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BODY FAT IN PREGNANT RATS AT MID- AND LATE-GESTATION

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Summary

Carcass fat content was estimated in fed 12- and 19-day pregnant rats and fed and 48 hour starved virgin females following both specific gravity determination and direct gravimetry of extracted lipids. No change in body fat accumulation was found in 12-day pregnant rats whereas in 19-day pregnant animals it increased significantly. A significant correlation was also found when the percentage of carcass fat was plotted against specific gravity considering values from all subjects. Results indicate that in spite of reported maternal anabolic changes in the rat at midgestation fat accumulation occurs later in pregnancy when the mother has the highest food intake, which makes available sufficient substrates to support both fetal growth and body lipidic deposition.

Carcass analysis has demonstrated that in the rat there is fat accumulation during gestation (1-5). This change is clearly related to food intake since it does not occur in food restricted (4,5) or food deprived rats (2,6). A similar fat accumulation has been reported in human pregnancy (7,8) accounting for most the maternal weight gain aside from the products of conception. There is, however, no agreement on when this increase in body fat starts during gestation. Beaton et al (6) estimated in samples from fasted rats that maternal fat accumulation began at day 12 of gestation whereas in fed 16-day pregnant rats Fain and Scow (2) found no increase in carcass fatty acid content. The subject deserves attention since on the basis of increases in both fatty acids synthesis in adipose tissue (9) and "in vivo" postheparin lipolytic activity in 12-day pregnant rats but no later (10) a biphasic change of fat metabolism in pregnancy has been proposed (9, 10). Using two different methods to measure body fat (gravimetry in lipid extracts (11) and specific gravity estimation (12)), in the present study carcass lipid content was estimated in fed 12- and 19-day pregnant rats. Results were

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compared to values in fed virgin controls and our methodology was validated by testing the same values in 48 hour starved virgin rats.

Materials and Methods

Female Wistar rats from our own colony were fed a Purina chow diet ad libitum (Panlab, Barcelona, Spain) and mated when weighing 160-180 g (the day spermatozoids appeared in vaginal smears was considered day 0 of gestation). Age matched virgin rats were used as controls. Animals were decapitated by guillotine and heads were discarded. Body hair was removed with electric clippers and the skin was rubbed with a depilatory cream. Following the method of Dahms and Glass (12), lungs, gastrointestinal tract, viscera and conceptus were removed whereas the mesenteric fat remained in each carcass which was suspended from the tail by a tared silk suture and consecutively weighed both in air and under water (24 C) until constant weight (within 0.03 g). Body specific gravity was calculated using the following formula:

$$\text{Specific gravity} = \frac{WA}{(WA-WW).SG}$$

, where WA= carcass weight in air ; WW= carcass weight in water; SG= specific gravity of water at 24ºC (0.997327). After the submerged weight was obtained each carcass was wiped on filter paper and homogenized with a meat mincer. Three 0.5 g aliquots of these homogenates were used for fat extraction and purification in chloroform-methanol (11). Lipid extracts from each rat were pooled in preweighed vials and allowed to evaporate completely, and the lipid content was determined gravimetrically. Results are expressed as means + SEM and statistical comparison between groups was done by the Student's "t" test.

Results

As shown in Table 1, body weight was significantly lower in 48 hour food deprived than in fed virgin rats. While carcass weight did not differ in the two groups, carcass specific gravity was significantly higher and fat content significantly lower in the food deprived animals. In the fed 12-day pregnant rats both body

TABLE 1

BODY WEIGHT AND CARCASS ANALYSIS IN VIRGIN AND LATE PREGNANT RATS

	Body weight (g)	Carcass Weight (g)	Specific gravity	Total fat (g)
Virgins	216±8	164±7	1.0726±.0016	14.7±0.6
Fasted virgins	190±8+	150±7	1.0809±.0009*	10.5±0.8*
12-day pregnants	242±7+	191±3‡	1.0763±.0013	14.3±0.7
19-day pregnants	329±7*	209±6*	1.0648±.0011*	24.0±1.5*

n=7-10 rats/group; Comparisons vs.Virgins: + =p<.05, ‡ =p<.01, * =p<.001

and carcass weight were significantly higher than in age matched virgin controls whereas neither carcass specific gravity nor lipid content differed in the two groups (Table 1). In fed 19-day pregnant rats, body and carcass weights and carcass lipid content were significantly ($p<0.001$) higher than in fed virgin controls, whereas carcass specific gravity was lower ($p<0.001$). As shown in Figure 1, a significant correlation was found between specific gravity and the percentage of carcass fat when all values were pooled together ($p<0.001$).

