Dietary and species influence on potential of plasma to stimulate differentiation and lipid accumulation in cultured adipocyte precursors

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Abstract   Sera and plasma from different species and from rats of various dietary statuses were compared with regard to effects on proliferation, glycerophosphate dehydrogenase (GPDH) activity, and lipid-filling of rat adipocyte precursors converting to adipocytes in primary cell culture. All of the tested sera and plasma samples were comparably supportive of cell multiplication, but their effects on elevation of GPDH activity (a key event in adipocyte differentiation) and lipid-filling varied greatly. Plasma supported a much greater increase in GPDH activity than serum, while serum from cats supported a much lower increase than serum from humans, calves, goats, or rats. Dietary status of rats did not affect the potential of plasma to support GPDH activity, but did affect plasma support of lipid-filling. A higher than normal degree of lipid-filling was promoted by plasma from rats fed a high-fat, high-sugar diet, while a lower than normal degree was promoted by plasma from fasted rats. Lipid-filling was also found to vary in response to changes in content of very low density lipoprotein (VLDL) in human plasma. This suggests that the influence of diet on the potential of plasma to promote adipocyte lipid-filling may be mediated by the effect of diet on plasma VLDL. The absence of a diet-dependent effect of plasma either on multiplication of adipocyte precursors or on degree of elevation of GPDH activity leaves unresolved the mechanism by which diet affects adipocyte production in animals. — **Björntorp, P., I. M. Faust, W. H. Miller, Jr., M. Karlsson, G. Sypniewska, and K. Dahlgren.** Dietary and species influence on potential of plasma to stimulate differentiation and lipid accumulation in cultured adipocyte precursors. *J. Lipid Res.* 1985. **26:** 1444–1454.

Supplementary key words   cat • fetal calf • glycerophosphate dehydrogenase • goat • human • pre-adipocyte • rat • VLDL • 3T3-F442A cells

Hyperplastic growth of adipose tissue occurs primarily during the first few months of life in rats fed only chow (1). There is practically no further growth in female rats after 35 weeks of age (2), whereas in males, in which overall body growth is continuous, expansion of the number of fat cells appears to continue at a slow rate indefinitely (3, 4). However, in both male and female adult rats, certain stimuli can cause hyperplastic growth of adipose

tissue to accelerate dramatically (5–7). Thus, in response to being fed a highly palatable high-fat or high-sugar diet, rats may double total body fat cell number, relative to controls, in only 6 months (5, 8). The events that lead to this phenomenon appear to be as follows. During the first few days of overfeeding, fat cells enlarge and synthesis of DNA in adipose tissue increases (9). Many of the cells produced during the first few days are connective tissue cells probably needed to support the growing adipose tissue mass, but some of the newly formed cells are adipocyte precursors (10, 11). The increased levels of DNA synthesis caused by overfeeding are seen in vivo (9, 10) as well as in adipose tissue fragments during brief incubation (10). The number of preadipocytes present in rat adipose tissue, as determined by a suspension culture technique, increases significantly between the second and eighth week of overfeeding (11). As overfeeding continues, the fat cells of the rat continue to enlarge until they each contain, on average, between 1.0 and 1.5 μg of lipid. At about the time that the fat cells attain this large size, increases in the number of mature fat cells begin to become readily apparent (5). Some of the increase may be due simply to the lipid-filling of very small or immature adipocytes formed prior to the initiation of overfeeding (12). However, it appears that most of the increase stems from the differentiation of adipocyte progenitor cells formed during the period of overfeeding (8, 10, 11).

Kuri-Harcuch and Green (13) reported finding great variation in the potential of sera from different species to stimulate adipose conversion of 3T3-F442A cells. Serum from fetal calves promoted substantial conversion while serum from cats did not. Similarly, Loffler et al. (14) found that serum from genetically obese rodents supports

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Abbreviations: GPDH, glycerophosphate dehydrogenase; VLDL, very low density lipoproteins; S-V cells, stromal-vascular cells; HFS, high-fat, high-sugar diet.

greater adipose conversion than serum from lean rodents. In preliminary experiments with primary cultures of rat adipocyte precursors, Björntorp (11) found wide variation in the lipid accumulation promoted by sera from different human donors. It is thus possible that there are factors in the blood that stimulate or inhibit proliferation or differentiation of adipocyte precursors and that vary not only across species but also across members of the same species. Systematic evaluation of effects of sera from different sources on proliferation and differentiation of adipocyte precursors and on the lipid-filling of these cells during differentiation might lead to the isolation and identification of such factors. In the present study, conditions for successful culture of adipocyte precursors were optimized and the effects of various sera and plasmas on proliferation, differentiation, and lipid-filling were evaluated. Emphasis was given to the examination of effects of plasma from Osborne-Mendel rats of differing dietary status, since adipose tissue growth in these animals is known to vary greatly as a function of diet (5, 9, 11).

METHODS

Animals and diets

Male Osborne-Mendel rats were obtained as weanlings from Rockland farms (Gilbertsville, PA); Sprague-Dawley weanlings were obtained from Charles River Breeding Laboratories (Wilmington, MA) and from Anticimex (Stockholm, Sweden). The Sprague-Dawley rats served as cell donors. They were killed for the purpose of tissue removal at 4 weeks of age. The Osborne-Mendel rats served as blood donors. They were bled at 4 or 7 months of age (approximately 450 and 650 g body weight, respectively). These rats always had ad libitum access to either Purina Laboratory chow #5001 (by weight: 4.5% fat and 23.4% protein) or to a similar chow made in Sweden (Ewos, Sodertalje, by weight: 5% fat and 22.5% protein). Some of the blood donors were fed a high-fat, high-sugar (HFS) diet consisting of chocolate chip cookies (Chips Ahoy, Nabisco, East Hanover, NJ) and sweetened condensed milk substitute (Melloream, Borden, Columbus, OH) in addition to chow for 10 or 28 days prior to bleeding. All rats were housed in polycarbonate group cages (two or three rats/cage) at constant temperature (23°C, New York; 20–22°C, Göteborg). Lights were programmed to cycle on and off every 12 hr.

