Metabolism of Exogenous Palmitic and Oleic Acids by Preimplantation Mouse Embryos

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Abstract: *In the present study, the oxidation of palmitic acid to carbon dioxide and the difference of incorporation rate of the same and oleic acid into embryo lipids were examined. To test the exact role of fatty acids for energy production, the oxidative rates of exogenous palmitic were assessed in M16 medium with and without carbohydrate substrates. The oxidation of palmitic acid in the medium with carbohydrates was lower than that without carbohydrates in the blastocyst stage. Incorporation rates of both palmitic and oleic acids into embryo lipids increased significantly from the 2-cell to the blastocyst stage ($P<0.05$), whereas the incorporation rates of palmitic acid were significantly higher than those of oleic acid at most of the cell stages except for the blastocyst stage ($P<0.05$). In the neutral lipid fraction, ^{3}H-palmitic acid was predominately distributed in triacylglycerol species and a little in the other glycerides ($P<0.05$). However, higher percentages of ^{14}C-oleic acid were recovered in fatty alcohols, diacylglycerols and monoacylglycerols. In the polar lipid fraction, the percentages of ^{3}H-palmitic acid in choline and ethanolamine phosphatides were significantly higher than those of oleic acid among all cell stages except the 2-cell stage ($P<0.05$), whereas the percentages of ^{14}C-oleic acid in inositol and serine phosphatides were significantly higher than those of ^{3}H-palmitic acid during preimplantation development ($P<0.05$). According to the comparison of incorporations into the embryo lipids and distributions in individual lipid classes between palmitic and oleic acids, it could be inferred that exogenous oleic acid is not a main energy substrate but a major intermediate for synthesis of various embryo lipids.*

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Several early investigators indicated that supplementation of some fatty acids had certain effects on the development of mouse and other mammalian embryos culturing *in vitro* [2, 6, 12]. Nanogaki *et al.* [11] reported that individual polyunsaturated fatty acids have some inhibitory effects on the development of early stage mouse embryos because of lipid peroxidation. However, Khandoker *et al.* [9] demonstrated that the mixed exogenous fatty acids bound to BSA played an important function in supporting and promoting the development of rat embryos *in vitro*. Flynn and Hillman [5] first revealed the characteristics of incorporation of ^{3}H-palmitic acid into the embryo lipids and its distribution in embryo lipids of 8-cell stage embryos. Khandoker *et al.* [7, 8] also characterized the metabolism of exogenous palmitic acid by preimplantation rat and rabbit embryos. Our previous work [17] has characterized the compositions of fatty acids in mouse oocytes and embryos, and found that palmitic and oleic acids were the main constituents in preimplantation mouse embryos of each cell stage. As yet no attempts have been made to characterize and compare the differences of incorporation of individual fatty acids into the embryo lipids and the metabolic abilities of the incorporated fatty acids in preimplantation mouse embryos developed *in vitro* at different cell stages.

In the present study, the oxidation of palmitic acid to carbon dioxide and the different rates of incorporation of the same and oleic acid into embryo lipids were examined. To test the exact role of fatty acids for energy production, the oxidative rates of exogenous palmitic

were assessed in M16 medium with and without carbohydrate substrates. With reference to the fatty acid composition of lipids in oviductal and uterine fluids of the mouse [17], palmitic and oleic acids were selected for incorporation in this experiment.

Materials and Methods

Animals and embryo collection

Six to eight week female mice of Slc:ICR strain (SLC Co. Japan) were used in the experiments. They were kept under controlled environmental conditions at room temperature with a cycle of 12 h light (light on from 6:00 a.m. to 6:00 p.m.) and 12 h dark. The females were superovulated by an i. p. injection of 5 IU pregnant mare serum gonadotrophin (PMSG) in physiological saline, then 48 h later by an 5 IU i. p. injection of human chorionic gonadotrophin (hCG). After hCG injection, the females were housed overnight with males of the same strain and checked for copulation plugs the following morning. Embryos were collected only from those females with copulation plugs at 24, 40, 65, 77 and 90 h after hCG injection. The oviduct and uterus were collected for flushing embryos of the one-cell, two-cell, eight-cell, compact morula and early blastocyst stages, respectively. The embryos collected were washed three times in M16 medium without BSA or carbohydrates and used for the following *in vitro* cultures.

