

The Effects of Ergogenic Compounds on Myogenic Satellite Cells

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ABSTRACT

VIERCK, J. L., D. L. ICENOGGLE, L. BUCCI, and M. V. DODSON. The Effects of Ergogenic Compounds on Myogenic Satellite Cells. *Med. Sci. Sports Exerc.*, Vol. 35, No. 5, pp. 769–776, 2003. **Purpose:** A series of studies were conducted in which compounds commonly shown to be ergogenic aids for strength athletes if taken orally were evaluated for their ability to directly induce postnatal muscle stem cell proliferation or differentiation/fusion *in vitro*. **Methods:** Compounds tested were creatine monohydrate, creatine pyruvate, L-glutamine, dehydroepiandrosterone (DHEA), androstenedione, Ma Huang (*Ephedra sinensis*) extract, and Zhi Shi (*Citrus aurantium*) extract. Dulbecco's modified eagle medium, supplemented with minimal levels of serum and antibiotics, was used as the initial vehicle for the test compounds. Subsequently, a defined treatment medium termed ITTC was used. Satellite cells were exposed to the test compounds for the indicated times and then evaluated by counting mononucleated and multinucleated (fused) cells. **Results:** In serum-containing media, none of the treatment groups displayed increased proliferation over that of the control. However, in the differentiation cultures, 0.10% creatine monohydrate increased differentiation over that of the control cultures. When 0.10% creatine monohydrate was added to defined media formulations, all treatments but one demonstrated increased differentiation over the 0.5% serum control. Time course experiments, which followed the effect of 0.10% creatine monohydrate contained in ITTC defined media over 120 h, suggested that cells exposed to this treatment differentiated earlier and to a greater level than cells exposed to ITTC alone. **Conclusions:** Creatine in the monohydrate form induced differentiation of myogenic satellite cells. Other agents examined did not increase satellite cell proliferation or differentiation. These results provide initial evidence for a mechanistic understanding of observed effects *in vivo* of increased muscular size and strength from creatine supplementation. **Key Words:** MUSCLE STEM CELL CULTURE, NUTRICEUTICALS, L-GLUTAMINE, CREATINE, EPHEDRINE ALKALOIDS, PYRUVATE, ANDROSTENEDIONE, DHEA

Many athletes desire increased performance during resistance, endurance, or low-impact training/events. A common practice among athletes seeking increased muscular mass and/or strength is the consumption of a variety of ergogenic aids (6,37). Dietary ergogenic supplements marketed as having benefits for increasing muscle mass and strength are numerous. Anabolic steroid administration has been associated with increases in muscle size and strength but is not acceptable for use by athletes because of legal issues, ethics, and safety (7,21,28,45–47). The most popular noncaloric ergogenic aids include creatine, pyruvate, beta-hydroxy-beta-methylbutyrate (HMB), L-glutamine, and prohormones (DHEA, androstenedione, norandrostenediones, norandrostendiols, and others) (4,10). Of these, only creatine and HMB have reproducible evidence of increases in muscle mass and

strength in individuals engaged in resistance exercise (2,3,5,13,18,20,23,24,26,27,34,35). Prohormones tested in exercising human subjects to date, DHEA and androstenedione, have not been associated with increased muscle mass and strength in human studies (8,9,25,36,40,44,48).

Previous studies of supplements utilized *in vivo* animal cell models. Dangott et al. (14) found that creatine supplementation in rats, in combination with an increased functional load, resulted in increased activity of muscle precursor cells called satellite cells. However, little research has been published that utilized *in vitro* experiments to examine the action of ergogenic compounds at the cellular level. One exception is the work by Ingwall et al. (22), which indicated that creatine is involved in the regulation of the synthesis of muscle proteins.

In vitro experiments utilizing cell cultures offer the advantages of being readily available in large numbers for ease in obtaining data, easy to manipulate and expose to specific external environmental factors and less expensive than *in vivo* studies. Proper interpretation when extrapolating cell culture data back to the whole animal allows this tool to be useful in deciphering the actions of nutritional supplements on skeletal muscle (41).

The following studies were undertaken to gain knowledge about the mechanisms through which ergogenic dietary supplements, commonly used by strength athletes for muscle hypertrophy and strength, work at the cellular level. We

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screened a group of seven compounds for their effects on altering satellite cell activity. These commercially available supplements included two creatine compounds, two steroids, two herbal alkaloids, and one amino acid. The hypothesis of this project was that the administration of ergogenic compounds may accelerate normal skeletal muscle hypertrophy by facilitating the proliferation and differentiation of satellite cells. The long-term goal of this project is to decipher the cellular mechanisms involved in the action of ergogenic aids used for increasing muscle mass and strength.

METHODS

Materials. Ovine satellite cells were obtained from a young adult sheep and stored in liquid nitrogen using routine isolation and handling protocols (11,16). The basal media were composed of Dulbecco's modified eagle medium (DMEM), antibiotics, and either horse serum (HS) or fetal bovine serum (FBS). The HS, FBS, DMEM, penicillin/streptomycin, and gentamicin were purchased from Invitrogen/Life Technologies (Carlsbad, CA). Pig skin gelatin (porcine skin, type A) and Giemsa stain (Accustain) were purchased from Sigma Chemical Company (St. Louis, MO). Creatine monohydrate (CM), creatine pyruvate (AMT Laboratories, North Salt Lake City, UT), L-glutamine, dehydroepiandrosterone (DHEA), androstenedione, *Ephedra sinensis* extract (Ma Huang, 6% alkaloids) and *Citrus aurantium* extract (Zhi Shi or Bitter Citrus, 6% alkaloids) were provided by Weider Nutrition International (Salt Lake City, UT). All cell cultureware was purchased from Nalge Nunc (Rochester, NY).

The ITTC defined medium consisted of DMEM:F12 (1:1) supplemented with insulin (850 nM), transferrin (10 $\mu\text{g}\cdot\text{mL}^{-1}$), triiodothyronine (0.2 nM), hydrocortisone (50 $\text{ng}\cdot\text{mL}^{-1}$), and antibiotics (43). The transferrin, triiodothyronine, and hydrocortisone were purchased from Sigma Chemical Company. The media and insulin were obtained from Invitrogen/Life Technologies.

