

**The Effects of Altering Dietary Fat  
on Fatty Acids in Adipose  
Tissue of Strong Strain A Mice**

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## Introduction

Research has suggested that the fatty acid composition of an individual's adipose tissue is a valid index for the habitual fatty acid composition of the diet of individuals. Current research involves identifying fatty acids of adipose tissue of animals on specifically prepared diets. Results from the literature indicate a mathematical relationship leading to a rather high correlation coefficient, between the fatty acids in the diet and those of the subcutaneous fat stores of an individual. The highest correlation coefficient was found when polyunsaturated fatty acids were present in both the dietary and adipose tissue parameter.

Studies are also being conducted relating fatty acids to the age of the subject. These investigations have determined there is an age-dependent decrease of unsaturated fatty acids and an increase in saturated fatty acids due to lipid peroxidation.

In our laboratory, the fatty acid composition of adipose tissue has been determined for mice of different ages fed high and low controlled amounts of stearic and linoleic acids. Mice fed a high fat (15%) stearic acid rich (SA-1) diet had large stores of oleic acid. Mice fed a high fat safflower oil (SAF) diet had large stores of linoleic acid. The SA-1 mice had larger percentages of palmitoleic and stearic acids than did the SAF mice. However, neither showed the elevated percentage of palmitoleic acid associated with obesity. The SAF mice had an average weight higher than that of SA-1 mice. As the SAF mice

aged, there were slight increases in palmitoleic and linoleic acids and slight decreases in stearic and oleic acids. The aging of the SA-1 mice caused decreases in palmitoleic and linoleic acids and a large increase in oleic acid. The percentage of stearic acid remained unchanged.

The purpose of this research project was to determine the effects of altering dietary fat on the rate of change of the fatty acid composition of adipose tissue in relationship to age and weight gain. As changes are made in dietary intake of fats, the composition of the adipose tissue is altered. The fatty acid profile of this tissue, showing the percentages of each fatty acid profile component, differs highly between lean and obese subjects. Thus, a specific relationship or trend is sought among an individual's dietary intake of fat, adipose tissue composition, and weight gain. A fourth factor considered in this project was the age of the mice and its effect on fatty acid composition.

### Review of Related Literature

Beynan and Katan (1) have devised a method for obtaining human adipose tissue without anesthesia. With their method, they collected over five hundred adipose samples and objectively determined the fatty acid composition of the diets of the subjects. Their investigation indicated that the fatty acid composition of adipose tissue is a valid index of the fatty acid composition of the diet over the preceding one to three years.

Beynan et. al. (2) found that the fatty acid profile of human subcutaneous adipose tissue is strongly influenced by the fatty acid composition of the dietary fat. Among the adult population, age played a minor role in determining the fatty acid pattern of adipose tissue. A major factor, however, for figuring the time required for an alteration in dietary fat type to be reflected in the fatty acid profile is the turnover rate of adipose fatty acids. Using seven studies previously done on dietary and adipose fat composition, a mathematical relationship was derived. The highest correlation coefficient, indicating the ability to predict the fatty acids found in the adipose tissue from the dietary fat intake, was calculated when polyunsaturated fats (PUFA) were present in both diet and adipose tissue. This may be related to the fact that PUFA are an essential component.

Similar findings were obtained by Plakke (3). In addition investigations implied that 25% of the variance of adipose tissue fatty acids between individuals is accounted for by differences in dietary fat composition.

Burns et. al. (4) studied the modification of the fatty acid composition of mouse tissues. Male DBA/2 mice approximately five weeks old were fed a diet enriched with either saturated or polyunsaturated fats for five weeks.

The saturated fats in the diet were numerous. The polyunsaturated fat was only linoleic acid. Tissues from animals fed the two experimental diets showed considerable variations. In general, tissues from animals fed the saturated diet were rich in monoenoics, mostly oleate. In contrast, tissues from animals ingesting the polyunsaturated diet contained larger amounts of polyenoics, especially linoleate. Conclusions stated that the modification of fatty acids of normal tissues induced by experimental diets differs in extent and type demonstrating selectivity amongst normal tissues.