Preparation of the labeled media

1) ^{14}C-palmitic acid medium without or with carbohydrates: ^{14}C-palmitic acid media were prepared according to the method of Khandoker *et al.* [7, 8]. Each 4 ml of M16 medium with carbohydrates (Glucose, sodium lactate and sodium pyruvate) or without carbohydrates contained 2.7 MBq [1-^{14}C] palmitic acid (specific activity: 1.85 TBq/mM, Moravek Biochemicas, Inc.), 0.1 mM non-radioactive palmitic acid and 5 mg/ml fatty acid-free BSA (FAF-BSA, Sigma Chemical Co. Ltd. St. Louis, USA). 2) ^{3}H-palmitic acid and ^{14}C-oleic acid media: ^{3}H-palmitic acid and ^{14}C-palmitic acid media were prepared essentially as described by Flynn and Hillman [5]. Either 12 MBq [9, 10 ^{3}H-] palmitic acid (specific activity: 1.85 TBq/mM; Moravek Biochemicals, Inc.) or 5.2 MBq [1-^{14}C] oleic acid (Specific activity: 2.04 TBq; American Radiolabeled Chemicals Inc.) was added to each 4 ml of M16 culture medium containing 0.1 mM non-radioactive palmitic acid or oleic acid and 5 mg/ml FAF-BSA, respectively. The prepared media were stored at 4°C before use.

Oxidation of ^{14}C-palmitic acid in mouse embryos

The determinations of exogenous palmitic acid oxidation were carried out according to the procedure described by Brinster [3] and Flynn *et al.* [4]. Twenty blastocysts were transferred into 150 μl of ^{14}C-palmitic acid medium without or with carbohydrates. The sample tube together with hyamine was put into a scintillation vial. The vial was sealed with a rubber stopper, and then cultured at 37°C for 4 h. The blank values of non-oxidation were obtained from control incubation without embryos.

Incorporation of exogenous fatty acids into embryo lipids

After the microdrops (100 μl each) ware overlaid with silicone oil of ^{3}H-palmitic acid or ^{14}C-oleic acid medium, an 1 h pre-incubation (37°C, 5% CO_2, 95% air) was carried out. Twenty embryos each of 1-cell, 2-cell, 8-cell, morula and blastocyst stages were transferred into each pre-incubated microdrop of the medium, and incubation was continued *in vitro* for 3 h. The labeled embryos were then washed 5 times by piping in non-radioactive M16 medium without BSA, pooled and stored at –20°C until measurement of radioactivity.

Analysis of incorporation of fatty acids into embryo lipids

Each sample of about 150~200 labeled embryos was extracted with 14.5 ml mixtures of chloroform, methanol and water (2:2:1.8, by vol.) for 3 h at room temperature according to the procedure of Bligh & Dyer [1]. The aqueous layer was removed from the chloroform layer. The radioactivity of both the chloroform and aqueous layers was determined. The chloroform layer was evaporated to dryness in vacuum, and then resolved in 100~250 μl hexane. Aliquots of the extract were used for distributive analysis of fatty acids as described below.

Analysis of distribution of exogenous fatty acids

Analysis of distribution of ^{3}H-palmitic or ^{14}C-oleic acid in the neutral and polar lipids was carried out by thin-layer chromatography on aluminum sheet silica gel 60 thin plates (Merck, Darmstadt, Germany) of 2.5 × 8.0 cm size according to the method described by Khandoker [7]. For fractionation of the neutral lipid, the lipid extracts were developed in a mixture of hexane-diethyl, ether-glacial and acetic acid (80:20:1, by vol.) at room temperature. Development of the polar lipid fraction was performed in a mixture of methyl acetate,-propan-1-ol,-chloroform,-methanol-0.25% and aqueous KCl (25:25:25:10:9, by vol.) [16]. Appropriate lipid standards

(Sigma Chemicals, USA) were added to the lipid extract as carriers for location of individual lipid species.

