Insulin-like growth factor I mediates high-fat diet-induced adipogenesis in Osborne-Mendel rats

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Insulin-like growth factor I mediates high-fat diet-induced adipogenesis in Osborne-Mendel rats. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R654–R662, 2000.—To determine if high-fat (HF) diet-induced changes in adipose tissue cellularity are associated with the presence of paracrine growth factor(s) that alter preadipocyte proliferation, Osborne-Mendel rats were fed either a HF (76% energy) or a low-fat (LF, 12% energy) diet for 85 days. HF-fed rats had greater (P < 0.05) fat pad size, total fat cell number, number of small (30–70 µm) and large (80–140 µm) adipocytes, and percentage of 100- to 140-µm adipocytes compared with LF-fed rats. Preadipocytes in primary cell culture treated with inguinal adipose tissue conditioned medium (ATCM) prepared from HF-fed rats had greater (P < 0.05) proliferation compared with cultures treated with ATCM from LF-fed rats. Proliferative capacity of ATCM prepared from HF-fed rats was attenuated after the stripping of the medium of insulin-like growth factor I using an immunomagnetic bead separation system. These data are consistent with the concept that insulin-like growth factor I is involved in the paracrine regulation of adipogenesis.

MATERIALS AND METHODS

Materials. Diet supplies were purchased from United States Biochemical (Cleveland, OH). Osmium tetroxide was ob-
were taken from Electron Microscopy Sciences (Fort Washington, PA). Collagenase type I was procured from Worthington Biomedical (Freehold, NJ). PBS was purchased from InterGen (Purchase, NY). Nylon mesh screens were obtained from Tetko (Briarcliff Manor, NY). Cell culture reagents were purchased from Sigma (St. Louis, MO). Supplies for tissue IGF-I double antibody RIA included anti-IGF-I (UB3–189), a gift from the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases (distributed by Ogden BioServices, Rockville, MD); recombinant human IGF-I from Bachem (Torrance, CA); standard and 125I-labeled IGF-I from New England Nuclear; and 4% formaldehyde (500 ml) from Electron Microscopy Sciences (Fort Washington, PA). Collagenase type I was procured from Worthington (Freehold, NJ). The second antibody, sheep, anti-rabbit, antiguinea pig, was a gift from the Animal Physiology Unit of the University of Pennsylvania (Philadelphia, PA). The second antibody, sheep, anti-rabbit, antiguinea pig, was a gift from the Animal Physiology Unit of the University of Pennsylvania (Philadelphia, PA). The second antibody, sheep, anti-rabbit, antiguinea pig, was a gift from the Animal Physiology Unit of the University of Pennsylvania (Philadelphia, PA).

Animals and diets. Male Osborne-Mendel rats (61–140 g) were obtained from National Institutes of Health Genetic Resources (Bethesda, MD) and were housed in individual wire-bottom cages in a room environmentally regulated for temperature (23 ± 3°C) and humidity (40–50%) with a 12:12-h light-dark cycle. All procedures for the care of the animals used in this study were approved by the University of Georgia Institutional Animal Care and Use Committee.

During an initial 10-day period of acclimation, the rats had free access to water and a nonpurified diet (Purina Rodent Chow; Ralston Purina, St. Louis, MO). They were then ranked by body weight and allocated into two dietary treatment groups (Table 1) such that mean body weight did not differ between groups (Table 2). Energy contribution by lipid was 76% for the HF diet and 11.7% for the low-fat (LF) diet. The casein content of the HF diet was adjusted so the protein-to-energy ratio was not different from the LF diet. Rats had free access to their respective diet and tap water for R rats had free access to their respective diet and tap water for R rats/

Table 1. Diet composition

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/100 g diet</th>
<th>Low Fat</th>
<th>High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>19.2</td>
<td>31.1</td>
<td></td>
</tr>
<tr>
<td>Cornstarch</td>
<td>55.8</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Tallow</td>
<td>0.0</td>
<td>47.8</td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Minerals</td>
<td>3.5</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Vitamins</td>
<td>10.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>L-cysteine</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Energy density, MJ/100 g diet</td>
<td>1.61</td>
<td>2.63</td>
<td></td>
</tr>
<tr>
<td>Percent energy</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Protein</td>
<td>19.9</td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>68.3</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td>11.7</td>
<td>76.1</td>
<td></td>
</tr>
</tbody>
</table>

Minerals, AIN-76 vitamin mix; Vitamins, AIN-76 mineral mix.