Takatori (5) discovered that changes in tissue lipid fatty acid composition are exhibited when trans-fatty acids are fed to mice as the sole source of unsaturated fat. Trans-fatty acids caused suppressed incorporation of eicosatrienoic acid into tissues. Characteristics of essential fatty acid (EFA) deficiency emerged, including reduced weight. When trans-fatty acids were fed in conjunction with adequate amounts of EFA, however, they had little effect on growth.

Research by Thomas revealed that trans-unsaturated fatty acids arose in large measure in adipose tissue via consumption of hydrogenated fats, such as margarine (6). This was not the case, though, when butter-fat was consumed. As stated previously,

trans-fatty acids may suppress incorporation of eicosatrienoic acid into tissues and cause reduced growth if not accompanied by an adequate amount of EFA.

Incorporation of monounsaturated fatty acids depends on the dietary concentration. The incorporation of these fatty acids is accompanied by increases in polyunsaturated fatty acids. Evidence also shows that linolenic acid rapidly substitutes C 22 polyunsaturated fatty acids of the linoleic acid family (n-6) with those of the linolenic acid family (n-3) (7).

Conclusions have been drawn, as well, pertaining to the correlation between dietary-induced changes in the fatty acid composition of human cheek cells and changes in the dietary polyunsaturated/saturated (p/s) fat ratio (8). The proportion of linoleic acid in cheek cell phospholipids was significantly increased by increasing the dietary p/s ratio. The change in ratio was achieved by substitution of saturated and monounsaturated fatty acids with linoleic acid. The increase occurred within six weeks of the introduction of the new diet. Changing from a high to a low p/s ratio diet resulted in only a small drop in the proportion of linoleic acid, suggesting that an increase in the acid may occur more quickly than a decrease in this specific tissue. This may further mean there is a minimum level of linoleic acid required for tissue.

In a related investigation (9), the profile of human cheek cells was shown to differ between vegetarians exhibited a significantly lower saturated fat intake than the nonvegetarians,

and this was associated with a relatively lower proportion of saturated fatty acids in their cheek cell phospholipids.

Current research also includes studies of obesity in connection with dietary and tissue fat compositions. Enser and Ashwell (10) reported that the subcutaneous adipose tissue of obese mice differs from that of lean mice littermates in fatty acid composition. The adipose of obese mice was characterized by a higher proportion of palmitoleic acid and a lower proportion of linoleic acid than that of adipose from lean mice. To determine whether these differences were inherent in fat cells, the adipose tissue from obese and lean mice was transplanted to lean and obese host mice, respectively. The fatty acid composition of the transplanted graft and of the host subcutaneous fat was determined one or two months later. The fatty acid composition of grafts from lean donors in obese hosts resembled that of the obese mice. Grafts from obese mice in lean hosts had fatty acid profiles like those of the lean mice. These results clearly demonstrated that differences in the fatty acid composition of adipose tissue neutral lipids between obese and lean mice depends on the physiological characteristics of the animal and not on genetic differences in the adipose tissue.

Weight gain is associated with a relatively rapid deposition of dietary fat in adipose tissue (11). During periods of negative energy balance, in corporation of dietary fatty acids into adipose tissue was found to be limited. In obese subjects, fat cell size and number of fat cells were increased.

One research team (12) divided obese women into hyperplastic (increase in fat cell number) and hypertrophic (increase in fat cells size) categories of obesity. The women were placed on an energy-reduced diet until weight loss ceased. At this point, enlarged fat cells in either of the two groups had decreased to the size of fat cells of controls. Fat cell number remained unchanged. Therefore, the hypertrophic patients ended up with a normal body fat and hyperplastic patients remained pronouncedly obese. This suggests that knowing the fat cell size and total fat cell number of an individual may help to predict the success of an energy-reduced dietary regimen in terms of remaining body fat and decrease in regional fat deposit.

Diets with a high fat content cause substantial expansion of the adipose tissue mass before the use of the fat by the body reaches a rate commensurate with the diet's fat content (13). Thus, obesity may be the result of the body's tendency to achieve the steady state, where the fuel mix oxidized has the same composition as the food consumed, only after an undesirable large expansion of the adipose tissue mass has take place.