Measurement of radioactivity

Each radioactive sample was placed in a scintillation vial, then a 5 ml scintillation cocktail containing 0.5% 2, 5-diphenyloxazole and 0.03% 2, 2-p-phenylene-bis (5-phenyloxazole) in toluene was added. The radioactivity of samples was determined by a liquid scintillation counter (LS-6500, Beckman Instruments, Inc., USA).

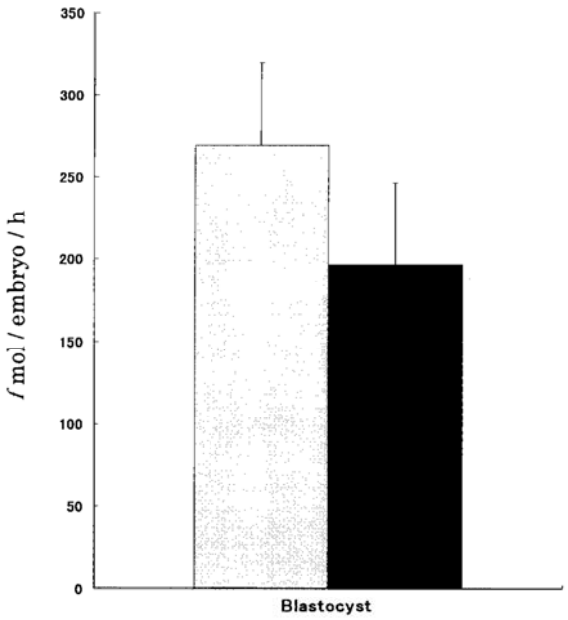

Fig. 1. The comparison of oxidation rates of exogenous ^{14}C-palmitic acid between the labeled M16 media without or with carbohydrates in mouse blastocysts. ▒ Without carbohydrates, ■ With carbohydrates.

Statistical analysis

The data obtained were statistically analyzed by Student's t-test and expressed as mean ±SEM.

Results

The oxidative rates of ^{14}C-palmitic acid by preimplantation mouse embryos are shown in Fig. 1. The oxidative rates of ^{14}C-palmitic acid in culture medium without carbohydrates were significantly higher than those in the medium with carbohydrates in the blastocyst stage ($P<0.05$).

The data in Table 1 show that ^{3}H-palmitic and ^{14}C-oleic acids were steadily incorporated into the embryo lipids and increased significantly with the preimplantation development ($P<0.05$). The incorporation rates (*f* mol/embryo/h) of ^{3}H-palmitic acid or ^{14}C-oleic acid into embryo lipids were shown to be significantly different from each other at the corresponding cell stages ($P<0.05$). The incorporation rates of ^{3}H-palmitic acid into the embryo lipids including the neutral and polar lipid fractions increased significantly from the 2-cell to the blastocyst stage, and those of ^{14}C-oleic acid increased significantly from the 8-cell to the blastocyst stage ($P<0.05$). The incorporation rates of ^{3}H-palmitic acid into the embryo lipids were significantly higher than those of ^{14}C-oleic acid at most corresponding cell stages except the blastocyst stage ($P<0.05$).

Distributions of ^{3}H-palmitic and ^{14}C-oleic acids in the neutral lipid fraction are shown in Table 2. The patterns of distribution between ^{3}H-palmitic and ^{14}C-oleic acids show significant differences from each other at most corresponding cell stages ($P<0.05$). The highest percentages of ^{3}H-palmitic acid were mainly found in triacylglycerols (TG) with lesser percentages in monoalkyldiacylglycerols (MADG), diacylglycerols (DG) and monoacylglycerols (MG) through the preimplanta-

Table 1. The incorporate rates of ^{3}H-palmitic and ^{14}C-oleic acids into embryo lipids from 1-cell to blastocyst stage in the single-labeling culture *in vitro* for 3 h

