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Effects of Diet, Age, Strain and Anatomical Site on Fat Depot Triglyceride and Fatty Acid Content in Rats (38915)[1,2]

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Obesity is one of the most common forms of malnutrition in the U.S. afflicting 30–40% of the population (1). Furthermore, untimely deaths from certain chronic illnesses such as diabetes and cardiovascular diseases are more common among the obese (2). Although the ultimate study of man is man, there are limitations to investigations with human subjects. Therefore, appropriate animal models are helpful in elucidating pertinent factors which eventually should be tested in man. The fact that Osborne Mendel (OM) rats become very obese when fed a high fat ration whereas S 5B/Pl (S) rats do not (3), makes comparisons between these two strains of rats valuable for nutritional studies which evaluate obesity.

The present investigation assesses the effects of diet, age, strain and anatomical site on weight, triglyceride and fatty acid composition of three selected fat depots in rats.

Materials and Methods. Forty-eight male Osborne Mendel (OM) and 48 male S 5B/Pl[3] (S) rats were weaned at 22 ± 1 days of age. Twelve rats from each strain were sacrificed at weaning in order to establish base lines for fat depot weights, triglyceride (TG) content and fatty acid composition of the inguinal, epididymal and perirenal–retroperitoneal fat depots. The remaining 36 rats from each strain were divided into two groups of 18 rats each according to litter number and body weight at weaning. One group was fed a high fat (60% fat w/w) while the other group was fed a grain (3% fat w/w) ration (4). All other nutrients were adequate. Six rats from each strain and each ration group were sacrificed at 4, 10 and 20 wk postweaning. To assure that no more than two littermates were killed at the same age for any one diet group, the time of sacrifice for each rat was designated at weaning.

Water and food were provided ad libitum to all rats. The animals were housed in individual wire screen cages in a laboratory lighted for 12 hr each day and with the temperature controlled at 23 ± 1°.

At sacrifice all rats were exposed to an overdose of ether. In order to decrease the quantity of blood retained in the fat depots, the rats were decapitated with a guillotine and bled. The inguinal, epididymal and perirenal–retroperitoneal fat depots were removed from all rats according to the procedure described by Schemmel *et al.* (5). Bilateral fat depots are the same weight (3, 6, 7) therefore, only the right fat depot was removed from postweanling rats. The fat depots weighed much less in the weanling rats than in the older rats; therefore, both the right and left depots from one anatomical site were removed from two weanling rats to provide one sample. Fat depots were placed in a polyethylene bag, atmospheric air was replaced with nitrogen and the bag was sealed and stored in a freezer (0° or below) until analyzed.

Total triglyceride (TG) in each fat depot was determined according to the method of Ostrander and Dugan (8). Instead of using the Waring Blendor as these authors did, the entire fat depot was macerated in an Omni-mixer. The capacity of this mixer was

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[2] NIH predoctoral trainee, GM 0810-02.

[3] The pedigreed S 5B/Pl Cr breeding stock was secured from Samuel M. Poiley, Head, Mammalian Genetics and Animal Production Section DR and D, Chemotherapy, National Cancer Institute, DHEW.

TABLE I. MEAN BODY AND SELECTED FAT DEPOT WEIGHTS OF OM AND S MALE RATS AT WEANING AND AFTER FEEDING GRAIN OR A HIGH FAT RATION.

