Study on the Adipose Tissue Cellularity and the Lipid Metabolism in Two Lines of Mice Selected for High and Low Resistance to Starvation

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Abstract In order to clarify the difference in the lipid metabolism between two lines of mice selected for high and low resistance to starvation (SH and SL), the changes of chemical body composition, total lipid, total cholesterol and glucose in plasma, cellularity and lipase activity of epididymal adipose tissue and lipid synthesis in the liver under starvation were investigated. Both under normal control (45 days of age) and starvation (48 hours from 45 days of age) conditions, there was no difference in body weight between SH and SL lines, while SH line had more body fat and less water, and statistically not significant but less fat free dry matter content than SL. In the normal control, SH line was higher than SL in plasma total cholesterol and glucose. It was clarified that SH was larger than SL in adipose fat cell size, but there was no significant difference in fat cell number between two lines. The total lipase activity of the epididymal adipose tissue of SH line was significantly higher than that of SL in the normal control. And under starvation condition, although the difference was not statistically significant, the activity of SH line was also higher than that of SL. There was no significant difference in liver lipid content between two lines but the incorporation of $1\text{-}^{14}\text{C}$ -acetic acid into liver lipid of SH line surpassed that of SL. From these results, it was concluded that the difference in body fat content between SH and SL lines selected for resistance to starvation was accounted for by the adipose tissue cell size which was brought about as the correlated genetic response in the lipid metabolism between SH and SL lines.

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If there is the genetic variation in the ability of adaptation to quantitative nutritional deficiency, it is very important to take the selection for this character into livestock improvement. For this purpose, the selection of mice for the resistance to starvation was conducted by YAMAGISHI¹⁾. The mice selected for high resistance (SH) had more fat, and less water and protein than those selected for low resistance (SL)²⁾. Furthermore, when the body weight of two lines of mice were restricted by food, SL line lost body fat, but SH line didn't²⁾. And SH line had more liver DNA content and less RNA/DNA than SL³⁾. These results may suggest that there is a difference in lipid metabolism between SH and SL lines.

In the present study, in order to clarify the difference in the lipid metabolism between SH and SL lines, the changes of chemical body composition, total lipid, total cholesterol and glucose in plasma, epididymal fat cell size and number, adipose tissue lipase activity and ^{14}C incorporation into liver lipid under starvation were investigated.

Materials and Methods

Animals: Experimental animals were derived from two lines of mice selected for high (SH) and low (SL) resistance to starvation. The selection procedure of these lines was described by YAMAGISHI¹⁾. Male mice of each line were sacrificed by decapitation at 45 days of age in normal control group and after 48 hours of starvation from 45 days of age in starvation group in following three experiments.

Experiment 1: After removing the gastrointestinal tract, liver and epididymal adipose tissue, carcasses were frozen until analysis. The carcasses were weighed and dried until constant weight was attained in vacuum dry oven at 70°C for 48 hours. The carcass fat was determined by ether extraction. Total lipid, total cholesterol and glucose in plasma were determined by the method of Sulfo-phospho-vanillin reaction⁴⁾, ZURKOWSKI⁵⁾ and Glucose Test Wako, respectively.

Liver lipid content was determined by weighing the lipid extracted by the method of BLIGH and DYER⁶⁾. About 0.1 g of wet liver tissue was dried in an oven for 24 hours at 90°C and liver moisture was determined.

The epididymal adipose tissue was divided into two portions: one for adipose cell size determination (about 100 mg) and the other for the determination of lipid content (about 100 mg) extracted by the method of BLIGH and DYER⁶⁾. Fat cells were isolated from epididymal adipose tissue of the mice by the method of ROBBELL⁷⁾. The diameter of the 200 fat cells from the same population was measured with a microscope using an eyepiece micrometer at a magnification of $\times 70$. The sizing of 200 fat cells was performed within 15 minutes. Fat cell volume ($\pi D^3/6$) was calculated by the data on diameters (D) of 200 fat cells in suspension. The number of fat cells of adipose tissue could be obtained by dividing the total lipid content of the tissue by the average lipid content of a fat cell. The average lipid content of a fat cell was derived from the mean cell volume $\times$ density (0.915) of lipid.