Bleeding and serum and plasma preparation procedures

Rats were anesthetized with intraperitoneal injections of a commercially prepared chloral hydrate/pentobarbital mixture (Chloropent, Fort Dodge Laboratories, Fort Dodge, IA) at a dose of 3 cc per kilogram body weight. Blood was drawn by means of a 1/16-in O.D. plastic cath-

eter (#18 TWTFE, Penntube Plastics, Clifton Heights, PA) inserted into the abdominal aorta. For each rat, the first blood sample (5 ml) was delivered into a tube containing 25 units of heparin (LipoHepin, Riker Laboratories, Northridge, CA). The next 5-ml sample was delivered into a tube containing sodium citrate at an amount calculated to give a final concentration of 0.38%. Remaining blood was delivered into a tube without additives for preparation of serum. After blood was drawn, the test tubes with additives were inverted for mixing and all tubes were placed on ice until processed (between 1/2 and 2 hr later).

All tubes were centrifuged at 1085 g for 15 min at 4°C to remove blood cells. Serum was transferred by plastic pipet to clean tubes and frozen at $-70°C$. Heparin and citrate plasmas were similarly transferred and then recentrifuged at 22,000 g for 30 min at 4°C to remove platelets. Heparin plasma was then decanted to another set of clean tubes and frozen at $-70°C$. Citrate plasma was recalcified with 20 $\mu mol/ml$ $CaCl_2$ and incubated at 37°C for 2 hr. The clot that formed was then removed by another centrifugation at 22,000 g for 30 min at 4°C. The recalcified citrate plasma was frozen at $-70°C$ (see reference 15). Very low density lipoproteins (VLDL) were separated from fresh plasma samples by ultracentrifugation (Beckman, L5-65, Palo Alto, CA).

Pooled human serum was obtained from the outpatient department of the Department of Medicine I at Sahlgren's Hospital, Göteborg, Sweden. Cat serum was purchased from the Colorado Serum Company, Denver, CO (lot number 47), and fetal calf serum was from Gibco, Grand Island, NY (control number 28 K 8005).

Harvesting, plating, and culturing of cells

Rats were decapitated and shaved and epididymal pads were exteriorized and removed under sterile conditions. Stromal-vascular (S-V) cells (including adipocyte precursors) were liberated from the tissue by collagenase treatment as previously described (16). The cells were plated at a density as close as possible to 1.5×10^5 cells per 1.6-cm diameter multi-well dish (Falcon, Oxnard, CA). The plating range obtained was $1.1–1.8 \times 10^5$ cells. Cultures were assessed as early as 48 hr after plating in the experiments in which lipid filling was studied. The cells were cultured in Dulbecco-Voigt's medium to which was added 5 mM glucose, 40 mU/ml insulin (regular Iletin, Lilly, Indianapolis, IN), 0.1 mg/ml cephalothin (Keflin, Lilly, Fegersheim, France), and serum or plasma as indicated below. The medium was changed every second day after inoculation. For analysis of glycerophosphate dehydrogenase (GPDH) activity, the inoculation density was about 10^4/well.

3T3-F442A cells were received as a gift from Dr. Howard Green, Department of Physiology, Harvard Medical School, Boston, MA.

Treatment of rats prior to bleeding

In one experiment, 18 4-month-old Osborne-Mendel rats were divided into three weight-matched groups of six. The first group was fed the experimental high-fat, high-sugar diet (HFS) for 10 days prior to bleeding, the second group was fasted for 4 days prior to bleeding, and the third group was fed chow and water at all times.

In the second and third experiments, five 7-month-old Osborne-Mendel rats were fed the experimental diet for 28 days prior to bleeding, while five control rats, matched by age and weight to experimental rats, were fed chow and water at all times.

A fourth experiment was identical to the second and third except that the rats were 4 months old and the number of rats studied was 12, 12, and 10 in HFS, chow-fed, and fasted groups, respectively.

Analysis of cultures

Within each experiment, cell plating density was identical across multi-well dishes since aliquots of suspended cells were taken from the same cell preparation. On the fourth day following plating (unless stated otherwise) four areas in each culture were selected at random for examination by light microscopy at low power. Those cells in each area that fell within the borders of a grid, established in the field of view by an etching in the microscope eyepiece, were counted and categorized on the basis of whether or not they contained lipid droplets. The medium was then removed and the culture was washed with 1 ml of medium containing no serum or plasma. One ml of a solution containing 1 mM EDTA and 25 mM Tris-buffer (pH 7.4) was then added, and the cells were removed and suspended by repeated aspirations with a Pasteur pipet. Aliquots were then taken for protein determination according to the method of Lowry et al. (17) and for analysis of lipid ester bonds (18).

The rate of cellular multiplication was examined by adding 1.0 μCi of [methyl-^{3}H]-thymidine (H net-02722, New England Nuclear, Dreieichenhain, West Germany) to the tissue culture medium, and determining levels of radio-label in DNA according to the procedure described by Roncari and Van (19) at 22, 28, and 47 hr after the time of plating.

Rate of formation of cells containing lipid was determined by counting cells containing visible amounts of lipid at different time points. This was performed in cultures to which a methyl-cellulose layer had been added for the purpose of preventing lipid-laden cells from detaching from the bottom of the culture dish (20).

Plasma triglyceride concentrations were determined by a commercially available enzymatic method (Testcombination, triglycerides, Boehringer Mannheim, West Germany). Degree of lipid accumulation in the cell cultures was determined either in terms of μg of triglyceride

per μg of protein or μg of triglyceride per 10^5 cells. In a preliminary comparison of these two ways of expressing the data, they were found to yield virtually the same information ($r = 0.97$). GPDH activity was determined by removing cells in a culture with Tris-EDTA and solubilizing them with sonication as described by Wise and Green (21). Activity is expressed in terms of units per mg of protein (one unit being equal to one ng of NADH oxidized per min).

Statistical methods used to analyze the results of the present experiments were Student's t test, linear regression analysis, and analysis of variance.