Strain and ration[a]	N	Body weights[b] (g)	Carcass fat[c] (g)	Fat depot weights		
				Inguinal (g)	Epididymal (g)	Perirenal–retroperitoneal (g)
		Time on experiment—0 wk Age—22 days				
OM	6	65 ± 4[d]	7.51	0.91 ± 0.21[d]	0.14 ± 0.04	0.13 ± 0.09
S	5	56 ± 4	3.63	0.44 ± 0.10	0.13 ± 0.04	0.05 ± 0.03
		Time on experiment—4 wk Age—50 days				
OM-G	6	267 ± 5	31.19	3.01 ± 0.19	1.30 ± 0.08	1.12 ± 0.13
OM-HF	5	339 ± 12*	83.77	9.70 ± 0.84*	4.86 ± 0.38*	4.63 ± 0.44*
S-G	6	157 ± 8	7.94	0.98 ± 0.06	0.47 ± 0.92	0.16 ± 0.03
S-HF	5	150 ± 6	15.54	2.16 ± 0.39	0.73 ± 0.17	0.55 ± 0.08
		Time on experiment—10 wk Age—92 days				
OM-G	5	399 ± 7	49.63	4.99 ± 0.29	2.86 ± 0.27	2.93 ± 0.22
OM-HF	5	535 ± 25*	187.30	17.66 ± 2.37*	8.95 ± 0.75*	10.32 ± 0.95*
S-G	5	254 ± 4	22.35	2.48 ± 0.13	1.27 ± 0.23	0.96 ± 0.05
S-HF	6	256 ± 5	30.94	3.27 ± 0.19	1.60 ± 0.12	1.41 ± 0.55
		Time on experiment—20 wk Age—162 days				
OM-G	5	494 ± 21	58.57	5.71 ± 0.55	3.59 ± 0.25	3.82 ± 0.33
OM-HF	5	724 ± 16*	268.03	28.58 ± 2.25*	13.57 ± 0.84*	29.93 ± 3.26*
S-G	5	306 ± 9	33.01	2.72 ± 0.43	1.73 ± 0.21	1.10 ± 0.16
S-HF	6	348 ± 9*	51.41	4.88 ± 0.75	2.43 ± 0.14	2.91 ± 0.44

[a] Strain refers to Osborne Mendel (OM) or S 5B/PlCr (S), an inbred strain of rat. The breeding stock for the latter animals was obtained from Samuel M. Poiley, Head, Mammalian Genetics and Animal Production Section, DR&D, Chemotherapy, National Cancer Institute. Rats were fed grain (G) or a high fat ration (HF).

[b] Live body weight.

[c] Grams of carcass fat were determined from appropriate prediction equations as published by Grewal, *et al.* (13) and verified by comparing to similar groups of rats analyzed for carcass fat (3).

[d] Mean ± SEM.

* When strain and age were constant, there was a significant difference between diets, $P < 0.01$. No statistical comparisons were done for carcass fat.

more appropriate for the small fat pad samples and thus, all reagents were reduced to one half the volume outlined in the method. One half of the reduced sample provided sufficient material to analyze each fat depot site for TG in triplicate. Instead of using steam evaporation procedures to eliminate the organic solvents as outlined by Ostrander and Dugan (8), the Golfisch apparatus was employed to recover the organic solvents from the fat. The latter material remained in the previously tared flask, was dried, weighed and the difference in weight represented the weight of the TG in the sample. The other half of the sample was used for fatty acid analyses. An aliquot of each sample was taken, the chloroform–methanol layer was removed by a flash evaporator and the remaining fat methylated. Fatty acid methyl-esters were kept in individual screw top glass bottles and frozen until analyzed. Composition of the methyl esters of the fatty acids was determined using a F and M gas-liquid chromatograph with flame ionization detector. A 6-foot column containing Diatoport S (80–100 mesh) coated with 5% DEGS was used. The instrument was programmed for 145°–190° at 5° per min. Peak areas were measured by triangulation and expressed as percent of the entire area. Seven fatty acids were identified by this method and taken to be 100% of the fatty acids of the triglyceride fraction of each depot.

Data were statistically evaluated by analyses of variance (9) and Duncan's test (10) for comparison of means.

Results and Discussion. Body weight. OM rats fed a high fat ration *ad libitum* were significantly heavier than littermate rats of the same age fed grain ($P < 0.01$; Table I). S rats were much lighter than OM rats ($P < 0.01$) and when they were fed a high fat diet they maintained a body weight similar to grain-fed S rats ($P < 0.05$) Since rats (3, 11) and mice (12) of many strains become overweight and frequently obese when fed a high fat diet, the ability of the S rats to maintain "normal" body weight when fed a high fat ration is unique and needs further investigation.