General cell culture methods. Satellite cells were plated into 24-well culture plates that had been previously coated with 0.1% pig skin gelatin (16). All of the cell cultures were incubated at 37°C in an atmosphere of 5% CO₂ and 95% air. In each experiment, the cells were initially plated in DMEM + 10% FBS for 24 h to achieve efficient cell attachment. The plating medium was then removed, the wells were rinsed three times with DMEM, and the appropriate treatments were added. Treatment media were changed every 24 h. At time intervals determined by the type of experiment, cultures were removed from incubation, fixed with methanol, and stained with Giemsa stain (16). Differentiation/fusion of satellite cells was determined when three or more nuclei were observed within a continuous cell membrane, forming a myotube (42). Total and fused nuclei densities were calculated by counting the number of nuclei in 10 randomly chosen areas (fields) of each well at 400 \times magnification (42).

Preparation of test compounds. CM, creatine pyruvate, and L-glutamine were diluted in basal media to levels of 0.1%, 0.25%, 0.5%, and 1.0% w·v⁻¹. The pH of creatine pyruvate was returned to 7.08. Androstenedione and DHEA were dissolved in ethanol, starting with 100 $\text{mg}\cdot\text{mL}^{-1}$, until the solution was saturated. Concentrations were determined by applying Beer's law and using an extinction coefficient of 16,200. Saturated androstenedione was calculated to be 1.751×10^{-4} M, and DHEA was 1.829×10^{-4} M. These two solutions were added to basal media using a 1:100 dilution. The first treatment level for androstenedione was 1.751×10^{-6} M, and DHEA was 1.829×10^{-6} M. Three subsequent concentrations were made by diluting each treatment stock 1:100, which resulted in a lowest concentration of androstenedione at 1.751×10^{-12} M and DHEA at 1.829×10^{-12} M. The controls for androstenedione and DHEA were basal media + 10 $\mu\text{L}\cdot\text{mL}^{-1}$ ethanol to control for any solvent effects. The last two compounds, Ma Huang and Zhi Shi extract, were dissolved in DMEM to make a 10% solution and centrifuged at 2700 rpm for 10 min to remove any insoluble particulate. The supernatants were then centrifuged at 12,500 rpm for 10 min, and the retentates were discarded. The treatment solutions were filtered twice, first with a 0.45- μm syringe filter and then with a 0.20- μm syringe filter. These two sterile extracts were next diluted in basal media to attain treatment application levels of 0.1%, 0.25%, 0.5%, and 1.0%. The final pH was adjusted to 7.08.

Proliferation and differentiation studies. For the proliferation experiments, satellite cells were plated at a density of 10 nuclei·mm⁻² and exposed for 96 h to treatments contained within a maintenance medium consisting of DMEM, 2.0% HS and antibiotics (Table 1). The differentiation studies were plated at a density of 100 nuclei·mm⁻² and exposed for 120 h to treatments contained within a maintenance medium of DMEM, 0.5% HS, and antibiotics (Table 1). The controls for both studies consisted of the maintenance medium without the test compound.

Subtraction studies. To expand on the observations derived from the initial screening experiments, 0.1% CM was used as a component in chemically defined treatment media. Satellite cells were plated at 100 nuclei·mm⁻² and exposed for 120 h to various defined treatment media: ITTC (insulin, transferrin, triiodothyronine, hydrocortisone), ITT (insulin, transferrin, triiodothyronine), IT³C (insulin, triiodothyronine, hydrocortisone), ITC (insulin, transferrin, hydrocortisone), and TTC (transferrin, triiodothyronine, hydrocortisone) (Table 1). Each of these treatments also contained 0.1% CM. The controls were either DMEM + 0.5%

TABLE 1. Experimental design of four studies.

Type of Study	Cell Density (nuclei·mm ⁻²)	Media	Fixed and Stained at
Proliferation	10	DMEM + 2.0% HS	96 h
Differentiation	100	DMEM + 0.5% HS	120 h
Subtraction	100	Five defined media + 0.1% CM	120 h
Time course	100	ITTC + 0.1% CM	24 h intervals up to 120 h

HS (the differentiation maintenance media) or ITTC with no CM. The wells were fixed and stained at the endpoint of 120 h.

Time course studies. The effects of ITTC plus 0.1% CM (ITTC-CM) on the proliferation and differentiation of satellite cells over time were then evaluated. Satellite cells were plated at $100 \text{ nuclei} \cdot \text{mm}^{-2}$, allowed to attach for 24 h in DMEM + 10% FBS, washed/rinsed, and then exposed to ITTC-CM (Table 1). The controls were either ITTC or DMEM + 0.5% HS. All wells were fixed and stained at intervals of 24 h up to a terminal point of 120 h.

Statistical analyses. Each experiment was performed twice for a total of six wells per treatment. At the conclusion of the experiments, all wells were counted, initial plating densities were subtracted, data were collated and reduced, and a one-way ANOVA was performed. The treatment means were separated and compared using the Tukey-Kramer multiple comparisons test (33). Significance for testing treatment effectiveness was established at ($P < 0.05$).

RESULTS

Proliferation studies. In the proliferation experiments with creatine monohydrate, creatine pyruvate, L-glutamine, androstenedione, and DHEA, none of the treatment groups induced proliferation over the control cultures ($P > 0.05$) (Fig. 1; Fig. 2, A and B; Fig. 3, A and B). Satellite cells exposed to 1.0% creatine pyruvate decreased in cell numbers, when compared with the control cultures ($P < 0.05$) (Fig. 2A). Androstenedione at $1.751 \times 10^{-6} \text{ M}$ and DHEA at $1.829 \times 10^{-6} \text{ M}$ precipitated when added to the basal media and subsequently induced cell death (Fig. 3, A and B). Androstenedione at $1.751 \times 10^{-8} \text{ M}$ and $1.751 \times 10^{-10} \text{ M}$ ($P < 0.05$) (Fig. 2A) and DHEA at $1.829 \times 10^{-8} \text{ M}$ (P

