Relations between Plasma Acetate, 3-Hydroxybutyrate, FFA, Glucose Levels and Energy Nutrition in Lactating Dairy Cows

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ABSTRACT. To clarify the implication of an energy nutrition on a metabolic alteration with advancing lactation, total 270 blood samples were taken from 16 lactating dairy cows. Amounts of dietary allowance and the refusals were measured daily, and the energy (TDN) intakes and a satisfaction (energy balance) of each cow were estimated. Plasma acetate, 3-hydroxybutyrate (3-HB), free fatty acid (FFA) and glucose levels were estimated. The data were divided into 3 groups depending on the days in milk; early (up to 70 days postpartum), mid (71 to 140 days), and late (after 141 days) lactation. There were many cases of higher FFA level in early lactation, especially with declining acetate and glucose levels. There were proportional elevations of 3-HB in connection with FFA levels in many samples of early lactation, though the 3-HB increased independently of FFA levels in the most cases of the mid and late lactations. Plasma 3-HB levels increased in many cases of decreased glucose level, especially in the early lactation. Plasma acetate level correlated positively with 3-HB level, but not correlated with glucose level. Higher FFA level and elevation of FFA/3-HB ratio were observed in the conditions of negative energy balance. This implies the metabolic importance of FFA in a ketogenesis of the early lactation.—KEY WORDS: blood acetate, dairy cattle, energy nutrition, ketone, plasma metabolite.

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There have been many cases of metabolic or nutritional disorders in high yielding dairy cows, especially in periparturitional (transitional) period. Confusions of lipid and carbohydrate metabolism have been risk factors for these metabolic disorders [10] which were mainly brought about by an insufficient intake of feed (nutrients) [4]. Recently, a metabolic profile testing based on blood analyzing have become of the center of wide interest for preventing metabolic and nutritional diseases, and finally for an efficient milk production and farm economics [9]. Blood metabolite levels have been influenced by many factors, such as a nutritional status of the animals, feed intakes and the nutrients requirements which have fluctuated largely by parturition and advancing lactation stage. Many workers measured the blood metabolite levels of dairy cows, however the informations of the cows used have not been in detail concerning to nutrient intakes, precise dietary chemical properties, body weight and nutritional satisfaction such as energy or protein balance. In the present study, changes of blood metabolite levels were monitored for the purpose of elucidating the implications of the actual nutritional (energy) balance and a lactational stages on lipid metabolism of dairy cows. Nutritional status of the cows were monitored during the experiment by measuring of feed intake, body weight, milk yields and milk quality and precise analysis of the diets.

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MATERIALS AND METHODS

The study was conducted from May 1990 until January 1992 using 16 lactating cows in Holstein dairy herd of Tohoku National Agricultural Experiment Station. The cows were individually tied in a cowshed, with about 5 hrs' freedom in an exercise yard, and they were milked twice daily. They were given ample amounts of grass silage or corn silage twice daily. Twenty lots of grass silage and 14 lots of corn silage were used during the experiment, and an alfalfa hay cube and a grass hay were given in fixed amounts. A concentrate diet was given on the basis of approximately 55 to 60% of total digestible nutrient (TDN) requirement [5]. The feed analyses (CP, fiber fractions, dry matter) were conducted for the silage diets in every 2 to 3 weeks intervals during the experiment. The TDN levels of the silages, the alfalfa cube and the hay were estimated by the regression equations depending on acid detergent fiber (ADF) levels [8]. The feed refusals were removed and weighed every morning. Nutrients levels of the diets used were given in Table 1. From actual measurements of the feed intakes, chemical compositions of the diets and nutritional requirements of each cow [5], an energy balance (as TDN) was calculated weekly.