RESULTS

Effects of different plasmas on rates of cell multiplication, adipose conversion, and lipid-filling of adipose tissue S-V cells in primary culture

The cells plated in each well of a multi-well dish attached and multiplied until confluence was attained. Virtually no differences were found in the numbers of cells in these wells 4 days following plating (**Fig. 1**), so it is clear that the various sera and plasmas can support cell multiplication equally well. Lipid accumulation usually began at about the time that confluence was attained and proceeded linearly with time until at least day 6 of culture
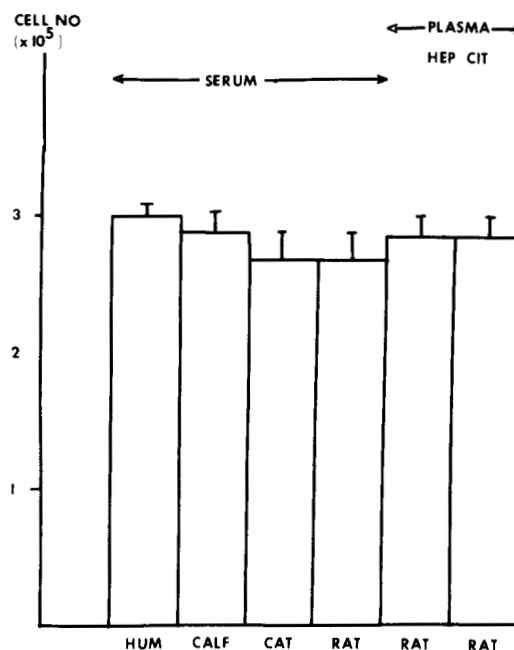

Fig. 1. Number of cells in each culture well 4 days after plating. Cells were cultured in the presence of either 20% serum from man, fetal calf, cat, or rat, or in the presence of 20% platelet-poor heparin or citrate plasmas from rats. Means ± SEM, n = six to eight cultures per serum or plasma sample. No differences are significant.

(Fig. 2). In the presence of cat serum, triglyceride accumulation/unit protein increased slowly with time, while the percentage of cells containing lipid droplets did not. In contrast, in the presence of rat plasma, triglyceride accumulation/unit protein as well as percentage of cells containing lipid droplets increased rapidly with time. Fig. 2 also shows that the lipid content of these cultures 4 days following plating is indicative of the lipid-filling rate. Thus, the day 4 data presented in Figs. 3 and 4 suggest that there is little variation in rate of lipid-filling as a function of the species source of serum, with the notable exception that cat serum promotes much less lipid-filling than serum from humans, calves, and rats. In addition, lipid accumulation is more rapid in cultures grown in the presence of rat plasma than in the presence of rat serum.

Fig. 3 compares the tested sera and plasmas with regard to effects on degree of lipid filling/10^5 cells while Fig. 4 compares them in terms of their effects on the number of cells that undergo adipose conversion. Comparison of the two figures reveals that serum or plasma effects on one parameter are comparable to effects on the other. In both sets of comparisons, cat serum had the least effect; human, fetal calf, and rat sera were all significantly more effective than cat serum; and rat heparin plasma was significantly more effective than any of the sera. In the present study, plasma samples were platelet-poor, but platelet-rich plasmas would probably have been just as effective. In parallel experiments (not shown), we found no change in the effectiveness of plasma as a function of platelet removal.

HFS-feeding for 10 or 28 days caused Osborne-Mendel rats to increase body weight considerably, while fasting for 4 days caused rats to lose a substantial amount of body weight. For example, in experiments 1 and 2, HFS-feeding
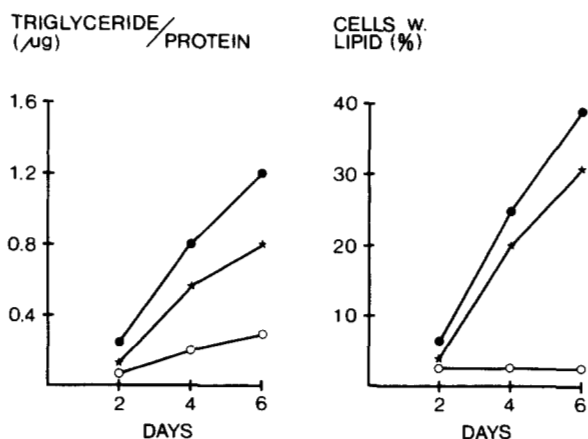

Fig. 3. Triglyceride/10^5 cells 4 days post-plating in cultures grown in the presence of either 20% serum from man, fetal calf, cat, or rat or in the presence of 20% platelet-poor heparin or citrate plasmas from rats. Cat serum produced results lower than all others ($P < 0.01$); rat serum and plasmas produced results higher than all others ($P < 0.05$); rat heparin plasma yielded results higher than rat serum and rat citrate plasma ($P < 0.05$). Means ± SEM; n = six to eight cultures per serum or plasma sample; same sera and plasmas as in Fig. 1.

for the two time periods caused Osborne-Mendel rats to gain 63 ± 4 and 132 ± 22 g body weight, compared to changes in body weight of +23 ± 8 and −11 ± 7 g seen in their respective chow-fed controls. (A gain in body weight of 23 g over 10 days is fairly common for 4-month-old male Osborne-Mendel rats fed chow; an 11-g loss in body weight over 28 days in older chow-fed rats is within the range of normal body weight fluctuation.) Fasting for 4 days caused rats to lose 68 ± 5 grams. **Fig. 5** describes levels of triglyceride accumulation in cell cultures incubated with varying concentrations of heparin plasmas from rats in the different dietary treatment groups. In order to minimize the variation that would be caused by differential rates of cell multiplication, cat serum (which supports cell multiplication but not differentiation of pre-adipocytes into adipocytes) was added to all cultures that received less than 20% plasma. The combination of cat serum and plasma was always 20%, by volume, of the incubation medium. For each combination of serum and plasma that was tested, the small degree of triglyceride accumulation seen in cultures with cat serum alone was subtracted from the total level of triglyceride accumulation. These corrected levels were then used to construct dose-response curves that depict the relationship between plasma concentration and triglyceride accumulation for each plasma sample (see Fig. 5).