Cell stage	*f*mol / embryo / h					
	In total lipids		In neutral lipids		In polar lipids	
	^{3}H-PA	^{14}C-OA	^{3}H-PA	^{14}C-OA	^{3}H-PA	^{14}C-OA
One-cell	183.5 ± 11.2^{a}	88.0 ± 9.4^{e}	145.0 ± 15.3^{a}	72.0 ± 6.4^{d}	38.5 ± 2.9^{a}	16.0 ± 1.2^{d}
Two-cell	238.4 ± 20.5^{b}	104.4 ± 12.1^{ef}	191.4 ± 11.8^{b}	86.3 ± 13.5^{d}	47.0 ± 3.2^{b}	18.1 ± 2.3^{d}
Eight-cell	261.9 ± 17.7^{bc}	184.3 ± 13.7^{g}	218.4 ± 13.2^{bc}	136.4 ± 10.6^{e}	43.5 ± 6.2^{b}	47.9 ± 3.0^{e}
Morula	286.6 ± 10.3^{bc}	240.5 ± 8.2^{h}	220.6 ± 10.9^{bc}	188.3 ± 12.6^{f}	66.0 ± 5.8^{c}	52.2 ± 2.4^{e}
Blastocyst	294.9 ± 21.5^{cd}	408.3 ± 9.8^{i}	244.0 ± 9.8^{bc}	328.3 ± 14.8^{g}	50.9 ± 4.3^{b}	80.0 ± 2.6^{f}

a~i: Different superscript values within the same column differ significantly from each other ($P<0.05\%$). All data were expressed as mean ± SEM of six determinations made in three independent experiments.

Table 2. Distributions of ^{3}H-palmitic acids and ^{14}C-oleic acids in the neutral lipid fraction in the single-labeling culture *in vitro* from 1-cell to blastocyst stage

Lipid class	% of the labeled neutral lipid species in the total neutral lipid fraction									
	One-cell		Two-cell		Eight-cell		Morula		Blastocyst	
	^{3}H-PA	^{14}C-OA	^{3}H-PA	^{14}C-OA	^{3}H-PA	^{14}C-OA	^{3}H-PA	^{14}C-OA	^{3}H-PA	^{14}C-OA
Triacylglycerol	64.4 ± 6.6^{a}	38.5 ± 3.5^{b}	70.5 ± 5.3^{a}	42.8 ± 5.3^{b}	67.1 ± 8.6^{a}	31.6 ± 3.5^{bc}	66.8 ± 5.7^{a}	36.1 ± 4.8^{b}	78.3 ± 6.4^{ad}	31.9 ± 4.2^{bc}
Diacylglycerol	7.2 ± 1.7^{a}	11.0 ± 1.3^{b}	6.9 ± 1.5^{a}	8.5 ± 1.2^{a}	0.2 ± 0.1^{c}	15.1 ± 2.0^{d}	1.8 ± 0.3^{e}	11.4 ± 2.5^{b}	2.4 ± 1.6^{e}	15.7 ± 0.8^{f}
M-acylglycerol	2.3 ± 1.2^{a}	21.2 ± 3.5^{b}	6.6 ± 2.3^{c}	5.0 ± 1.8^{c}	5.8 ± 1.4^{c}	12.8 ± 3.6^{d}	9.6 ± 2.0^{cd}	17.4 ± 3.0^{e}	4.0 ± 1.8^{c}	18.2 ± 2.7^{e}
MA-diglycerol	10.0 ± 2.7^{a}	4.0 ± 0.8^{b}	8.4 ± 1.7^{a}	13.7 ± 2.4^{ac}	15.9 ± 3.1^{ac}	7.3 ± 1.7^{a}	9.3 ± 2.8^{a}	9.1 ± 2.6^{a}	1.2 ± 0.9^{d}	1.6 ± 0.9^{d}
Fatty alcohol	4.9 ± 1.0^{a}	16.6 ± 3.0^{b}	2.2 ± 1.5^{c}	20.0 ± 3.2^{bd}	3.4 ± 1.1^{ae}	19.4 ± 2.4^{b}	2.8 ± 1.2^{ae}	17.3 ± 1.9^{b}	5.0 ± 1.2^{a}	18.5 ± 3.4^{b}
Fatty acid	4.6 ± 1.3^{a}	6.2 ± 1.5^{a}	3.9 ± 1.6^{ab}	9.5 ± 2.3^{ac}	6.7 ± 2.2^{a}	10.1 ± 3.4^{ac}	8.5 ± 2.3^{ac}	6.6 ± 1.3^{a}	8.6 ± 2.9^{ac}	11.5 ± 2.9^{ac}
Sterol ester	6.6 ± 1.8^{a}	2.5 ± 0.5^{b}	1.5 ± 1.0^{bc}	0.5 ± 0.1^{cd}	0.9 ± 0.5^{cd}	3.7 ± 0.8^{be}	1.2 ± 0.5^{bc}	2.1 ± 0.6^{bc}	0.5 ± 0.4^{cd}	2.6 ± 0.7^{bc}