Venous blood samples were taken into the heparinized tube at about 4 hrs after the morning feeding. The samples were chilled in an ice bath immediately, and the resulting plasma samples were stored at −30°C until analysis. Individual cows were used for 4 to 32 samplings with an interval of more than 2 weeks. No samples were taken from sick cows including mastitis. Acetate and 3-hydroxybutyrate (3-HB) were analyzed enzymatically on a deprotenized supernatant of the plasma (F-kit for acetate,

Table 1. Diets and their nutritional evaluations during the feeding trials

Diet	Number of lots	DM (%) Mean	DM (%) Range	TDN* Mean	TDN* Range	Fiber (NDF)* Mean	Fiber (NDF)* Range	Crude protein* Mean	Crude protein* Range
Concentrate	1	88		80		21		21	
Hay cube	3	88	87–89	57	57–58	47	46–48	15	14–15
Grass hay	3	87	84–91	57	55–58	65	58–71	8	6–12
Grass silage	20	25	14–44	57	44–69	63	40–82	10	5–19
Corn silage	14	31	26–35	71	64–76	40	34–60	6	4–12

*% on DM basis,
NDF: Neutral detergent fiber.

Table 2. Milk yield, plasma metabolite levels and nutritional satisfaction in early, mid and late lactation

Weeks in lactation		Body wt. kg	Milk kg/d.	Blood plasma Acetate (mM)	3-HB (mM)	FFA (mM)	Glucose (mM)	Nutrient intake TDN Int/Req[2)]	CP %	DMI/BW[1)] %
~10	Mean	644[a]	31.4[a]	1.01	0.74[a]	0.16[a]	3.56[a]	92[a]	108[a]	2.91[a]
(n=81)	SD	42	6.6	0.25	0.32	0.12	0.31	14	18	0.40
10<~20	Mean	655[ab]	27.5[b]	0.92	0.60[b]	0.09[b]	3.68[b]	105[b]	121[b]	2.90[a]
(n=73)	SD	40	5.8	0.26	0.24	0.05	0.27	12	15	0.39
20<~	Mean	666[b]	20.2[c]	1.04	0.67[ab]	0.08[b]	3.68[b]	105[b]	119[b]	2.55[b]
(n=116)	SD	36	3.7	0.27	0.24	0.04	0.27	11	19	0.31

1) Dry matter intake/Body weight, 2)Dietary intake/requirement
Means with different superscripts in the same row were significantly different ($p<0.05$).

and F-kit for D-3-hydroxybutyric acid; Boehringer Mannheim, Germany). Free fatty acid (FFA) and glucose were determined by using the clinical chemistry kits (NEFA-C test, and Glucose-B test; Wako Pure Chemical, Japan).

The data were divided into 3 groups (lactation stages) depending on the days postpartum of sampling; early (up to 70 days postpartum), mid (71 to 140 days), and late (after 141 days) lactation. First period of 10 weeks was assumed as early lactation in the present study, depending on the situation of negative energy balance owing to an insufficient recovery of appetite in that period in many dairy cows [6]. Statistical significance among the groups was determined by one-way ANOVA using Stat View (Abaccus Concepts, U.S.A.). Relations among the blood metabolite levels and the energy status (TDN balance) were calculated by regression analysis. Regression lines were given in the cases of $R^2>0.1$ in Figs. 2–4. Molar ratio of FFA/3-HB was estimated to elucidate the relative significance of lipolysis and ketogenesis.

RESULTS

Mean and SD of the plasma metabolite levels, nutritional satisfaction, milk yield and body weight at the blood sampling were shown in Table 2. The means of FFA and 3-HB levels were higher and mean glucose level was lower in the early lactation. Many cases of insufficient energy nutrition were observed in the early lactation.

There were two tendencies for the relationship between plasma FFA and 3-HB levels; one is an elevation of 3-HB without FFA increase (line A in Fig. 1), and the other trend is an elevation of 3-HB in connection with increasing FFA levels (line H). Many cases of the proportional elevation of 3-HB with FFA levels were observed in the samples of the early lactation.

