# Increasing Adipocyte Number as the Basis for Perirenal Depot Growth in Adult Rats

Abstract. *The mass of the perirenal adipose depot in male Fischer 344 rats increases between 6 and 18 months of age. This increase is due to an increase in the number of adipocytes in this depot, in contrast with the concept that adipocyte number is constant throughout adult life. The epididymal depot increases in mass between 6 and 18 months of age by adipocyte hypertrophy alone.*

The idea that the number of adipocytes in fat depots remains constant during the adult life of mammals is based on two lines of evidence. First, factors that acutely alter adipose tissue mass such as fasting and experimentally induced adult-onset obesity do not change the number of adipocytes in a depot, but rather alter the mean volume of the adipocyte population (*1–3*). Second, the number of adipocytes in rat epididymal and perirenal depots does not change between 12 and 26½ and between 16 and 26½ weeks of age, respectively (*3*). However, these studies were performed during short periods of the animals' total life-span. Data from research involving longer periods of the life-span disagree with this concept (*4–6*). Lemmonier (*7*) showed that feeding a high fat diet (41 percent fat, by weight) to adult mice and rats increased the number of adipocytes in certain depots. We now report that in male rats (Fischer 344 strain) the number of adipocytes in a depot can markedly increase in adult life without dietary manipulations.

Specific pathogen–free male rats of the Fischer 344 strain (28 days old) were individually housed in a barrier facility and given free access to a diet consisting of 21 percent casein, 15 percent sucrose, 43.65 percent dextrin, 3 percent Solka-Floc, 10 percent corn oil, 0.15 percent DL-methionine, 0.2 percent choline chloride, 5 percent Ralston Purina mineral mix, and 2 percent Ralston Purina vitamin mix. At 6, 12, or 18 months of age, ten rats were fasted for 15 hours, weighed, and killed by decapitation. The epididymal and perirenal adipose tissue depots were excised and analyzed for mass, distribution of adipocyte sizes, and number of adipocytes per depot by a modification of the method of Stiles *et al.* (*6*). The modification consisted of freeing adipocytes from collagenase by three successive flotations at 1*g* in medium containing no collagenase, and sizing cells stained with crystal violet. This procedure did not influence the cell size distribution since the same pattern was obtained when measured before and after washing.