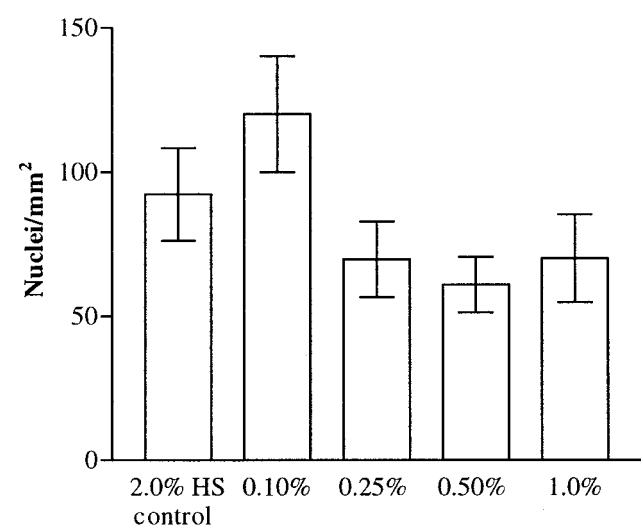
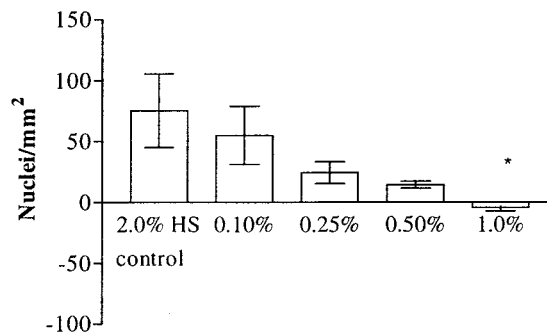


FIGURE 1—Effects of creatine monohydrate on satellite proliferation. Satellite cells (two experiments) were plated at a density of $10 \text{ nuclei} \cdot \text{mm}^{-2}$ and exposed for 96 h to a proliferative treatment medium containing DMEM, 2.0% HS, antibiotics, and either 0 (control), 0.10, 0.25, 0.50, or 1.0% creatine monohydrate. Each bar represents the mean \pm SEM of six wells.

Creatine Pyruvate A



L-Glutamine B

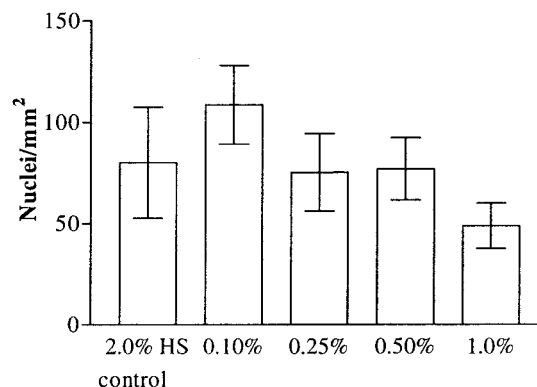


FIGURE 2—Effects of (A) creatine pyruvate and (B) L-glutamine on satellite cell proliferation. Satellite cells (two experiments per compound) were plated at a density of $10 \text{ nuclei} \cdot \text{mm}^{-2}$ and exposed for 96 h to a proliferative treatment medium containing DMEM, 2.0% HS, antibiotics, and either: 0 (control), 0.10, 0.25, 0.50, or 1.0% treatment compound. Each bar represents the mean \pm SEM of six wells. *Significantly different from 2.0% HS control.

$P < 0.05$) (Fig. 3B) decreased satellite cell numbers. Extracts of Ma Huang and Zhi Shi induced cell death at all concentrations tested.

Differentiation studies. Only the 0.1% creatine monohydrate treatment induced differentiation (albeit low) of satellite cells when compared with control cultures ($P < 0.05$) (Fig. 4A). Creatine pyruvate, L-glutamine, androstenedione, and DHEA produced no detectable fusion; thus, no graphs are presented.

Under differentiation conditions, the 1.0% ($P < 0.05$) and 0.5% ($P < 0.05$) creatine monohydrate treatment cultures decreased in cell numbers compared to controls (Fig. 4B). The control and the 0.10% and 0.25% creatine monohydrate treatment groups did not proliferate but maintained their cell numbers. These results suggest that the decrease in number of nuclei was not due to the basal media but to an increase in concentration of creatine monohydrate above 0.10%.

The 1.0% creatine pyruvate treatment group ($P < 0.05$, Fig. 5A), all of the glutamine treatment groups ($P < 0.05$, Fig. 5B), and the $1.829 \times 10^{-8} \text{ M}$ DHEA treatment group ($P < 0.05$, Fig. 6B) all decreased in cell number when compared with control. Androstenedione at 1.751×10^{-6}

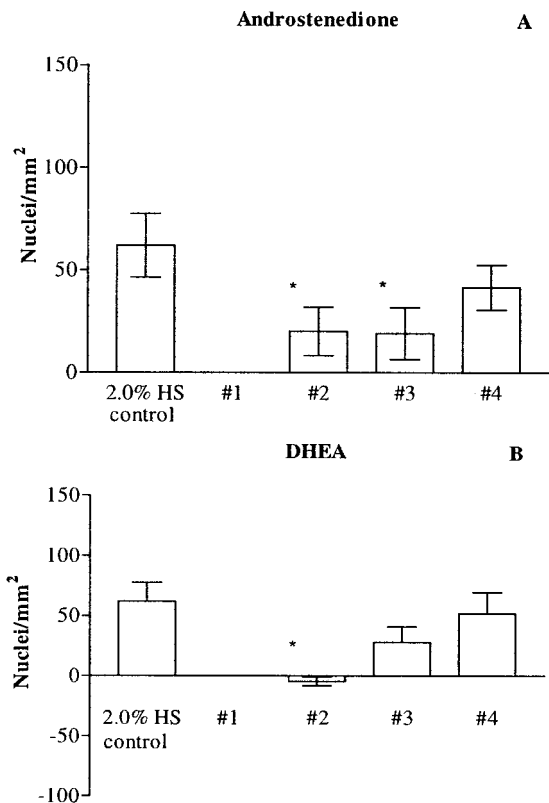


FIGURE 3—Effects of (A) androstenedione and (B) DHEA on satellite cell proliferation. Satellite cells (two experiments per compound) were plated at a density of 10 nuclei·mm⁻² and exposed for 96 h to a proliferative treatment medium containing DMEM, 2.0% HS, antibiotics, and either: (A) #1 = 1.751 × 10⁻⁶ M, #2 = 1.751 × 10⁻⁸ M, #3 = 1.751 × 10⁻¹⁰ M, #4 = 1.751 × 10⁻¹² M androstenedione; (B) #1 = 1.829 × 10⁻⁶ M, #2 = 1.829 × 10⁻⁸ M, #3 = 1.829 × 10⁻¹⁰ M, #4 = 1.829 × 10⁻¹² M DHEA. The controls for both androstenedione and DHEA consisted of basal media + 10 μL ETOH. Each bar represents the mean ± SEM of six wells. *Significantly different from 2.0% HS control.