Relations between plasma acetate and 3-HB or FFA levels were shown in Fig. 2. There were many cases of higher FFA level accompanying with decreased acetate level. Plasma acetate and 3-HB levels showed a positive relationship. Many samples of the early lactation showed rather higher 3-HB with lower acetate levels compared to the other stages. There were no clear correlations between acetate and glucose levels.

Relations between plasma glucose and 3-HB or FFA levels were shown in Fig. 3. The 3-HB levels increased accompanying with decreasing glucose level in the early, mid and late lactations. However, increased FFA levels in connection with the decreased plasma glucose level were observed only in the early lactation.

Relations between the energy status of the cows and their plasma metabolite levels were shown in Fig. 4. Plasma FFA levels increased in the lower energy balance, however energy status caused a little influence on glucose or no clear influence on acetate or 3-HB levels. The molar FFA/3-HB ratio was lower in the conditions of higher energy status, and the much cases of higher FFA/3-HB ratio were observed in negative energy balance, especially in the early lactation (Fig. 5).

DISCUSSION

Large amount of 3-HB is produced not only by hepatic ketogenesis from FFA which mobilized from the adipose tissue, but also by butyrate metabolism in the rumen wall (alimentary ketogenesis) accompanying with volatile fatty acids (VFA) absorption [2, 4]. A case of plasma 3-HB level of more than 1.2 mM (12.5 mg/d*l*) have been considered to be subclinical ketosis [3]. There was a report that 16.4% of healthy dairy cows showed the level of 3-HB more than 1.2 mM in some area of Canada [3]. In the present experiment, 6% of the samples (16/270) showed the level of 3-HB more than 1.2 mM. The proportional elevation of 3-HB and FFA levels (line H in Fig. 1) reflects the accelerated hepatic ketogenesis from the FFA supplied by lipid mobilization from the adipose tissue. On the other, the higher 3-HB concentrations with lower FFA level, as indicated by line A of Fig. 1, might be brought about by an alimentary ketogenesis from the butyrate produced in the rumen, not from the FFA by lipid mobilization.

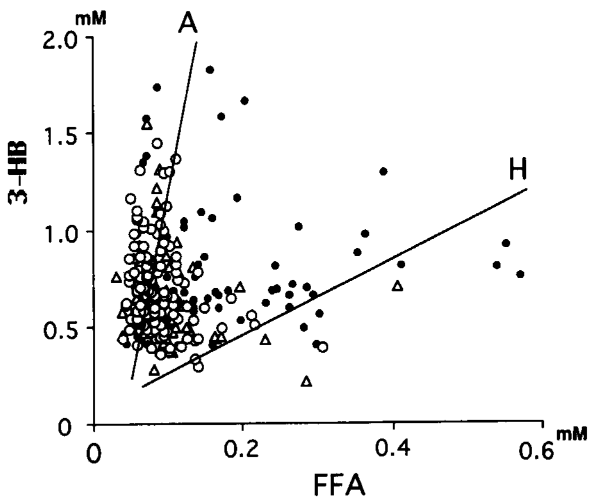

Fig. 1. Relations between plasma FFA and 3-HB levels. Line A---Elevation of 3-HB without FFA increase (mainly by alimentary ketogenesis). Line H---Elevation of 3-HB in connection with FFA (mainly by hepatic ketogenesis). ● Early lactation, △ Mid lactation, ○ Late lactation.

Ruminants have two sources of blood acetate, *i.e.*, the gut (exogenous), and tissue metabolism mainly in the liver (endogenous) [1]. However, much acetate is produced by rumen fermentation in fed status (not starved condition), and the acetate is supplied to the peripheral tissue *via* systemic circulation [4]. It is reported that blood acetate level has been increasing with higher feed intake, especially dietary fiber intake [7]. The trend of higher FFA level with declining acetate (Fig. 2) or declining glucose level (Fig. 3) suggest that the lipid mobilization from the adipose tissue might be more activated under the lower feed intake or

