Adipose tissue cellularity in woodchucks: effects of season and captivity at an early age[1]

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Abstract The objectives of this study were to determine the roles of adipocyte hypertrophy and hyperplasia in the prehibernatory weight gain of adult woodchucks and in the increased body weight of woodchucks born in captivity. The seasonal increase in weight in wild adult woodchucks was associated with an increase approaching tenfold in both body fat and in subcutaneous and retroperitoneal adipocyte size. There was no increase in total adipocyte number. Four groups of woodchucks were used in the study of the effect of captivity: I) animals born to females bred in the laboratory; II) those born to females captured just before parturition; III) those captured at weaning; and IV) animals captured at 12 months of age. At 14 months non-fat body weight and subcutaneous adipocyte size were equal in the four groups. The males but not the females in Groups I, II, and III had both an increased body fat content and a significantly increased total adipocyte number in comparison to the males in Group IV and the adults in the seasonal study. ■ This study demonstrates that captivity at an early age, unlike prehibernatory weight gain, is associated with an increased adipocyte number in male woodchucks, and this increase can occur after weaning.—**Young, R. A., L. B. Salans, and E. A. H. Sims.** Adipose tissue cellularity in woodchucks: effects of season and captivity at an early age. *J. Lipid Res.* 1982. **23:** 887–892.

Supplementary key words adipocyte hypertrophy • adipocyte hyperplasia • sex differences • hibernation

Many hibernators deposit large amounts of body fat as they prepare for hibernation. The woodchuck or groundhog, *Marmota monax*, may double in weight from spring to fall (1, 2). Findings in other hibernators including chipmunks and ground squirrels (3) and bats (4) show that the seasonal weight increase in those species is in the form of fat, while the non-fat body mass remains constant. Woodchucks raised in captivity continue to show these annual cycles of body weight, and presumably body fat, but they attain considerably higher body weights than do wild animals of comparable age (2).

The growth of adipose tissue can occur by increases in the number of fat cells, the sizes of the individual fat cells, or both. It was believed until recently that, in normal chow-fed rats and mice, a maximum number of adipose cells was attained during the postnatal, prepubertal growth period, and further growth was the result of cell enlargement rather than cell proliferation (5). Similarly in humans, adipose tissue cells were assumed to be formed early in life, and after adolescence fat cell number remained fixed (6). Weight gain and weight loss in adult rats (7) and in adult humans (8) are usually associated with changes in the sizes of the individual fat cells rather than increases or decreases in the number of body fat cells. It is now apparent, however, that under certain circumstances increased adipose tissue cell numbers can occur in both rats (9) and man (10) at an older age.

The objectives of the present study were to determine whether the permanent weight gain induced by captivity affects adipose tissue morphology differently from the transient weight gain that occurs prior to hibernation.

MATERIALS AND METHODS

Animals

For the study of seasonal changes, adult (greater than 14 months of age) woodchucks were captured in the wild during May, June, August, and October. They were housed in individual rabbit cages in a room maintained at 16–20°C with a light cycle synchronized with time of sunrise and sunset and were fed Purina Laboratory Chow ad libitum. All were studied after a 14-day holding period which served to stabilize the animals' body weights and allow the elimination of diseased individuals.

Abbreviations: TBW, total body water; RP, retroperitoneal; SC, subcutaneous.

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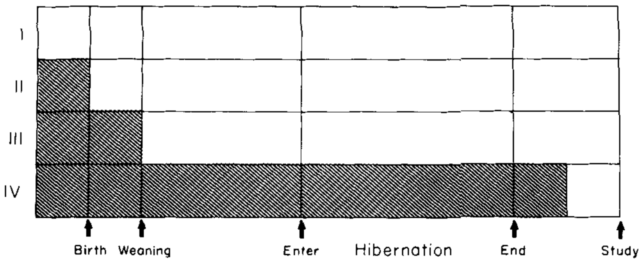

Fig. 1. Schematic representation of the four groups in the study of the effects of captivity. The open areas represent the time spent in captivity. I. Woodchucks born to females bred in the laboratory. II. Animals born to females captured just prior to parturition. III. Animals captured at weaning. IV. Animals captured at 12 months of age.

