〈原著論文〉

Genetic Aspects for Fatty Acid Components of Adipose Tissues in Syrian Hamster

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Abstract

To study the genetic aspects of fatty acid components in adipose tissues of Syrian Hamsters, a total of ninety animals of three lines were used. One inbred line (GN) was always fed cereals (Diet F) and inbred to the closed herd. Another selected line (ALF) was always fed with alfalfa meal pellet (Diet A) and selected to increase the weaning number. Another control line (GNR) was mated at random, with one of its group always fed cereals without selecting for litter size, and the second group fed an alfalfa pellet. Adipose tissues were taken from three parts of the subcutaneous round of back legs, round of kidney and generative organs. Rates of fatty acid components of total lipids extracted from their tissues were determined by Gas Chromatography and compared statistically to average out random effects of the line, sex, age in weeks, body part and diet. To research for genetic variation of fatty acid components and $\Delta 9$ -desaturase activity index ($\Delta 9$ -desat.), genetic parameters of each effect were estimated.

ALF seems to increase the animal's productive ability of C18:1, a measure of the efficiency of biosynthesis for mono-unsaturated fatty acids (MUSFA). So, ALF might be introducing a genetic variation by increasing the capacity to make use of energy in reproductive behavior. Estimated heritabilities of

MUSFA and Δ^9 -desat. were 0.73 and 0.61 respectively. Estimated genetic correlation between total saturated fatty acid(SFA) and MUSFA or Δ^9 -desat. were negative values. Considering the circumstances mentioned above, amounts of fatty acid components in adipose tissues of every part might be induced to genetically. To determine that, it might be useful to use Δ^9 -desat. as an indicator.

KEY WORDS: syrian hamster•alfalfa•fatty acid•heritability•genetic correlation
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Introduction

Recently, for evaluation of meat quality, it is known to be important to research the fatty acid components of total lipids in adipose tissues and muscle. However, it is not clear what factors affected the fatty acid components. So, research of the genetic variations will not account for the full effect. Especially, in today's Japan, it is impossible to make a meat which include high rate of total unsaturated fatty acid (USFA) without feeding much the cereals imported from the other country. So it is important to improve the strains genetically and increase their adipose tissues and muscles by considering many factors^{1,2,3,4,5,6,7}).

Syrian Hamster is a grassing animal that has two stomachs. A fore-stomach has specific fungi stocks that produce some volatile fatty acids (VFA) and a back-stomach that secretes some digestive enzymes. Also, they have simple fatty acid components that most resemble those of beef cattle⁸). So, for their structural and functional aspects, they resemble other ruminant animals, and can thus be examined easily without economic loss instead of large sized ruminants⁹).

In the future, improvement of meat quality is important for a suitable response to many requests from the market. But, there is only one published report for genetic aspects of the fatty acid components in adipose tissues of meat^{10,11,12}). Since genetic parameters are population attributes that require large data sets involving the lengthy laboratory procedures involved in fatty acid analysis, large data sets are hard to come by¹³). So, in this study, the genetic aspects of

fatty acid components in adipose tissues were researched by estimation of genetic parameter, the $\Delta 9$ -desaturase activity index ($\Delta 9$ -desat.) using the three lines of Syrian Hamster as examples of the ruminant animals.

In the study, the fatty acid components in adipose tissues of lines that were selected toward increasing fertilized ability under feeding with an alfalfa pellet and an unselected line were compared respectively. Genetic differences for fatty acid components, therefore, were discussed. The possibility to improve the herd genetically with crude diets and produce meat that has more acceptable fatty acid components in animal production was researched ¹⁴⁾.

Materials and Methods

Animals and Management

A total of ninety Syrian Hamsters of three lines were used in this study. The one line was GNR that was maintained with random mating in the closed herd during fifty-two generations. And the second line was GN that was maintained during one hundred forty eight generations with mating between siblings of GNR line in closed herd. And the third line was ALF that was produced by selecting during forty generations toward increasing number able to wean mating between siblings of GNR in closed herd fed the alfalfa leaf pellet (Diet A). Every number and the construction and feeding condition of comparison group are shown in the Table 1.

Table 1.