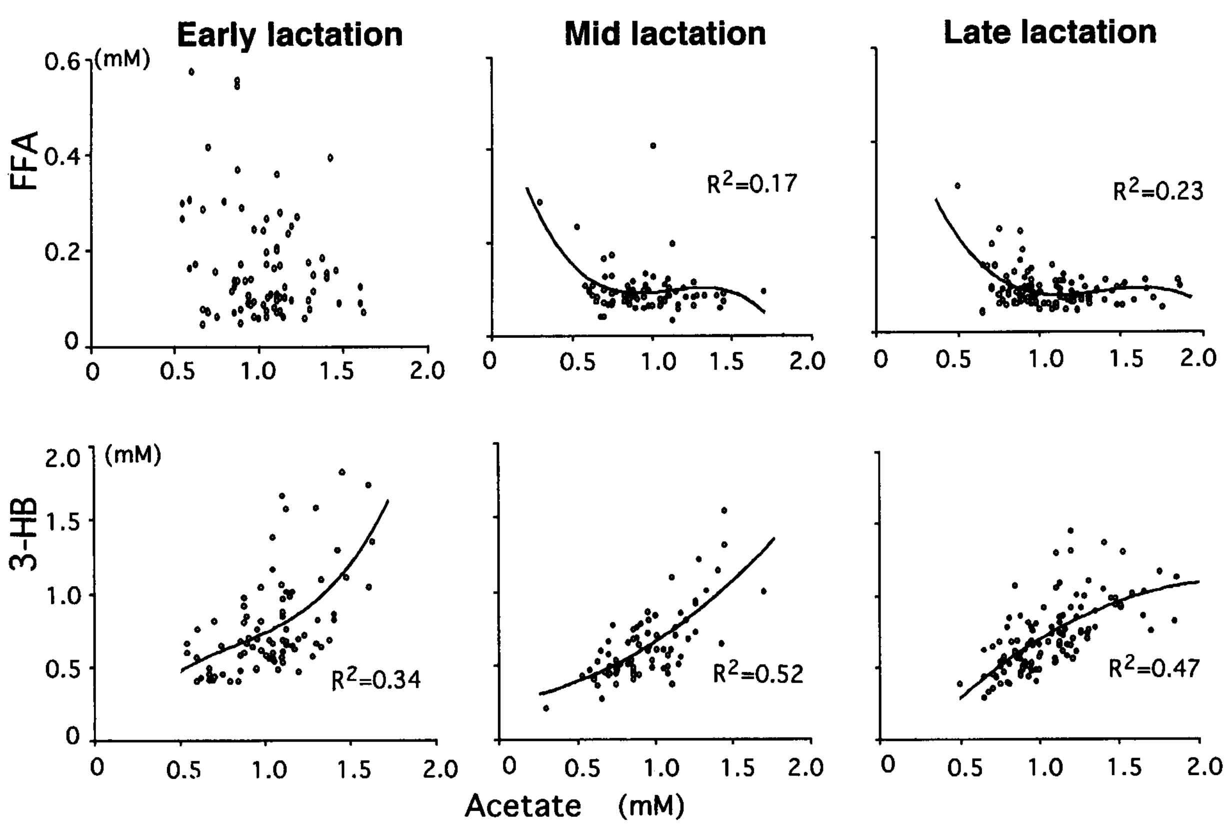

Fig. 2. Relations between plasma acetate and 3-HB or FFA levels. Regression line was given on the case of R^2>0.10.