Four groups of animals were used in the studies of the effects of captivity. Group I consisted of woodchucks born to dams that had been in captivity during the entire gestational period (30–32 days). The average litter size was 4.7, and all pups survived until weaning (5 to 6 weeks of age). Nine animals from three litters were studied. Group II consisted of woodchucks born to dams captured just a few days before they gave birth. As woodchuck pups are born during the month of April in Vermont, it was possible to time the trapping accordingly. The average litter size for this group was four, all survived until weaning, and eight animals from three litters were studied. Group III consisted of animals captured at weaning in early June. Young woodchucks leave the burrows for short intervals at this time and are relatively easy to catch. Seven animals from five litters are included in this group; two pups were captured at each of two burrows, and those captured from the same burrow can be assumed to be littermates. Group IV consisted of woodchucks captured at 1 year of age. Their age was determined using the criteria of Snyder, Davis, and Christian (1). The four groups are shown schematically in **Fig. 1.**

Animals not born in the laboratory were trapped in four adjacent counties in northwestern Vermont and transported immediately to the laboratory. Animals in Groups I and II were nursed by their own dams and weaned at 5 weeks. Litter sizes and dates of birth and weaning were comparable to those in the wild (11). Pregnant and nursing dams were fed Purina Laboratory Chow ad libitum, as were the pups after they were weaned. The pups were housed two or three per cage during their first year and individually thereafter. The room was kept at 21°C with a seasonally adjusted light cycle from March through September. Groups I, II, and III were placed in a 6°C coldroom without food or water from October through February, during which time all hibernated normally. All animals in the four groups were studied at 14 months of age.

Estimation of body composition and body size

An isotope dilution technique was used for the determination of total body water (TBW). Biological quality tritiated water (^3H_2O, sp act 0.25 mCi/g, New England Nuclear) was diluted to a final concentration of 10 μCi/ml with sterile water and stored at 4°C.

After an overnight fast, the woodchucks were weighed and given approximately 1 μCi ^3H_2O per kilogram body weight intraperitoneally. Serial samples of blood showed that equilibration of the isotope was complete at 2.5 hr. After 3 hr the animals were anesthetized with sodium pentobarbital, and blood was collected by cardiac puncture (seasonal study) or from the toes (captivity study) in heparinized tubes. The plasma was separated and stored at −20°C until assayed.

One hundred-microliter aliquots of either plasma or an appropriate dilution of the injected solution were counted in Aquasol (New England Nuclear) in a Packard Tricarb Scintillation Spectrometer. A known amount of tritiated toluene internal standard (Packard Instrument Co.) was added, the samples were recounted, and the original counts were corrected for quenching. Fifty-microliter aliquots of plasma were weighed and then dried to constant weight, the percent water in the plasma was calculated, and the specific activity of the plasma water was determined. The TBW of the animals was calculated from the dilution of the isotope in the body:

$$\%\ \text{water} = \frac{\text{dps per ml plasma water/dps given}}{\text{body weight (g)}} \times 100$$

The percent body fat was estimated using the formula of Pace and Rathbun (12): % fat = 100 − (% water/0.732). Body fat and non-fat body weight were calculated from the above. The accuracy of this indirect method for the determination of body composition was checked by direct carcass analysis of three comparable animals using a method described previously (13). It was found that the percentage of body fat was underestimated by an average of only two percentage points by the isotope dilution technique.

Determination of adipose tissue cellularity

Samples of fat were taken at the same time as blood was taken for the TBW determinations. For the seasonal study, the anesthetized animals were killed by exsanguination and samples of subcutaneous (SC) fat were taken from the lower abdomen, and retroperitoneal (RP) fat samples were taken from near the kidneys. In the study of the effects of captivity, small incisions were made in the skin of the lower abdomen and of the back approximately over the scapulae. Subcutaneous fat was obtained, and the incisions were closed with stainless steel wire.

The dissected tissues were washed with warm saline. Fragments of adipose tissue were weighed on tared nylon sieves and placed either in 2% osmium tetroxide in a 0.05 M collidine buffer for fixing and cell counting according to the Method III of Hirsch and Gallian (14) or in chloroform–methanol 2:1 for lipid extraction and subsequent triglyceride determination by the method of Rapport and Alonzo (15). All analyses were performed in duplicate. Fat cell size was expressed as μg triglyceride/cell. The total estimated fat cell number was obtained by dividing the total body fat by the average lipid content per cell. The contribution of brown fat to body fat and fat cell number was ignored as brown fat constitutes only 1–5% of the total body fat stores at any one time (1, 16).

The dangers involved in extrapolating the total number of fat cells in an animal from the average adipocyte size in one or two sites in one depot are realized, and have been discussed in a previous paper (8). The exact error in this case is not known, but the subcutaneous fat depot is the largest fat depot in the woodchuck, and the sizes of the fat cells in this depot parallel the total amount of fat in the animal during the seasonal changes in body weight, indicating that the subcutaneous cell size in this species may provide a reasonable estimate of the average adipocyte size of the body.