Carcass fat. These data were obtained from the weight of one or three selected fat depots (5) and the appropriate prediction equation (13) for groups of rats. For weanling rats, the weights of the inguinal, genital and perirenal fat depots in the age-ration-sex equations were used for predicting body fat. For 50-day old rats the weights of the same three fat depots were used but the strain-sex equations were applied. There was no prediction equation for S rats therefore the equation for the smallest rats (Gray) was used. This may have contributed to a slight overprediction of body fat in S rats of this age group. At both 92 and 162 days of age, only the inguinal fat depot weights were used and the appropriate age-ration-sex equations were applied to determine carcass fat. The over-all results indicated that OM rats accumulated a great deal of body fat when fed a high fat ration whereas S rats did not (Table I) and were consistent with previously analyzed data (3).

Fat depot weights. The inguinal, epididymal and perirenal–retroperitoneal fat depots were 3–4 times heavier in OM rats fed the high fat ration than they were in the same strain of rats fed grain. (Table I). In all cases, diet made a significant difference in weight ($P < 0.01$). That this is true for most rats was demonstrated in our laboratory when we observed similar results in six other strains of rats.[4] Wistar rats under the investigation of Lemmonier (14) responded similarly when fed a high fat diet. The same phenomenon was observed in Swiss mice (15). Although there was a tendency for S rats fed a high fat ration to have heavier fat depots than those fed grain, comparison of mean weights of fat depots between the two dietary groups but of the same anatomical site and age were not significantly different as tested by analysis of variance and Duncan's multiple range test.

A reasonable explanation for the large differences in strain response to the high fat diet could be that genetic factors intrinsic to the fat depots regulate incremental growth and developmental patterns of the lipid-free mass and lipid content. This has also been suggested by others (7, 16).

In general, the inguinal fat depot weighed more than the epididymal or perirenal-retroperitoneal fat depots. The one exception was the perirenal–retroperitoneal fat depot of OM rats fed a high fat ration. For this group of rats the mean weight gain of the perirenal–retroperitoneal fat depot between 10 and 20 wk postweaning was 16.4 g whereas the mean weight gain of the inguinal fat depot was 9.0 g for the same period of time. In older OM rats, the perirenal–retroperitoneal fat depot was heavier than the inguinal (5). Apparently, the variation in developmental patterns associated with the anatomical site of the fat depot as shown in rats also exist in man, since Škerjl (16, 17) and Young, *et al.* (18) reported that intra-abdominal fat depots represented a higher proportion of body weight in older than in younger women.

When weights of fat depots (same strain and ration) from any one site were compared for two successive ages, i.e., 4 vs 10 and 10 vs 20 wk, OM rats fed the high rat ration showed a consistent and significant ($P < 0.01$) increase in weight for all six comparisons. In all other cases, there were no significant differences in fat depot weights between successive age groups, although there was an overall tendency to increase in weight with age. In general, for the entire 20 weeks of the study, the incremental growth of all fat depots, regardless of ra-

[4] Schemmel, R. and O. Mickelsen. Unpublished data.

TABLE II. TRIGLYCERIDES EXPRESSED AS GRAMS PER 100 g OF WET ADIPOSE TISSUE FOR THREE FAT DEPOT SITES IN OM AND S MALE RATS AT WEANING AND AFTER FEEDING GRAIN OR A HIGH FAT RATION.

