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Diurnal variations in polyunsaturated fatty acid contents and expression of genes involved in their de novo synthesis in pigs

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ABSTRACT

The daily variations in circulating fatty acid (FA) contents and lipid metabolism have been well documented. However, whether long chain polyunsaturated FA (PUFA) contents and expression of genes involved in their de novo synthesis exhibit daily rhythms are yet unknown. We conducted the present study to investigate the daily variations in PUFA contents in plasma and liver of pigs. Moreover, diurnal expression of genes encode fatty acid desaturases and elongases, which are key enzymes catalyzed de novo synthesis of long chain PUFA, were also explored. The results showed that long chain PUFA contents in plasma and liver both exhibited diurnal rhythms. Diurnal variations were also observed in mRNA expression of FASD1 (Delta 5-desaturase), FASD2 (Delta 6-desaturase), ELOVL5 (fatty acid elongase 5) and ELOVL2 in liver, with an unexpectedly high level at night. Moreover, our results showed a similarity between the diurnal patterns of FASD1, FASD2, ELOVL2, ELOVL5 and Period 2. These results indicated a high activity of the desaturase-elongase pathway at night in pigs. These findings have important physiological and pathophysiological implications, since long chain PUFA are essential for cell function and closely involved in the development of metabolic syndrome.

1. Introduction

Most circulating nutrient levels have been reported to fluctuate over the course of the day and a large number of metabolic pathways have been demonstrated to be regulated by the circadian clock, directly and/or indirectly [1]. These rhythms in metabolic processes are not only in associated with daily behavioral influences, but also are mediated by cell autonomous circadian clocks. It is well documented that lipid metabolism exhibits daily rhythm. Most genes such as apolipoprotein B (apoB) and intestinal fatty acid binding protein (FABP) involved in lipid uptake and transportation in the intestine display time-of-day dependent rhythms and these rhythms are mediated by functioning of the circadian clock [2,3]. Also, lipid turnover and fatty acid β-oxidation are diurnally regulated [1]. Moreover, plasma lipids including circulating free fatty acids (FFA) are proved to maintain within a narrow physiologic range and display daily variations in both human [4] and rodents [5,6]. Evidence suggested that these rhythms are not simply secondary to sleep/wake and feeding/fasting cycles [7–9].

However, no reports yet have shown the daily rhythms of long chain polyunsaturated fatty acids (PUFA) in plasma and liver and

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expression rhythms of genes involved in de novo synthesis of PUFA in liver. The n-3 and n-6 long chain PUFA are essential to healthy mammalian cell function [10] and the ratio of n-3 to n-6 PUFA is a key factor involved in the development of metabolic syndromes such as obesity, insulin resistance and cardiovascular diseases [11]. Thus understanding the diurnal variations of PUFA would be helpful in devising methods to prevent these diseases. The purpose of the present study was to see whether PUFA contents in plasma and liver display diurnal variations. Furthermore, the diurnal pattern of expression of genes related to de novo synthesis of long chain PUFA were also investigated.

2. Materials and methods

2.1. Animals and experimental design

Piglets (Duroc × Landrace × Large Yorkshire) weaned at 28 d were selected for the experiment. All piglets at the age of 35 d were fed a corn-soybean based diet (Supplementary Table 1) at 8:00 a.m., 12:00 a.m. and 5: 00 p.m. every day for 3 weeks. Pigs were fed ad libitum for 30 min every time and the residual were collected and weighed. During the experiment, piglets were housed individually under a 12:12-h light dark cycle (lights on at 7:00 and lights off at 19: 00) and given free access to water. Sixty pigs with an average body weight (BW) of 16.5 ± 0.23 kg were selected at the age of 56 days for sample collection at 11: 00 a.m. (Clo11), 15: 00 p.m. (Clo15), 19: 00 p.m. (Clo19), 23: 00 p.m. (Clo23), 3: 00 a.m. (Clo3) and 7: 00 a.m. (Clo7). At each time point, ten pigs were chosen for blood collection by jugular puncture. Then four pigs were anaesthetized with Zoletil (15 mg tiletamine/kg BW, 15 mg zolazepam/kg BW, i.m.) and bled by exsanguination, and then liver samples were collected. Blood and liver samples were collected within 30 min at each time point.