a~f: Different superscript values within the same row differ significantly from each other ($P<0.05$). ^{3}H-PA: ^{3}H-palmitic acid; ^{14}C-OA: ^{14}C-oleic acid; M-acylglycerol: monoacylglycerol; MA-diglycerol: Monoakyldiglycerol. All data are expressed as mean percentage ± SEM.

Table 3. Distributions of ^{3}H-palmitic and ^{14}C-oleic acids in the polar lipid fraction in the single-labeling culture *in vitro* from 1-cell to blastocyst stage

Lipid class	% of each labeled polar lipid species in the total polar lipid fraction									
	One-cell		Two-cell		Eight-cell		Morula		Blastocyst	
	^{3}H-PA	^{14}C-OA	^{3}H-PA	^{14}C-OA	^{3}H-PA	^{14}C-OA	^{3}H-PA	^{14}C-OA	^{3}H-PA	^{14}C-OA
Phospholipids										
P-choline	40.9 ± 2.0^{a}	21.7 ± 3.4^{b}	40.1 ± 4.2^{a}	32.4 ± 2.8^{ac}	42.9 ± 4.2^{a}	16.6 ± 2.9^{d}	45.7 ± 3.6^{a}	26.6 ± 2.3^{bc}	35.0 ± 7.3^{a}	22.6 ± 2.1^{b}
P-ethanolamine	30.6 ± 5.4^{a}	1.7 ± 0.8^{b}	29.7 ± 2.2^{a}	24.2 ± 3.5^{c}	23.1 ± 2.4^{c}	4.5 ± 1.2^{d}	19.9 ± 1.9^{ce}	2.8 ± 0.7^{b}	22.0 ± 5.8^{c}	4.8 ± 1.0^{d}
P-serine/Inositol	4.3 ± 1.0^{a}	28.3 ± 2.5^{b}	9.7 ± 3.1^{c}	23.8 ± 3.8^{bd}	6.6 ± 1.4^{a}	13.5 ± 2.9^{ce}	2.5 ± 1.4^{af}	25.7 ± 3.2^{b}	10.3 ± 2.1^{c}	23.1 ± 0.9^{bd}
Lp-choline	1.4 ± 0.5^{a}	UD^{b}	7.0 ± 1.4^{c}	6.1 ± 1.2^{c}	8.3 ± 1.8^{c}	18.1 ± 3.2^{d}	11.9 ± 2.5^{e}	6.4 ± 1.3^{c}	10.9 ± 2.0^{cf}	UD^{b}
Sphingomyecin	UD	13.3 ± 2.0^{a}	UD	3.4 ± 0.3^{b}	UD	2.1 ± 0.3^{b}	UD	4.6 ± 1.3^{bc}	UD	7.1 ± 2.2^{cd}
Glycolipids										
Mono-GG	UD	8.3 ± 1.2^{a}	UD	UD^{b}	UD	16.6 ± 2.1^{c}	UD	10.1 ± 1.7^{a}	UD	20.2 ± 3.5^{c}
Sulfatide	10.0 ± 2.3^{a}	11.7 ± 2.6^{a}	9.5 ± 2.6^{a}	UD^{b}	9.8 ± 1.9^{a}	28.6 ± 3.7^{c}	16.2 ± 3.6^{d}	2.8 ± 0.5^{e}	15.9 ± 3.9^{d}	UD^{b}
Cerebroside	12.8 ± 1.8^{a}	15.0 ± 2.3^{a}	4.0 ± 1.6^{b}	8.1 ± 1.6^{bc}	9.3 ± 2.1^{bc}	UD^{d}	3.8 ± 1.3^{b}	21.0 ± 3.4^{e}	5.9 ± 1.0^{b}	22.2 ± 2.5^{e}

a~f: Different superscript values within the same row differ significantly from each other. ($P<0.05$) All data are expressed as mean percentage ± SEM. UD: Undetectable.