Strain and ration[a]	N	Inguinal	Epididymal	Perirenal–retroperitoneal
		Triglycerides (g/100 g adipose tissue)		
Time on experiment—0 wk Age—22 days				
OM	6	76.43	78.61	80.66
		±4.82[b]	±2.18	±2.46
S	5	67.40	77.43	78.81
		±1.73	±5.02	±3.98
Time on experiment—4 wk Age—50 days				
OM-G	6	67.59	85.62	90.73
		±0.85	±2.29	±1.80
OM-HF	5	82.87	93.36	93.12
		±1.32	±1.14	±0.72
S-G	6	64.81	86.08	89.12
		±1.94	±1.53	±3.77
S-HF	5	76.39	93.35	91.03
		±1.74	±2.21	±1.78
Time on experiment—10 wk Age—92 days				
OM-G	5	56.40	89.60	92.48
		±2.28	±2.03	±1.81
OM-HF	5	79.66	94.55	92.74
		±3.07	±1.04	±1.15
S-G	5	66.52	91.96	94.03
		±2.61	±0.61	±1.38
S-HF	6	74.08	91.50	92.35
		±2.09	±0.76	±0.38
Time on experiment—20 wk Age—162 days				
OM-G	5	64.53	91.61	92.82
		±3.53	±1.58	±1.20
OM-HF	5	86.63	92.78	94.86
		±0.44	±0.65	±0.99
S-G	5	61.15	91.36	93.23
		±2.16	±0.92	±1.17
S-HF	6	72.58	92.97	94.51
		±2.25	±0.78	±1.05

[a] See Table I for explanation of strain and ration.

[b] Mean + SEM.

tion, strain or anatomical site was greater than that of the whole body. However, by 20 wk postweaning, the incremental weight gain of the inguinal fat depot in high fat-fed OM rats had slowed. It had been previously observed that the relative weight gain of the inguinal fat depot was proportional to the gain in body weight of older OM rats fed a high fat ration (5).

Triglyceride (TG) content of fat depots. The absolute weight of triglyceride for any one fat depot increased as the weight of the fat depot increased. In general, the inguinal fat depot contained less TG than either the epididymal or perirenal-retroperitoneal when the weight of TG was expressed as grams per 100 g adipose tissue (Table II). The differences between sites were significant for all groups of rats fed grain ($P < 0.01$) except for the comparisons between the inguinal and epididymal sites at 4 wk postweaning. Earlier, Hausberger and Hausberger (6) made a similar observation on a very limited number of rats. In contrast, when rats were fed a high fat ration, the only significant difference observed ($P < 0.01$) in the percentage of TG among fat depots was that between the inguinal and perirenal–retroperitoneal for S rats at 162 days of age. Although there was a fairly consistent tendency for rats fed a high fat ration to have a higher percentage of TG in adipose tissues than rats fed grain, the values were never significant as long as age, anatomical site and strain were kept constant. Strain had no significant effect on the percentage of TG in the fat depot. As long as site, ration and strain were constant, the percentage of TG in adipose tissue was the same between 4 and 20 wk postweaning. Inguinal fat depots of rats fed grain tended to decrease in the percentage of TG from that observed at the time of weaning but not significantly so. For older rats fed a high fat ration, the percentage TG in inguinal fat depots was higher but not significantly so from that observed at weaning. There was a consistent but insignificant increase in the percentage TG in the epididymal and perirenal–retroperitoneal fat depots from 22 to 50 days of age regardless of strain or ration.

Fatty acid composition. The fatty acid composition of the TG of the inguinal, epididymal and perirenal-retroperitoneal fat depots in both strains of rats and fed the experimental rations for 10 wk are presented in Table III. In this study, when strain and

TABLE III. MEAN FATTY ACID COMPOSITION EXPRESSED AS g PER 100 g FATTY ACIDS FOR THREE SELECTED FAT DEPOT SITES IN OM AND S MALE RATS FED EITHER A GRAIN OR A HIGH FAT RATION FOR 10 WEEKS FROM WEANING.