The experimental protocol was approved by the Protocol Management and Review Committee of the institute of Subtropical Agriculture, Chinese Academy of Science and pigs were cared for and slaughtered according to the guidelines of the institute of Subtropical Agriculture on Animal Care (Changsha, China).

2.2. Plasma total free fatty acid and glucose analysis

Plasma samples were thawed at 4 °C, and then glucose and total FFA content were analyzed by commercial kits (Beijing Strong Biotechnologies, Inc. Beijing, China).

2.3. Fatty acid contents analysis

FA contents was analyzed according to previously reported methods (Demirel et al., 2004) except that 19: 0 methyl ester were added as internal standard. FAs were identified through comparisons to the retention time of standard esters, and FA content were calculated according to peak area. FA methyl ester standard mixtures (No. 47885-u) were purchased from Sigma-Aldrich.

2.4. Gene expression analysis

Total RNA was isolated from liquid nitrogen-frozen and ground liver samples with the TRIZOL reagents (Invitrogen, Carlsbad, CA, USA). Primers were designed with Primer 5.0 and presented in Supplementary Table 2. Real time-PCR was performed and results were calculated as previously described [12].

2.5. Statistical analysis

Data were analyzed by the one-way analysis of variance and a mixed procedure for repeated measures (PROC MIXED RM) using SAS software version 9.2 (SAS Institute Inc., Cary, NC), considering time point as fixed effect and animal as randomized effect. And data were presented as Least Squares Means ± SEM. Mean values were considered to be significantly different when $P < 0.05$.

3. Results and discussion

3.1. Diurnal variations in FFA, glucose and PUFA contents in plasma of pigs

Total FFA content reached the peak at Clo3 and Clo7 while reached the nadir at Clo19 (Fig. 1A). In general, total FFA content in plasma were lower during daytime (Clo11, Clo15 and Clo19) than the night (Clo23, Clo3 and Clo7). These results were in agreement with previous experiments in human [4] and might be due to the inhibition of lipolysis by high glucose (Fig. 1B) and insulin levels during daytime [8]. Furthermore, the diurnal variation in plasma FFA content in pigs and humans were opposite to that in rodents which exhibit a nocturnal feeding behavior [6].

The content of arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3)) in plasma showed diurnal changes and they were lower during daytime than the night (Fig. 1E, F, G, H). These daily variations were similar to the diurnal pattern of total FFA. However, we did not see any significant diurnal variations in linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 3n-3) contents (Fig. 1C and D). These results indicated that circulating LA and ALA from daily diets might be rapidly used by peripheral organs.

3.2. Diurnal variations in PUFA and triglycerides concentration in liver of pigs

The content of PUFA and triglycerides (TG) in liver during 24 h were presented in Fig. 2. Hepatic LA and ALA were higher during daytime while they were lower at night (Fig. 2B and C). Since LA and ALA are essential FAs, these results further confirmed our speculation that circulating LA and ALA absorbed from diets immediately entered into liver during daytime. ARA, EPA and DHA were slightly higher during the night than daytime (Fig. 2D, E, F). Total TG content in liver did not show daily rhythm (Fig. 2A), which suggested that hepatic lipid metabolism might be inclined to keep lipid content unchanged during 24 h. However, other studies on adult rodents showed an oscillation of hepatic TG [13,14]. These difference may be caused by that we used the young growing pigs.

3.3. Diurnal variations in hepatic expression of FA metabolism related genes and comparison to the patterns of circadian clock genes in pigs

Previous studies on rodents have reported the diurnal expression of certain genes involved in lipid metabolism. However, discrepancies were observed in these results. Filiano et al. showed that carnitine palmitoyltransferase 1a (CPT1a) mRNA expression was higher during daytime [15] while CPT1a was lower during daytime according to the results of Alenghat et al. [16]. Furthermore, Alenghat et al. showed that expression of fatty acid elongase 6 (Elovl 6) and fatty acid synthesis genes such as acetyl-CoA carboxylase 2 (ACC2) reached the peak in the daytime [16], while Le Martelot et al. showed that expression of Elovl 6 and fatty acid synthase (FAS) were higher during the night than daytime [14]. Our results showed that mRNA expression of genes (ACC2, FAS and stearoyl-CoA desaturase (SCD)) involved in de novo lipogenesis were higher during the night than daytime (Fig. 3C, D, E). It is noteworthy that mRNA expression of CPT1a also reached the peak at Clo3 and Clo7

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