Fig. 2. Lipid accumulation (expressed as triglyceride/protein and % cells with lipid) in cultures of adipose tissue stromal/vascular cells grown in the presence of 20% cat serum (open circles) or 20% platelet-poor plasma from male Osborne-Mendel rats fed chow. Platelet-poor heparin plasma (closed circles); platelet-poor citrate plasma (stars). The figure describes the average results of three separate experiments.
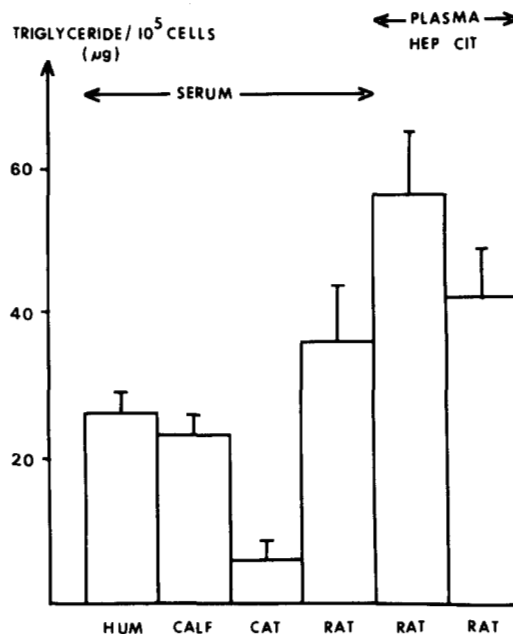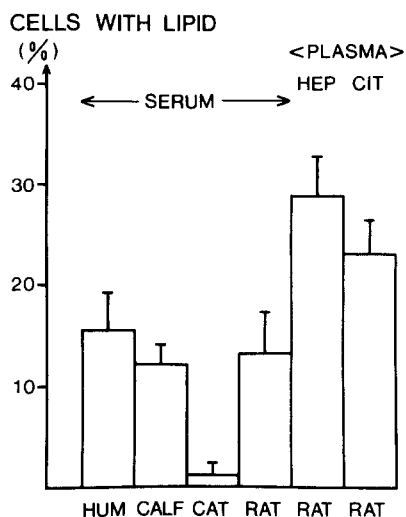
CELLS WITH LIPID
(%)

Fig. 4. Percentage of cells with lipid droplets 4 days post-plating in cultures grown in the presence of either 20% serum from man, fetal calf, cat, or rat or in the presence of 20% platelet-poor heparin or citrate plasmas from rats. Cat serum produced results lower than all others ($P < 0.01$); rat plasmas produced results higher than all others ($P < 0.01$). Means ± SEM; n = six to eight cultures per serum or plasma sample; same sera and plasmas as in Fig. 1.

Within the range tested, the resulting response curves (describing effects on triglyceride/protein) were approximately linear. Heparin plasmas from the chow-fed control rats for the first two dietary treatment groups (controls for rats fed HFS for 10 and 28 days) produced essentially identical curves, so the data from the two groups were pooled. Significant increases in triglyceride/protein occurred in response to the three highest plasma concentrations tested; plasma from rats fed HFS for 10 or 28 days promoted significant increases at the four highest concentrations. Plasma from fasted rats did not promote a significant increase at any concentration. Comparisons of the regression coefficients of the response curves for the different plasmas reveals that plasma from fasting rats promoted significantly less lipid accumulation than all other plasmas, while plasma from rats fed HFS for 28 days promoted significantly more accumulation than all other plasmas. Plasma from rats fed HFS for 10 days did not promote significantly more lipid accumulation than plasma from rats fed chow.

Analysis of DNA synthesis in cultures incubated with the plasmas obtained in the fourth dietary experiment confirmed that rate of cell multiplication does not vary as a function of the diet of the plasma donor (**Fig. 6**). Incorporation of labeled thymidine into DNA in cultures incubated with plasma from rats fed HFS for 28 days was comparable to that seen in cultures incubated with plasma from fasted rats or rats fed chow. Triglyceride content per 10^5 cells as well as the percentage of cells containing lipid droplets were again seen to vary significantly on day 4 of culture as a function of the dietary status of the plasma

donor. Lipid-filling was greatest with plasma from HFS-fed rats and least with plasma from fasted rats. However, unlike the first dietary experiment in which plasma from fasted rats supported virtually no lipid-filling, plasma from fasted rats in the fourth experiment did support lipid-filling. The basis for diet-dependent variation in the adipogenic or lipid-filling activity of rat plasma is addressed next.

Effects of plasma components on lipid-filling of adipose tissue S-V cells in culture

The dietary treatments did not differentially affect plasma glucose levels, but they did affect plasma concentrations of insulin and triglyceride. Correlation analysis revealed that differences in insulin concentration were unrelated to the above effect, whereas differences in tri-
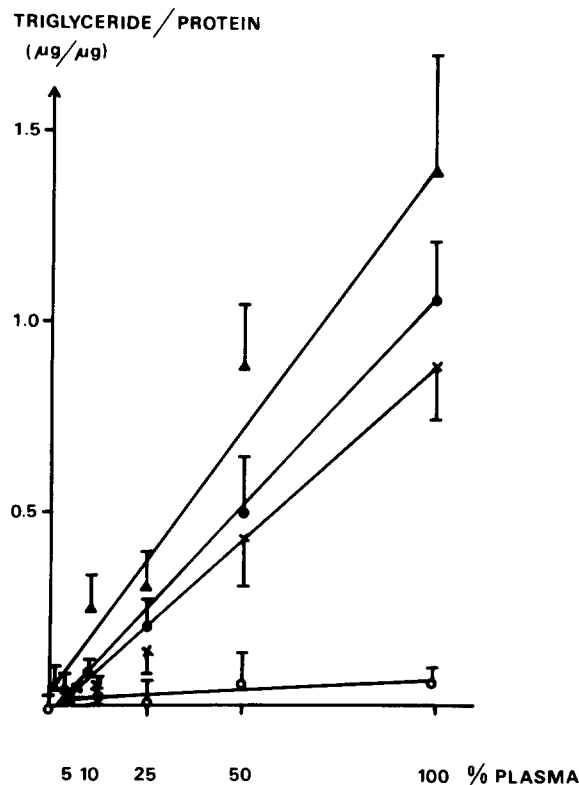

TRIGLYCERIDE / PROTEIN
($\mu g/\mu g$)