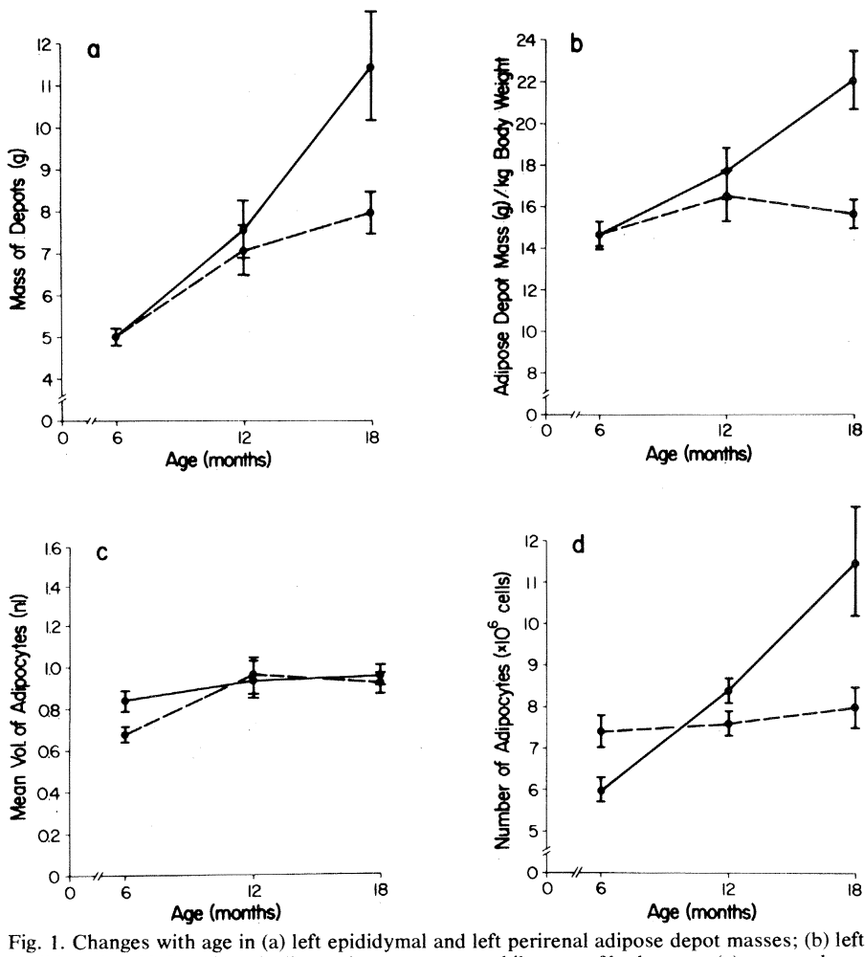


Fig. 1. Changes with age in (a) left epididymal and left perirenal adipose depot masses; (b) left epididymal and left perirenal adipose depot masses per kilogram of body mass; (c) mean volume of adipocytes in epididymal and perirenal depots; (d) number of adipocytes in left epididymal and left perirenal depots. The circles connected by broken lines refer to the epididymal depot and the circles connected by solid lines refer to the perirenal depot. The data reported are mean values ± standard errors for each group of ten animals.

A significant increase was observed in the mass of the epididymal depot (Fig. 1a) between 6 and 12 months ($P < .01$), but not between 12 and 18 months of age. The mass of the perirenal depot markedly increased both between 6 and 12 months ($P < .01$) and between 12 and 18 months of age ($P < .02$).

The data on mass of depot per kilogram of body mass of the rat (Fig. 1b) indicated that the change in epididymal depot mass with age was proportional to the change in body mass. However, there was a significantly greater percentage increase in the perirenal depot mass than in body mass between the ages of 6 and 18 months ($P < .001$).

The mean volume of the adipocytes in the epididymal depot (Fig. 1c) significantly increased between 6 and 12 months of age ($P < .02$) with no further change between 12 and 18 months of age. These changes in mean volume are consistent with the age-related changes in total epididymal depot mass. However, in the perirenal depot, there was no significant change in the mean adipocyte volume between 6 and 18 months of age. By 12 months of age, the adipocytes of both depots have the same mean volume.

No significant change in the number of adipocytes in the epididymal depot occurred between 6 and 18 months (Fig. 1d). In contrast, there was a significant increase between 6 and 18 months ($P < .001$) in the number of adipocytes in the perirenal depots. This increase occurred entirely by increasing cell number.

Our data establish that the number of adipocytes in the perirenal depot is not constant during the adult life of the rat, but can increase markedly during young


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adulthood to late middle age. The mean life-span on the Fischer 344 strain of male rats is 29 months with a maximal survival of 35 months under barrier conditions (*8*). This increase in number of adipocytes need not imply that the cells are derived from mitotic activity of stem cells, but rather they could originate from preadipocytes becoming lipid laden (*9*).

The perirenal depot is of great importance in this strain since it accounts for a large fraction of the total adult adipose mass. A study of the effect of age on lean body mass and adipose tissue mass suggests that the epididymal and perirenal depots each account for about 20 percent of the total adipose tissue mass at 6 months of age (data not shown). However, between 6 and 18 months, the perirenal depot mass increased 2.3-fold compared to a 1.35-fold increase in total adipose mass and a 1.56-fold increase in epididymal depot mass.

The studies on which constant adipocyte theories are based in rats were usually terminated at about 6 months of age (*3*) since an asymptote in adipocyte number seemed to have been reached by that time. However, only a small part of the adult life-span had been studied. The experimental investigations in humans involved a time span of only about 6 months (*1*). Although most data on clinical obesity support the concept that adipocyte number is fixed in adults, there have been some reports of adipocyte hyperplasia in what appears to be adult-onset obesity (*10*).