Statistical analyses

The data were analyzed using both one-way analysis of variance and two-way analysis of variance with group and sex as covariables. Comparisons between groups were made using the Student-Newman-Keuls test (17), reporting significance at $P < 0.05$.

RESULTS

Seasonal study

Body weight and total body fat increased progressively in both males and females from May to October (**Table 1**). The male woodchucks were heavier than the females in June ($P < 0.05$) but not in August and October. There were no significant seasonal or sex differences in non-fat body weight.

The seasonal increases in body fat were reflected in the increasing fat cell sizes in both the retroperitoneal and subcutaneous depots. The average fat cell sizes for the cells of the RP depot were significantly less than those for the SC depot in both June ($P < 0.05$) and August ($P < 0.05$). Total fat cell number was greater in May ($P < 0.05$) than at the other three times. This increase is probably an artifact due to the errors involved in indirectly measuring body fat in animals that are very thin as well as the problems involved in counting very small fat cells. In two of the male woodchucks, no subcutaneous fat could be found.

Effects of captivity

The body weight records for the four groups are presented in **Fig. 2.** Weight records were not kept on the woodchucks until August of the first year. At that time, the animals in Group I were significantly heavier than those in Group II and III ($P < 0.05$). By late August the weights for Groups II and III, which were not significantly different, were also not significantly different from those of Group I.

During the second year the relationships between the mean weights for Groups I, II, and III were maintained.

TABLE 1. Body weight, body composition, and adipocyte size and number in adult woodchucks at four times during the year

		Body Weight	Fat	Non-fat Body Weight	Adipocyte Size[a] Retroperitoneal	Adipocyte Size[a] Subcutaneous	Total Adipocyte Number × 10^6
		kg	*kg*	*kg*	*μg triglyceride/cell*		
May	Males (4)[b]	2.40 ± 0.15[c]	0.16 ± 0.06	2.24 ± 0.15	0.07 ± 0.02	0.16 ± 0.05[d]	2504 ± 492
	Females (1)	2.79	0.36	2.43			
June	Males (5)	3.32 ± 0.14	0.50 ± 0.11	2.82 ± 0.14	0.30 ± 0.03	0.49 ± 0.04	1251 ± 161
	Females (7)	2.85 ± 0.14[e]	0.38 ± 0.04	2.47 ± 0.15			
August	Males (4)	3.61 ± 0.36	0.87 ± 0.40	2.74 ± 0.06	0.70 ± 0.21	1.18 ± 0.25	1496 ± 184
	Females (3)	3.74 ± 0.29	1.24 ± 0.26	2.49 ± 0.10			
October	Males (3)	4.51 ± 0.10	1.73 ± 0.10	2.78 ± 0.20	1.72 ± 0.37	1.46 ± 0.21	1149 ± 109
	Females (4)	4.01 ± 0.32	1.64 ± 0.31	2.37 ± 0.10			

[a] Adipocyte size and number in male and female woodchucks did not differ in June, August, and October, The data are therefore pooled.
[b] The number of animals is in parentheses.
[c] Mean ± SEM.
[d] Data from two males and one female. No subcutaneous fat could be found on the other two males.
[e] Data for female woodchucks significantly different from that for males ($P < 0.05$).