Table 2. Food intake, body weight, and carcass composition

<table>
<thead>
<tr>
<th></th>
<th>Low Fat</th>
<th>High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative food intake, kcal</td>
<td>7,010 ± 224*</td>
<td>7,022 ± 235*</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>424.1 ± 16.2*</td>
<td>469.3 ± 17.0*</td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>268.3 ± 13.3*</td>
<td>331.7 ± 13.9*</td>
</tr>
<tr>
<td>Carcass composition, %</td>
<td>17.7 ± 0.7*</td>
<td>17.3 ± 0.7*</td>
</tr>
<tr>
<td>Protein</td>
<td>9.4 ± 0.5*</td>
<td>15.4 ± 0.6†</td>
</tr>
<tr>
<td>Lipid</td>
<td>71.1 ± 0.9*</td>
<td>65.8 ± 0.9†</td>
</tr>
<tr>
<td>Ash</td>
<td>1.6 ± 0.2*</td>
<td>1.6 ± 0.1*</td>
</tr>
</tbody>
</table>

Note: Data are least squares means ± SE for 11 (low fat) or 10 (high fat) rats/group. Values in a row not sharing a superscript are significantly different (P < 0.05).

were taken for cellularity studies and determination of IGF-I content. The remaining portion of the inguinal pad was used to prepare adipose tissue conditioned media. The remaining eviscerated carcass, with skin and fur attached, was used for determination of carcass composition, as previously described (8). Carcass dry matter, lipid, and ash were determined directly, with carcass protein calculated by difference.

Adipose tissue cellularity. Fat cell size and number were determined through electronic quantification using the method of Hirsch and Gallian (13) as modified by Cartwright (4). Triplicate adipose tissue samples were fixed in a solution containing 0.12 M osmium tetroxide in 50 mM collidine (2,4,6-trimethylpyridine) buffer. Samples were fixed for at least 1 wk at room temperature, rinsed with 0.9% NaCl, and then placed in 8 M urea for several days to facilitate separation of cells from the tissue. Fixed adipocytes were rinsed with 0.9% NaCl through a 240-µm nylon screen and then were collected on a 20-µm nylon screen. Samples of cells that met quality standards during the fixation process were analyzed on a Coulter (model ZM; Coulter Electronics, Hialeah, FL) electronic particle counter. Sizing was accomplished by Dual Threshold analysis at nine defined size ranges (36–240 µm) calibrated with microsphere standards (Coulter Electronics). Counts in all size ranges were performed in triplicate. Number of adipocytes per pad was calculated by multiplying the mean cells per milligram of sample by the total mass of the corresponding depot.

Adipose tissue IGF-I content. Frozen tissue samples were ground under liquid nitrogen and then extracted two times in 1 M acetic acid (20). Tissue extracts were frozen at −80°C and lyophilized until dry. Freeze-dried samples were reconstituted in 0.5 M acetic acid and then centrifuged at 600 g for 15 min. The supernatant was removed and stored at −20°C until assayed. A double-antibody RIA was used to determine IGF-I content of the tissue extracts as previously described (20). Sensitivity of the IGF-I assay was 10 pg/assay tube with 40% B/B0 (% maximum binding). The intra-assay coefficient of variation was 6.42%. Whole pad IGF-I content was calculated by multiplying the total mass of the depot by the nanograms IGF-I per milligram tissue.

