal β -oxidation constituted a greater proportion of total β -oxidation capacity in liver homogenates in early lactation (wk 3) than at peak lactation or mid-lactation; this proportion then increased again by wk 42 of lactation. Peroxisomal β -oxidation was not altered by supplemental fat or NA fed between wk 4 and 42 of lactation. Long-term supplementation of fat or NA also did not impact contents of total lipid, TG, or glycogen in liver. For samples obtained at wk 3 postpartum, capacities for both total β -oxidation and peroxisomal β -oxidation of palmitate per unit of liver tissue mass were negatively correlated with concentrations of total lipid and TG in liver. The ratio of peroxisomal β -oxidation capacity to total β -oxidation capacity in liver homogenates is related negatively to energy balance throughout the lactation cycle. We suggest, therefore, that peroxisomal β -oxidation may be part of a strategy

to deal with increased NEFA flux during negative energy balance.

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