Experiment 2: The epididymal adipose tissues of normal control and starvation groups from each line were quickly removed and weighed. Epididymal adipose tissue was homogenized in a 0.06 M phosphate buffer (pH 7.4). Lipase activity was measured by adding 0.5 ml of this homogenate to 0.45 ml of 0.06 M phosphate buffer containing 5% albumin and 0.05 ml of Ediol (a commercial emulsion of 50% coconut oil) solution diluted with 4 volumes of water. Incubation was performed at 37°C for 60 minutes. After incubation, free fatty acids were measured by the method of NEFA Test Wako. The enzyme activity was defined as μEq of free fatty acid released per g of wet adipose tissue and expressed as the total activity in the whole tissue.

Experiment 3: One μCi radioactive acetate was injected intraperitoneally per 20 g of body weight of normal control group animals. The dose was doubled in the starvation group animals. In the present experiment, the animals were killed at 30 minutes after the injection, as VAN BRUGGEN *et al.*⁸⁾ have shown that in mice maximum incorporation of an injected tracer amount of acetate-1-¹⁴C occurred within 30 minutes after

the injection with no further change up to 8 hours. The lipid extraction from liver was done by the method of BLIGH and DYER⁶⁾. After evaporating the chloroform in the vials, ten ml of scintillation fluid (5g PPO and dimethyl-POPOP per liter of toluene) was added and radioactivity in total lipid was measured by a fluid scintillation counter.

For statistical analysis, analysis of variance was adopted to detect line differences.

Results

The chemical body composition, total lipid, total cholesterol and glucose in plasma of SH and SL lines of mice of normal control and starvation groups are given in Table 1. There were no differences in body weight and FFDM (Fat Free Dry Matter) between SH and SL lines and between the treatment groups. But SH line had significantly more fat and less water than SL. In the starvation group, body weight and body fat percentage were reduced and FFDM percentage was increased as compared with the normal control group in both lines. In the normal control group, SH line had more total lipid, total cholesterol and glucose in plasma than SL. Plasma total lipid was unchanged but plasma total cholesterol increased whereas plasma glucose decreased under starvation in two lines.

The epididymal fat pad weight, fat cell diameter, fat cell volume and number of both lines and groups are given in Table 2. The SH line had significantly heavier epididymal fat pad weight and larger fat cell diameter and cell volume than SL. And there was no difference in fat cell number between two lines. In the starvation group, fat pad weight, fat cell diameter and cell volume but not fat cell number were smaller than in the normal group. The phenotypic correlations among body weight, epididymal fat pad weight and adipose cellularity traits pooled within group-line subclasses

Table 1. Chemical body composition, plasma lipid and glucose concentration of SH and SL lines.

Treatment	Line	Number of mice	Body weight	Chemical body composition			Plasma		
				Water	Fat	FFDM ¹⁾	Total lipid	Total cholesterol	Glucose
			(g)	(%)	(%)	(%)	(mg/dl)	(mg/dl)	(mg/dl)
Normal	S H	6	28.03 ²⁾	61.88	15.12 ^{**3)}	23.01	618.40	218.40 ^{***}	213.67 ^{**}
			0.79	1.53	2.23	0.54	86.93	14.33	14.02
	S L	6	27.44	66.35 ^{***}	9.84	23.81	608.69	174.03	185.89
			0.68	1.71	3.20	1.56	47.76	12.99	10.89
Starvation	S H	6	22.29	61.84	12.33 ^{***}	25.83	608.69	248.49(5)	71.17(4) ⁴⁾
			0.71	1.43	1.53	0.87	105.28	33.16	13.15
	S L	6	22.25	66.52 ^{***}	5.63	27.86	574.26(4)	216.44(4)	79.33(2)
			1.16	1.33	2.46	1.83	141.78	10.96	16.97

1): FFDM: Fat Free Dry Matter. 2): Mean $\pm$ S.D. 3): Significant difference between SH and SL lines. ^{**}P < 0.01. ^{***}P < 0.001. 4): Number of mice. 5): The mice of normal control group were killed at 45 days of age and of starvation group were after 48 hours starvation from 45 days of age.

Table 2. Epididymal adipose tissue weight, cell diameter, cell volume and cell number of SH and SL lines.

Treatment	Line	Number of mice	Body weight (g)	Epididymal adipose tissue weight (mg)	Epididymal adipose tissue		
					cell diameter (μ m)	cell volume (pl)	cell number ($\times 10^6$)
Normal	S H	6	28.03 ¹⁾	474.57 ^{***2)}	72.96 ^{***}	229.05 ^{***}	1.99
			0.79	50.08	1.57	14.24	0.27
	S L	6	27.44	257.70	56.77	108.83	2.12
			0.68	45.50	2.55	15.87	0.16
Starvation	S H	6	22.29	377.67 ^{**}	65.11 ^{***}	169.40 ^{***}	2.11
			0.71	66.66	4.46	34.77	0.12
	S L	6	22.25	191.80	48.42	76.49	2.00
			1.16	76.62	6.02	27.94	0.21

1): Mean $\pm$ S.D. 2): Significant difference between SH and SL lines. ^{**}P < 0.01. ^{***}P < 0.001.
 3): The mice of normal control group were killed at 45 days of age and of starvation group were after 48 hours starvation from 45 days of age.