Conditioned media preparation. Inguinal adipose tissue was quickly removed, further dissected to remove visible blood vessels, finely minced, rinsed three times in fresh 37°C Hanks’ balanced salt solution (HBSS), blotted dry, and weighed. Tissue aliquots (1 g tissue±5 ml media) were incubated in DMEM-Ham's F-12 (DMEM-F-12) containing 72 mM gentamicin sulfate, 120 mM cefazolin, and 27 mM amphotericin B for 4 h at 37°C in a humidified 5% CO2 atmosphere. The
adipose tissue conditioned media was sterile filtered and stored frozen at −20°C. Primary cell culture. S-V cells, including preadipocytes, were obtained from the inguinal adipose tissue of specific pathogen-free male Sprague-Dawley rats (80–100 g; Harlan Sprague Dawley, Indianapolis, IN) by methods previously described (29). Briefly, animals were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg), and inguinal adipose tissue was removed aseptically and pooled. Each gram of minced adipose tissue was incubated with 5 ml of a digestion buffer consisting of 0.1 M HEPES and 6.21 × 10−3 M collagenase for 90 min in a 37°C shaking water bath. Digests were filtered through nylon mesh screens with 240- and 20-µm openings, resuspended in DMEM-F-12, and centrifuged for 10 min at 600 g. Floating fat-filled cells were discarded. Cells in the S-V pellet were washed with plating medium (DMEM-F-12 with 10% FBS), centrifuged, and then resuspended in plating medium. An aliquot of the suspension was mixed with Rappaport’s stain, and cells were counted on a hemocytometer. Cells were diluted with plating medium and were seeded on 25-cm² tissue culture flasks at a density of 4.8 × 10⁶ cells/cm² for proliferation studies and in 35-mm tissue culture plates at 1.0 × 10⁵ cells/cm² for differentiation studies. Cultures were incubated at 37°C in a humidified 5% CO₂ atmosphere. Differentiation assay. Plating medium was removed after 24 h and was replaced with DMEM-F-12 containing the following serum-free supplements: 872 nM insulin, 65 nM transferrin, 29 nM sodium selenite, and 2 nM triiodothyronine. On days 4 and 6, test media comprised of 25% adipose tissue conditioned media and 75% serum-free supplemented DMEM-F-12 was applied to the cells. Cultures were harvested on day 8 into sucrase buffer (0.25 M, pH 7.4). The cellular homogenates were analyzed for sn-glycerol-3-phosphate dehydrogenase (GPDH, EC 1.1.1.8) activity by the method of Wise and Green (34) as modified by Ramsay et al. (29) and for protein (Bio-Rad, Melville, NY). Proliferation assay. Day 1 after seeding, the plating medium was removed and replaced with DMEM-F-12 until treatment media was applied on day 2. Proliferation of preadipocytes and S-V cells in response to test media was determined through the use of [3H]thymidine incorporation during the exponential growth phase (26). Cultures were treated with basal control medium (DMEM-F-12, 0.5% porcine serum) or test media (25% adipose tissue conditioned media, 75% DMEM-F-12, 0.5% porcine serum) containing 0.2 µCi/flask [3H]thymidine for days 2–5 of culture. On day 5, the flasks were rinsed and refed with lipid filling medium (1.0% porcine serum, 1.0% porcine insulin, and 10,000 U/l heparin in DMEM-F-12) to promote fat accretion in the preadipocytes. Lipid filling medium was changed every other day through day 13. On day 15, the cells were enzymatically harvested using HBSS containing 0.5% BSA, 4,185 U/l trypsin, and 1.05 × 10² U/l collagenase. The lipid-filled cells (preadipocytes now fully differentiated) and the non-lipid-filled S-V cells were separated by density gradient centrifugation through Percoll as described by Novakofski (26). Both cell fractions were assayed for incorporation of [3H]thymidine by scintillation counting. IGF-I stripping from media by immunomagnetic bead separation. IGF-I was isolated and removed from the conditioned media using the MiniMac magnetic separation system (19) and antibodies directed against IGF-I. Basal control medium containing 10 nM IGF-I and pooled samples of conditioned media (4 ml each) were treated with mouse anti-human IGF-I (500 ng/ml) and gently mixed on a rotator at 4°C. After 30 min, goat anti-mouse IgG microbeads (2 µ/ml) were added to the treated media and then were incubated for an additional 30–45 min at 4°C on a rotator. MiniMac columns were placed on the MACS magnet assembly and were prepared with 500 µl of PBS supplemented with 0.5% BSA and 2 mM EDTA. Residual buffer was expelled, and treated media were placed on the columns. Media were allowed to flow through the columns by gravity. IGF-I/IGF-I antibody complexes were retained on the magnetic columns, and the IGF-I-stripped flow-throughs were collected in fresh tubes. Statistical analysis. Data were subjected to ANOVA using SuperANOVA (version 1.11; Abacus Concepts, Berkeley, CA) to determine the effect of diet treatment on adipose tissue cellularity parameters, IGF-I content, and the proliferation and differentiation of preadipocytes in culture. Least-squares contrasts were used for comparisons between means. Differences were accepted at the P < 0.05 level. RESULTS Food intake and body weight and composition. There was no significant difference in cumulative energy intake for the 85-day study between the two diet treatment groups (Table 2). However, final body weight and body weight gain were both significantly greater in rats fed the HF diet compared with those rats fed the LF diet (P < 0.05 and P < 0.005, respectively; Table 2). The increase in body weight gain in the HF diet-fed rats was associated with a significant increase in percent carcass lipid (P < 0.0001) and corresponding reduction in percent carcass water (P < 0.001). Percent carcass protein and percent carcass ash were not influenced by the dietary treatment. Adipose depot development. The size of both the inguinal and epididymal adipose tissue depots was significantly greater (P < 0.05) in rats fed the HF diet (Table 3). Inguinal and epididymal fat pad depot weights were 78 and 130% greater, respectively, in HF-fed rats compared with the LF-fed rats. Cellularity data reflect only those fat cells with diameters of 30–240 µm; thus, these results may underestimate the actual fat cell number, as adipocytes with diameters <30 µm were excluded from the analysis. The mean number of adipocytes per unit weight of adipose tissue was not significantly different (P > 0.1) between diet treatment groups for either fat pad (Table 3). However, the mean number of fat cells per pad was significantly greater (P < 0.05) for both depots in rats fed the HF diet (Table 3). Fat cell number in the inguinal pads was 177%
greater in rats consuming the HF diet compared with those fed the LF diet. In the epididymal depot, fat cell number was 199% greater for rats in the HF group compared with the LF-fed rats.