The aging process brings about tan increased production of superoxide radical responsible for lipid peroxidation. The result of the continuing lipid peroxidation is an age-dependent decrease of unsaturated fatty acids and an increase in saturated fatty acids (14).

To summarize, the fatty acid composition in the diet has been shown to determine the fatty acid profile of adipose tissue.

The percentages of certain fatty acids in the profile of adipose are distinctly different in obese mice than those of lean mice. Age also brings about changes in the fatty acid profile due to chemical processes accompanying it. Thus, the dietary intake, adipose tissue fatty acid profile, weight gain, and age of individuals appear to be interrelated. It is the purpose of this investigation to determine the rate at which these changes take place in young and old mice.

## Materials and Methods

### Dietary Alteration

Two groups of six strong A/st mice fed a high fat stearic acid diet, containing 13.2% stearic acid and 0.8% linoleic acid, from weaning were placed on a high fat safflower oil diet, containing 0.4% stearic acid and 11.4% linoleic acid. One group was ten months old and the other group was twelve months old. Two groups of six mice, five and ten months of age, fed the safflower oil diet from weaning were placed on the stearic acid diet. The mice were weighed weekly to monitor changes in weight.

### Tissue Collection

Two, four and six weeks after mice were placed on new diets, two mice in each of the four groups were killed by exsanguination under light ether anesthesia and the adipose tissue was surgically removed. The tissue was stored in 0.25% acetic acid at -20 C until ready for fatty acid analysis.

### Saponification, Extraction, and Methylation of Fatty Acids

Tissues were placed in 15 mL conical test tubes with approximately 2 mL of 15% KOH in 85% methanol. The samples were refluxed in a hot water bath (80 C) for one and a half hours. A volume of 2:1 hexane: ether equal to the volume of the cooled solution was added and the aqueous layer was retained sufficient concentrated HCl was added to make the solution acidic to pH paper. The acidified fatty acids were extracted into an equal

volume of hexane and the aqueous layer was removed. An equal volume of saturated KCl solution was added twice and the aqueous layer was removed both times. A spatula tip full of anhydrous  $\text{Na}_2\text{SO}_4$  was added to the sample to remove any excess water. The solution was then transferred to another centrifuge tube and evaporated under  $\text{N}_2$ .

An 80:20 mixture of ether: methanol was added to redissolve the fatty acids. The samples were methylated with the diazomethane procedure of Schlenk and Gellerman (15). The ether was evaporated under  $\text{N}_2$  and the methylated fatty acids were stored at  $-20\text{ C}$  until gas liquid chromatographic (GLC) analysis.

#### Chromatographic Analysis

The fatty acids were redissolved with 500-600 mL of hexane. The hexane was evaporated under  $\text{N}_2$  to concentrate the fatty acids in the bottom of the centrifuge tube. The fatty acids were redissolved in 50-100 mL of hexane and 0.6-0.8 mL of sample was drawn up with a syringe. The sample was then injected into a column of a Varian 3600 gas chromatograph for fatty acid analysis

## Results and Discussion

The largest changes in fatty acid composition of the adipose tissue were seen palmitoleic, stearic, oleic and linoleic acids. The trends followed the expected patterns of change that should accompany the dietary alterations made during this project. Overall, the five month SAF mice tended to make a smoother transition toward the expected profile of SA-1 mice. The ten month SAF mice, however, were closer to the expected fatty acid profile of SA-1 mice at the end of six weeks. Thus, the older mice seemed to have a faster rate of change. The younger SAF mice had a problem during the first two weeks with weight loss, probably due to adjustment to the new diet. After the six weeks, however, both the young and old mice appeared to have lost the same average amount of weight. The rates of change for the ten and twelve month SA-1 mice were extremely similar, as was their pattern of weight gain. The SA-1 mice had profiles very close to the expected SAF profile. They had the fastest rate of change, again showing that the older mice change their fatty acid profiles more rapidly than the younger mice. (Tables 1-4, Fig. 1)

More specifically, the following trends were observed:

- (1) Both five and ten month SAF mice showed rapid incorporation of palmitoleic acid (16:1) and rapid loss of linoleic acid (18:2).
- (2) The ten month SAF mice had a much quicker increase in percentage of oleic acid (18:1) than the five month mice.
- (3) The ten and twelve month old SA-1 mice had high rates of decreasing stearic and oleic acid percentages. They had a very rapid rate of linoleic acid incorporation.