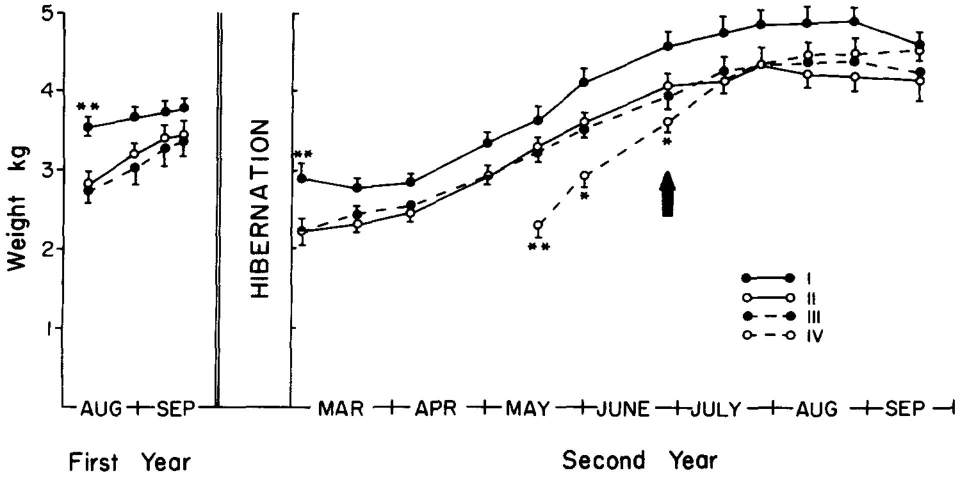

Fig. 2. Body weight curves for the woodchucks in the study of the effects of captivity. The group designations are the same as those in Fig. 1. The arrow shows the time of study and the asterisks represent significant differences of $P < 0.01$ (**) and $P < 0.05$ (*) between the indicated group and the other groups. The mean ± SEM is shown.

The mean weight of Group IV, which was initially lower, reached the others by mid-July.

At the time of the study (late June), the male woodchucks in Group I were significantly heavier than those in Group IV (**Table 2**). There were no significant differences in the body weights of the females. The male woodchucks, but not the females, in Group IV had a significantly lower body fat content than the animals in Groups I and II. There were no statistically significant differences in non-fat body weight or fat cell size among the four groups.

The male woodchucks in Group IV also had significantly lower total fat cell numbers than the animals in the other three groups. This relationship did not hold for the female woodchucks. When the adipocyte number data of males and females were analyzed using a two-way analysis of variance, a significant treatment effect ($P < 0.004$) and a significant sex effect ($P < 0.030$) were found. When the data from the males and females were analyzed separately, it was determined that the treatment effect was the result of the effect on the males ($P < 0.01$), but not on the females ($P < 0.50$).

DISCUSSION

This study indicates that the seasonal accumulation of fat in the adult woodchuck is due to deposition of triglyceride in existing adipocytes, rather than an increase in the number of body fat cells. These results are

TABLE 2. Body weight, body composition, and adipocyte size and number in woodchucks born in the laboratory or captured at different ages

		Body Weight	Fat	Non-fat Body Weight	Adipocyte Size	Adipocyte Number × 10^6
		kg	*kg*	*kg*	*µg triglyceride/cell*	
Group I[a]	Males (6)[b]	4.57 ± 0.09[c]	2.01 ± 0.08	2.55 ± 0.07	0.99 ± 0.07	2108 ± 164
	Females (3)	4.59 ± 0.55	1.91 ± 0.52	2.67 ± 0.15	1.38 ± 0.38	1454 ± 116
Group II	Males (4)	4.34 ± 0.16	1.78 ± 0.14	2.55 ± 0.03	0.90 ± 0.07	1983 ± 59
	Females (4)	3.79 ± 0.16	1.62 ± 0.07	2.16 ± 0.09	0.97 ± 0.05	1695 ± 150
Group III	Males (2)	4.08 ± 0.68	1.71 ± 0.41	2.36 ± 0.27	0.84 ± 0.13	2025 ± 186
	Females (5)	3.88 ± 0.17	1.66 ± 0.22	2.22 ± 0.13	0.95 ± 0.05	1731 ± 161
Group IV	Males (5)	3.62 ± 0.22[d]	1.15 ± 0.09[e]	2.47 ± 0.14	0.91 ± 0.08	1298 ± 151[f]
	Females (4)	3.61 ± 0.29	1.20 ± 0.23	2.42 ± 0.13	0.86 ± 0.19	1453 ± 105

[a] The composition of the groups is as follows: Group I, woodchucks born in the laboratory to females bred in the laboratory; Group II, animals born in the laboratory to wild-caught pregnant females; Group III, animals captured at weaning; Group IV, animals captured at 1 year of age. All were studied at 14 months of age.

[b] The number of animals is in parentheses.

[c] Mean ± SEM.

[d] Significantly different from the males in Group I, $P < 0.05$.

[e] Significantly different from the males in Groups I and II, $P < 0.05$.

[f] Significantly different from the males in Groups I, II, and III, $P < 0.05$.

what one would expect in an animal that gains and loses large amounts of fat every year. Earlier studies have shown that adult woodchucks (2) and other hibernators (18) do not reach progressively higher weights in successive pre-hibernatory weight gain cycles, suggesting that pre-hibernatory weight gain involves no adipocyte hyperplasia. Mrosovsky (18) has described these weight cycles and has postulated that the control of body weight in hibernators depends on a changing "set-point" for body weight. Although it is possible that average fat cell size could decline to compensate for a cell number increase, this is unlikely (9).