Fig. 5. The effect of increasing volumes of platelet-poor heparin plasmas from rats of differing recent dietary experience on lipid content of primary cultures of adipose tissue stromal-vascular cells 4 days post-plating. Plasmas were combined with appropriate amounts of cat serum so that total plasma and serum volume always equalled 20%. 100%, 100% plasma and no cat serum; 50%, 50% plasma and 50% cat serum; 5%, 5% plasma and 95% cat serum, etc. "Background" activity of cat serum was subtracted from each measure. Plasmas were obtained from adult male Osborne-Mendel rats. Filled triangles (▲), rats fed HFS for 28 days; filled circles (●), rats fed HFS for 10 days; crosses (×), rats fed chow; open circles (○), rats fasted for 4 days. Comparisons of regression coefficients: lower activity in plasmas from fasted rats than in all other plasmas ($P < 0.05$); higher activity in plasmas from rats fed HFS for 28 days than in all other plasmas ($P < 0.05$). Means ± SEM; n = five to ten per group.
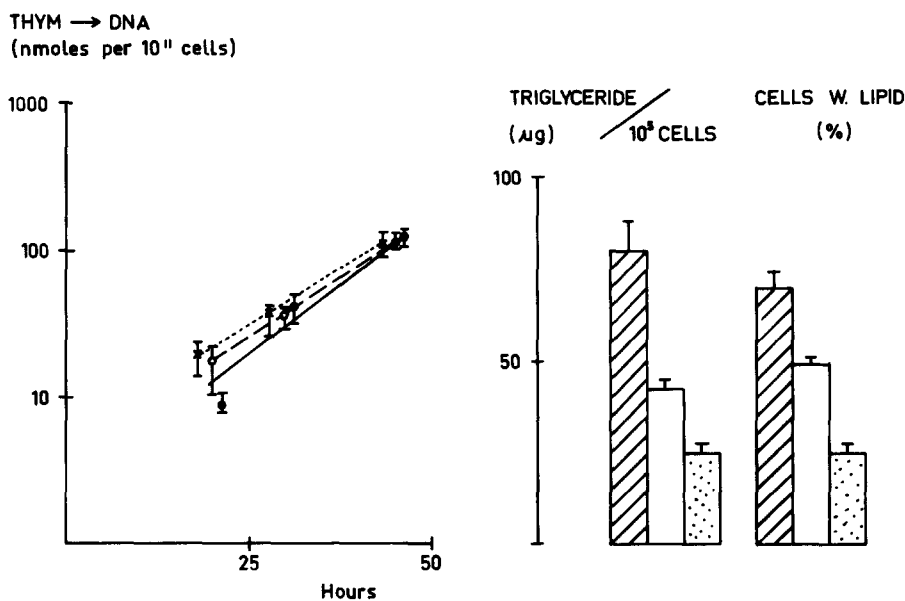
THYM → DNA
(nmoles per 10" cells)

Fig. 6. Proliferation and lipid-filling of cultured adipose tissue stromal-vascular cells incubated with heparin plasma from rats either fed chow, HFS (for 28 days), or starved for 4 days. Left: labeled thymidine incorporation into DNA (chow: filled circles (●), solid line; HFS: open circles (○), broken line; starved: crosses (×), dotted line). No differences are significant. Right: triglyceride content and percentage of cells containing lipid droplets after 4 days in culture (chow: open columns; HFS: hatched columns; starved: dotted columns). All differences are significant, $P < 0.01$. Means ± SEM; n = six in each group.

glyceride concentration covaried with a high degree of significance with intracellular lipid accumulation (plasma insulin vs. triglyceride content of cultured cells: $r = 0.12$; plasma triglyceride vs. triglyceride content of cultured cells: $r = 0.92$). The next experiment examines the relationship between level of plasma triglyceride VLDL and lipid-filling of cultured adipocyte precursors.

Human plasma, with an abundance of VLDL, was used instead of rat plasma. The results are summarized in **Fig. 7.** As seen in the figure, plasma recombined from VLDL and non-VLDL fractions was not different from whole plasma with regard to promoting lipid accumulation in the cell cultures in this experiment. However, VLDL-free plasma was much less effective than whole plasma or recombined plasma. Thus, the VLDL in plasma is at least partially responsible for the plasma's effectiveness in promoting lipid-filling in cultures of preadipocytes undergoing adipose conversion.

The VLDL fraction, alone, supported only minimal cellular multiplication, so its effects on differentiation and lipid-filling were tested in medium supplemented with VLDL-free plasma. As is depicted in **Fig. 8**, degree of lipid accumulation was found to vary as a function of the level to which the culture medium was supplemented with VLDL. As VLDL concentration in the medium was increased, triglyceride/protein increased accordingly. Effects of VLDL on GPDH activation in adipocyte precursors is addressed below.

Effects of different sera and plasmas on GPDH activity

If effects of serum or plasma on preadipocyte differentiation are assessed solely on the basis of triglyceride per cell or percent cells with lipid, the results can be mislead-
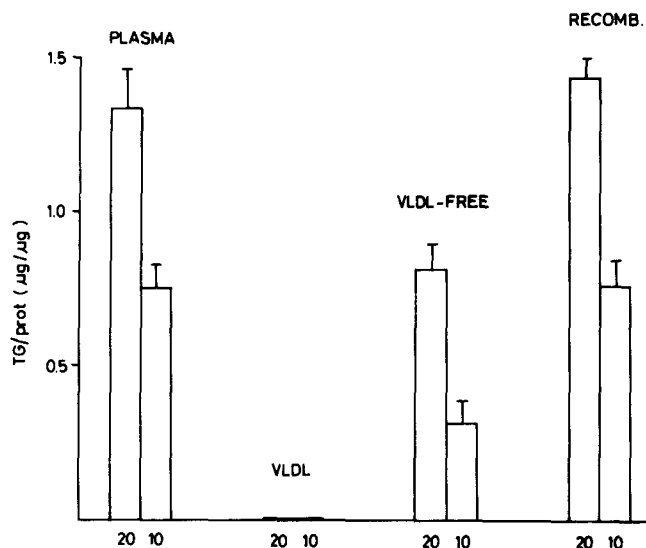