The data clearly revealed that dietary fat intake affects the fatty acid composition of the adipose tissue. At the end of six weeks, the SA-1 mice had shown a quicker rate of transition to the new SAF profile they were expected to exhibit than the SAF rate of transition to the SA-1 profile. The ten and twelve month mice of each group had quicker rates of change.

Patterns also emerged relative to weight changes and dietary intake. The mice began their patterns of weight gain or loss after consuming the new diet for one week. After the six week period, the SAF mice of both ages had lost an average of approximately two grams. The ten and twelve month SA-1 mice followed the same weight gain pattern for the first four weeks. During the last two weeks, however, the rate of weight gain for the twelve month mice increased over that of the ten month mice. In general, the rates of weight gain of the SA-1 mice uses the same as the rates of weight loss of the SAF mice and age did not appear to appear to affect the rate either. The exception was the one cage of five month SAF mice who adjusted poorly to the new diet during the first two weeks. These results support those observed previously in our laboratory. (Fig. 2)

Putting data from fatty acid composition and weight gain together, led to conclusions contradictory to those of Enser and Ashwell (10). SAF mice showed a weight loss, yet had an increased proportion of 16:1 and a decreased proportion of 18:2. The SA-1 mice gained weight, but had a decreased proportion of 16:1 and an increased proportion of 18:2. These findings may

differ from other research due to the fact that the mice used in this project merely gained weight, and were not considered obese.

Although age did not appear to affect the weight change of the mice, it did seem to play a role in the change from the initial fatty acid profile of the mice to the profile indicative of the new diet as mentioned previously. A lesser percentage of 16:1 and a greater percentage 18:1 was found in ten month SAF mice than in five month SAF mice. This observation had been made during other investigations in our laboratory.

TABLE 1

FATTY ACIDS IN ADIPOSE TISSUE OF 5 MONTH OLD SAF MICE  
FED AN SA-1 DIET FOR 2, 4 AND 6 WEEKS

ID	1SF-5	2SF-5	AVE	3SF-5	4SF-5	AVE	5SF-5	6SF-5	AVE
<16	2.3	4.1	3.2	12.3	4.1	8.2	15.3	31.5	23.4
16:0	10.7	13.2	12.0	15.2	12.8	14.0	13.6	6.9	10.3
16:1	3.7	5.9	4.8	12.6	7.7	10.2	9.1	27.1	18.1
16:2	0.4	0.5	0.5	1.1	0.0	0.6	0.0	0.0	0.0
18:0	2.4	2.9	2.7	3.9	3.2	3.6	6.2	1.8	4.0
18:1	26.8	27.7	27.3	27.4	24.6	26.0	29.8	16.6	23.2
18:2	49.2	40.9	45.1	25.4	46.6	36.0	23.1	12.9	18.0
18:3W	1.3	1.2	1.3	0.6	0.6	0.6	0.0	0.0	0.0
20:2	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
20:3	0.3	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.0
20:4	0.3	0.3	0.3	0.3	0.3	0.3	0.0	0.0	0.0