Adipocyte size distribution. Determination of a whole pad fat cell size distribution profile was used to ascertain which specific sizes of adipose cells were increased throughout the depot in response to HF feeding. As seen in Fig. 1A, rats fed the HF diet had significantly more (P < 0.05) inguinal fat cells in both small (30–70 µm), large (80–140 µm), and very large (180–240 µm) size ranges when compared with rats receiving the LF diet. Figure 1B shows the total adipocyte cell size distribution for the epididymal fat depots. Compared with rats in the LF diet group, HF-fed rats had more (P < 0.05) epididymal adipocytes for all but the very largest size range. When viewed as a percent distribution (Fig. 2), both the inguinal and epididymal depots of HF-fed rats exhibited a significant increase (P < 0.05) in the number of 100- to 140-µm fat cells per gram of tissue.

Adipose tissue IGF-I content. Feeding an HF diet increased the total fat pad IGF-I content of both the inguinal and epididymal depots (Table 4). An overall 80% increase in IGF-I content was observed in the inguinal fat pad of HF-fed rats compared with rats fed the LF diet (P < 0.001). Epididymal fat pad IGF-I content of the HF-fed rats was also significantly (P < 0.001) increased compared with the LF-fed rats. There was no significant difference between the treatment groups for nanograms IGF-I per gram of inguinal or epididymal adipose tissue (data not shown).