tion developmental stages. In addition, only a little radioactivity from ^{3}H-palmitic acid was distributed in fatty alcohol and sterol ester classes during preimplantation development. However, the distribution of ^{14}C-oleic acid in TG class was significantly less than that of ^{3}H-palmitic acids at all cell stages ($P<0.05$). The percentages of MG and DG species labeled by ^{14}C-oleic acid were significantly higher than those labeled by ^{3}H-palmitic acid at the 1-cell, 8-cell, morula and blastocyst stages ($P<0.05$). In addition to this, higher percentages of fatty alcohols labeled by ^{14}C-oleic acids than those labeled by ^{3}H-palmitic acid were also found at all cell stages ($P<0.05$). The percentages of TG, fatty alcohol and fatty acid classes labeled by ^{3}H-palmitic acid or ^{14}C-oleic acid were relatively constant from the 1-cell to the blastocyst stage ($P>0.05$). But, those of the other neutral lipid species varied markedly at the different cell stages of preimplantation development.

The distributive characteristics of ^{3}H-palmitic and ^{14}C-oleic acids in the polar lipid fraction are shown in Table 3. In the labeled phospholipid species, the highest percentages of ^{3}H-palmitic acid existed in choline phosphatides (PC) and ethanolamine phosphatides (PE), while, lower percentages of ^{3}H-palmitic acid were found in serine phosphatides (PS)-inositol phosphatides (PI) and lysophosphatidylcholines at all cell stages, and the percentages of PC class labeled by ^{14}C-oleic acid were significantly lower than those labeled by ^{3}H-palmitic acid at each corresponding cell stage, except the 2-cell stage ($P<0.05$). In contrast, percentages of ^{14}C-oleic acids were higher than those of ^{3}H palmitic acid in the PS-PI class, but were significantly lower in the PE class at

each corresponding cell stage except the 2-cell stage ($P<0.05$). Furthermore, higher percentages of sulfatides and lower percentages of cerebrosides labeled by ^{3}H-palmitic acid than those labeled by ^{14}C-oleic acid were also found at the 2-cell, morula and blastocyst stages ($P<0.05$), whereas the percentages of sulfatides and cerebrosides labeled by both ^{14}C-oleic and ^{3}H-palmitic acids were almost identical to each other at the 1-cell stage ($P>0.05$).

Discussion

Mouse embryos can oxidize palmitic acid to carbon dioxide in culture medium with or without carbohydrate substrates, although carbon dioxide production is relatively higher without carbohydrate substrates than with them in blastocyst stages. It has been reported that carbohydrates, such as glucose, are mainly converted to the glycerol skeletons of TG rather than the fatty acids in the abundance of fatty acids [4]. The higher production of carbon dioxide without carbohydrate substrates indicated that the fatty acid was undoubtedly used for the energy production. A similar phenomenon was also observed in preimplantation rabbit embryos [8] suggesting that essential oxidation of both fatty acids and carbohydrates might be maintained simultaneously to supply metabolic energy by different biochemical pathways in preimplantation mouse embryos and other species.

The results in the present study revealed that preimplantation mouse embryos at different cell stages could utilize exogenous palmitic and oleic acids by incorporating them into various embryo lipid fractions, and demonstrated that the active metabolic pathway for lipid synthesis already functioned in preimplantation mouse embryos from the 1-cell to early blastocyst stage. Similar results were also found in preimplantation rat and rabbit embryos by Khandoker *et al.* [7, 8].