TABLE 2

FATTY ACIDS IN ADIPOSE TISSUE OF 10 MONTH OLD SAF MICE  
FED AN SA-1 DIET FOR 2, 4 AND 6 WEEKS

ID	1SF-10	2SF-10	AVE	3SF-10	4SF-10	AVE	5SF-10	6SF-10	AVE
<16	5.3	3.3	4.3	10.0	3.5	6.8	1.8	15.1	8.5
16:0	12.1	11.7	11.9	10.6	14.1	12.4	16.5	14.8	15.7
16:1	5.9	5.4	5.7	10.2	11.1	10.7	7.6	10.6	9.1
16:2	0.7	0.0	0.4	1.1	0.0	0.6	0.4	0.0	0.2
18:0	3.1	3.8	3.5	2.6	2.4	2.5	4.8	5.3	5.1
18:1	26.2	29.2	27.7	19.0	28.3	23.7	47.0	30.2	38.6
18:2	43.6	40.6	42.1	44.1	39.7	41.9	20.0	24.0	22.0
18:3W	0.8	2.3	1.6	0.4	0.5	0.5	1.1	0.0	0.6
20:2	0.2	0.7	0.5	0.0	0.0	0.0	0.1	0.0	0.1
20:3	0.2	0.5	0.4	0.0	0.0	0.0	0.0	0.0	0.0
20:4	0.3	0.6	0.5	0.2	0.2	0.2	0.3	0.0	0.2

TABLE 3

FATTY ACIDS IN ADIPOSE TISSUE OF 10 MONTH OLD SA-1 MICE  
FED A SAF DIET FOR 2, 4 AND 6 WEEKS

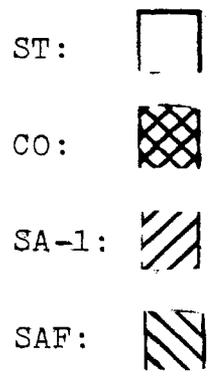
ID	1SA-10	2SA-10	AVE	3SA-10	4SA-10	AVE	5SA-10	6SA-10	AVE
<16	21.2	4.4	12.8	25.9	2.8	14.4	2.0	1.5	1.8
16:0	13.8	13.0	13.4	11.9	13.1	12.5	11.4	11.9	11.7
16:1	9.6	6.7	8.2	12.8	7.3	10.1	5.2	6.1	5.7
16:2	2.0	0.0	1.0	0.0	0.3	0.2	0.0	0.0	0.0
18:0	4.5	3.9	4.2	1.7	2.6	2.2	1.9	2.2	2.1
18:1	29.1	50.8	40.0	21.6	33.2	27.4	30.5	34.2	32.4
18:2	12.3	18.7	15.5	24.9	38.9	31.9	47.2	42.4	44.8
18:3W	0.8	1.3	1.1	0.0	0.8	0.4	0.8	0.8	0.8
20:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:3	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.0
20:4	0.0	0.0	0.0	0.0	0.5	0.3	0.5	0.6	0.6