Strain and ration[a]	N	Fatty acid composition (g/100 g fatty acids) 14 : 0	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	18 : 3
		Inguinal						
OM-G	5	2.81 ±0.22[b]	24.58 ±0.62	14.06 ±0.98	1.86 ±0.25	31.02 ±0.47	24.45 ±0.75	0.99 ±0.12
OM-HF	5	0.61 ±0.06*	15.15 ±0.75*	3.05 ±0.34*	7.74 ±0.55*	46.56 ±0.49*	25.17 ±0.89	2.06 ±0.28*
S-G	5	3.32 ±0.39	24.68 ±1.09	9.69 ±0.38	2.60 ±0.43	30.10 ±0.18	28.95 ±0.55	0.79 ±0.11
S-HF	6	1.31 ±0.15*	12.32 ±0.21*	3.74 ±0.31*	8.22 ±0.30*	44.86 ±0.55	28.22 ±0.62	1.31 ±0.13
		Epididymal						
OM-G	5	1.92 ±0.16	25.83 ±0.58	13.60 ±0.99	1.99 ±0.35	30.49 ±0.90	24.34 ±0.48	1.82 ±0.17
OM-HF	5	1.47 ±0.12*	16.59 ±0.57*	3.38 ±0.26*	6.75 ±0.50*	44.05 ±0.37*	25.70 ±0.63	2.06 ±0.08
S-G	5	3.40 ±0.22	22.16 ±0.44	11.60 ±0.47	2.36 ±0.16	30.67 ±0.87	28.95 ±0.61	1.48 ±0.14
S-HF	6	1.58 ±0.24*	12.40 ±0.32*	3.68 ±0.22*	5.84 ±0.19*	45.18 ±0.32*	29.45 ±0.26	1.89 ±0.12
		Perirenal-retroperitoneal						
OM-G	5	2.51 ±0.44	25.96 ±1.41	11.49 ±1.19	2.77 ±0.40	32.97 ±0.42	23.20 ±0.38	1.16 ±0.14
OM-HF	5	0.61 ±0.06*	16.38 ±0.30*	3.24 ±0.10*	6.19 ±0.17*	46.02 ±0.43*	26.34 ±0.34	1.22 ±0.13
S-G	5	3.24 ±0.18	27.86 ±0.42	14.60 ±0.81	2.75 ±0.46	30.45 ±1.22	20.07 ±0.72	1.05 ±0.08
S-HF	6	1.04 ±0.18*	12.53 ±0.27*	2.22 ±0.24*	9.14 ±0.69*	45.83 ±0.35*	28.20 ±0.34	1.04 ±0.16

[a] See Table I for explanation of strain and ration.

[b] Mean ± SEM.

* Ration caused a significant difference ($P < 0.01$) in fatty acid composition providing strain and fat depot site were held constant.

ration were constant, site caused no statistical differences in the fatty acid composition of the fat depot. However, several investigations suggest that fatty acid composition varies from subcutaneous to intra-abdominal fat for other species such as humans (19), rabbits (20) and cattle (21).

For rabbits (20) it was reported that subcutaneous fat was more highly unsaturated than perinephric fat. However, the quantities of saturated and unsaturated fatty acids in the inguinal and perirenal–retroperitoneal fat depots of the rats investigated in this study were similar.

Even though the quantitative distribution of specific fatty acids in fat depots of rats fed the high fat ration did not accurately reflect the dietary composition, it was strongly influenced by the diet. The percentage of stearic acid was slightly lower in carcass fat than in dietary fat, whereas the percentage of myristic, palmitoleic and oleic acids were higher in carcass than in dietary fat. This observation is not new, since several investigators evaluating various species (19, 22, 23) have noted that the composition of body fat was influenced by dietary fat. However, when dietary fat was

TABLE IV. FAT AND FATTY ACIDS ACCUMULATED IN THE CARCASS FROM DAYS 22–92 OF OM AND S MALE RATS FED GRAIN OR A HIGH FAT RATION.