Construction and number for the experimental animal

Line	Diet	Male	Female
GN	A	10	10
	F	10	10
ALF	A	10	10
	F	10	10
GNR	A	5	—
	F	5	—

DietA:alfalfa meal pellet, DietF:Cereal diet for laboratory animals

GNR and GN lines were always fed *ad libitum* cereal (Diet F). ALF line was fed *ad libitum* Diet A. Tap drinking water was always freely available for each animal. Each ten heads of male and female on GN were fed on Diet A from starting of weaning. Residual animals of GN were given Diet A. Also, each ten heads of male and female on ALF were fed on Diet F from starting of weaning. Residual animals of ALF were given Diet F. And five of GNR were fed continuously on Diet A and residuals were given Diet F.

Sampling of Tissues Preparation

Animals were slaughtered by absorbing ether. Adipose tissues in the carcass of animals were taken from subcutaneous tissue of jointing part of back legs, tissues of the reproductive organ and kidney of round at the age of three weeks (weaning period), eight and thirteen weeks as point of maturing. Obtained samples were stored at -20°C until analyzing them.

Fatty Acid Component Analysis

Extraction of total lipid from samples (10mg) was carried out by the method of Folch *et al*¹⁵⁾. They were methylated in 600 μ l methanol-choleric acid for 20 second. at 50°C. The fatty acid methylated esters were extracted by hexane and analyzed using a dual column gas chromatograph (HITACHI 163, Japan) equipped with a glass column (J&W DB-23). The injection port and detector temperature was 215°C. The oven temperature was 100°C. The flow rate of nitrogen was 6kg/min. A standard of known composition was analyzed to verify the identity of fatty acids in the samples. The fatty acids peak areas were quantified with an electric integrator (C-R6A, Chromatopac, Shimazu Co., Kyoto, Japan).

Detected Fatty Acids and Calculated Index

Fatty acids which were detected in this study were myristic acid(C14:0), myristoleic acid (C14:1), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1) and linoeic acid (C18:2). Δ 9-desat. was calculated using an equation described by Siebert *et al.* and shown as the ratio of sum of C18:0 and C18:1 to C18:1 ¹⁶⁾.

Statistical Analysis

All data were analyzed with least squares analysis of variance using PROC

GLM Type III Sums of Squares of SAS¹⁷⁾. The model for analysis of variance of all data was

$$X_{ijklmn} = \mu + L_i + S_j + A_k + P_l + D_m + e_{ijklmn}$$

where X_{ijklmn} : data of 1th individual in i^{th} line, j^{th} sex, k^{th} age, l^{th} part and m^{th} diet

μ : over all mean

L_i : effect of i^{th} line

S_j : effect of j^{th} sex

A_k : effect of k^{th} age

P_l : effect of l^{th} part

D_m : effect of m^{th} diet

e_{ijklmn} : random error in X_{ijklmn} .

SFA was a sum of each ratio of saturated fatty acids. USFA was a sum of each ratio of unsaturated fatty acids. PUSFA was a sum of each ratio of monounsaturated fatty acids, and MUSFA was a sum of polyunsaturated fatty acids.

Heritabilities and genetic correlations as genetic parameters were computed by multiple trait derivative free restricted maximum likelihood computer program (MTDFREML) of Boldman *et al.*¹⁸⁾ with genetic mixed model of animal model. In matrix notation, the genetic mixed model for an observation vector, Y , is

$$Y = X\beta + Zu + e, \text{ where}$$

β = vector of fixed effects of line, age and diet in Y by X ,

u = vector of random effects associated with the additive genetic effect for fatty acid components in Y by Z , and

e = vector of residuals that were impossible to explain as an effective factor.

Results

Chemical components of the experimental diets

The chemical composition (%) of the experimental diets is shown in Table 2. The nitrogen free extract of 65% in Diet F was at a

Table 2.

Chemical composition of the experimental diets(%)

	Diet A		Diet F
Dry material(DM)	91.6	in DM	93.3
Crude protein	18.7		21.7
Crude fat	3.5		5.3
NFE	44.5		65.3
ADF	24.1		3.0
Crude ash	9.1		4.4
Calcium	1.8		0.6
Phosphorus	0.2		0.6
Magnesium	0.4		0.2
NDF	52.4		15.5

NFE:Nitrogen free extract, ADF: Crude fiber, NDF: Total Fiber, DietA: Alfalfa meal pellet, DietF:Cereal diet for laboratory animals, DM: Dry material

high level as compared to 44.5% in Diet A. Crude fiber of 24% in Diet A was higher in level than 3.0% in Diet F. Crude ash in Diet A was about two times that of Diet F. The mineral component of calcium and magnesium in Diet A comprised about twice that of Diet F. Additionally, the total fiber of 52.4% in Diet A was much higher than 15.5% for Diet F.

