

Intercellular Communication Between Muscle Cells and 3T3-L1 Preadipocytes

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Abstract

As specific factors involved in the intercellular communication between muscle and fat cells are ill-defined, the present project was initiated to study the interaction between myogenic satellite cells and preadipocytes with reference to the insulin-like growth factor (IGF)/IGF binding protein (IGFBP) system. Sheep satellite cell strain J-6 and 3T3-L1 preadipocytes were used in the development of a defined co-culture system. Cells were plated in individual cultures, or in co-cultures, and allowed to condition medium for 0-24, 0-48, 0-72, 0-96, 0-120 hr *in vitro*. At the times indicated, cultures were terminated and cell numbers determined. Conditioned media were removed from all cultures and evaluated for IGF-I and IGF-II by RIA, and IGFBP by western ligand blot. 3T3-L1 cell numbers in individual cultures declined immediately upon exposure to the defined medium. In co-cultures 3T3-L1 cell numbers initially declined ($P < .05$), but by 48 hr in co-culture 3T3-L1 cell numbers were stabilized at approximately 65-70% of 24 hr plating density. This density (~ 375 nuclei per mm^2) was maintained throughout the remainder of the study ($P > .05$). J-6 satellite cell numbers (~ 200 nuclei per mm^2) were constant in individual cultures and in co-cultures ($P > .05$). IGF-I levels in conditioned medium samples were low for all cell types and culture systems (1-2 ng/ml). IGF-II levels (2-8 ng/ml) were higher than IGF-I levels in conditioned media samples. IGF-II was not produced by 3T3-L1 cells to any appreciable level, but was produced by J-6 cultures and in co-cultures to an equivalent level ($P > .05$). The J-6 satellite cell strain secreted relatively high levels of IGFBP-2 and smaller amounts of IGFBP-3 and IGFBP-4. When co-cultured with 3T3-L1 preadipocytes, there was an enhanced secretion of all IGFBPs, but especially IGFBP-3 and -4. Secretion of the IGFBPs increased over time in culture and was positively correlated with the degree of differentiation of the J-6 cells. The current study suggests that the growth of 3T3-L1 preadipocytes is positively influenced by sheep-derived satellite cells and that the presence of preadipocytes induced an increase of IGFBP secretion by differentiating satellite cells.

Key words: satellite cell, 3T3-L1 cell, sheep muscle, IGFs, IGFBP, paracrine.

BAM 6 (6): 503-512, 1996

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Satellite cells [29] from meat animals may be isolated from postnatal skeletal muscle and propagated in cell culture [13, 16, 17], providing a system to study satellite cell activation [4], satellite cell involvement in muscle regeneration and all aspects of postnatal myogenesis [2, 16, 44]. This laboratory has isolated pure populations of satellite cell strains from sheep skeletal muscles [17, 33, 34] that display different degrees of proliferation and differentiation *in vitro* [33, 44]. These non-transformed satellite cell strains are derived from single cell clones and maintain

stable phenotypes for months in culture [44]. In conjunction with the use of serum-free defined culture media [14, 16, 44], these strains allow the examination of satellite cell growth in a well-controlled environment, free from exogenous influences from serum. The J-6 satellite cell strain, used for the present studies, remains viable for numerous generations, *in vitro*, and expresses moderate differentiation levels (30-40% fusion) [33, 34]. A subsequent report showed that exposure to a defined fusion medium enhanced the differentiation amount of the J-6 cells to nearly

60% [44]. For the present studies, this satellite cell strain possessed attractive growth and differentiation properties, which could be monitored for changes (either proliferation or differentiation) due to co-culture with a preadipocyte cell line (3T3-L1 cells).

Adipocytes develop from primordial cells termed preadipocytes, and the extent of postnatal cellular differentiation is genetically and environmentally controlled [1, 7, 23, 24, 41]. Few fat-type stem cells initiate the complete differentiation program or form distinguishable adipocytes until postnatal stimuli induce them to do so [1, 23]. Cell cultures have been used to address questions about preadipocyte activation and adipocyte maturation [1, 6, 7, 23, 24, 41]. For the purposes of the present studies, 3T3-L1 cells were chosen based on the large amount of literature published in which these cells were used as fat cell models, on our experience in using 3T3-L1 cells for other projects, on the potential use of these cells as references for subsequent studies with muscle-derived preadipocytes, and so that we would not sacrifice substantial numbers of muscle-derived preadipocytes [42], which we are currently characterizing, for co-culture system development. 3T3-L1 cells respond to mitogens but also may differentiate and fill with lipid in a similar manner to preadipocytes *in vitro*.

The endocrine system regulates the growth of tissues, organs and cells. Although the endocrine system functions, in part, to coordinate total body nutrient use and metabolism, factors that induce local changes in growth, specific to site, are still not well characterized. For example, different skeletal muscles possess different degrees of adiposity, which are regulated by inherent genetic mechanisms. However, does a local communications system also exist within specific muscles that regulates and coordinates the proportion of fat in these muscles? Are these locally identified agents from the systemic regulatory system or are they paracrine regulators?