M and DHEA at 1.829 × 10⁻⁶ M precipitated when added to the basal media and subsequently induced cell death (Fig. 6A, B). All levels of Ma Huang and Zhi Shi extracts caused cell death in the satellite cell cultures.

Subtraction studies. Only ITTC-CM promoted an increase in differentiation over that of the ITTC control (*P* < 0.05) (Fig. 7A). All of the treatment groups displayed an increase in differentiation over the 0.5% serum control (*P* < 0.05) with the exception of TTC-CM (*P* > 0.05), suggesting that insulin was a necessary component for the creatine effect. All of the treatments were able to maintain similar numbers of cells (*P* > 0.05) when compared with the ITTC defined medium control (Fig. 7B), except for the TTC-CM treatment (*P* < 0.05), which decreased satellite cell numbers.

Time course studies. The time course experiments suggested that cells exposed to ITTC + 0.1% CM tended to differentiate earlier, and to a greater extent, than cells exposed to ITTC alone (Fig. 8). At 120 h, there was an increase in fusion of the ITTC-CM treatment group over the reduced serum control (*P* < 0.05) but not of the ITTC treatment group over the serum control (*P* > 0.05). In the

0.5% horse serum control, the initial cell numbers were maintained over 120 h (Fig. 8A). The low numbers of fused nuclei and low percent fusion rate in the serum control provided a base for detecting the effects of creatine supplementation on differentiation and cell density. In the ITTC control, as the number of mononucleated cells decreased, the number of fused nuclei increased, resulting in an increase in the percent fusion (Fig. 8B). In the ITTC-CM treatment group, the number of mononucleated cells decreased over time and were replaced by fused nuclei (Fig. 8C).

DISCUSSION

At the cellular level, it is generally assumed that growth of normal skeletal muscle cells (myofibers) occurs by two distinct processes, hyperplasia and hypertrophy. In most mammals, myofiber hyperplasia is associated with embryonic muscle, and myofiber hypertrophy is associated with postnatal muscle growth. The number of muscle fibers present in mammals is generally fixed at birth (19,30,38,39),

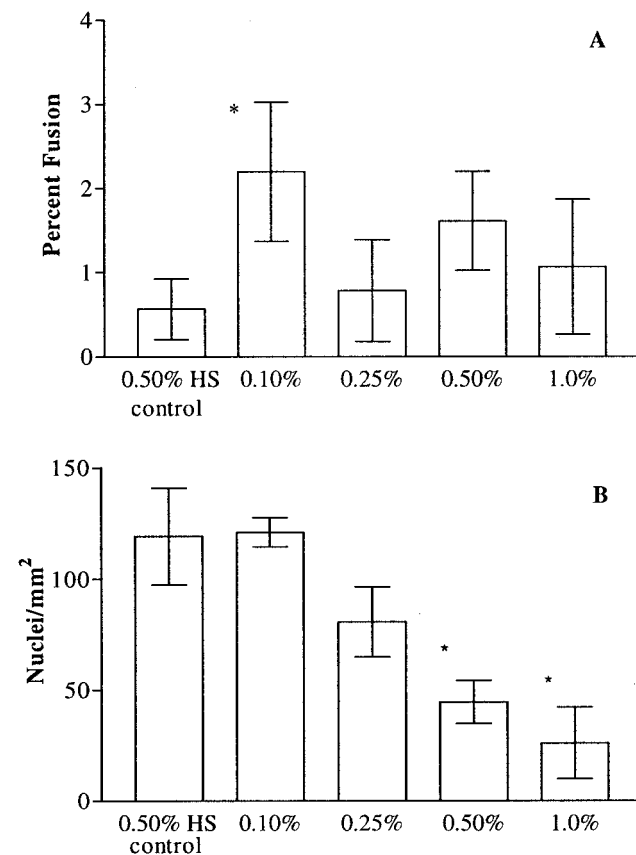


FIGURE 4—Effects of creatine monohydrate on the differentiation of satellite cells under conditions that were permissive for cell fusion. Satellite cells (two experiments) were plated at a density of 100 nuclei·mm⁻² and exposed for 120 h to a differentiation treatment medium containing DMEM, 0.5% HS, antibiotics, and either 0 (control), 0.10, 0.25, 0.50, or 1.0% creatine monohydrate. (A) Graph depicts the percent differentiation at the end of 120 h for four treatment levels and control. Each bar represents the mean ± SEM of six wells. (B) Graph depicts the total nuclei number at the end of 120 h for four treatment levels and control. Each bar represents the mean ± SEM of six wells. *Significantly different from the 0.50% HS control.