Adipose tissue conditioned media and preadipocyte development. The potential role of paracrine substances from adipose tissue on altering fat cell size and number was investigated using adipose tissue conditioned media and primary preadipocyte cell culture. Results from two separate primary cell culture experi-
ments have been combined to yield \( n = 10 \) for each treatment group and are expressed as the difference in uptake of \( ^{3}H \) thymidine from the DMEM-F-12 basal control medium. As shown in Fig. 3, both preadipocyte and S-V cell proliferation in primary cell culture was significantly stimulated (\( P < 0.05 \)) in cultures treated with 25% inguinal adipose tissue conditioned medium prepared from rats fed the HF diet. Preadipocyte and S-V cell proliferation was not stimulated by treatment of the cultures with conditioned medium prepared from rats receiving the LF diet. There was no significant effect of diet treatment on the ability of inguinal adipose tissue conditioned media to influence the specific activity of GPDH, a late marker of preadipocyte differentiation, in primary cell culture (control 1,528 \( \pm \) 211, LF 1,567 \( \pm \) 100, and HF 1,469 \( \pm \) 95 nmol NADH \( \cdot \) min\(^{-1} \) \( \cdot \) mg protein\(^{-1} \)).

Neutralization of proliferation by IGF-I stripping from conditioned media. To determine whether the increase in proliferative activity observed with the conditioned media from the HF rats may have been associated with IGF-I, samples of conditioned media from the HF-fed rats were pooled and treated with an IGF-I antibody. The IGF-I/IGF-I antibody complexes were subsequently removed by immunomagnetic bead separation before testing the stripped conditioned media in the primary preadipocyte cell culture system. As

Table 4. Adipose tissue IGF-I content

<table>
<thead>
<tr>
<th>IGF-I, ng/pad</th>
<th>Low Fat (11)</th>
<th>High Fat (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inguinal</td>
<td>204 ( \pm ) 42*</td>
<td>368 ( \pm ) 44†</td>
</tr>
<tr>
<td>Epididymal</td>
<td>116 ( \pm ) 42*</td>
<td>201 ( \pm ) 42†</td>
</tr>
</tbody>
</table>

Data are least squares means \( \pm \) SE; nos. in parentheses are no. of rats. Values in a row not sharing a superscript are significantly different (\( P < 0.001 \)).
a control, samples of basal control medium (0 IGF) and basal control medium supplemented with 10 nM IGF-I were also stripped using the antibody/immunomagnetic bead separation technique. As shown in Fig. 4, proliferation was increased in preadipocytes exposed to basal control medium supplemented with either 10 nM IGF-I (P < 0.02) or 25% adipose tissue conditioned media from the HF-fed rats (pooled samples; P = 0.067) compared with unsupplemented basal medium. The stripping protocol had no influence on proliferative activity of the basal medium. In contrast, proliferative activity of the basal medium supplemented with either 10 nM IGF or pooled HF conditioned media was significantly attenuated (P < 0.05) after treatment with the IGF-I antibody and immunomagnetic bead separation.

**DISCUSSION**

We have determined that HF diet-induced obesity is associated with changes in adipose tissue cellularity, cell size distribution profile, and adipogenic activity. The feeding of an HF diet had a profound effect on adipose tissue cellularity by increasing the number of fat cells in both the inguinal and epididymal depots. This increase in fat cell number was the result of substantial increases in the number of both large and small adipocytes. Furthermore, we have demonstrated that inguinal adipose tissue from the HF-fed rats had an increase in the percentage of adipocytes in the 100- to 140-µm size range and that conditioned medium prepared from this depot had the ability to significantly stimulate the proliferation of preadipocytes in primary cell culture. The observed changes in adipose tissue cellularity and in the stimulation of in vitro preadipocyte proliferation are consistent with the concept that expanding adipocytes may secrete local growth factors involved in the regulation of adipose tissue expansion.