A recent review from this laboratory describes present knowledge about extrinsic factors that regulate meat animal satellite cell proliferation and differentiation [16]. Of the factors known to influence meat animal satellite cells, the insulin-like growth factors (IGFs) seem to be the most dynamic, as the source of the IGFs may be from both endocrine and paracrine sites. Further, the IGFs not only interact with cellular receptors but also with six IGF-specific binding proteins (IGFBPs), located in both the systemic circulation and in the extracellular milieu, which modulate the actions of the IGFs [31]. As both preadipocytes and satellite cells are target cells for endocrine IGFs, and as they are thought to produce locally-acting IGFs/IGFBPs themselves, a goal of this research to define the interaction of the IGF/IGFBP system in providing intercellular regulation between these cells. As such we have extended present knowledge with satellite cells into the development of a defined co-culture system for myogenic and adipogenic cells [27, 42, 43]. IGF-I and -II and IGFBP secretion by muscle satellite cells and 3T3-L1 preadipocytes, grown alone and in co-culture, was examined in this study.

Materials and Methods

Materials

The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO): pig skin gelatin (PSG; G-1890), leupeptin (L-2884), pantothenic acid (P-5155), triiodothyronine (T-5516), transferrin (T-5391), biotin (B-4639), HEPES (H-9136), Giemsa stain (GS-1L), oil red O stain (O-0625), polyethylene glycol (P-1458), rabbit gamma globulin (G-0261) and prestained standards (C-3437). Fetal bovine serum (FBS; 26140-020), penicillin-streptomycin (15140-015), gentamicin (15170-023), Dulbecco's Modified Eagle Medium (DMEM; 12800-017), and Ham's F-12 Nutrient Mixture (F-12; 21700-075) were purchased from Gibco/Life Technologies, Inc. (Grand Island, NY). Bovine insulin (40205) was obtained from Collaborative Biomedical Products (Bedford, MA), and IGF-I and IGF-II were purchased from Bachem (Torrance, CA). Anti-IGF-II antibodies were obtained from Amano Enzyme (Troy, VA). Plastic cultureware, including 10 mm cell culture inserts (No. 162243), was purchased from Nunc Inc. (Naperville, IL). Centricon-10 concentrators (No. 4205) were obtained from Amicon, Inc. (Beverly, MA).

Defined medium

A defined medium termed ITT [11], which has been validated for use with sheep satellite cells [45] was used in all experiments. This medium consisted of 500 nM insulin, 0.2 nM triiodothyronine, 10 µg/ml transferrin, 33 µM biotin, 15 mM HEPES, and 17 µM pantothenate in a base of DMEM and F-12 [1:1].

Animals and cells

Satellite cell strain J-6 originated from clones which were obtained from the semimembranosus and semitendinosus muscles of a black-face wether lamb [33, 34]. The 3T3-L1 cell line was purchased from the American Type Culture Collection (ATCC; CCL 92.1; Rockville, MD). J-6 cells of passage number 5 through 7 and 3T3-L1 cells of passage number 6 through 12 were used in all experiments. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% O₂.

Co-culture procedures

In all experiments the 3T3-L1 cells were plated into the uncoated wells of 24-well culture plates at a density of 200,000 cells per well (995 nuclei/mm²). Cells of the J-6 satellite cell strain were plated onto 0.5% pig skin gelatin-coated [15] membranes at a density of 20,000 nuclei per insert (200 nuclei/mm²). Both 3T3-L1 cells and satellite cells were grown in separate wells for 24 hours in DMEM supplemented with 10% fetal bovine serum (FBS) to facilitate attachment. After the wells and inserts were thoroughly washed with serum-free DMEM [27], inserts containing J-6 cells were placed into wells containing 3T3-L1 cells, and the defined medium was added. Control cultures consisted of J-6 cells grown alone on inserts in

wells with no 3T3-L1 cells and 3T3-L1 cells grown alone in wells with inserts, but no J-6 cells. All media contained 100 U per ml penicillin-streptomycin and 50 µg per ml gentamicin. Samples of conditioned media were removed from the wells after the following time intervals of conditioning by the cells: 0-24 h, 0-48 h, 0-72 h, and 0-120 h post treatment initiation with no further media changes. The conditioned media samples were analyzed for IGF-I, IGF-II, and IGFBPs. Evaluation of the cultured cells was as described [44], following oil red O staining of the 3T3-L1 preadipocytes and Giemsa staining of the J-6 satellite cells at the times that conditioned media samples were collected. The nuclear density in 10 randomly chosen fields of each culture well or insert was determined by counting cells at 400 X magnification. Cells were classified as myotubes if they contained three or more myonuclei [44].

Collection of conditioned media

Conditioned media were removed from the wells, pooled by treatment [3 wells (3 ml being pooled together into one sample)], and leupeptin was added to each pooled sample (75 µg per 3 ml sample) on ice. Conditioned media samples were then centrifuged at low speed to remove cellular debris. Aliquots of 300 µl were taken from all sample vials and frozen (-70°C) for IGF quantitation. The remaining conditioned medium was concentrated 4 - 5 X using Centricon-10 concentrators (mw cutoff = 10,000) for IGFBP analyses.

IGF/IGFBP quantitation

A double-antibody radioimmunoassay (RIA) was used to analyze conditioned media samples for IGF-I [25]. Specific activity of the radiolabeled standard was 50-100 Ci/g. Antisera (UBK 487) to IGF-I, obtained from the National Hormone and Pituitary Program (Baltimore, MD), did not react with IGF-II and only minimally with insulin at 1 µmol/l.