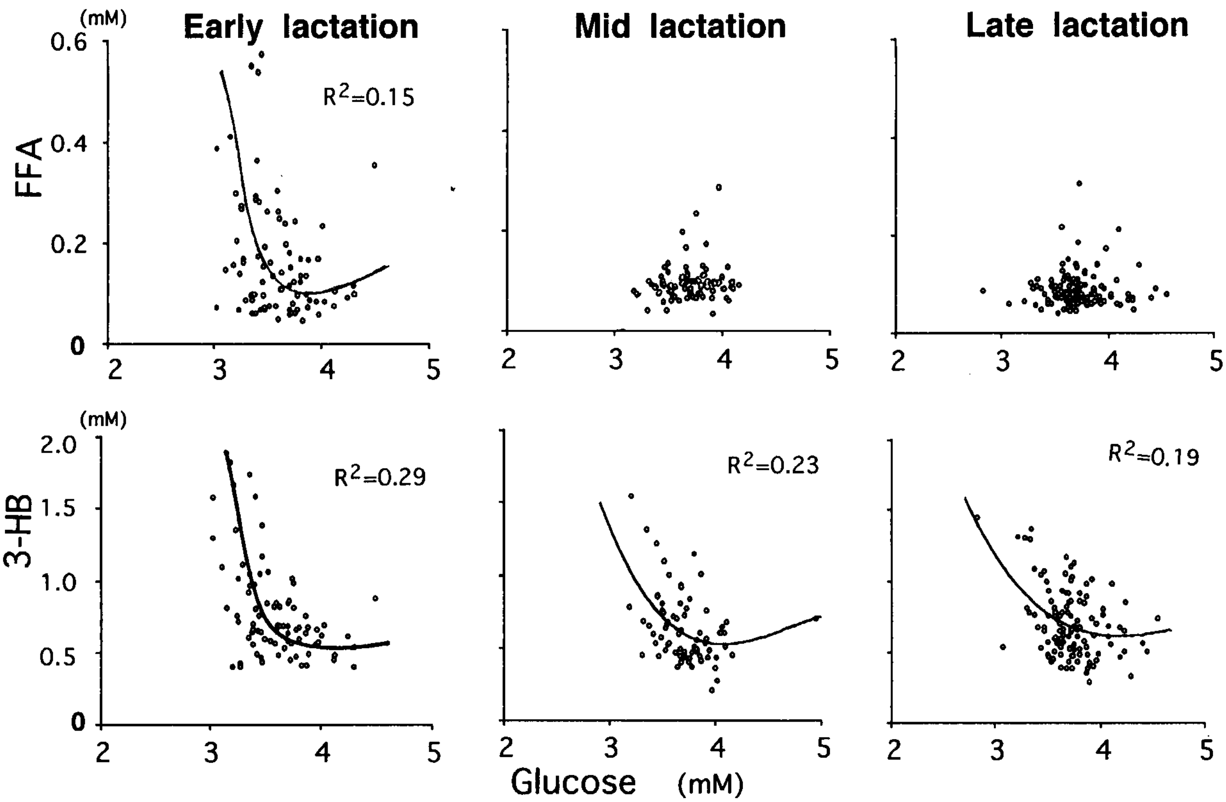

Fig. 3. Relations between plasma glucose and 3-HB or FFA levels. Regression line was given on the case of R^2>0.10.

hypoglycemic status, especially in the early lactation.

Positive correlation between acetate and 3-HB level (Fig. 2) indicates an accelerated alimentary ketogenesis and an accompanying acetate production by the active ruminal fermentation in vigorous appetite. However, the higher 3-HB level compared to acetate in the early lactation (Fig. 2) implies a facilitated hepatic ketogenesis in that period even not in patients. If sick cows were used for the experiment, a clear elevation of 3-HB accompanying with lower acetate levels might be observed owing to an accelerated hepatic ketogenesis by a diminished feed intake.

Depending on the data of Figs. 2–4, plasma FFA levels seemed to be largely influenced by energy status of the animals, rather than plasma acetate or glucose levels. Lower correlations between plasma FFA and acetate or glucose levels might be owing to weak influence of an energy status on acetate or glucose levels, especially in mid and late lactation (Figs. 2–3). Plasma 3-HB level have shown to elevate in the conditions of lower appetite (by endogenously) or higher feed intake (by exogenously) as discussed above [1, 10]. Therefore, no correlation between the 3-HB level and the energy status (feed intake) in the present experiment seems to be reasonable [4].