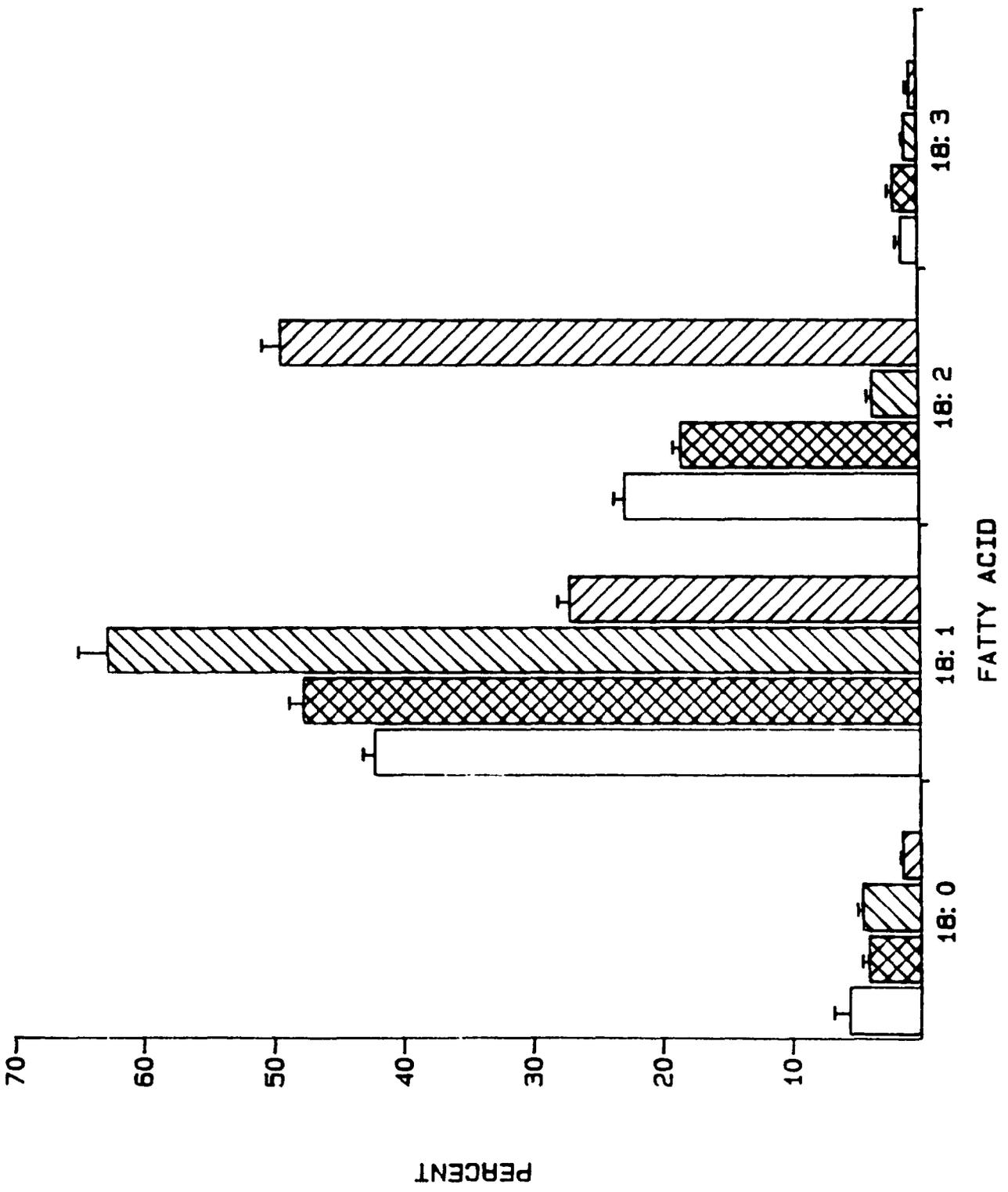
TABLE 4

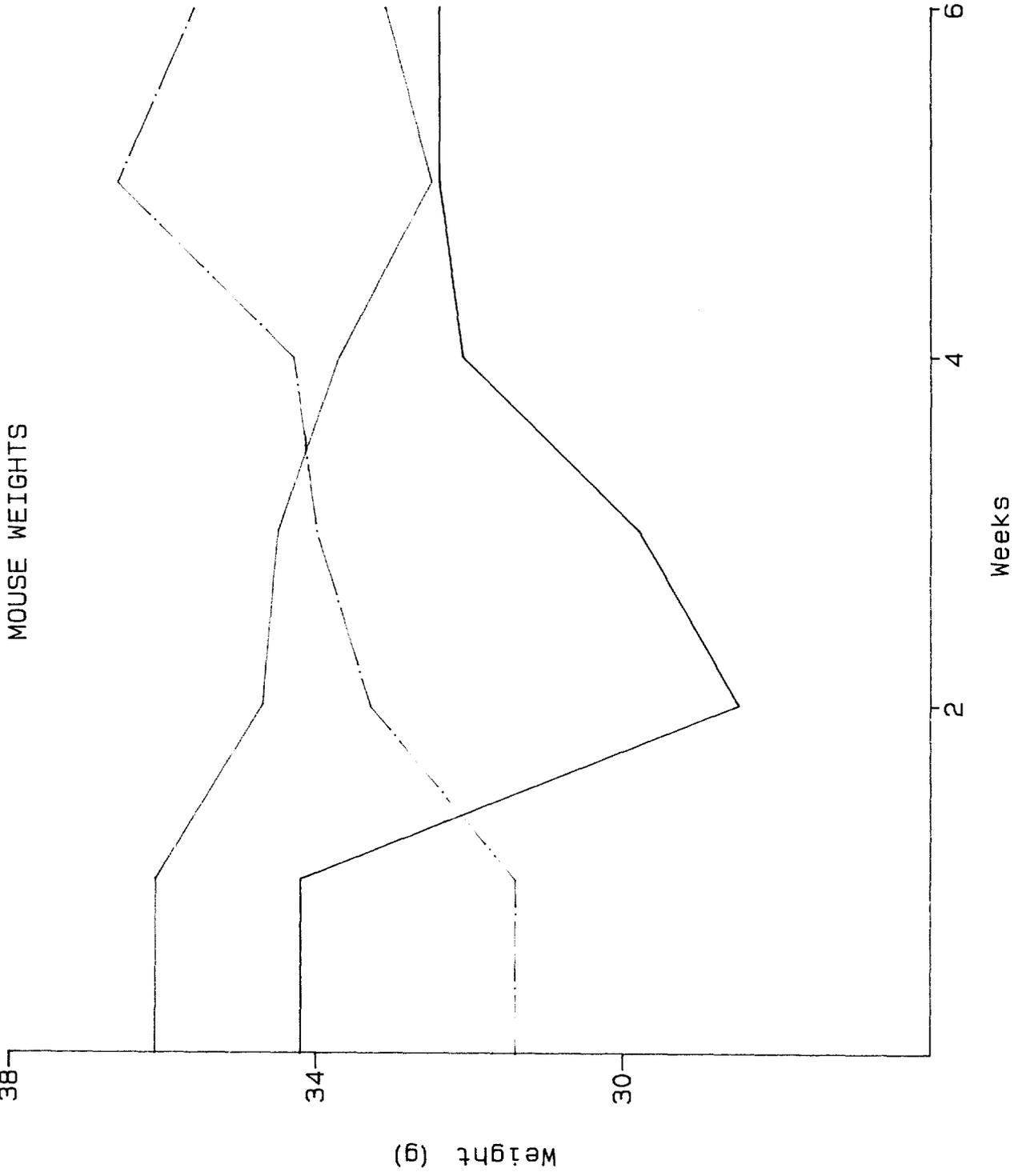
FATTY ACIDS IN ADIPOSE TISSUE OF 12 MONTH OLD SA-1 MICE  
FED A SAF DIET FOR 2, 4 AND 6 WEEKS