The incorporation rates of ^{3}H-palmitic acid into embryo lipids significantly differed from those of ^{14}C-oleic acid at the corresponding cell stages. The percentages of radioactivity in the embryo lipids including the neutral and the polar lipid fractions labeled by ^{3}H-palmitic acid were significantly higher than these labeled by ^{14}C-oleic acid through preimplantation development. In our previous work [17], we also found that the oleic acid content was always lower in mouse embryos than that of palmitic acid at the corresponding cell stages, but was higher in the mouse oviduct and uterine fluids. This finding might imply that the different incorporation rates of palmitic and oleic acid are consistent with the requirements of quantity for different fatty acids during preimplantation development of mouse embryos, and the preimplantation mouse embryos have the ability of selectively incorporating various fatty acids. Spector *et al.* [13] suggested that the utilization of different fatty acids by Ehrlich ascites tumor cells was based on their structural differences and concentrations in the culture medium.

About 64.4~78.3% of ^{3}H-palmitic acid incorporated into the neutral lipid fraction was used to synthesize TG species, and only a little was found in the other glycerides from the 1-cell to blastocyst stages. These results agree with Flynn and Hillmans' early finding [5]. Khandoker [7] also observed a similar result that about 72% of ^{3}H-palmitic acid incorporated into neutral lipid fractions was distributed in TG species in 8-cell rat embryos. Urade *et al.* [15] also found that about 70% of TG species in Chinese Hamster V79-R cells were those containing three saturated palmitic acids or two saturated and one unsaturated oleic acids while palmitic and oleic acids were simultaneously present in culture medium. In our present study, only about 31.9~42.8% of ^{14}C-oleic acid incorporated into the neutral lipid fraction was distributed in the TG class, while higher percentages of ^{14}C-oleic acid than those of ^{3}H-palmitic acid were widely found in fatty alcohol, DG and MG species. These significant differences may imply that the preimplantation mouse embryos could chemically recognize exogenous palmitic and oleic acids and convert them to different neutral lipids as intermediates of energy substrates and their cellular constituents according to the requirements for fatty acid substrates during preimplantation development. It may also suggest that palmitic acids are predominantly incorporated into the TG class for mainly serving as a major storage form of metabolic energy, but not oleic acid. The higher percentages of exogenous oleic acid distributed in the other glycerides such as MG and DG may be potential substrates for further phospholipid synthesis.

Some significant differences of ^{3}H-palmitic and ^{14}C-oleic acids distributed in individual polar lipid classes were also observed. The differences were mainly embodied in predominant distribution of exogenous palmitic acid in PC and PE species, with high percentages of exogenous oleic acid in PS-PI species. This may suggest that individual phospholipid synthesis in embryos culturing *in vitro* could be regulated effectively by addition of different fatty acids in medium. The results also showed that the ability of synthesis of different phospholipid species varied significantly dependant on the various cell stages of mouse embryos. The differences

between the two fatty acids distributed in various phospholipid species may have some important physiological values for regulating the fluidity of membrane lipid [14].

Other significant differences between palmitic and oleic acids distributed in various glycolipids were also found at most corresponding cell stages. Glycolipid species are an important component of antigens, especially surface antigens, of early embryos as well as somatic cells [18]. Nagai *et al.* [10] also demonstrated that glycolipids with different constituents of fatty acids took important roles in the early development of mammalian cells.

The results in the present study showed that the incorporations of palmitic and oleic acids into the embryo lipids and their distributions in various lipid fractions were significantly different from each other at most corresponding cell stages of preimplantation mouse embryos cultured *in vitro*. It might reflect that saturated fatty acids such as palmitic acid have some different physiological significance from unsaturated fatty acids such as oleic acid during development of preimplantation mouse embryos. Palmitic acid has been demonstrated as a main energy substrate of mouse embryos [5] and other mammalian cells [7, 15]. According to the comparison of incorporations into the embryo lipids and distributions in individual lipid classes between palmitic and oleic acids, it could be inferred that exogenous oleic acid is not a main energy substrate but a major intermediate for synthesis of various embryo lipids. These differences between incorporation and distribution of saturated and unsaturated fatty acids as mentioned above may contribute to effects on the development of preimplantation mouse embryos. Further study will be necessary to understand in what roles these fatty acids take during preimplantation development.

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