In the present study, the molar ratio of FFA/3-HB was chosen to evaluate the physiological significance of FFA as a precursor for ketogenesis. It is considered that the elevated FFA/3-HB ratio might be a reflection of accelerated ketogenesis from mobilized FFA; *i.e.*, an increased significance of FFA as a ketogenic precursors. On the other hand, the lower FFA/3-HB ratio in the positive energy status seems to be an expression of increasing ketogenesis from butyrate (produced in the rumen).

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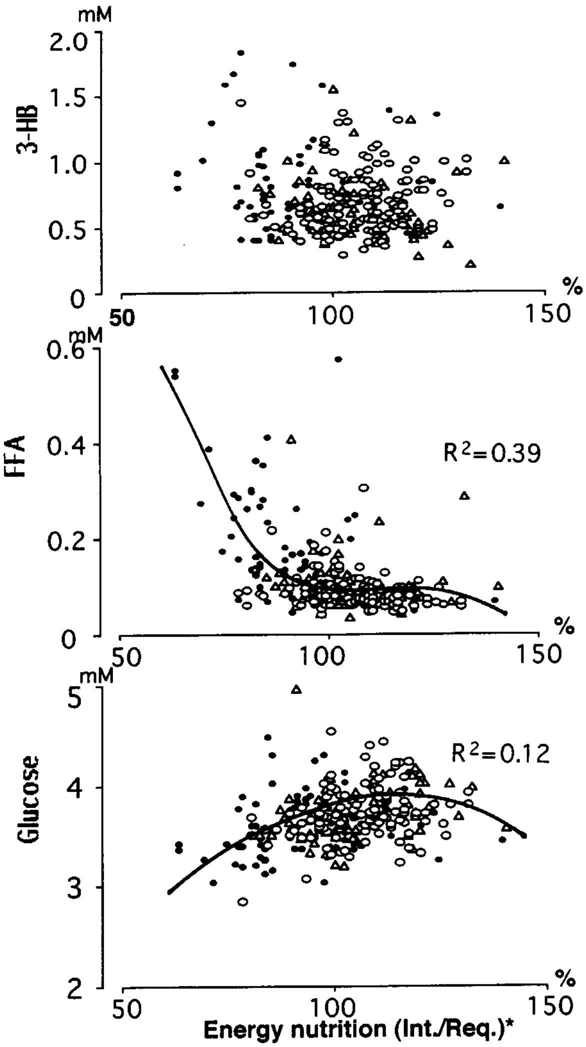

Fig. 4. Relations between energy nutrition and plasma metabolite levels. * Dietary intake/requirement (TDN). Regression line was given on the case of $R^2>0.10$. ● Early lactation, △ Mid lactation, ○ Late lactation.

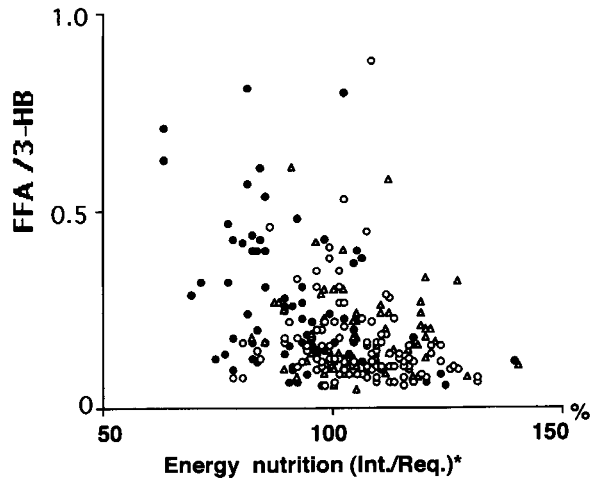

Fig. 5. Relations between energy nutrition and FFA/3-HB ratio. * Dietary intake/requirement (TDN), ● Early lactation, △ Mid lactation, ○ Late lactation.

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