Table 3. Phenotypic correlations among body weight, epididymal adipose tissue weight, cell diameter, cell volume and cell number pooled within treatment-line subclasses.

	Epididymal fat pad weight	Cell diameter	Cell volume	Cell number
Body weight	0.410*	0.457*	0.416*	0.082
Epididymal fat pad weight		0.961 ^{**}	0.957 ^{**}	0.152
Cell diameter			0.986 ^{**}	-0.015
Cell volume				-0.101

Degrees of freedom=22. *P < 0.05. ^{**}P < 0.01.

are given in Table 3. Body weight was positively correlated ($P < 0.05$) with fat pad weight and fat cell size. Fat pad weight was highly and positively correlated ($P < 0.01$) with fat cell diameter and cell volume. But there were no significant correlations between fat cell number and other characters. The total fat weight in the epididymal fat pad could be approximated by the product of fat cell number $\times$ fat cell volume $\times$ lipid density. Therefore, the difference in fat accumulation between SH and SL lines was accounted for by the fat cell volume.

Table 4 shows the epididymal adipose lipase activities of SH and SL lines of mice. Although there was no significant line difference in lipase activity expressed per g wet weight, its activity expressed in the whole tissue basis was significantly higher in SH than in SL. The lipase activities of both lines were significantly decreased under starvation.

The liver weight and composition, and the incorporation of ¹⁴C into liver lipid are given in Table 5. There were no significant line differences in the weight, moisture,

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Table 4. Epididymal adipose tissue lipase activity of SH and SL lines.

Treatment	Line	Number of mice	Body weight (g)	Epididymal fat pad weight (g)	Lipase activity	
					$\mu\text{Eq/g wet tissue}$	$\mu\text{Eq/whole tissue}$
Normal	SH	6	26.42 ¹⁾	0.493 ^{**2)}	12.44	6.03 ^{***}
			1.03	0.080	2.39	0.88
	SL	6	27.38	0.322	11.03	3.47
			1.06	0.076	2.10	0.73
Starvation	SH	6	21.18	0.377	6.63	2.16
			0.44	0.112	0.88	0.49
	SL	5	22.30	0.288	6.12	1.65
			0.79	0.087	1.56	0.20

1): Mean $\pm$ S.D. 2): Significant difference between SH and SL lines. ^{**}P < 0.01. ^{***}P < 0.001. 3): The mice of normal control group were killed at 45 days of age and of starvation group were after 48 hours starvation from 45 days of age.

Table 5. The weight, moisture, lipid and FFDM of the liver and the incorporation of 1-¹⁴C-acetate into liver lipid of SH and SL lines.

Treatment	Line	Number of mice	Liver weight (g)	Liver composition			Number of mice	Liver weight (g)	Incorporation	
				Moisture (%)	Lipid (%)	FFDM ¹⁾ (%)			$\text{cpm} \times 10^3$ g liver	$\text{cpm} \times 10^3$ whole tissue
Normal	SH	6	1.65 ²⁾	72.25	3.58	24.18	4	1.22	5.69	6.79 ^{*3)}
			0.15	0.92	0.19	0.89		0.12	2.57	2.62
	SL	6	1.65	73.55	3.14	23.32	4	1.07	2.60	2.80
			0.15	0.62	0.45	0.26		0.05	0.68	0.84
Starvation	SH	6	0.89	67.58	7.43	24.99	6	0.81	4.61 [*]	4.04 [*]
			0.06	0.46	1.02	0.97		0.02	0.63	0.88
	SL	6	0.88	68.47	6.40	25.14	6	0.81	3.06	2.47
			0.10	2.08	2.59	0.82		0.08	0.85	0.71

1): FFDM: Fat Free Dry Matter. 2): Mean $\pm$ S.D. 3): Significant difference between SH and SL lines. ^{*}P < 0.05. 4): The mice of normal control group were killed at 45 days of age and of starvation group were after 48 hours starvation from 45 days of age.

lipid and FFDM of liver of both treatment groups. In the starvation group, liver weight and liver moisture were smaller, but liver lipid was larger than in the normal control group. The incorporation of 1-¹⁴C-acetate expressed per g liver into liver lipid was higher in SH than in SL in both groups. And the incorporation of 1-¹⁴C-acetate into liver lipid expressed in the whole liver basis was significantly higher in SH than in SL in both treatment groups. In the starvation group, although 1-¹⁴C-acetate was injected twice as much as the normal control group, the ¹⁴C incorporation into liver lipid was reduced in two lines.