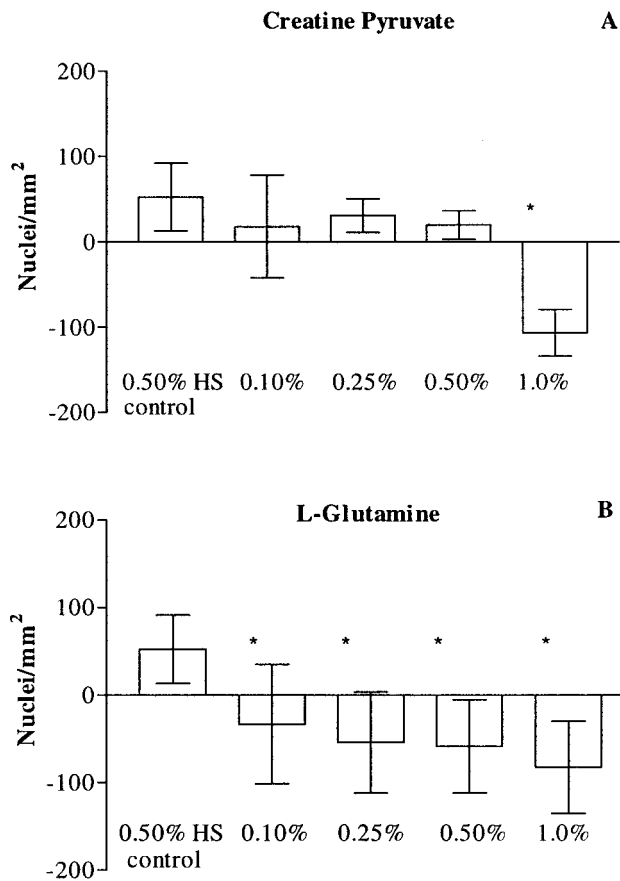


FIGURE 5—Effects of (A) creatine pyruvate and (B) L-glutamine on the differentiation of satellite cells under conditions that were permissive for cell fusion. Satellite cells (two experiments per compound) were plated at a density of 100 nuclei·mm⁻² and exposed for 120 h to a differentiation treatment medium containing DMEM, 0.5% horse serum, antibiotics, and either: 0 (control), 0.10, 0.25, 0.50, or 1.0% treatment compound. The graph for each compound depicts the total nuclei number at the end of 120 h for four treatment levels and control. Each bar represents the mean ± SEM of six wells. *Significantly different from the 0.50% HS control.

and normal postnatal growth of skeletal muscle is attributed to the hypertrophy of existing myofibers. Therefore, it is reasonable to suggest that the administration of ergogenic factors may accelerate normal skeletal muscle hypertrophy.

Because postnatal myofiber nuclei are amitotic (32), in order to undergo hypertrophy, myofibers must acquire from 60 to >99% of their adult myonuclei (31,32) from satellite cells (1,12,17). Satellite cells are postnatal myogenic stem cells that normally exist in a quiescent state between the basal lamina of the myofiber and the plasma membrane (29). When activated, these cells proliferate, differentiate, and finally fuse with myofibers to allow DNA accretion, which is required for increases in myofiber hypertrophy. This fusion of satellite cells to form myotubes is a morphological endpoint in satellite cell involvement in muscle differentiation (15). When isolated from postnatal skeletal muscle and propagated in cell culture, satellite cells provide a system to study satellite cell regulation. Sheep satellite cells were used in the assay system because they are easy to acquire from only one muscle, rather than from numerous pooled muscles as in the case of rodent satellite cells. Also,

methods and tools were in place to study sheep satellite cells in a completely defined environment (42). Human satellite cells could have been used for these studies, but we would have spent considerable time in developing a workable defined system, similar to the one employed. It should be noted that if any of the data derived from our studies are to be used for humans, then a variety of animal and human performance studies would likely need to be designed and conducted.

Experimental concentration ranges for the dietary supplements were selected to coincide with toxic levels and physiological levels to which the cells might be exposed *in vivo*. Screening of the test compounds utilized two culture systems. The levels of serum used in the serum-containing system, as well as the defined media chosen for these studies, were designed to keep cells at a level similar to that when initially plated and also to maintain cell health. This meant that any increase in cell number or fusion was due to a direct effect of the treatment compound tested. The chemically defined treat-

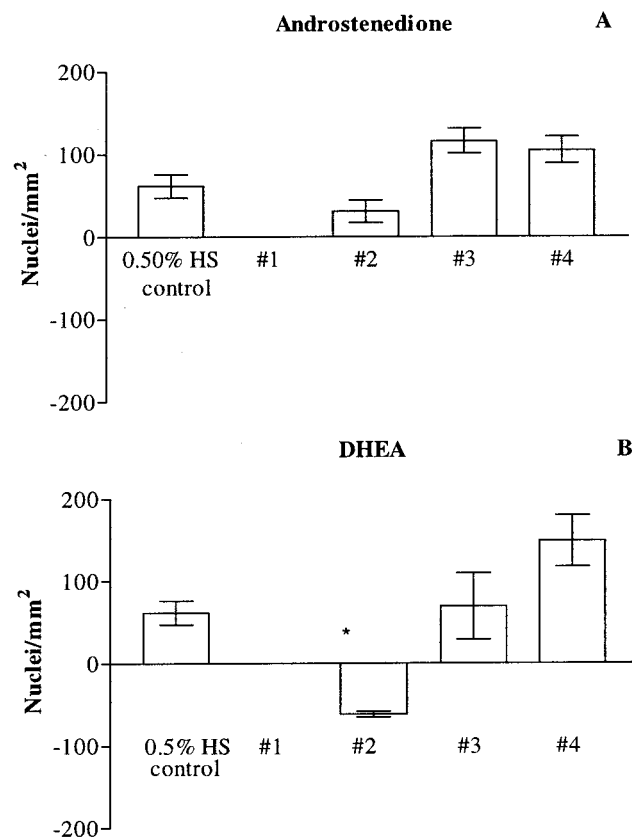


FIGURE 6—Effects of (A) androstenedione and (B) DHEA on the differentiation of satellite cells under conditions that were permissive for cell fusion. Satellite cells (two experiments per compound) were plated at a density of 100 nuclei·mm⁻² and exposed for 120 h to a differentiation treatment medium containing DMEM, 0.5% horse serum, antibiotics, and either: (A) #1 = 1.751 × 10⁻⁶ M, #2 = 1.751 × 10⁻⁸ M, #3 = 1.751 × 10⁻¹⁰ M, #4 = 1.751 × 10⁻¹² M androstenedione; (B) #1 = 1.829 × 10⁻⁶ M, #2 = 1.829 × 10⁻⁸ M, #3 = 1.829 × 10⁻¹⁰ M, #4 = 1.829 × 10⁻¹² M DHEA. The controls for both androstenedione and DHEA consisted of basal media + 10 μL ETOH. The graph for each compound depicts the total nuclei number at the end of 120 h for four treatment levels and control. Each bar represents the mean ± SEM of six wells. *Significantly different from the 0.50% HS control.