Concentrations of IGF-II in conditioned media were measured after a modified formic acid-acetone extraction based on the original procedure of Bowsher et al. [8]. In order to accurately assess the low levels of IGF-II in the media, the extracts were evaporated under a stream of nitrogen gas, lyophilized overnight and neutralized by the addition of 100 µl 0.1 M Tris-HCl (pH 8.6) and 0.5% BSA. After the addition of radioactive IGF-II and 1 ng monoclonal antibody to IGF-II, samples were incubated for 16 h at 4°C in 0.1 M Tris-HCl (pH 7.4), and 0.5% BSA (300 µl total volume). Bound radioactivity was precipitated with 1 ml 16% polyethylene glycol after addition of 300 µl of 5 mg/ml rabbit gamma globulin. Human IGF-II was used as standard. Standards were extracted and neutralized exactly as conditioned media samples. Under these conditions, 47% of the ¹²⁵I-IGF-II was specifically bound, and extracted samples were parallel to the standard curve. The use of the formic acid-acetone extraction system has been validated for sheep serum [38].

The separation of proteins was accomplished with SDS polyacrylamide gel electrophoresis in the absence of reducing agent [28] in 12% gels loaded with 75 µl of conditioned media. A semi-dry electrophoretic transfer apparatus (BioRad Transblot, Richmond, CA) was used to transfer the separated proteins to nitrocellulose membranes. The membranes were then fixed overnight with glutaraldehyde [37], hybridized to ¹²⁵I-IGF-I (5 x 10⁵ cpm/ml) as described by Hossenloep et al [26] and exposed to Kodak X-OMAT AR5 X-ray film at -70°C for 3-5 days. Linear regression analysis of molecular weight standards was used to determine the molecular weights of all radioactive bands. As antisera to the IGFBPs are not readily available, identification of IGFBP was based on comparison of estimated molecular weights to those published previously [21], after subtraction of the molecular weight of IGF.

Statistical design and data analyses

Data were analyzed by analysis of variance of unequal subclass numbers [39]. Separation of treatment means was by t-test, and unequal variances were accounted for in this test. Graphs were plotted on Cricket® graph or Sigma Plot®. The level of significance was estimated using the value (P < .05).

Results

Sheep satellite cells of the strain J-6 were plated onto inserts and grown in ITT defined medium for 120 hr. When grown alone or together with 3T3-L1 cells, J-6 cell numbers were constant (P > .05) throughout the treatment period (Figure 1 A). J-6 satellite cells, grown alone or in co-culture, began to fuse after 24 hr in culture (Figure 1 B). Alternatively, 3T3-L1 cell numbers dropped substantially between 24 hr and 72 hr, if cultured alone (Figure 1 A). By 72 hr, *in vitro*, 3T3-L1 cell cultures displayed a negligible cell density. By comparison, co-cultures of 3T3-L1 and J-6 cells maintained a relatively high 3T3-L1 cell density (approximating 65-70% of 24 hr density) after 48 hr *in vitro*.

Figure 2 summarizes the IGF-I and IGF-II levels in conditioned media samples from cultures of J-6 and 3T3-L1 cells, grown alone and in co-culture. Sensitivity of the two assays was approximately 1.5 ng/ml for both IGF-I and IGF-II. IGF-I levels in conditioned media samples from J-6 cultures were below or very near the sensitivity of the IGF-I RIA. Levels of IGF-I were low and unaltered by time in culture, cell type or co-culture. Levels of IGF-II increased over time in culture in media conditioned by J-6 cells, and co-culture with 3T3-L1 cells did not alter the accumulation of IGF-II in the medium. 3T3-L1 cells did not secrete IGF-II.

The pattern of IGFBP secretion by the cells is shown in Figure 3. There was no detectable IGFBP in conditioned medium from 3T3-L1 cells at any time. The J-6 cells secreted detectable amounts of IGFBP-2 at 24 hr. Levels of IGFBP-2 in J-6 cell conditioned medium increased with

