Research note

**Origin of Odd-numbered Fatty Acids
of Adipose Tissue Fats of Rats
Fed on Various Diets†**

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In our previous paper, we noticed that the accumulation of small amounts of odd-numbered fatty acids in rats fed a diet containing whole egg powder. To clarify whether odd-numbered acids in body fats of rats are derived from diet or not, rats were fed a diet containing soybean and tuna for 3 weeks and the relation between odd-numbered acid contents in diet and the accumulation of those into body fats were analyzed. Total contents of odd-numbered acids in tissue fat of rats fed on soybean and tuna diet were 0.78 and 0.33% of total acids, respectively. On comparing the amounts of odd-numbered acids intake during feeding period with gain of such acids during the period, it was evident that a large portion of the odd-numbered acid gain during feeding period was not caused by diets.

In a previous paper, the authors reported the accumulation of odd-numbered fatty acids in adipose tissue fats of rats fed on yeast grown on *n*-alkanes¹⁾. However, small amounts of odd-numbered fatty acids were also found in adipose tissue fats of rats fed on control diet containing whole egg powder.

So, it may be necessary to elucidate the contents of odd-numbered fatty acids in rats

fed on various diets to study a metabolism of odd-numbered fatty acid derived from feeding with *n*-alkane assimilating yeast.

Contents of odd-numbered acids in higher animals and plants, such as vegetable oils²⁾, fishes³⁻⁴⁾, chicken⁵⁾, and human tissue fats⁶⁾ were determined by several workers and they reported that contents of odd-numbered acids were 0-4% of total acids in these materials. Odd-numbered fatty acid in liver fat of rat was determined by J.J. CASSAL *et al.*⁷⁾, in relation to the effect of high carbohydrate diet. Recently, H. IKUMO and M. YOSHIDA reviewed⁸⁾ the distribution and metabolism of odd-numbered fatty acids in domestic animals and poultry. They pointed out that such acids were metabolized in the same manner as even-numbered acids. However, there is no report which discusses the relationship between odd-numbered acid contents in diet and those in body fats.

In this study, we determined the contents of odd-numbered fatty acids in adipose tissue fats of rats fed on various diets and analyzed the relation between odd-numbered acid contents in diet and that in the adipose tissue.

Materials and Methods

Animals: Young rats of Wistar strain weighing about 80 g were used. The diets were fed to groups of 6 rats each for 3 weeks.

Diets: The composition of the diet is shown in Table 1. Soybean and tuna were selected as protein source. Soybean was selected as a representative of conventional food and tuna was selected by its large odd-numbered acid contents⁹⁻¹⁰⁾. The variety of soybean was "Amsoy" which was provided by Dr. K. Saio of our Institute. The tuna "Honmaguro" variety was purchased from the market. They were freeze-dried and made to powder using a mill. Fatty acid composition of the diets were also shown in

† Evaluation of Single-cell Protein, Part IV. Part III, this journal, 23, 562 (1976).

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Table 1. Composition of Diets (%)

	Soybean diet	Tuna diet
Soybean	19.2 ^{a)}	0
Tuna	0	8.5 ^{a)}
Starch	44.8	55.4
Sugar	30.0	30.0
Cellulose powder	1	1
Minerals ^{b)}	4	4
Vitamines ^{c)}	1	1
Total fat	3.24	0.63
Fatty acid composition (% of total acids)		
14=0	0.14	2.01
16=0	12.26	26.68
16=1	0.02	2.87
18=0	6.58	10.21
18=1 + 18=2	79.35	32.58
20=0	0.24	0.42
20=1	0.17	4.69
20=2	0.14	4.79
22=2	0.00	8.14
15=0	0.07	1.17
15=1	0.04	0.00
17=0	0.10	0.76
17=1	0.10	0.88
21=0	0.04	0.00
Total odd	0.35	2.81

a) Nitrogen level was adjusted to 1.2%.

b) Johoson salt mixture.

c) A, D, E, K, and water soluble vitamins.

the Table. Total contents of odd-numbered fatty acid of soybean and tuna diet was 0.35 and 2.81% of total fatty acid, respectively.

Analytical method: At the end of feeding period, the rats were killed and freeze-dried immediately.

Lipids of adipose tissue and diets were extracted and fractionated by Folch's method. These lipids were saponified by 1N potassium hydroxide in ethanol and methylated with 15% boron trifluoride in methanol. Gas-chromatography was performed in accordance with the procedure previously described by us¹⁾. Chain length of fatty acids was determined by plotting the retention

time against the carbon number after hydrogenation using platinum oxide as a catalyst¹¹⁾.

The relative portions of fatty acids were calculated from the peak areas estimated using a digital integrater.