ID	1SA-12	2SA-12	AVE	3SA-12	4SA-12	AVE	5SA-12	6SA-12	AVE
<16	5.8	1.7	3.8	5.3	3.3	4.3	3.1	6.4	4.8
16:0	14.1	12.1	13.1	12.8	13.8	13.3	12.1	11.3	11.7
16:1	5.5	5.9	5.7	9.7	8.2	9.0	5.8	4.9	5.4
16:2	0.7	0.3	0.5	0.0	0.0	0.0	0.0	0.6	0.3
18:0	6.8	3.0	4.9	3.9	2.7	3.3	3.9	3.0	3.5
18:1	59.0	47.9	53.5	36.2	29.7	33.0	40.0	26.0	33.0
18:2	0.0	24.3	12.2	29.2	41.6	35.4	32.9	46.6	39.8
18:3W	2.0	1.3	1.7	0.4	0.0	0.2	0.9	0.7	0.8
20:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
20:3	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1
20:4	0.0	0.3	0.2	0.5	0.7	0.6	0.3	0.3	0.3

FIGURE 1: The expected fatty acid profiles of the adipose tissue of mice fed different diets.







### Summary

Fatty acids analysis showed that alteration of dietary fat intake caused changes in the adipose tissue fatty acid composition. The rate of change was more rapid in the older mice than in the younger mice. The SA-1 mice had a quicker rate of change than the SAF mice. At the end of six weeks, the SA-1 mice had a fatty acid profile very close to that of SAF mice. The SAF mice, after six weeks, were not as close to the SA-1 profile, the ten month being closer than the five month.

The SAF mice lost weight, accompanied by increases in stearic, oleic, and palmitoleic acids and a decrease in linoleic, the polyunsaturated fatty acid. The SA-1 mice followed the opposite pattern. Thus, diets high in PUFA led to high percentages of PUFA in the adipose tissue and weight gain. Diets high in stearic acid, a saturated fatty acid, led to large stores of stearic acid in the adipose and weight loss.

In conclusion, an interrelationship between dietary intake, fatty acid composition of adipose tissue and weight gain has been demonstrated by this project. Age was also shown to play a minor role in the incorporation of fatty acids and the rate of change.

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