Several studies (1, 6, 11) have noted that an increase in mean fat cell size precedes permanent increases in fat cell number. Thus it has been proposed that reaching a critical fat cell size will trigger events that result in the proliferation of adipocytes (6). However, noting an increase in mean fat cell size alone does not clearly support this hypothesis. It is necessary to examine fat cell size distribution patterns and cellularity changes over time to ascertain if an increase in the relative number of large adipocytes is followed by a subsequent increase in overall fat cell number. Adipocyte size distribution profiles (17, 18) provide an excellent technique to investigate the quantitative changes of both large and small fat cells. In a recent study (24), we observed alterations in fat cell size distribution during the development of obesity in the genetically obese Zucker rat that were correlated with proliferative activity of conditioned media prepared from the representative adipose tissue depots. In turn, proliferative

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Fig. 3. Incorporation of [3H]thymidine into preadipocyte (A) and stromal-vascular (B) fractions of rat primary preadipocyte cell cultures treated with 25% inguinal adipose tissue conditioned media from rats fed diets differing in fat content. Values from 2 separate experiments have been combined to yield n = 10 for each treatment group and are expressed as the difference in uptake of [3H]thymidine compared with cultures treated with the basal control medium. Data shown are least squares means ± SE. Values not sharing a letter are significantly different (P < 0.05).

Fig. 4. Incorporation of [3H]thymidine into preadipocyte fraction of rat primary cell cultures treated with 0 or 10 nM IGF-I or with 25% conditioned media from inguinal adipose tissue of HF-fed rats, pooled samples (0 IGF, 10 nM IGF, and HF pool controls), or with similar samples that had been stripped of IGF with IGF antibody/magnetic bead separation. Data shown are the least squares means ± SE (n = 3 or 4) and are expressed as the difference in uptake of [3H]thymidine compared with cultures treated with a basal control medium. *Significant difference (P < 0.05) from the 0 IGF control.
activity of the conditioned media was associated with changes in fat cell number in vivo during the subsequent 3-wk interval. The present study demonstrated that the obesity that resulted from consumption of an HF diet also altered fat pad cellularity by increasing the percentage of large fat cells and the total number of fat cells in several size ranges for both the inguinal and epididymal depots. Proliferative activity was also greater in conditioned media prepared from the adipose tissue of the HF-fed rats compared with conditioned media from adipose tissue of the LF-fed rats. This would suggest that the increased presence of large adipocytes in rats fed an HF diet might have produced local growth factors that enhanced the adipogenic activity in these depots. However, as only one time point measurement was taken in the present study, it is not known definitively whether the HF diet-induced changes in fat cell distribution profile and proliferative activity translated into a subsequent increase in fat cell number in vivo.

As previously mentioned, it had been proposed earlier by Faust et al. (6) that new fat cell proliferation may be stimulated during the course of obesity development as existing fat cells reach their peak capacity to store lipid. Although accepted readily, this "critical fat cell size" hypothesis had not been tested thoroughly for its applicability across animal models of human obesity. In our previous study in genetically obese Zucker rats (24), it was determined that the percentage of 140- to 180-µm fat cells contributed to 76% of the variability in proliferative activity in conditioned medium from the inguinal adipose tissue of the obese rats. Furthermore, the proliferative activity of the conditioned medium in the obese animals was significantly correlated with subsequent changes in fat cell number. In the present study, it was demonstrated that HF feeding resulted in a specific increase in the percentage of inguinal adipocytes in the 100- to 140-µm size range and that conditioned medium prepared from this depot had the ability to significantly stimulate preadipocyte proliferation. Taken together, results of these two studies provide further evidence that enlarging adipocytes may secrete growth factors that induce the proliferation of preadipocytes. However, there is a slight discrepancy between the two studies with regard to the specific fat cell size range most closely associated with proliferative activity and/or subsequent changes in fat cell number. This would suggest that the critical fat cell size associated with an increase in proliferative activity during obesity development may 1) represent a continuum of cell size enlargement rather than a specific cell diameter or volume per se and/or 2) be species, animal model, or depot dependent.