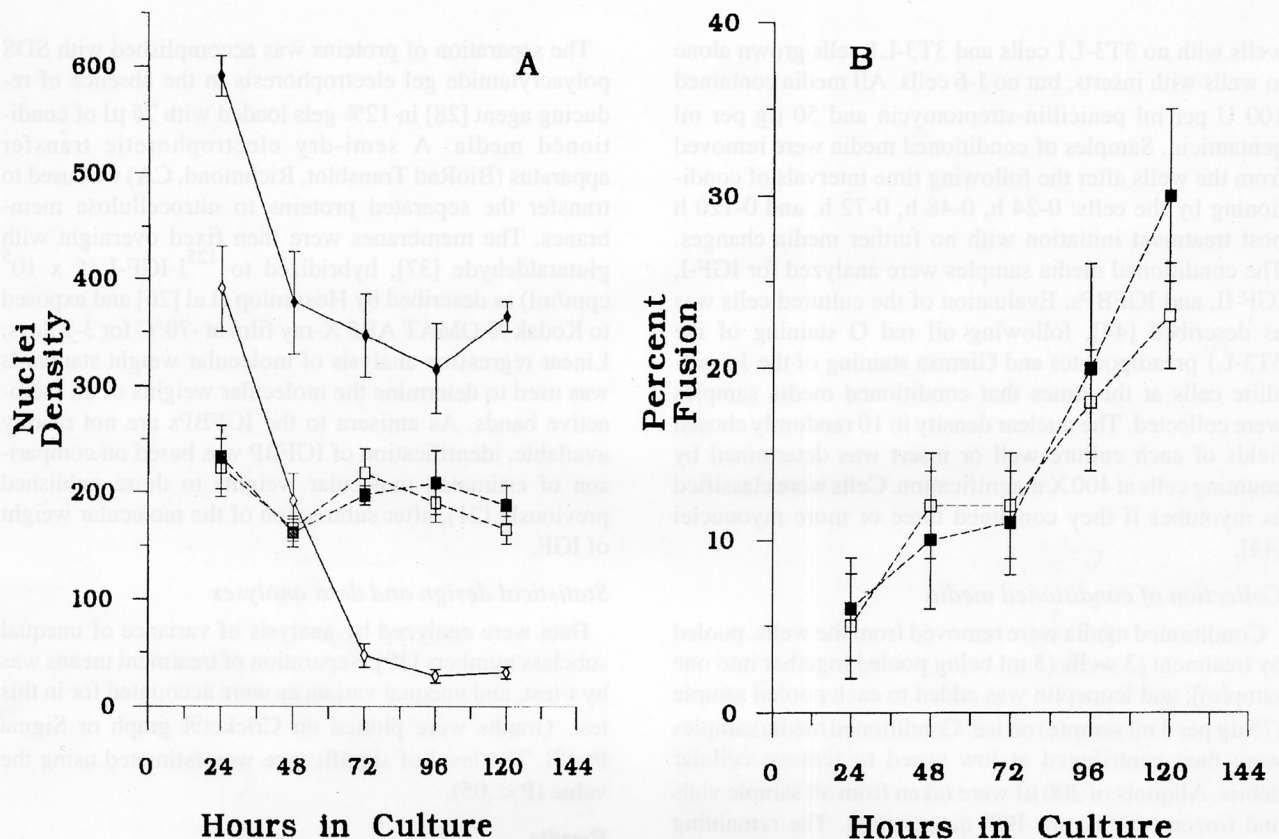


Figure 1. (A) Cell status of individual and co-cultures of 3T3-L1 cells and J-6 satellite cells (SEM). Cultures were initiated individually. At time 0, J-6 cells on inserts were introduced into 3T3-L1 cultures. Control cultures were treated similarly where appropriate (ex. J-6 control cultures were initiated on inserts and 24 hr later were placed into new wells with no 3T3-L1 cells). Nuclei density refers to the average nuclei per mm² detected in ten randomly chosen microscope fields (per well or insert). (◇) 3T3-L1 cells in individual cultures, N = 8-9 ea; (◆) 3T3-L1 cells in co-cultures, N = 6 ea; (□) J-6 satellite cells in individual cultures, N = 6-9; (■) J-6 satellite cells in co-cultures, N = 6-9. (B) Fusion levels of J-6 satellite cells alone (□), or in co-culture (■).

time, *in vitro*, and IGFBP-2 was the predominant IGFBP secreted by J-6 cells. In addition, smaller amounts of IGFBP-3 and IGFBP-4 were present in the J-6 conditioned medium after 2-3 days *in vitro*. The binding of ¹²⁵I-IGF-I to IGFBP-2, -3, and -4 in conditioned medium was enhanced when the cells were co-cultured with 3T3-L1 cells. Co-culture was especially effective in inducing IGFBP-3 and -4. As shown by the densitometer tracings in Figure 4, there was a progressive increase with time in total ¹²⁵I-IGF-I binding of co-cultures over J-6 satellite cells grown alone. While co-culture enhanced IGFBP-2 binding by almost 2-fold (over J-6 cells alone) after 120 hr, the binding by IGFBP-3 and IGFBP-4 was enhanced 2.7-fold and almost 8-fold, respectively. Thus the presence of 3T3-L1 cells appears to enhance IGFBP secretion by J-6 satellite cells. Although IGFBP-2 remains the primary IGFBP of co-cultured cells, IGFBP-3 also binds a large proportion of IGF-I in medium conditioned for 120 hr by the co-cultured cells.

Discussion

Sheep satellite cells of the strain J-6 and 3T3-L1

preadipocytes were used for this study, in part, to establish conditions which might be useful for future co-culture of sheep satellite cells and preadipocytes. Myogenic satellite cells provide substantial numbers of myonuclei to postnatal skeletal muscle fibers and are the cells responsible for muscle fiber regeneration following myotrauma [2, 9, 16, 29, 35, 36]. Alternatively, 3T3-L1 cells were chosen for the stromal cell component, because they possess similar characteristics to muscle-derived preadipocytes.