Fatty acids components of contained in the experimental diets

Fatty acid components for the lipids contained in the two diets are shown in Table 3. The Diet A contained 15.1% C16:0 of SFA, but Diet F did not. Diet F

Table 3.

Fatty acid components(%) of lipid contained in diets

Fatty acid	Diet A	Diet F
C14:0	0.60	0.40
C14:1	0.30	0.13
C16:0	15.07	(trace)
C16:1	0.90	13.72
C18:0	12.47	25.27
C18:1	19.83	51.10
C18:2	37.87	5.07
C18:3	7.27	1.27
SFA	28.14	25.67
USFA	66.17	71.29

SFA:Total saturated fatty acids,USFA: Total unsaturated fatty acids, DietA:alfalfa meal pellet, DietF:Cereal diet for laboratory animals

had contained some kinds of high level USFA, but, in Diet A, C18:2 and C18:3 were present in higher levels at 37.9% and 7.3%, respectively, than those of Diet F, 71.3%, was higher in level than that of Diet A, 66.2%. So, considering various fatty acid components, the specific kinds that were part of the rich USFA in both diets differed a great deal.

Analysis of variances for fatty acid components etc.

Analysis of variance for the fatty acid component ratios is shown in Table 4. The effect of breeding line was significant for C16:1(P<0.001), C18:0(P<0.01),

Table 4.
Analysis of variances for fatty acid components and
 Δ^9 -desat. in adipose tissues of Syrian Hamsters

	Line	Sex	Age	Part	Diet
C16:0	ns	*	ns	ns	*
C16:1	* * *	ns	ns	*	* * *
C18:0	* *	ns	ns	ns	ns
C18:1	* *	ns	* * *	ns	*
C18:2	ns	ns	ns	* *	* *
C18:3	* * *	ns	* * *	* * *	* * *
SFA	ns	ns	* * *	ns	* *
USFA	ns	*	ns	ns	ns
U/S	ns	*	*	ns	*
MUSFA	* * *	ns	* * *	ns	ns
PUSFA	* * *	ns	ns	ns	ns
Δ^9 -desat.	* * *	ns	* * *	ns	*
C18:1/18:0	*	ns	ns	ns	ns

SFA:Total saturated fatty acids, USFA:Total unsaturated fatty acids, U/S:USFA/SFA, MUSFA:Total monounsaturated fatty acids, PUSFA:Total polyunsaturated fatty acids,
 Δ^9 -desat.: Δ^9 -desaturase activity index(=C18:1/(18:1+18:0)),
*:P<0.05, * *:P<0.01, * * *:P<0.001, ns:ns letters are not significant

C18:1(P<0.01), C18:3(P<0.001), MUSFA(P<0.001), PUSFA(P<0.001), Δ^9 -desat.(P<0.001) and C18:1/C18:0(P<0.05). The effect of hamster age was significant, highly, for C18:1(P<0.001), C18:3(P<0.001), SFA(P<0.001), U/S(P<0.05), MUSFA(P<0.001) and Δ^9 -desat(P<0.001). The effect of body part was significant for C16:1(P<0.05), C18:2(P<0.01) and C18:3(P<0.001). The effect of the type of diet was significant for C16:0(P<0.05), C16:1(P<0.001),

C18:1(P<0.05), C18:2(P<0.01), C18:3(P<0.001) and SFA(P<0.01), U/S(P<0.05) and Δ^9 -desat.(P<0.05).

Differences between lines for calculated index on fatty acid components

SFA, USFA, MUSFA and PUSFA at the age of ten weeks for animals fed on Diet A and Diet F in three lines out of all the lines are shown in Table 5. and Table 6., respectively. When Diet F was fed to all lines until the age of ten

Table 5.

Least square of means(%) and standard errors
of fatty acid components in adipose tissues of GN, ALF
and GNR at the age of ten weeks fed to Diet F

	GN	ALF	GNR
SFA	23.2(0.3)	25.7(0.3)	23.6(0.3)
USFA	75.3(0.3) ^a	72.2(0.2) ^b	76.0(0.3) ^a
MUSFA	50.0(0.9) ^b	51.7(0.8) ^b	56.7(0.8) ^a
PUSFA	25.2(0.3) ^a	21.1(0.4) ^b	26.3(0.3) ^a

SFA:Total saturated fatty acids, USA:Total unsaturated fatty acids, MUSFA:Total monounsaturated fatty acids, PUSFA:Total polyunsaturated fatty acids, DietF:Cereal diet for laboratory animals, ():Standard error
Different superscripts between lines is significant in the same low(P<0.05).