In this study, the proliferation of preadipocytes in primary cell cultures was significantly increased upon exposure to medium conditioned by the inguinal adipose tissue from rats fed the HF diet. Other investigators also noted that media conditioned by the addition of mature fat or cells isolated from adipose tissue could alter both differentiation (22, 32) and proliferation (5, 22) of preadipocytes in primary cell culture. However, the specific paracrine/autocrine factors responsible for inducing these effects had not been delineated. IGF-I is both expressed and secreted by adipose tissue cells (7, 27, 33) and participates as an autocrine or paracrine regulator of preadipocyte development (10). Because exogenous IGF-I is known to exert both a mitogenic (31, 35) and adipogenic (9, 31) effect on preadipocytes in culture, it is reasonable to investigate its presence in the enlarged fat depots characteristic of obesity. Direct adipose tissue analysis of growth factor content is confounded by the means of expressing the data. The elevated lipid content of adipose tissue in relation to the small quantity of protein may distort comparisons of growth factor data expressed on a per unit of protein basis (28), especially when comparing tissue from lean and obese animals. In this study, we have shown that total adipose IGF-I content of both the inguinal and epididymal depots increased in response to HF diet-induced obesity in Osborne-Mendel rats. A recent study in a model of overfeeding (30) noted that, in contrast to systemic IGF-I, adipose tissue expression of IGF-I mRNA is not reduced in the obese animals. Thus IGF-I may be a significant growth factor in the paracrine milieu of adipose tissue.

We have ascertained that treatment of the conditioned media with an IGF-I antibody and subsequent removal of the IGF-I/IGF-I antibody complex by immunomagnetic separation attenuated the increase in proliferation seen when the cells were exposed to inguinal adipose tissue conditioned media prepared from the HF-fed rats. Treatment of basal medium supplemented with IGF-I with the IGF-I antibody/immunomagnetic bead separation protocol likewise attenuated the increase in preadipocyte proliferation induced by IGF-I. This would imply that the inguinal adipose tissue conditioned media from the HF-fed rats contained a proliferative factor that reacted with the IGF-I antibody. Even though IGF-I is biologically active in inducing preadipocyte proliferation, it must exert this paracrine effect at extremely low concentrations, as we have been unable to detect measurable levels of IGF-I in the conditioned media samples from the HF-fed rats using conventional RIA techniques (unpublished observations).

Our study has demonstrated that animals fed HF diets have an increase in the number of very large adipocytes and that inguinal adipose tissue conditioned media from these animals can significantly increase the proliferation of preadipocytes in primary culture. These observations expand the critical fat cell size hypothesis in that they demonstrate increased fat cell number, a shift in the fat cell distribution profile, and the ability of conditioned media from tissue with an increased percentage of 100–140 µm cells to stimulate preadipocyte proliferation. The changes in adipose tissue IGF-I content and the alteration of preadipocyte proliferation in response to removing the in vitro activity of IGF-I are consistent with the concept that IGF-I is an autocrine/paracrine regulator of adipose tissue expansion induced by HF feeding.
Perspectives

Fat cell enlargement is present at any level of obesity, and those with extreme obesity also exhibit increased adipocyte number (2, 12). Hypertrophic obesity, especially that of the internal depots, is associated with metabolic disorders that contribute to adverse health consequences (2). Hyperplastic obesity develops, subsequent to persistent increases in fat cell size (2, 6, 12) and is the most severe form, having an extremely poor prognosis for therapeutic intervention (2, 3). The data from our study imply that an increased percentage of moderately enlarged adipocytes can alter the paracrine environment of adipose tissue sufficiently to permit proliferation of preadipocytes and the development of hyperplastic obesity.

Our data expand the critical fat cell size hypothesis originally proposed by Faust and colleagues (6) by demonstrating the concomitant presence of adipocyte hypertrophy and a mitogenic factor. However, it still does not definitively prove that large adipocytes are the source of this factor. Future studies should be conducted utilizing separation of adipose cells by size to determine if enlarged cells actually secrete IGF-I or other growth factors. Additional studies would be needed to determine if adipocyte size alone is sufficient to regulate the production and secretion of adipogetic growth factors or if other regulators of transcription are involved. Elucidation of the paracrine environment that regulates adipose tissue expansion may allow for the development of effective therapeutic interventions for obesity.

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