Fig. 7. Lipid-filling (triglyceride/protein) in day-4 primary cultures of adipose tissue stromal-vascular cells incubated with media containing 10 or 20% whole human plasma, VLDL, VLDL-free human plasma, or recombined VLDL and VLDL-free components of human plasma. VLDL-free produced results lower than plasma and recombined ($P < 0.01$). Means ± SEM; n = six to eight cultures per plasma sample.
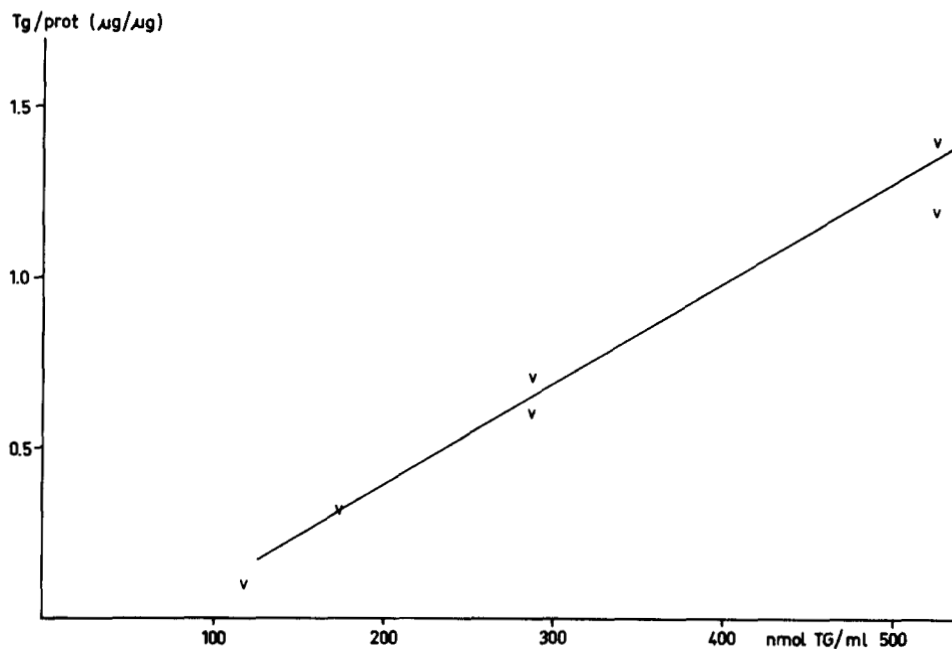
Fig. 8. Lipid-filling (triglyceride/protein) in day-4 primary cultures of adipose tissue stromal-vascular cells as a function of amount of VLDL added to culture media containing 20% VLDL-free human plasma; n = six cultures.

ing. Differentiated adipocytes do not always fill with lipid (22, 23). GPDH activity is a more sensitive indicator of differentiation of cultured 3T3-F442A cells into adipocytes (21, 23). Therefore, GPDH activity was measured in the present experiment in primary cultures of preadipocytes and, for purposes of comparison, in cultures of 3T3-F442A cells. These experiments were performed in cultures in which the cells were in the post-confluent stage. Very high GPDH activity was found in the cultures of 3T3-F442A cells grown in the presence of fetal calf serum (920 ± 10 units/mg protein, n = 11) while very low activity was found when these cultures were grown in the presence of cat serum (40 ± 3, n = 5). This is consistent with previously reported observations of serum-dependent adipose conversion of 3T3-F442A cells (13). GPDH activity in the primary cultures of cells from rat adipose tissue was about one-third that seen in the 3T3-F442A cultures.

Relative effects of sera and plasmas on GPDH activity are shown in **Fig. 9a**. Of the various sera and plasmas tested, cat serum is unique in promoting only a very low level of GPDH activity. Human, fetal calf, and goat serum are each seen to promote activity that is about six times greater than that which occurs in the presence of cat serum. Heparin plasma is seen to promote about twice the activity of serum. However, as seen by the results depicted in Fig. 9b, the superiority of plasma to serum in the promotion of GPDH activity is not due to the presence of heparin in the plasma. Heparin added to serum does not stimulate GPDH activity. On the contrary, in-

creasing heparin concentration is seen to be associated with decreasing GPDH activity.

Onset of differentiation in preadipocyte cultures incubated with plasmas from rats of differing dietary status was analyzed next. Initial experiments established cell and plasma concentrations that would allow monitoring of the development of GPDH activity with time. A cell concentration of 10^3 per cm^2 and a test plasma concentration of 2% (supported by 3% cat serum) was found to be optimal, yielding maximal GPDH activity on about the 7th day of culture (**Fig. 10a**). Plasmas from rats given different dietary treatments yielded the results described in Fig. 10b. In the presence of plasmas from fasting rats, somewhat higher values for GPDH activity were seen than in cultures grown with plasmas from HFS or chow-fed rats. In initial trials with 10% plasma, dietary status had no effect on maximal GPDH level and increases in activity were too rapid to allow meaningful comparisons. When VLDL-free plasma from HFS-fed rats was tested and compared with plasma reconstituted with the original VLDL contents and with plasma containing twice the original amount of VLDL, again no differences in GPDH activity were seen (Fig. 10c).

DISCUSSION

The culture conditions selected for the present set of experiments are known to support the proliferation of S-V cells from adipose tissue and the differentiation of some of
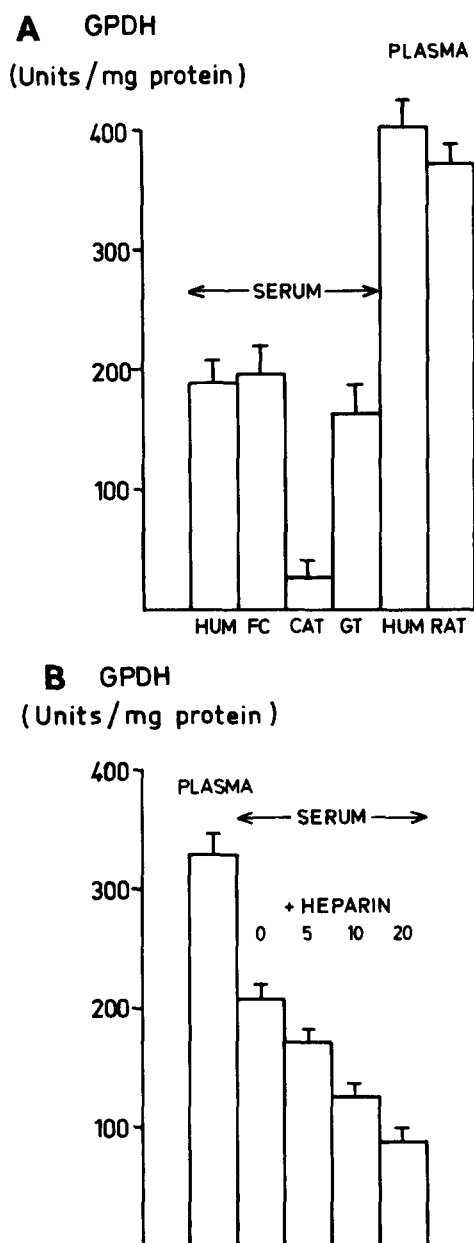
A GPDH
(Units / mg protein)

B GPDH
(Units / mg protein)