Table 6.

Least square of means(%) and standard errors
of fatty acid components in adipose tissues of GN, ALF
and GNR at the age of ten weeks fed to Diet A

	GN	ALF	GNR
SFA	34.0(0.3) ^a	24.5(0.3) ^b	30.3(0.4) ^b
USFA	55.2(0.2) ^b	71.0(0.2) ^a	69.5(0.3) ^a
MUSFA	12.1(0.9) ^c	36.0(0.8) ^a	29.2(0.8) ^b
PUSFA	27.0(0.3) ^b	36.8(0.3) ^a	41.1(0.3) ^a

SFA:Total saturated fatty acids, USA:Total unsaturated fatty acids, MUSFA:Total monounsaturated fatty acids, PUSFA:Total polyunsaturated fatty acids, DietA:alfalfa meal pellet, ():Standard error
Different superscripts between lines is significant in the same low(P<0.05).

weeks, MUSFA tended to increase on ALF. And $\Delta 9$ -desat. was highest in ALF, but the differences of means between GN and ALF was not significant on MUSFA. This was because USFA of ALF was lowest of three lines, significantly. There were not significant differences between GN and ALF, but ALF tended to increase the biosynthesis ability of MUSFA. So, $\Delta 9$ -desat. of ALF had been high level, compared to the other lines ($P < 0.01$). Generally, C18:1 is a high ratio in mammalian adipose tissues, that is, more than C16:1 in the composition of feeding in both diets. So, occupied MUSFA in total ratio of fatty acid components was highest percentage. Also when GN was fed with Diet A, MUSFA was significantly low ($P < 0.01$). But, for ALF and GNR, the difference between their means was not significant.

To estimate genetic parameter of calculated index on fatty acid components

To determine statistically the genetic influences of SFA, USFA, MUSFA, PUSFA, $\Delta 9$ -desat. and C18:1/C18:0 in adipose tissues, genetic parameters for each were estimated using the MTDFREML computer program by Van Veleck *et al*⁽¹⁸⁾. To estimate these parameters, a genetic mixed model that contained the fixed effect of line, age, diet and the additive genetic effects from sire and dam as the random effect was used. Their estimated parameters are shown in Table 7. Heritabilities of SFA, USFA, MUSFA, and C18:1/C18:0 were 0.22,

Table 7.

Estimated genetic parameters of SFA, USFA, MUSFA, PUSFA, $\Delta 9$ -desat. and C18:1/18:0

	Heritability	Genetic correlation
SFA	0.22	(1.00)
USFA	0.60	-0.34
MUSFA	0.73	-0.27
PUSFA	0.30	-0.85
$\Delta 9$ -desat.	0.61	-0.88
C18:1/C18:0	0.27	-0.88

SFA:Total saturated fatty acids, USA:Total unsaturated fatty acids, MUSFA:Total monounsaturated fatty acids, PUSFA:Total polyunsaturated fatty acids, $\Delta 9$ -desat.:

$\Delta 9$ -desaturase activity index(=C18:1/(C18:1+C18:0))

0.60, 0.73 and 0.27, respectively. And, for Δ^9 -desat., heritability was estimated at 0.61. The genetic correlation among USFA, MUSFA, PUSFA, Δ^9 -desat., C18:1/C18:0 with SFA was estimated to be -0.34, -0.27, -0.85, -0.88 and -0.88, respectively.

Discussion

The components of the lipids in adipose tissues are mainly triglycerides. And C18:1 contained the richest amount of triglyceride and tended to increase, slightly, with age of animal on our information from nonpublic reports. Because the ratio of C18:1 in a triglyceride was different significantly between the three lines, the significance for genetic effect was predicted as a biosynthesis ability of C18:1. That is, USFA might be able to reflect the genetic variance. Additionally, considering the effect of diet, the interaction between diet and line must be researched to reveal its genetic aspects. Suda *et al.* reported in other research that a relationship between the effect of diet and line revealed a change in fatty acid components in adipose tissues¹⁹). However, the relationship was not quantifiable, significantly, between both effects in that study. The effect of diet has been known to be a factor that might influence the fatty acid components. It was reported that diet had an influence on the rumen microbial population *in vivo* and *in vitro*⁸). That investigation agreed with the results of this examination. Most of lipids in the diet are absorbed in the intestine. The C18:1 has inserted a double bond at the Δ^9 -position in C18:0, so synthesized by Δ^9 -desat. in the liver or alternative it is absorbed from the diet and stored along with the other triglyceride components. Δ^9 -desaturase activity or the expression of a gene coding the enzyme might be able to estimate this genetic variance, because the effect of line was significant for C18:1 ($P < 0.01$) and, highly, for MUSFA ($P < 0.001$), PUSFA ($P < 0.001$). Also, because the effect of breeding line was significant for C18:0, the influence for bio-synthesis to C18:1 might significantly vary significantly the fatty acid components in adipose tissues. So, to reveal the interaction between the effect of diet and line is very difficult and complex, but the concept of a peptide of a specific enzyme synthesizing MUSFA affecting the absorption of lipids must be easy to