Studies indicating that adipocyte number may not be constant in the adult have been largely ignored either because a marked dietary perturbation was involved (*7*), or the data could be otherwise interpreted, or the changes noted were not significant. For example, DiGirolamo and Mendlinger (*4*) found that the perirenal depots from 1-year-old guinea pigs have a much larger number of adipocytes than did those from 6-week-old animals; however, it was not clear that this increase occurred during the adult stage of life. A similar problem exists in interpreting the work of Enesco and Leblond (*11*) and Zingg *et al.* (*5*). Although this difficulty is not involved in interpreting the work of Stiles *et al.* (*6*), the age-related increase in the number of epididymal adipocytes was not great.

The data in our report demonstrate that the sole mode of increasing the mass in the perirenal depot of the adult Fischer 344 male rat, a strain not prone to obesity, involves increasing the number of adipocytes either by the genesis of new adipocytes or by the maturation of preadipocytes. In contrast in the same rats the sole mode of increasing the epididymal depot mass is one of adipocyte hypertrophy. However, even in this depot, hyperplasia can occur in adult life; in an earlier study a 30 percent increase in adipocyte number was seen in this depot between 24 and 30 months of age, during senescence. To what extent our findings apply to depots other than the epididymal and perirenal and to other rat strains remains to be explored.

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## Processes Controlling Arm Movements in Monkeys

Abstract. *The experiments identify some of the processes underlying arm movements in rhesus monkeys. Three monkeys were trained to point to a target with the hand and forearm and to hold that position for about 1 second to obtain a reward. Forearm movements were performed without sight of the arm before and after bilateral dorsal rhizotomy. In both intact and deafferented animals, we unexpectedly displaced the forearm prior to movement initiation and observed that the arm moved accurately to the target. These results are relevant to the question of what is being controlled by motor commands. The controlled variable appears to be an equilibrium point between agonist and antagonist muscles. The findings suggest that the feedback system plays a major role in updating and adjusting the central programs subserving the execution of learned motor patterns.*

Certain limb movements elicited by visual stimuli are currently assumed to be controlled by "programs" that generate instructions appropriate for activating the spinal motoneurons. Nothing is known, however, about the organization and the characteristics of these programs; in particular, it is not yet clear which aspects of movement they may control. The experiments reported here are addressed to the latter question. Three adult rhesus monkeys were trained in a pointing task. The monkeys sat in a primate chair with the right forearm fastened to an apparatus that permitted flexion and extension of the forearm about the elbow in the horizontal plane. The pointing task required that the monkey position its limb in front of a small target light. Ten lights were spaced at 5° intervals along a small perimeter arc centered around the axis of rotation of the elbow. The monkeys were trained to point to whichever light was on and to hold the arm at that position for about 1 second. To obtain a reward, the monkey had to point to an electrically defined target zone centered on the target light. The zones were 12° to 15° wide. This width was found to make the task moderately difficult without requiring the monkey to hunt for the target zone with a zigzag approach. In the intertrial interval (3 to 5 seconds) the monkey was free to choose any arm position. The experiments were conducted in a dark room to restrict visual cues to the target; at no time during an experiment was the animal able to see its forearm.

In each trial, the arm was either loaded or unloaded; loads were applied on about 20 percent of the trials by way of a torque motor in series with the shaft of the apparatus. The load most often used was a constant torque load whose onset time, duration, amplitude, and direction were randomized. In most instances, the load was applied within the reaction time of the monkey. Hence, when the motor command specifying a given forearm movement occurred, the positional disturbance had altered the length of the agonist and antagonist muscles, and the proprioceptive stimulation resulting from this disturbance had altered their state of activation. In spite of these changes, the intended final arm position was always reached; this was true


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