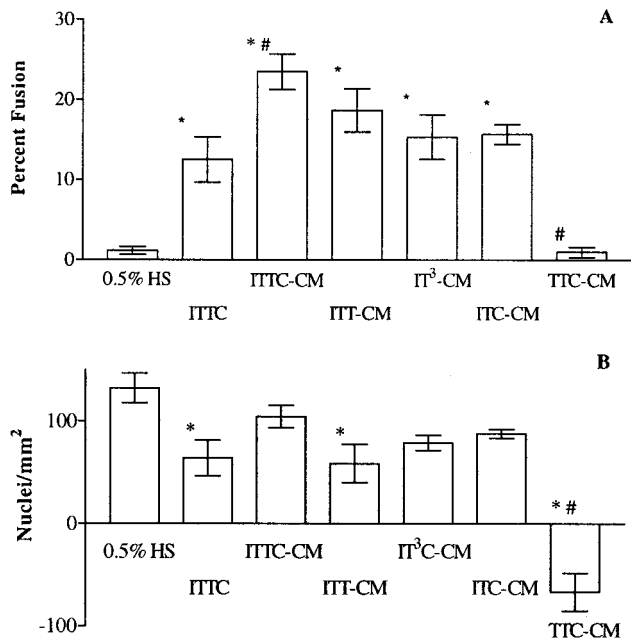


FIGURE 7—Effects of 0.1% creatine monohydrate on the differentiation of satellite cells under defined conditions that were permissive for cell fusion. Satellite cells (two experiments) were plated at a density of 100 nuclei·mm⁻² and exposed for 120 h to five defined treatment media: ITTC (insulin, transferrin, triiodothyronine, hydrocortisone), ITT (insulin, transferrin, triiodothyronine), IT³C (insulin, triiodothyronine, hydrocortisone), ITC (insulin, transferrin, hydrocortisone), and TTC (transferrin, triiodothyronine, hydrocortisone). All of these defined treatments also contained 0.1% creatine monohydrate (CM). Controls consisted of either DMEM + 0.5% horse serum or ITTC (no CM). (A) Graph depicts the percent fusion for the defined treatments and controls at the end of 120 h. (B) Graph depicts total nuclei·mm⁻² for the defined treatments and controls at the end of 120 h. Each bar in (A) and (B) represents the mean ± SEM of six wells. *Significantly different from 0.50% HS control. #Significantly different from ITTC control.

ment system also allowed the examination of the effects of creatine monohydrate without the confounding effects of the undefined serum milieu. The results of these studies suggested that a narrow concentration window of creatine monohydrate has the potential to positively affect one mechanism of muscular hypertrophy—satellite cell proliferation and differentiation. A similar concentration window has been shown for other agents. For example, dexamethasone, which is normally thought to exert catabolic effects on cells, actually induces satellite cell proliferation, but only at a discrete, narrow concentration (17).

Creatine in the monohydrate form had more of an effect on satellite cells than creatine pyruvate. At the pH of culture media, creatine monohydrate is most likely in a form recognizable by muscle cell receptors. As demonstrated in the subtraction studies using different defined media containing creatine monohydrate, insulin was a necessary component for increased fusion and cell number. The fact that insulin helped is evidence that a normal cellular uptake was seen, as insulin is required for enhancing cellular creatine uptake *in vivo* (L. Bucci, unpublished data). Insulin at 850 nM was likely interacting with the insulin receptor in a metabolic fashion and not the IGF-I receptor, which may occur at