understand.

In this study, an index for C18:1 on animals fed Diet F until the age of ten weeks was 47.8% in GN, 45.1% in ALF and 46.5% in GNR. And C18:1 for ones fed Diet A were 15.4% in GN, 30.4% in ALF and 26.8% in GNR. Considering the results and views mentioned above, the ALF line might be able to reproduce under the feeding condition of low energy. And they might get a high demanding volume of the diet according to increasing biosynthesis of USFA for the energy efficiency *in vivo*, because the selected line to increase the weaning number for reproduction was fed Diet A. Reed *et al.* reported that a shift in energy supply from forage to concentrate had a minimal effect on digestion and reproduction in first-calf beef heifer²¹). So, it is necessary to research the demanding volume of their diets. The synthesis ability of the animals for MUSFA might be increased indirectly, using selection to increase their reproductive ability under the feeding condition of the crude diet. However, in the line without the selection, GNR, the fact that SFA, USFA, and PUSFA were the same level might indicate that the GN line was fitted to a high energy diet, genetically.

Therefore, considering the significant difference between ALF and GN on USFA, MUSFA and $\Delta 9$ -desat., USFA and MUSFA might be altered by working with genetic factors. Also, they might be able to be used to select for genetic improvement of the fatty acid components, and that would be an important factor to improve the meat quality. Especially, C18:1 and $\Delta 9$ -desat. should be noted as efficient indicators. $\Delta 9$ -desaturase has been recognized as an enzyme that synthesizes C18:1 *in vivo* or *in vitro*. So, if $\Delta 9$ -desat. has a large genetic variance, its level could be increased, and one may thus hope to realize a genetic gain. In this research, because heritability of $\Delta 9$ -desat. was estimated at 0.61, a genetic gain will be realized gotten by selection using $\Delta 9$ -desat. as an index. However, the relationships between each of the fatty acids in many tissues are very complicated, both phenotypically and genetically. Therefore, correlations for each of them have to be considered, to improve the normal balance without producing an abnormal state. The estimated genetic correlation between USFA and SFA was -0.34. So, to proceed toward improvement by

decreasing SFA, we suggest that it is best to increase USFA in adipose tissues, and that might be able to realized. This might closely associate to the estimated(-0.88) genetic correlation between Δ^9 -desat. and SFA. The genetic change of each fatty acid component depends on the genetic variation of desaturase activity. But, biologically, the degree that USFA can be increase in adipose tissues must only be accomplished *in vivo*.

Considering the views mentioned above, a result that the selected line, ALF included better MUSFA than the other lines suggests that we are able to improve the fatty acid components genetically by using Δ^9 -desat. or USFA, indirectly. Realistically, in the case of animals of which will born many pups per one progeny, to select the full sibs would be most efficient, while, in case of beef cattle, the selection in half-sibs would still be useful. Four beef cattle, it was reported that the fatty acid components in adipose tissues differ between the breeds²⁰). The ability to use fatty acids as the energy source might differ between lines or breeds with a different genetic background. So, it is important for the meat quality to be improved by using Δ^9 -desat. and USFA as the indicators.

Acknowledgements

This study indicates clearly that line, age and diet are important factors for significant variance of fatty acid components in adipose tissues. Because the Syrian Hamsters used in this study were under the same management conditions, these results suggest that some factors of genetic import are the differences of the fatty acid components in them. Especially, the variance by an effect of line might be as high as it is for the diet, so the genetic character of fatty acid components in them would be related by information from the interactions between the two effects.

We think that, because this may be controlled by a few environmental factors, one would be able to make the desired traits in meat quality without the effect of diet using the genetic technique of selection, but alfalfa diet produces better meat quality and should also in cattle.

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