Discussion

Present results showed that the two way selection for resistance to starvation had brought about correlated response of body fat, fat free dry matter and water content without changing body weight, as reported before²⁾.

Recently, many investigations about adipose tissue cellularity of mice and rats were reported. JOHNSON and HIRSCH⁹⁾ classified the genetic and experimental obesity in mice and rats into either hypertrophic or hypertrophic-hyperplastic form. And EISEN *et al.*¹⁰⁾ reported that the mice selected for rapid growth have larger cell size and more cell numbers than the mice of unselected control. From these reports, it is generally concluded that fat accumulation was attained by the increase of adipose tissue fat cell number and cell size. Therefore, it was necessary to investigate whether the difference in fat accumulation between SH and SL lines was accounted for by the fat cell number or cell size or both of them. Present results showed that the two way selection for resistance to starvation had brought about the correlated increase or decrease of adipose fat cell size without change of fat cell number. It was known that fat cell number of mice was determined before weaning⁹⁾, and that the nutritional factor at the early process of development was more important to determine adipose fat cell number than genetical factor¹¹⁾. From the result that there was no difference in litter weight gain during suckling between SH and SL lines (unpublished), it was estimated that the milking ability of SH was similar to SL. Therefore, the difference in fat cell size may be resulted rather from the correlated genetic response in lipid metabolism than from the nutritional condition for pups.

As the adipose tissue weight is very variable with the change of physiological condition, for example, in case of starvation, it is important to select the index to express the lipase activity, such as whole tissue, cell number, DNA etc., so as to reflect the physiological condition¹²⁾. In the present study lipase activity was expressed in the whole tissue basis. The higher total activity in SH than in SL was suggested. MIZUNUMA *et al.*¹³⁾ reported that the adipose tissue lipase activity expressed in the whole tissue basis was significantly higher in aged rats, which had larger cell size than young rats, than in young ones. Furthermore, from the comparison of lipoprotein lipase activity and hormone-sensitive lipase activity both expressed on a cellular basis (DNA) between genetical obese and lean breed pigs, it was suggested that difference in obesity between these breeds was not the result of differences in ability to mobilize fat but the result of an increase rate of lipid deposition in the obese pigs¹⁴⁾. Judging from these facts, it is suggested that the lipase activity of SH line with larger fat cell size is higher than that of SL with smaller fat cell size. Generally, adipose tissue contains at least two triacylglycerol lipases; one is a lipoprotein lipase and the other is a hormone-sensitive lipase. But recently, the idea was reported that these two lipases might be identical, not different enzymes. That is, these lipases might have immunochemically identical enzyme molecules and the adipose tissue lipase measured by adding serum was named lipoprotein lipase, while one measured without adding

serum was named hormon-sensitive lipase¹⁵⁾. Accordingly, in the present experiment, whole adipose lipase activity was measured regardless of the existence of these two lipases.

There was no difference in liver lipid accumulation between SH and SL lines but the incorporation of 1-¹⁴C-acetate into liver Lipid was higher in SH than in SL line. From these results, it is suggested that the turnover of lipid in the liver of SH line may be higher than that of SL. In a series of investigations related to lipid metabolism in experimental and genetic obese mice, BATES *et al.*¹⁶⁾ showed that the incorporation of ¹⁴C into liver and carcass fatty tissues was higher in obese mice than in normal control. Furthermore, these obese mice were classified into "regulatory" obesity, where the impairment was in the central mechanism regulating food intake, and "metabolic" obesity, where the primary lesion was an inborn or acquired error in the metabolism of tissues, per se, as described by MAYER¹⁷⁾. Although SH line is not "so called" obese mice, body fat content and body fat percentage of SH line were higher than those of SL. And not only in the normal control group but also in the starvation group, the incorporation of 1-¹⁴C-acetate into the liver lipid was higher in SH than in SL. According to the definition of MAYER *et al.*, it may be said that SH line was similar metabolic mechanism to "metabolic" obesity form as compared with SL.