Strain and ration[a]	N	Fat (g)	Fatty acids (g)						
			14 : 0	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	18 : 3
OM-G	5	42.1[b]	0.95	10.90	5.12	0.88	12.82	11.69	0.35
OM-HF	5	179.8	3.28	26.52	6.08	13.58	84.94	50.80	2.87
S-G	5	18.7	0.41	4.57	2.28	0.39	5.70	5.21	0.17
S-HF	6	27.3	0.27	3.44	0.60	0.31	12.90	7.62	0.44

[a] See Table I for explanation of strain and ration.

[b] Mean values for five or six rats were calculated by subtracting mean total carcass fat and fatty acids in weanling rats (22 days) of the appropriate strain from the mean total carcass fat and fatty acids of 92 day-old rats fed grain or a high fat ration.

minimal as in the grain ration, the carcass fatty acid composition bore little relationship to the dietary fatty acid composition. In that case, only two fatty acids, stearic and oleic, were similar whereas the percentage linoleic acid was much lower in carcass TG than in the ration. On the other hand, the percentage of myristic, palmitic and palmitoleic were higher in fat depot TG than in the grain ration. It is of interest that approximately 31% of the fatty acids were saturated and approximately 68% were mono- or polyunsaturated in rats fed grain whereas for rats fed the high fat ration, the values were approximately 22 and 77%, respectively.

In most human studies, it is difficult to evaluate whether strain or environment (wherein diet is one of the major factors) contributes more extensively to the differences seen in fatty acid composition (24–26). However, for two strains of rats which vary widely in weight gain when fed a high fat ration, there was no variation in the percentage fatty acid composition of triglycerides (See comparisons for OM-HF and S-HF rats, Table III). This is true even though the S-HF rats deposited only 15% as much fat in their bodies as did OM rats (27 g for S-HF rats vs 180 g for OM-HF rats; Table IV). For the major fatty acid constituents of the fat depot, the ratio was approximately the same; for example, oleic acid at 12.9 g for S-HF rats and 84.9 g for OM-HF rats (Table IV). It is of interest that S-HF rats ate 67% as much fat as OM-HF rats during the same time interval.

A comparison of the fatty acid composition of fat depots of 50, 92 and 162 day-old OM rats fed the high fat ration indicated that there were no statistical differences related to age. However, for rats fed the high fat ration, there was a significant alteration ($P < 0.01$) in the fatty acid composition of the fat depots between 22 and 92 days of age. It would appear that this change had already occurred by 50 days of age since there were no statistical differences in fatty acid composition between rats fed the high fat ration for 4 and 10 wk. Since the diet for these rats was extensively altered at 22 days of age, the changes in fatty acid composition are more apt to be related to dietary differences than age *per se*.

A comparison between the fatty acid composition of weanling rats and rats fed grain for 10 weeks revealed no statistical differences. However, the rats used in this study were raised in our laboratory and nursed by dams fed grain. Nursing animals are known to begin to eat solid food by 16 days of age and this may have accounted for a failure to observe changes in fatty acid composition of the 22 and 92 day-old rats fed grain. Whether or not there was an alteration in fatty acid composition of the fat depots between 16 and 22 days of age was not evaluated in this study.

Summary. OM rats fed a high fat ration were heavier, fatter and had fat depots which weighed at least three times as much as those fed a low fat (grain) ration. S rats fed a high fat ration showed a slight increase in fat depot weights and no increase in body weight or fat when compared to rats of the same strain fed grain. For both strains of

rats fed either diet for 4, 10 or 20 wk following weaning, there was a lower percentage of TG in the inguinal depot than in the epididymal or perirenal–retroperitoneal fat depots. There was a tendency for rats fed a high fat ration to have a higher percentage of TG in adipose tissues than rats fed grain, but the differences were not significant as long as age, anatomical site and strain were constant. Strain and age had no significant effect on the percentage of TG in fat depots. The fatty acid composition of the fat depots was effected by ration, but not by age, strain or anatomical site of the fat depot.

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