The one female studied in May weighed more and had a larger amount of body fat than any of the males in the May group. These differences probably reflect the reserve of fat that the female woodchuck must have for the spring reproductive period. Snyder et al. (1) reported that the wild male woodchucks started to gain weight sooner in the spring than did the females. This was also true in the present study, as the male woodchucks were heavier than the females in June but not in August or September.

The data from the study of the effects of captivity indicate that captivity at an early age results in increased body weight due to increased amounts of adipose tissue, which in turn is due to increases in the numbers of adipocytes in male, but not in female woodchucks. At the time of study (June), the animals in Groups I, II, and III were heavier, fatter, and had larger fat cells than the animals studied in June for the seasonal study. In addition, the males in Groups I, II, and III had an increase in fat cell number when compared to both the animals in the seasonal study ($P < 0.05$) and the males in Group IV. The findings in the woodchuck are in contrast to data obtained with the desert sand rat (*Psammomys obesus*), which also develops obesity in captivity. In the sand rat the obesity appears to be associated with an increase in fat cell size but not in fat cell number (19).

Studies in rats and mice suggest that the nutrition of dams during gestation (20) and the nutrition of pups while nursing (21, 22) may influence the fat cell numbers of pups, the differences persisting into adult life. The diets of the woodchuck dams during pregnancy and lactation might therefore have played a role in the development of the increased numbers of adipocytes found in the male woodchucks born in the laboratory in this study. While animals in the laboratory have free access to food during pregnancy and lactation, animals in the wild have limited access to food during these periods since vegetation is very limited. Therefore, the woodchucks in Groups I and II were relatively overnourished during gestation and nursing as compared to the animals in Groups III and IV.

In view of such differences in the diets of the wild and captive woodchuck dams, it is not surprising that the animals in Groups I and II had more fat and more fat cells than those in Group IV. What is surprising, however, is the elevated fat cell number in the males in Group III, animals that were captured after weaning. The male woodchucks in Groups I and II had little or no increase in fat cell number over the animals in Group III.

Until recently it was thought that post-weaning influences had little or no effect on the ultimate fat cell number of the normal rat (7); the majority of new adipocytes in these animals were formed before weaning (5). However, several studies suggest that under certain circumstances the number of body fat cells can increase in the adult. Adult mice (23, 24), rats (9, 25, 26), and pigs (27) all show increases in adipocyte number in one or more fat depots if fed a palatable diet (high fat or high carbohydrate) for a sufficient length of time. Increases in adipocyte number have also been reported to occur spontaneously in adult male rats fed standard commercial rat diets (28–30).

Faust et al. (9) have postulated that when adipocytes reach a certain critical size during weight gain, this is a stimulus for the production of new adipocytes. The critical adipocyte size in the adult rat appears to be approximately 1.6 μg (9). If this same process is true for the woodchuck, one would expect the critical adipocyte size for adult woodchucks to be greater than approximately 1.72 μg, the maximum size measured in the seasonal study.

Probably none of the groups of woodchucks studied was restricted as to the quantity of food after weaning. Woodchucks in the wild have green vegetation in abundance right outside the burrow from May until the time of hibernation. Purina Laboratory Chow, however, has a somewhat higher protein content, 31.2% (Ralston Purina Co.), than a typical food in the wild such as red clover, which contains 16.5% of protein (31). The Purina Chow has a greater gross energy/g, containing 75% total digestible nutrients and 4.25 kcal/g as opposed to 17% total digestible nutrients and 0.75 kcal/g for red clover. If one takes the water content of the two diets into account, however, they are not that dissimilar, and the dietary alteration caused by feeding the chow diet is much milder than that caused by feeding a high fat or a very high carbohydrate diet to laboratory rats and mice to cause obesity. It is possible that the woodchucks responded to the chow diet in a manner similar to the rats, mice, and pigs fed the palatable diets in the studies mentioned above. One cannot rule out the possible effects of restriction of exercise in this case, however. Regular, forced exercise of rats from before weaning leads to fewer fat cells in the exercised rats compared to the controls kept at comparable weights (32). The animals in Groups I, II, and III were confined to cages, whereas the animals in Group IV were free in the wild during their first year.

The present study demonstrates that captivity in the male woodchuck at an early age is associated with an increase in fat cell number. This can occur after weaning. The reasons for the cell number increases are probably related to an altered energy balance because of changes in the composition or amount of food eaten or decreased physical activity. The hyperplastic obesity in the male woodchuck is not secondary to a neurologic or metabolic disorder and occurs with relatively mild dietary perturbations. The woodchuck therefore has potential as an animal model for the study of the influences of early nutrition and energy balance on the development of hyperplastic obesity.

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