A goal of this project was to establish a test system for determining the influence of co-cultured cells on each other. To this end we were only partially successful. For example, differentiation of myogenic cells is commonly observed in dense cultures [3]. Therefore, the density of the satellite cells (40 to 60% confluency) allowed us to monitor both proliferation and differentiation if triggered by the presence of 3T3-L1 cells in co-culture. Similarly, the density of the 3T3-L1 cells, 80 to 90% confluency, allowed us to measure both proliferation and differentiation, if triggered by the presence of the satellite cells in the co-culture environment. However, less than expected dif-

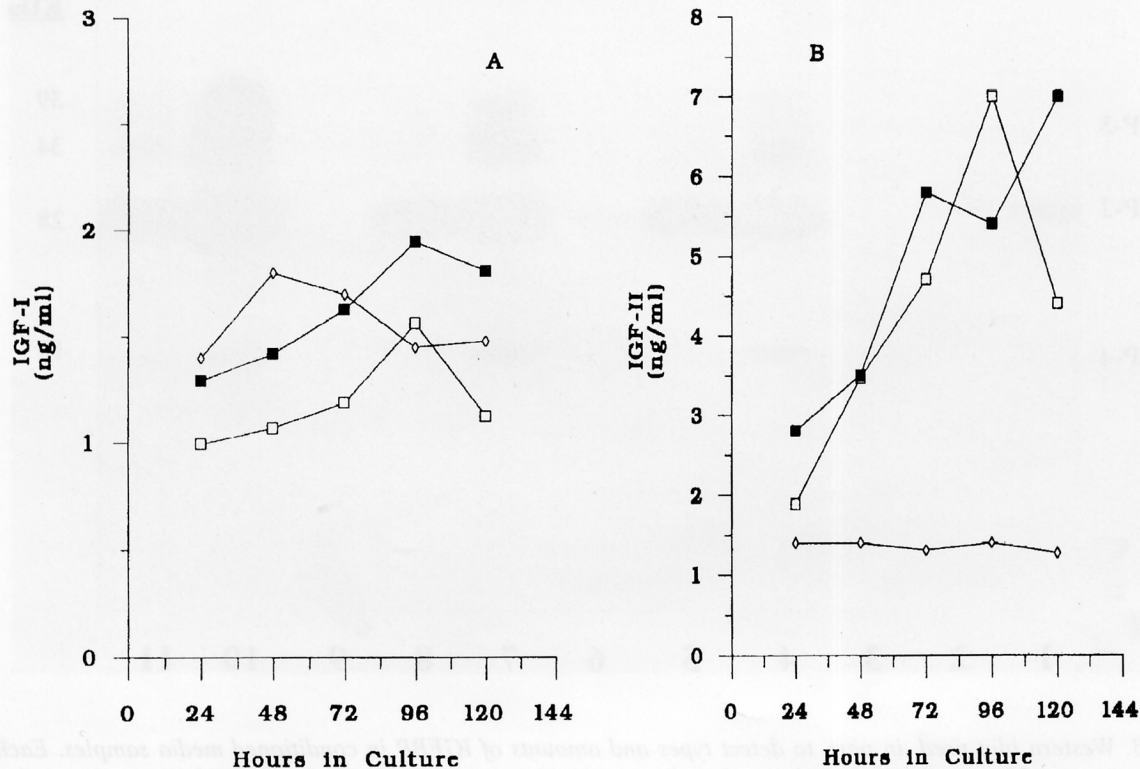


Figure 2. IGF-I (A) and IGF-II (B) levels (ng/ml) in conditioned media of individual and co-cultures. Conditioned media samples were evaluated for IGFs by radioimmunoassay, which had a sensitivity of 1.5 ng/ml. (□ J-6 cells in individual cultures; ◇ 3T3-L1 cells in individual cultures; ■ co-culture of J-6 and 3T3-L1 cells). For IGF-I, each point represent the means of two - three separate experiments. IGF-II values represent the mean of 3 observations per point. Standard error of the means ranged from .01 to .37 ng/ml for IGF-I and .2 to 2 ng/ml for IGF-II.

ferentiation was observed in the J-6 muscle cell cultures and few differentiated fat-like cells were observed in the 3T3-L1 component of the co-cultures. Thus, differentiation was not triggered by intercellular communication in this culture system.

As this study progressed, other incongruent observations arose with culture of the J-6 and 3T3-L1 cells. 3T3-L1 cells would not remain attached to the inserts, even with the use of numerous substrata to coat the membranes (data not shown). Detachment of 3T3-L1 cells was partially prevented through dipping [27] the inserts, rather than by using traditional rinsing techniques. 3T3-L1 cell detachment, although sporadic, also occurred at the time of fixing and staining of the cells, which meant that cell loss could occur just prior to evaluation of the cultures. For these reasons 3T3-L1 cells were plated onto the culture wells, and the J-6 satellite cells, were plated onto the inserts.

Another difficulty arose when we exposed the 3T3-L1 cells to the ITT medium. When plated alone, 3T3-L1 cells showed reduced viability and progressive cell death (Figure 1). Therefore, while the ITT medium facilitated satellite cell viability, it did not support 3T3-L1 cell growth and development. This was also apparent by the fact that 3T3-L1 cells displayed only spontaneous levels of differentiation in this medium. The ITT defined medium was

originally formulated for use in human preadipocyte cell cultures [11]. The formulation is very much like other media for fat-like precursor cells, including 3T3 cells [44]. The observation that 3T3-L1 cell numbers began to fall when cultured alone and exposed to this medium was surprising. In retrospect, other media like SFG and 4F [44] may have been better vehicles for sustaining the 3T3-L1 cells. However, other media such as SFG and 4F contain regulatory components like epidermal growth factor, fibroblast growth factor and fetuin that may have interfered with the IGF/IGFBP system [32]. In addition, these alternative media promoted only minimal fusion of satellite cells, and we were looking for a medium that promoted substantial fusion of the muscle cells in order to establish a myofiber model.