Fig. 9. A: GPDH activity (units/mg protein) on days 4–7 post-confluence in primary cultures of adipose tissue stromal-vascular (S-V) cells incubated in the presence of 20% human (HUM), fetal calf (FC), cat, and goat (GT) serum, and human and rat plasma. Cat serum produced results lower than all other sera and plasmas ($P < 0.001$). Sera produced results lower than plasmas ($P < 0.01$). Means ± SEM; n = six to eight cultures per serum or plasma sample. B: Effect of heparin added to media containing 20% rat serum on GPDH activity (units/mg protein) in primary cultures of adipose tissue S-V cells 4–7 days post-confluence. Serum produced results lower than plasma ($P < 0.01$). Addition of heparin was associated with diminished activity at each step ($P < 0.05$). Means ± SEM; n = six to eight cultures per plasma or serum sample.

these cells into adipocytes (16). Except for the serum or plasma contents of the culture media, these conditions were maintained in all experiments. In addition, all primary cell cultures used in these experiments were derived

in identical fashion from an identical source (epididymal pads of 4-week-old Sprague-Dawley rats). Thus, all observed differences among cultures in these experiments, with regard to effects on proliferation and differentiation of adipocyte precursors and the rate at which adipocytes fill with lipid, should have been due solely to differences among the sera and plasmas that were compared.

The experiments in this study were designed to assess whether sera and plasmas from different species and from rats of differing dietary status have differential effects on primary cultures of adipose tissue S-V cells. Several such differential effects were found. With regard to the species comparisons, all tested sera and plasmas were comparable with regard to effects on cellular proliferation. However, they were not all comparable in their ability to support differentiation of adipocyte precursors into mature adipocytes.

The number of cells containing lipid droplets increased with time in all cultures except those incubated with serum from cats. These cultures did show increases in total accumulation of triglyceride, but in just a small and constant number of cells. This suggests that most, or all, of the lipid-filling cells in these cultures had differentiated in vivo prior to the time that the S-V cells were harvested and plated, and the degree of differentiation was sufficient to allow full maturation in culture in the presence of just cat serum. In contrast to the high GPDH activity seen in cultures incubated with serum or plasma from other species, those incubated with serum just from cats showed practically no GPDH activity. The evidence is thus compelling that cat serum does not support differentiation in primary culture of preadipocytes freshly harvested from the adipose tissue of rats. This is consistent with the observation that cat serum fails to stimulate adipocyte conversion of cultured 3T3-F442A cells (a cell line with well documented potential for adipose conversion) (13, 23).

Among the sera and plasmas from different species that were tested, cat serum was unique in its inability to support adipocyte differentiation. Rat plasma (especially plasma prepared with heparin) was much more supportive of differentiation and somewhat more supportive of lipid-filling than any type of serum tested, including serum from rats. There are several possible explanations for the superiority of plasma to serum.

First, the substances that are added to blood to prevent coagulation during the process of plasma preparation may stimulate adipocyte lipid-filling. Indeed, the concentrations of heparin used in the present study have been shown to have such effects (20, 24). Thus, the small degree to which heparin plasma was superior to citrate plasma as a promoter of triglyceride accumulation in the cultures in the present study may have been due to the presence of heparin in the heparin plasma. However, as shown by the data depicted in Fig. 9b, heparin inhibits GPDH activity. Therefore, the enhancing effect of heparin
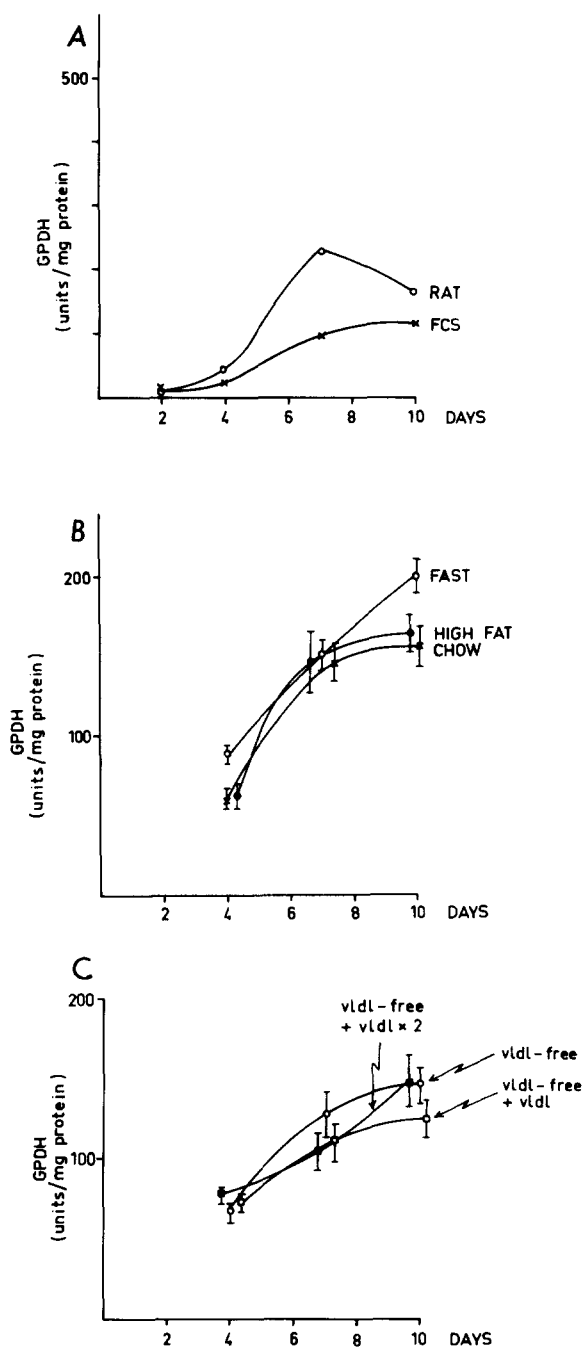
Fig. 10. A: GPDH activity in adipose tissue stromal-vascular (S-V) cells cultured in the presence of 2% rat heparin plasma (plus 3% cat serum) or 2% fetal calf serum (FCS) (plus 3% cat serum). Inoculation density: 10⁵ cells/cm² (one representative experiment out of three performed). B: GPDH activity in adipose tissue S-V cells cultured in the presence of 2% heparin plasma (plus 3% cat serum). Plasmas from fasting rats (fast), rats fed a high-fat, high-sugar diet (high fat), or rats fed chow. Means ± SEM; n = five to seven cultures per group at each time point. C: GPDH activity in adipose tissue S-V cells cultured in the presence of 2% rat plasma (plus 3% cat serum) containing a normal level of VLDL, twice the normal level of VLDL, or no VLDL. No significant differences; n = five to seven cultures per group at each time point.

on triglyceride accumulation seen in this study, and previously by others (24), may be due just to enhanced lipid-filling of differentiated cells and not to enhanced differentiation.