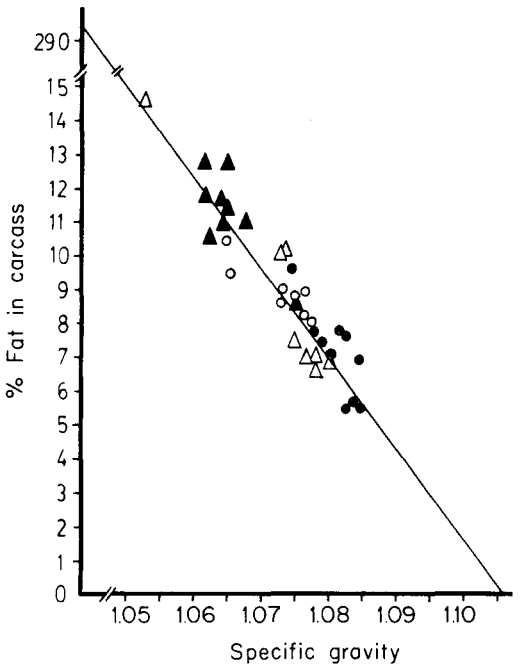

Figure 1.-Relationship between percentage of carcass fat and specific gravity between values of all animals studied. The equation for the regression line is: %Fat= -266.82(Specific gravity) + 295.32,($r=0.91$,$p<0.001$). (O)= Fed virgins, (●)= 48-hour starved virgins , (Δ)= 12-day pregnants , (▲)= 19-day pregnants.

Discussion

Present findings show that at late gestation in the rat there was an increase in fat content in agreement with previous reports (1-5) and that body fat content was not modified by 12 days of gestation, indicating that at this stage of gestation maternal fat storage has not yet increased. This conclusion is supported by measurements of body fat by two different methods: direct gravimetry and estimation of specific gravity. Recorded values are similar to those previously reported for both nonpregnant (12,13) and late pregnant rats (5). Significant reductions in carcass fat, increments in specific gravity found in 48 hour starved virgin animals, and the observed correlation between these two parameters in pooled values from all subjects validate the methodology used and indicate its high sensitivity. In a previous measurement of carcass fat content in 12 day pregnant rats, although values were obtained from pooled samples, they tended to be already augmented (6). This study was performed in

starved animals older than those used here which, together with differences in rat strain and habits, could explain the different results. In another study conducted in fed rats, at the 16th day of gestation no change in body fat was detected (2), which together with present findings indicate that maternal body fat accumulation in the rat does not occur during the first half of gestation. This conclusion is compatible with reported increments in lumbar fat pad size by the 12th gestational day (14) as it is known that changes in the various fat deposits during pregnancy do not occur simultaneously (3,15). An inter-tissular readjustment of fat deposits may occur during the first half of gestation since enhanced lipogenesis (9) and lipoprotein lipase activity (16) in some tissues by midgestation does not occur in others (17). Maternal structures are, however, generally hypertrophied at this stage of gestation, as indicated by the observed increase in maternal carcass weight, and these anabolic changes may be related to the increments in circulating insulin levels known to occur at this gestational time (9). Maternal body fat accumulation probably starts soon after the 12th gestational day and in the present study it is clearly manifest on the 19th day. At this stage of gestation in the rat, maternal metabolic changes tend to favor depletion rather than accumulation of fat, as indicated by reductions in adipose tissue lipoprotein lipase activity (16,18) and increments in adipose tissue lipolysis (19,20). These changes are counteracted by augmented food intake, known to peak at 18-19 days of gestation (5,10), and also by the increased activity of lipogenic enzymes (21) and esterification (19,20) in adipose tissue. The resulting increased availability of substrates allows the mother to synthesize and accumulate lipids even during activation of catabolic pathways of lipid metabolism. The not well understood significance of maternal fat accumulation at late gestation may be due to one or both of the following reasons: a) It guarantees sufficient substrate availability for both maternal and fetal metabolic needs whenever food is scarce. The accelerated response to fasting occurring at late gestation (22) produces increased maternal expenditure of endogenous resources. Augmented ketonemia (23) and use of glycerol as a major gluconeogenic substrate (24) in the fasted late pregnant rat support this hypothesis. (b) It serves to promote lactation. Decrements in both body fat storage (25) and maternal hypertriglyceridemia (26) occur prior to parturition, coinciding with increments in mammary gland lipoprotein lipase activity (26), both changes driving tissue accumulated fat and circulating triglycerides to the mammary gland for milk synthesis.

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