When animals were starved, in general, they undergo metabolic adaptation in order to derive energy from adipose tissue and to conserve their protein reserves as efficiently as possible¹⁷⁾. From this, it is possible that the selection for resistance to starvation is the indirect selection for body fat accumulation. And present investigation suggested that the difference in body fat content between SH and SL lines selected for resistance to starvation was accounted for by the adipose tissue cell size which was brought about as the correlated genetic response in the lipid metabolism between SH and SL lines.

References

- 1) YAMAGISHI, T., Jpn. J. Zootech. Sci., **46**: 219-225. 1975.
- 2) SUZUKI, K., T. YAMAGISHI and Y. MIZUMA, Jpn. J. Zootech. Sci., **49**: 243-249. 1978.
- 3) SUZUKI, K., T. YAMAGISHI and Y. MIZUMA, Tohoku J. Agr. Res., **29**: 79-87. 1978.
- 4) FRINGS, C.S. and R.T. DUNN, Am. J. Clin. Path., **53**: 89-91. 1970.
- 5) ZURKOWSKI, P., Clin. Chem., **101**: 451-453. 1964.
- 6) BLIGH, E.G. and W.J. DYER, Can. J. Biochem. Physiol., **37**: 911-917. 1959.
- 7) RODBELL, M., J. Biol. Chem., **239**: 375-380. 1964.
- 8) VAN BRUGGEN, J.T., T.T. HUTCHENS, C.K. CLAYCOMB, W.J. CATHEY and E.S. WEST, J. Biol. Chem., **196**: 389-394. 1952.
- 9) JOHNSON, P.R. and J. HIRSCH, J. Lip. Res., **13**: 2-11. 1972.
- 10) EISEN, E.J., J.F. HAYES, C.E. ALLEN, H. BAKKER and J. NAGAI, Growth, **42**: 7-25. 1978.
- 11) MUTO, Y. and N. SHINOHARA, Metabolism., **9**: 935-948. 1972.
- 12) OKUDA, H. and S. FUJII, Yukagaku. **23**: 281-289. 1974.
- 13) MIZUNUMA, T., F. SATO, Y. KISHINO and H. OKUDA, Food and Nutrition, **30**: 7-13. 1977.
- 14) WEISENBURG, C.L. and C.E. ALLEN, J. Anim. Sci. (Abs), **37**: 293. 1973.
- 15) OKUDA, H. and S. FUJII, J. Biochem., **73**: 1195-1203. 1973.
- 16) BATES, M.W., C. ZOMZELY and J. MAYER, Am. J. Physiol., **181**: 187-190. 1955.
- 17) MAYER, J., in the Handbook of Physiology, Section 5 Adipose tissue: 645-651. American Physiological Society, Washington, D.C. 1965.

飢餓抵抗性選抜系マウスの脂肪組織の細胞 特性と脂質代謝に関する研究

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飢餓抵抗性高, 低選抜系マウス (SH, SL) の脂質代謝の違いを明らかにするため, 両系統のマウスについて 45 日齢の時点の正常対照時と, 45 日齢から 48 時間飢餓条件下に置かれた時の体の化学的構成, 血しょう中総脂質, 総コレステロールおよびグルコース, 副睪丸脂肪組織の脂肪細胞の数と大きさ, 副睪丸脂肪組織のリパーゼ活性および肝臓中脂質への $1\text{-}^{14}\text{C}$ -酢酸の取り込みを比較検討した. 正常対照群, 飢餓群とも体重では SH と SL の間に差は認められないが, SH は SL より脂肪含量が多く, 水分と脱脂乾物含量は少なかった. 正常群では, 血しょう中総コレステロールとグルコース濃度について SH が SL より高かった. 副睪丸脂肪組織の細胞の数と大きさの比較から, 飢餓抵抗性に関する選抜は, 相関反

応として脂肪細胞の数は変えず, 細胞の大きさを変えることが明らかとなった. 副睪丸脂肪組織全体あたりで表示した脂肪組織リパーゼ活性は, 正常群では SH が SL より有意に高く, 飢餓群でも有意ではないが SH が SL より高かった. 肝臓中脂質含量では両系統間に差は認められなかったが, 肝臓中脂質への $1\text{-}^{14}\text{C}$ -酢酸の取り込みは SH が SL より有意に高かった. 以上から, SH と SL 系のマウスは脂質代謝において遺伝的に明らかに異なっており, この差が両系統のマウスの脂肪組織の細胞の大きさの差を引き起こし, さらに体脂肪蓄積の違いをもたらしたものと考えられる.

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