A second possible reason for greater lipid-filling in cultures grown with plasma is that adipogenesis might be stimulated by the fibrinogen present in plasma and/or inhibited by activated coagulation and fibrinolytic factors present in serum. Whether or not these factors do indeed effect adipogenesis must be answered by further experiments.

Finally, the lower levels of lipid accumulation in cultures grown with serum, relative to the levels seen in cultures grown with plasma, may be a response to factors released by platelets during the process of coagulation (15) which either inhibit adipocyte differentiation (H. Green, personal communication) or promote more rapid proliferation of fibroblasts relative to preadipocytes.

If platelet factors do inhibit adipocyte differentiation, comparisons of serum samples may fail to reveal differences among blood donors that might be detected by comparisons of samples of plasma. Furthermore, while plasma constitutes an environment similar to that to which cells are exposed in vivo, serum prepared from whole blood does not. For these reasons, the examination of the effects of diet on adipogenic potency was limited to comparisons of samples of plasma. Platelets were removed from these plasma samples, but the comparison of heparin plasmas with and without intact platelets, mentioned above, suggests that this precaution was unnecessary.

Comparisons of effects of plasmas from Osborne-Mendel rats with differing recent dietary histories show that the levels of lipid-filling in cells cultured with plasma from chow-fed rats and plasma from rats fed HFS for 10 and 28 days are approximately proportional to the weight increases shown by these rats during the HFS feeding period. This suggests that the plasma concentration of some substance that affects or reflects weight gain (such as VLDL) also affects lipid-filling of cultured fat cells. There was either no lipid-filling or a relatively low level of lipid-filling in cells cultured with plasma from fasted rats, although this plasma supported normal rates of cell multiplication and GPDH activation. Thus, a 4-day fast may eliminate or diminish some factor (or factors) in the blood that normally promotes adipocyte lipid-filling. The near absence of lipid-filling in cultures grown with plasma from fasted rats in the first experiment suggests that the effect of food deprivation on lowering the ability of plasma to support lipid-filling may involve more than just a reduction in blood VLDL.

The ability of plasma to stimulate lipid-filling does not correlate with plasma glucose or insulin concentrations. Addition of glucose or insulin to plasma does not alter the ability of plasma to stimulate differentiation or lipid-filling

(ref. 20 and unpublished data). However, there is a strong correlation between plasma triglyceride level and degree of lipid accumulation in the cell cultures. In addition, as seen in this study, as well as previously by others (24), lipid accumulation increases in concert with increases in levels of VLDL added to the culture medium. These effects are not due to increased stimulation of cell multiplication because plasmas from fasting rats and from cats, both essentially devoid of adipogenic activity, show no deficiency in ability to promote cell proliferation. Also, it does not appear to be caused by increased differentiation of cells. Plasmas from HFS-fed rats, and plasmas with added VLDL do not promote greater GPDH activity than other plasmas. Moreover, the absence of VLDL does not prevent differentiation of 3T3-F442A or 3T3-L1 cells (22) or, as seen in the present study, differentiation of preadipocytes freshly harvested from the adipose tissue of rats. The increased triglyceride accumulation seen in cultures grown with plasma from HFS-fed rats and in cultures grown with added VLDL seems to be due solely to enhanced cellular lipid-filling of cells that have undergone at least one major differentiation event (elevation of GPDH activity). Whether the observed effects of diet and VLDL on lipid-filling resulted from differential rates of substrate utilization by fully mature fat cells or were due to differential activation of some enzyme (or enzymes) other than GPDH (25) remains to be determined.

In summary, the present study shows that freshly harvested rat preadipocytes in primary culture are comparable in some ways, but not in others, to adipose-converting 3T3-F442A cells studied by Green and his associates (13, 22, 23, 25). Proliferation of neither the 3T3 cells nor rat preadipocytes varies as a function of the source of the serum component of the culture medium. Both cell types fail to show adipose conversion when the only serum in the culture medium is cat serum. And in both 3T3 and rat preadipocytes, activation of GPDH does not diminish with removal of the VLDL fraction of the serum. However, while degree of lipid-filling in cultures of rat preadipocytes is greatly affected by the quantity of VLDL in the culture medium, 3T3-F442A cultures have been seen to accumulate roughly the same amount of lipid in the presence of lipid-free serum as in the presence of whole serum (22). The 3T3-F442A and rat preadipocyte cultures compared in the present study differed markedly in degree of GPDH activity. However, this difference may be due, at least in part, to the presence in the primary cultures of adipose tissue S-V cells that were not preadipocytes.

The recent dietary status of rats used as serum or plasma donors did not affect the rate of proliferation of cultured preadipocytes or the degree of elevation of GPDH activity in the cultures around the time of confluence. However, it did affect the rate at which converted cells filled with lipid. Since the VLDL content of plasma varies as a function of diet, and lipid-filling of cultured preadipocytes varies as a function of plasma VLDL, it is very likely that the observed effects of diet on lipid-filling in the present study were mediated by variations among the tested plasmas in their VLDL contents. The effects of diet and VLDL on lipid-filling may stem from an effect on some differentiation event that is independent of elevation of GPDH activity (25) or they may result from effects on substrate utilization by fat cells that are fully mature. The relative contributions of these two possibilities to the effects seen in the present study remain to be determined. Since diet had no measurable effect on plasma support of either multiplication of preadipocytes or on GPDH activation in these cells following confluence, the manner in which diet influences the production of adipocytes in animals remains unresolved. Perhaps, diet-dependent variation of some plasma component affects fat cell maturation via an influence on the activation of an enzyme (or enzymes) other than GPDH. Alternatively, locally acting tissue factors, perhaps released by fat cells as a function of the degree to which they are filled with lipid, may stimulate or inhibit preadipocyte proliferation or differentiation (11, 26). ⬛

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