Our initial desire to use the ITT medium was based on the ability of ITT to support the differentiation of sheep satellite cell strains. We found that the ITT medium stimulated substantial fusion of many satellite cell strains if the cells were plated onto plastic cultureware [44]. Our intent was to subject the co-cultures to conditions that promoted satellite cell fusion (on inserts) and evaluate the ability of satellite cell-derived myotubes to elicit growth regulation on 3T3-L1 cells. We were surprised, however, because the J-6 satellite cells did not fuse to form extensive myotube

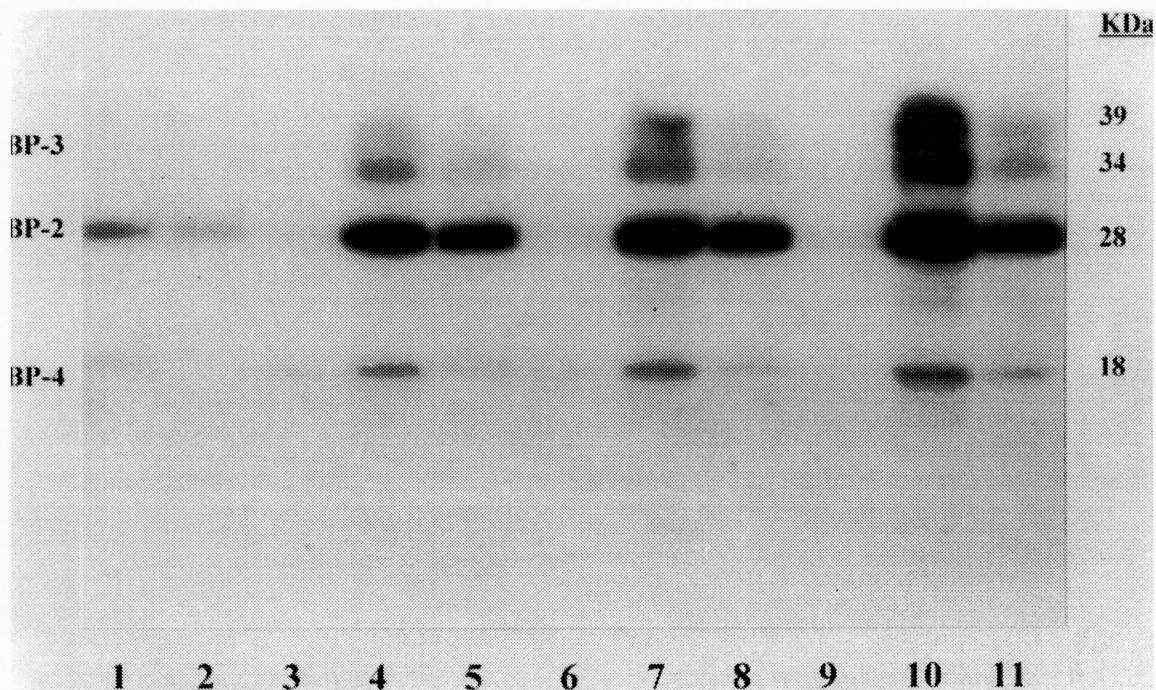


Figure 3. Western blot used, in part, to detect types and amounts of IGFBP in conditioned media samples. Each lane represents 75 microliters of conditioned media samples. Lanes 1, 4, 7, 10, 13; J-6 + 3T3-L1 (co-culture); Lanes 2, 5, 8, 11; J-6 cells (alone); Lanes 3, 6, 9; 3T3-L1 cells (alone). Lanes 1-2, 0-24 hr; Lanes 3-5, 0-48 hr; Lanes 6-8, 0-72 hr; Lanes 9-11, 0-120 hr.

structures on the inserts, as was described for satellite cells on other cell cultureware supports [44]. For example, while the J-6 satellite cell strain previously demonstrated 50 to 60% fusion when exposed to ITT in plastic culture wells, when these cells were plated onto the gelatin-coated membrane inserts, they only fused to 20 to 30%. Reasons for the modest amount of fusion by the J-6 satellite cells on the inserts, in the presence of the ITT defined medium, or 3T3-L1 cells, are unknown.

When grown alone, 3T3-L1 cell numbers dropped to near zero after 72 hr. However, when co-cultured with J-6 satellite cells, a significant number of the 3T3-L1 cells survived at 48 - 120 hr. This enhancement of preadipocyte viability by satellite cells suggests that the J-6 cell strain is secreting a soluble factor which rescues the 3T3-L1 cells from death.

The IGFs, in general, promote proliferation and differentiation of cultured satellite cells [12, 16, 19]. Proliferation and differentiation of myogenic cells seems to be mutually exclusive events [reviewed in 19]. In the present studies, both IGF-I and IGF-II levels in conditioned media samples were relatively low (Figure 2). The modest amount of IGF-I, or IGF-II, detected likely came from the J-6 satellite cells. IGF-II production by J-6 satellite cells was not affected by co-culture with 3T3-L1 cells. An explanation for the rising IGF-II levels observed in the present study, might be that IGF-II facilitated J-6 cell fusion [18-20].