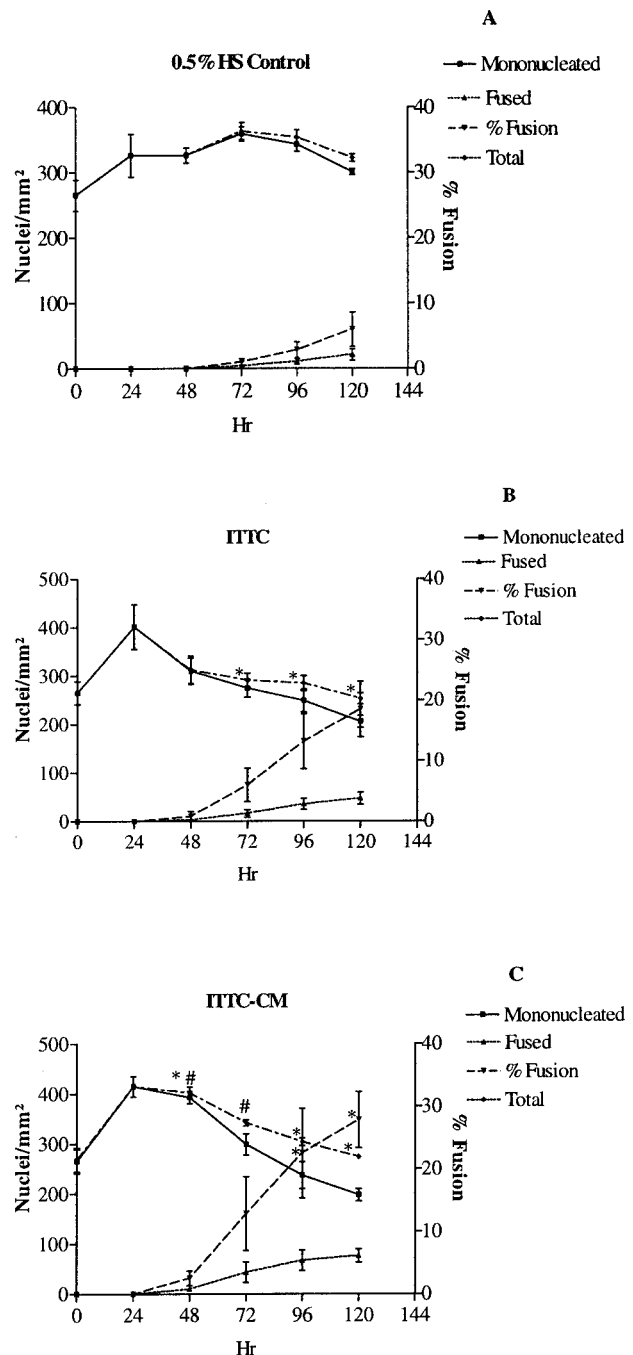


FIGURE 8—Effects over time of a defined treatment medium containing 0.1% creatine monohydrate on the mononucleated and fused cells counts (nuclei·mm⁻²) and percent fusion of satellite cells. Satellite cells (two experiments) were plated at a density of 100 nuclei·mm⁻², grown for 24 h in serum-containing media, and then exposed for 120 h to a treatment medium (ITTC-CM) consisting of 0.1% creatine monohydrate, which was contained in a defined medium termed ITTC. ITTC consisted of a basal medium, DMEM: F12 (1:1), supplemented with insulin (850 nm), transferrin (10 μg·mL⁻¹), triiodothyronine (0.2 nm), and hydrocortisone (50 ng·mL⁻¹) plus antibiotics. Controls were either ITTC without creatine monohydrate or a traditional differentiation medium of DMEM + 0.5% horse serum. At 24-h intervals, wells were fixed and stained. (A) Graph depicts the results at 120 h for the 0.5% horse serum control. (B) Graph depicts the results at 120 h for the ITTC control. (C) Graph depicts the results at 120 h for the ITTC-CM treatment. Each time point represents the mean ± SEM of six wells. *Significantly different from 0.50% HS control. #Significantly different from ITTC control.

higher levels (17). It is possible that the creatine pyruvate used in these studies had deteriorated, as pyruvate salts are known to be relatively unstable and capable of forming parapyruvate and hard-to-characterize polymers (L. Bucci, unpublished data). Also, clinical studies with creatine pyruvate (body composition and exercise performance) by various manufacturers found only an effect from the known amount of creatine, with little synergy or additive effects from pyruvate, when compared with creatine alone and pyruvate alone (L. Bucci, unpublished data).

Although we presently do not know the specific biochemical reason as to how creatine monohydrate affected

satellite cell activity, if this mechanism is operative *in vivo*, some of the enhancements in muscle mass and anaerobic exercise performance seen in human clinical trials could be explained. Future studies designed to evaluate additional ergogenic compounds, as well as combinations of the compounds tested in this paper, are in progress.