Results and Discussion

Concentration of odd-numbered acids in adipose tissue fat: Fatty acid composition of rats are shown in Table 2. Small amounts of odd-numbered fatty acid $C_{15=0}$, $C_{17=0}$, and $C_{17=1}$, were found in all cases. Total contents of these acids in tissue fat of rats fed on soybean diet and tuna diet were 0.78 and

Table 2. Results of Feeding Test and Fatty Acid Composition of Adipose Tissue Fat

	Initial	Soybean diet	Tuna diet	Yeast diet ^{a)}
Feed intake (g)		93	295	135
Fat intake (g)		3.01	1.86	1.21
Odd-numbered acid contents in diet (%)		0.0096	0.015	0.325
Odd-numbered acid intake (mg)		8.96	44.4	439
Body fat (g)	6.9	20.4	28.6	22.6
Body fat gain (g)		13.5	21.7	15.7
Fatty acid composition (% of total acids)				
14=0	1.12	2.11	1.98	
16=0	2.70	31.60	33.22	
16=1	0.06	9.27	10.95	
18=0	30.98	4.27	5.20	
18=1+18=2	8.98	47.99	46.56	
20=0	4.05	0.08	0.08	
20=1	50.16	0.46	0.43	
20=2	—	0.19	0.02	
15=0	0.01	0.30	0.06	
17=0	—	0.11	0.06	
17=1	0.11	0.37	0.21	
Total odd	0.12	0.78	0.33	2.04
Odd-numbered acid in body fat (mg)	6.45	135	80.2	392
Odd-numbered acid gain (mg)		129	73.7	386

a) TAJIMA M. *et al*, reference (1). Two weeks of feeding period.

0.33% of total acids, respectively. These figures indicate the increase of contents of these acids during feeding period, because of the fact that content of these acids was only 0.12% of total acids at the initial time of feeding test.

Relationship between amounts of odd-numbered acid intake and accumulation of those in body fat: To clarify whether the increase of odd-numbered acid levels during feeding period was caused by diet or not, following calculation was made. At first, amounts of odd-numbered acids intake during feeding period were calculated.

$$\text{Odd-numbered acid intake} = \text{Feed intake} \times \text{Fat content (\% of diet)} \times \text{Fatty acid contents (\% of fats)} \times \text{Content (\% of odd-numbered acid of total acid)} \times 10^{-6}$$

In the equation, fatty acid contents of fats (the third term) was assumed to 85%¹²⁾.

As odd-numbered acid intake, 8.96 and 44.4

mg were obtained for feeding of soybean and tuna diet, respectively.

Subsequent calculation was to obtain the gain of odd-numbered acid in body fat during feeding period.

Gain of odd-numbered acid

$$= (\text{Contents of odd-numbered acid in body fat at the end of test period}) - (\text{Contents of odd-numbered acid in body fat at the initial time of test period})$$

and

$$\text{Contents of odd-numbered acid in body fat} = \text{Body fat} \times \text{Fatty acid content (\% of fats)} \times \text{Contents (\% of odd-numbered acid of total acids)} \times 10^{-4}$$

In the equation, it was assumed that fatty acid pattern of body fat was equal to that of adipose tissue shown in Table 2. As the results, 129 and 73.7 mg was obtained as odd-numbered acid gain during feeding period

of sobean and tuna diet, respectively.

On comparing these values with those of intake, it is evident that odd-numbered acid gain during feeding period were much grater than intake of such acids from diet. From this fact, it would be concluded that a large part of odd-numbered acid gain is not depended on intake by diet.

However, it is interested that the odd-numbered acid gain by tuna diet was lower than that by soybean diet in opposition to the fact that the intake of such acids by tuna diet was five-times as much as that by soybean diet. Furthermore, when rats were fed on *n*-alkane assimilating yeast the increase of contents of odd-numbered acids in adipose tissue fat was observed¹⁾ as indicated in Table 2.

So, it must be necessary to carry out further study in order to elucidate the state and mechanism of storage of odd-numbered fatty acids in animals.

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各種飼料で飼育したラットの皮下脂肪中の奇数炭素数脂肪酸量*

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幼ラットを大豆及びマグロをタンパク源とした飼料で飼育し、皮下脂肪中に蓄積される奇数炭素数脂肪酸量を分析した。3週間の飼育後の皮下脂肪中の全脂肪酸中の奇数炭素数脂肪酸割合は、大豆区、0.78%、マグロ区、0.33%であった。飼育期間中に摂取した奇数炭素数脂肪

酸量と、飼育中に蓄積した体脂肪中の奇数炭素脂肪酸量を比較した結果、蓄積した奇数炭素数脂肪酸は飼料によらない部分が多いことが分った。

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