The IGF-I and IGF-II levels in the conditioned media

may have been influenced by the presence of the relatively high insulin concentration contained in the defined medium (500 ng/ml). Use of insulin at levels exceeding that in the ITT medium have been reported in other defined media formulations, even those used for IGF-I studies [16]. However, insulin at this concentration will compete for the type I, IGF receptor [30], and may generate a physiological stimulus to both cell types [30]. By doing so, IGF-I or IGFBP production by the cells may have been changed [30, 31]. Future studies will have to be conducted to analyse the effects of different levels of insulin on IGF and IGFBP secretion by J-6 satellite cells and preadipocytes.

The IGF system is only one of a variety of regulatory systems that might be facilitating the viability of the 3T3-L1 preadipocytes in co-cultures. Activated J-6 satellite cells may produce and secrete agents that alter the binding of 3T3-L1 cells to the plastic cultureware [22], other growth factors [10], and unknown agents that may aid 3T3-L1 cell viability *in vitro*. However, not all types of satellite cell strains possessed the capability of enhancing 3T3-L1 cells in similar co-culture systems [data in preparation].

3T3-L1 cells and J-6 satellite cells were different in their production of IGFbps (Figure 3, Figure 4). This was not surprising, as not only do different myogenic cells produce different patterns of IGFBP [30, 32], but proliferating myogenic cells secrete IGFBP in different patterns than differentiating cells [18, 20, 30, 31]. For example, myo-

Communication between muscle and fat cells

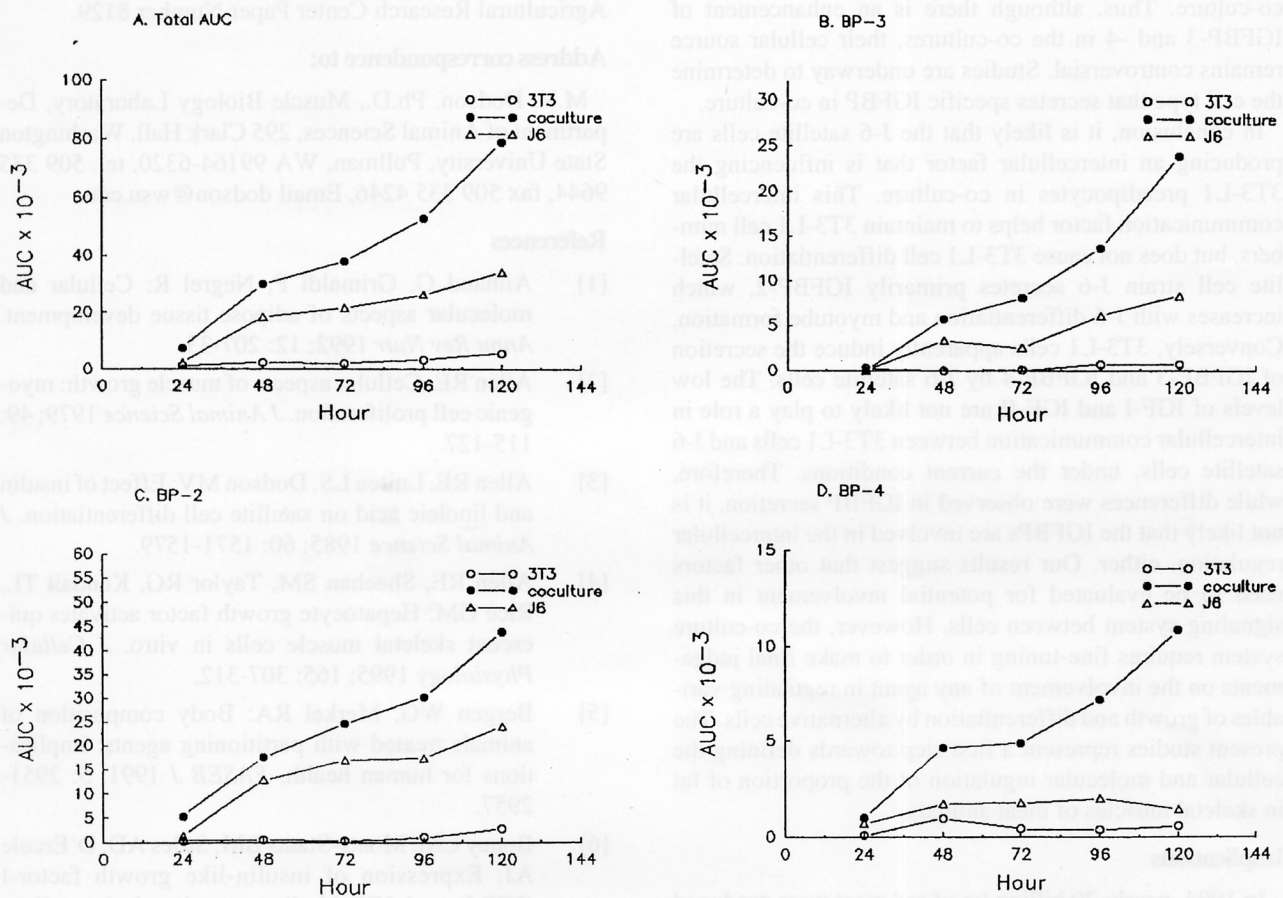


Figure 4. Results from densitometer evaluation of western blots for IGFBP definition. Values are area under the curve units ($\times 10^{-3}$).