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REFERENCES

- ALLEN, R. E., R. A. MERKEL, and R. B. YOUNG. Cellular aspects of muscle growth: Myogenic cell proliferation. *J. Anim. Sci.* 49:115–127, 1979.
- AMERICAN COLLEGE OF SPORTS MEDICINE Roundtable on the physiological and health effects of oral creatine supplementation. *Med. Sci. Sports Exerc.* 32:706–717, 2000.
- ANDRES, L. P. A., J. SACHECK, and T. SANTIAGO. A review of creatine supplementation: side effects and improvements in athletic performance. *Nutr. Clin. Care* 2:73–81, 1999.
- ANTONIO, J., and J. R. STOUT. *Sports Supplements*. Philadelphia: Lippincott, Williams & Wilkins, 2001, pp. 44–58.
- ANTONIO, J., and J. R. STOUT. *Supplements for Strength-Power Athletes*. Champaign, IL: Human Kinetics, 2002, 133 pp.
- BAZZARE, T. L. Nutrition and strength. In: *Nutrition in Exercise and Sport*, 3rd Ed., I. Wolinsky (Ed.). Boca Raton, FL: CRC Press, 1998, pp. 369–419.
- BLUE, J. G., and J. A. LOMBARDO. Steroids and steroid-like compounds. *Clin. Sports Med.* 18:667–689, 1999.
- BROEDER, C. E., J. QUINDRY, and K. BRITTINGHAM. The Andro-Project: physiological and hormonal influences of androstenedione supplementation in men 35 to 65 years old participating in a high-intensity resistance training program. *Arch. Intern. Med.* 160:3093–3104, 2000.
- BROWN, G. A., M. D. VUKOVICH, T. A. REIFENRATH, et al. Effects of anabolic precursors on serum testosterone concentrations and adaptations to resistance training in young men. *Int. J. Sport Nutr. Exerc. Metab.* 10:340–359, 2000.
- BUCCI, L. R. *Nutrients as Ergogenic Aids for Sports and Exercise*. Boca Raton, FL: CRC Press, 1993, 106 pp.
- BURTON, N. M., K. M. BYRNE, J. L. VIERCK, and M. V. DODSON. Methods for satellite cell culture under a variety of conditions. *Methods Cell Sci.* 22:51–61, 2000.
- CAMPION, D. R. The muscle satellite cell: a review. *Int. Rev. Cytol.* 87:225–251, 1984.
- CASEY, A., and P. L. GREENHAFF. Does dietary creatine supplementation play a role in skeletal muscle metabolism and performance? *Am. J. Clin. Nutr.* 72:607S–617S, 2000.
- DANGOTT, B., E. SCHULTZ, and P. E. MOZDZIAK. Dietary creatine monohydrate supplementation increases satellite cell mitotic activity during compensatory hypertrophy. *Int. J. Sport Med.* 21:13–16, 2000.
- DAYTON, W. R., and M. R. HATHAWAY. Autocrine, paracrine, and endocrine regulation of myogenesis. In: *Animal Growth Regulation*, D. R. Campion, G. J. Hausman, and R. J. Martin (Eds.). New York: Plenum Press, 1989, pp. 69–90.
- DODSON, M. V., B. A. MATHISON, and B. D. MATHISON. Effects of medium and substratum on ovine satellite cell attachment, proliferation and differentiation *in vitro*. *Cell Diff. Dev.* 29:59–66, 1990.
- DODSON, M. V., D. C. MCFARLAND, A. L. GRANT, M. E. DOUMIT, and S. J. VELLEMAN. Extrinsic regulation of domestic animal-derived satellite cells. *Dom. Anim. Endocrinol.* 13:107–126, 1996.
- GALLAGHER, P. M., J. A. CARRITHERS, M. P. GODARD, K. E. SCHULZE, and S. W. TRAPPE. β -hydroxy- β -methylbutyrate ingestion. Part I: effects on strength and fat free mass. *Med. Sci. Sports Exerc.* 32:2109–2115, 2000.
- GOLDSPIK, G. Studies on postembryonic growth and differentiation of striated muscle. In: *Structure and Function of Muscle*, C. H. Bourne (Ed.). London: Academic Press, 1972, pp. 179–236.
- GREENHAFF, P. L. Creatine and its application as an ergogenic aid. *Int. J. Sport Nutr.* 5(Suppl.):100–110, 1995.
- HAUPT, H. A., and G. D. ROVERE. Anabolic steroids: a review of the literature. *Am. J. Sports Med.* 12:469–484, 1984.
- INGWALL, J. S., M. F. MORALES, and F. E. STOCKDALE. Creatine and the control of myosin synthesis in differentiating skeletal muscle. *Proc. Natl. Acad. Sci. USA* 69:2250–2253, 1972.
- JOWKO, E., P. OSTASZEWSKI, M. JANK, J. SACHARUK, A. ZIENIEWICZ, J. WILCZAK, and S. NISSEN. Creatine, and β -hydroxy- β -methylbutyrate (HMB) additively increase lean body mass and muscle strength during a weight-training program. *Nutrition* 17:558–566, 2001.
- JUHN, M. S., and M. TARNOPOLSKY. Oral supplementation and athletic performance: a critical review. *Clin. J. Sport Med.* 8:286–297, 1998.
- KING, D. S., R. L. SHARP, M. D. VUKOVICH, et al. Effects of oral androstenedione on serum testosterone and adaptations to resistance training in young men: a randomized controlled trial. *JAMA* 281:2020–2028, 1999.
- KRAEMER, W. J., and J. S. VOLEK. Creatine supplementation: its role in human performance. *Clin. Sports Med.* 18:651–666, 1999.
- KRAMER, K., M. WEISS, and H. LIESEN. Creatine, physiology and exercise performance. In: *Nutraceuticals in Health and Disease Prevention*, K. Kramer, P. P. Hoppe, and L. Packer (Eds.). New York: Marcel Dekker, 2001, pp. 165–186.
- KUHN C. M. Anabolic steroids. *Recent Prog. Horm. Res.* 57:411–434, 2002.
- MAURO, A. Satellite cell of skeletal muscle fibers. *Biochem. Biophys. Cytol.* 9:493–496, 1961.
- MONTGOMERY, R. D. Growth of human striated muscle. *Nature* 195:194–195, 1962.
- MOSS, F. P., and C. P. LEBLOND. Nature of dividing nuclei in skeletal muscle growth of rats. *J. Cell Biol.* 44:459–462, 1970.
- MOSS, F. P., and C. P. LEBLOND. Satellite cells as the source of nuclei in muscles of growing rats. *Anat. Rec.* 170:421–436, 1971.
- MOTULSKY, H. *Analyzing Data with GraphPad Prism*. San Diego, CA: GraphPad Software Inc., 1999, pp. 68–69.
- NISSEN, S., R. SHARP, M. RAY. Effect of leucine metabolite β -hydroxy- β -methylbutyrate on muscle metabolism during resistance-exercise training. *J. Appl. Physiol.* 81:2095–2104, 1996.
- PERSKY, A. M., and G. A. BRAZEAU. Clinical pharmacology of the dietary supplement creatine monohydrate. *Pharmacol. Rev.* 53:161–176, 2001.
- RASMUSSEN, B. B., E. VOLPI, D. C. GORE, and R. R. WOLFE. Androstenedione does not stimulate muscle protein anabolism in young healthy men. *J. Clin. Endocrinol. Metab.* 85:55–59, 2000.
- SOBAL, J., and L. F. MARQUART. Vitamin/mineral supplement use among athletes: a review of the literature. *Int. J. Sport Nutr.* 4:320–334, 1994.
- SWATLAND, H. J. Morphometry of pectoral development in turkey breeding stock. *Br. Poult. Sci.* 30:785–795, 1989.

39. TIMSON, B. F., and G. A. DUDENHOEFFER. Skeletal muscle fibre number in the rat from youth to adulthood. *J. Anat.* 173:33–36, 1990.
40. VAN GAMMEREN D., J. UELMEN, L. EHLER, et al. Effect of legal androgens on strength and body composition in bodybuilders. *Med. Sci. Sports Exerc.* 32(Suppl. 5):S177, 2000.
41. VIERCK, J. L., and M. V. DODSON. Interpretation of cell culture phenomena. *Methods Cell Sci.* 22:79–81, 2000.
42. VIERCK, J. L., J. P. MCNAMARA, K. L. HOSSNER, and M. V. DODSON. Characteristics of ovine skeletal muscle satellite cell strains in a defined culture medium formulated to enhance differentiation: differentiation and the IGF-I system. *Basic Appl. Myol.* 5:11–20, 1995.
43. VIERCK, J. L., D. DAL PORTO, and M. V. DODSON. Induction of differentiation of adipofibroblasts using a defined treatment medium without DMI. *Basic Appl. Myol.* 11:99–104, 2001.
44. WALLACE, M. B., J. LIM, A. CUTLER, and L. BUCCI. Effects of dehydroepiandrosterone vs androstenedione supplementation in men. *Med. Sci. Sports Exerc.* 31:1788–1792, 1999.
45. WRIGHT, J. E. anabolic steroids and athletes. *Exerc. Sports. Sci. Rev.* 8:149–202, 1980.
46. WRIGHT, J. E., and M. H. STONE. Position statement: anabolic-androgenic steroid use by athletes. *Natl. Strength Condit. Assoc. J.* 15:9–28, 1993.
47. YESALIS, C. E. (Ed.) *Anabolic Steroids in Sport and Exercise*, 2nd Ed. Champaign, IL: Human Kinetics, 2000, 472 pp.
48. ZEIGENFUSS, T. N., J. M. BERARDI, and L. M. LOWERY. Effects of an androgen mixture on testosterone, estradiol, and anaerobic performance in men. *Med. Sci. Sports Exerc.* 32(Suppl. 5): S122, 2000.