genic cells like L-6 cells commonly secrete IGFBP-4 while proliferating, but secrete IGFBP-5 during the process of differentiation [31]. Western ligand blot analysis of IGFBPs in the media conditioned by satellite cells or 3T3-L1 preadipocytes showed possible cell-specific patterns of secretion and suggested that co-culture of these cells influenced IGFBP secretion. When grown alone, the 3T3-L1 cells did not secrete detectable amounts of IGFBP. In light of the rapidly declining cell numbers, this observation was not surprising. Boney *et al* [6] showed that proliferating 3T3-L1 cells secrete IGFBP-3 and -4, and that differentiation into adipocytes is accompanied by secretion of a 34 kDa IGFBP. Medium conditioned by J-6 satellite cells contained primarily IGFBP-2 (28 kDa), which was detected after 24 hr and which increased in intensity over the course of the experiment. Smaller amounts of IGFBP-3 and -4 were also detected. The intensity of these three bands was enhanced several fold when J-6 cells were co-cultured with 3T3-L1 cells.

Studies of IGFBP secretion by C₂C₁₂ myoblasts showed that IGFBP-2 is the major binding protein present and that IGFBP-2 secretion decreases as the cells differentiate [18]. Our results show that IGFBP-2 levels increase progressively throughout the culture period.

While different conditioned media sampling routines make comparisons difficult, our observations are consistent with a variety of muscle cell systems where it has been proposed that IGFBP-2 levels are related to differentiation of muscle cells [19, 30]. The production of IGFBP-3 by the J-6 satellite cells, and by co-cultures, is consistent with other muscle cell culture systems.

The effects of co-culture with 3T3-L1 preadipocytes on apparent IGFBP secretion by J-6 satellite cells suggest that the 3T3-L1 cells are inducing IGFBP secretion. There was a 2-fold increase in IGFBP-2 binding by medium conditioned by co-cultured cells, and there was an 3- and 8-fold increase in IGFBP-3 and -4 secretion when 3T3-L1 cells were co-cultured with J-6 satellite cells. Although these data provide evidence to indicate an induction of J-6 cell IGFBP secretion by 3T3-L1 preadipocytes, the cellular source of the IGFBP is difficult to determine for two reasons. First, the permeable nature of the membrane insert, separating the two cell populations, allows free diffusion of molecules the size of the binding proteins. In addition, the enhanced viability of the 3T3-L1 cells when co-cultured also makes the interpretation of the data difficult. As proliferating 3T3-L1 cells secrete IGFBP-3 and -4 [6], the 3T3-L1 cells may be the source of these IGFBP in

co-culture. Thus, although there is an enhancement of IGFBP-3 and -4 in the co-cultures, their cellular source remains controversial. Studies are underway to determine the cell type that secretes specific IGFBP in co-culture.

In conclusion, it is likely that the J-6 satellite cells are producing an intercellular factor that is influencing the 3T3-L1 preadipocytes in co-culture. This intercellular communication factor helps to maintain 3T3-L1 cell numbers, but does not cause 3T3-L1 cell differentiation. Satellite cell strain J-6 secretes primarily IGFBP-2, which increases with J-6 differentiation and myotube formation. Conversely, 3T3-L1 cells apparently induce the secretion of IGFBP-3 and IGFBP-4 by J-6 satellite cells. The low levels of IGF-I and IGF-II are not likely to play a role in intercellular communication between 3T3-L1 cells and J-6 satellite cells, under the current conditions. Therefore, while differences were observed in IGFBP secretion, it is not likely that the IGFBPs are involved in the intercellular regulation, either. Our results suggest that other factors need to be evaluated for potential involvement in this signaling system between cells. However, the co-culture system requires fine-tuning in order to make final judgments on the involvement of any agent in regulating variables of growth and differentiation by alternative cells. The present studies represent a first step towards defining the cellular and molecular regulation of the proportion of fat in skeletal muscles of meat animals.

Implications

In 1994, nearly 20 billion kg of red meat were produced in the United States [40]. This remarkable amount of meat animal production was not without cost, however. After considering losses from kidney fat, fat that stays on retail cuts to assure eating quality, and fat in edible rendered products, nearly 2 billion kg of fat was discarded or directed into by-product production in 1994 [40]. A reason for the waste fat accumulation in meat animals may be explained, in part, by the fact that intramuscular fat (marbling) is not deposited until late in maturity [5]. Marbling is important for flavor, texture and juiciness of meat products. However, considerable fat accumulation occurs (developmentally) in other body fat depots prior to the appearance of marbling in skeletal muscle [5]. From a production standpoint, extended feeding of meat animals in order to obtain a sufficient level of marbling to insure proper meat quality variables is an inefficient use of dietary energy and, therefore, costly. Determination of the intercellular, cellular and molecular regulation of muscle cells and muscle-derived preadipocytes would result in ways to change the timing of fat deposition in muscle and reduce the cost associated with extended feeding of meat animals prior to slaughter.

Acknowledgements

Supported by the Washington State Agricultural Research Station, Project 0913, and the Colorado State Agricultural Research Station, Project 0629. Washington State

Agricultural Research Center Paper Number 8129.

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