

# Early-age heat exposure affects skeletal muscle satellite cell proliferation and differentiation in chicks

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**Halevy, Orna, Alon Krispin, Yael Leshem, John P. McMurtry, and Shlomo Yahav.** Early-age heat exposure affects skeletal muscle satellite cell proliferation and differentiation in chicks. *Am J Physiol Regulatory Integrative Comp Physiol* 281: R302–R309, 2001.—Exposure of young chicks to thermal conditioning (TC; i.e., 37°C for 24 h) resulted in significantly improved body and muscle growth at a later age. We hypothesized that TC causes an increase in satellite cell proliferation, necessary for further muscle hypertrophy. An immediate increase was observed in satellite cell DNA synthesis in culture and in vivo in response to TC of 3-day-old chicks to levels that were significantly higher than those of control chicks. This was accompanied by a marked induction of insulin-like growth factor-I (IGF-I), but not hepatocyte growth factor in the breast muscle. No significant difference between treatments in plasma IGF-I levels was observed. A marked elevation in muscle regulatory factors on *day 5*, followed by a decline in cell proliferation on *day 6* together with continuous high levels of IGF-I in the TC chick muscle may indicate accelerated cell differentiation. These data suggest a central role for IGF-I in the immediate stimulation of satellite cell myogenic processes in response to heat exposure.

thermal conditioning; muscle differentiation; growth factors; myoblasts; stress

IN VERTEBRATES, SHORTLY AFTER birth or hatch, the skeletal myofibers are permanently differentiated and incapable of mitosis. However, myofibers undergoing hypertrophy appear to require an external source of new nuclei to maintain a constant myonucleus-to-fiber size ratio (reviewed in Ref. 4). The unique source of these new myonuclei is attributed to the satellite cells, located underneath the basal lamina of the skeletal muscle (reviewed in Ref. 11). At birth or hatch, skeletal muscle consists of a high percentage of proliferating satellite cells, an initial event that decreases rapidly toward the end of the growth period; thereafter, satellite cells become mitotically quiescent (12, 23, 29, reviewed in Ref. 11). Terminal differentiation of myoblasts during embryo development as well as satellite

cells postnatally involves the coordinate regulation of cell cycle withdrawal and upregulation of muscle-specific gene expression. The MyoD family, containing four basic helix-loop-helix (bHLH) transcription factors (MyoD, Myf5, myogenin, and MRF4), positively regulates myogenesis (reviewed in Ref. 52). These factors form heterodimers with ubiquitous bHLH nuclear proteins (E proteins) and act in collaboration with the MCM1-agamous-deficiens-serum response factor box proteins of myocyte enhancer factor-2 (MEF2) to direct skeletal muscle differentiation (reviewed in Refs. 41, 44). Except of myf5, none of the MyoD family members are expressed in quiescent satellite cells (10, 17, 53). However, on activation of these cells after injury or in culture, these members are expressed in a sequential pattern in proliferating myoblasts and in newly formed myotubes (16, 17, 26, 28, 53).

Several growth factors have been implicated in the recruitment of satellite cells for skeletal muscle growth and regeneration. Some of these factors, such as members of the fibroblast growth factor family (FGF; reviewed in Refs. 25, 42) and hepatocyte growth factor (HGF) have been reported to promote proliferation and to inhibit differentiation of primary cultures of satellite cells (5, 27, 37). HGF has the unique property of being able to activate quiescent satellite cells (5, 27) and has been shown to be the activating factor in extracts of crushed muscle for these cells (50). Insulin-like growth factor-I (IGF-I), on the other hand, has been shown to promote proliferation, differentiation, and fusion of satellite cells (3, 19, 34, reviewed in Ref. 25). Moreover, overexpression of IGF-I correlates with muscle hypertrophy in transgenic mouse lines (8, 15), the high levels being found locally in the muscle with no elevation in circulating IGF-I concentration. Consistent with this, localized infusion of IGF-I resulted in skeletal muscle hypertrophy in rats (2), suggesting a direct effect of IGF-I on satellite cell myogenesis in muscle.

Satellite cells can be activated in skeletal muscle under stress conditions such as mechanical stress

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(overload), injection of toxic agents and muscle injury (cold, crushing, mincing; reviewed in Refs. 13, 28). However, heat stress resulting from environmental conditions and/or excessive metabolic heat production (e.g., extensive muscle exercise) may lead to irreversible thermoregulatory events that can cause muscle damage (14, 35) or even be lethal for the animal. Birds and mammals are homeotherms and as such are able to maintain their body temperature within a narrow range. Exposure to heat-stress conditions results in most cases in hyperthermia, which involves a significant increase in the inducible heat-shock proteins (HSPs), mainly those belonging to the HSP70 family. This HSP70 induction has been found in many tissues, including skeletal (45, 46) and heart muscle (22).

Thermal conditioning (TC) is a process in which chicks are exposed during their first week of life to mild environmental heat stress for 24 h (7, 58, reviewed in Ref. 55), taking advantage of the immaturity of temperature regulation in young chicks at that age (20, 39). Such heat exposure results in significantly increased body temperature and temporary growth halt followed by immediate compensatory growth (56–58). TC on the third day posthatch has been found optimal in causing maximal weight gain in body and breast muscle of 42-day-old chicks (54).

On the basis of these data, we hypothesized that exposure to mild heat stress (i.e., TC) evokes an immediate response in satellite cell activity. To test this, we monitored the myogenic process of satellite cells *in vivo* and *in culture*, as well as changes in the expression of mitogenic growth factors during the first week of life. We found that mild heat exposure at an early age results in the acceleration of satellite cell myogenesis mediated by specific local growth factor expression.

## MATERIALS AND METHODS

**Animals and experimental design.** Male broiler chicks (Cobb) were obtained from a commercial hatchery ( $n = 160$ ) and divided into two experimental groups ( $n = 80$ ). Chicks were raised in battery brooders at ambient temperature ( $T_a = 33.0 \pm 1.0^\circ\text{C}$ ) situated in a temperature-controlled room ( $T_a = 27.0 \pm 1.0^\circ\text{C}$ ). On the third day of life, the TC group was transferred to another temperature-controlled room and was exposed for 24 h to  $37.5 \pm 0.1^\circ\text{C}$ , then transferred back to the battery brooders. The control group remained at  $33.0 \pm 1.0^\circ\text{C}$  for the entire period (56). Thereafter both groups were raised to the age of 42 days under standard conditions (56). Water and feed were provided *ad libitum*. Feed was designed according to specifications of the National Research Council (40). Each experiment was repeated three times. All experimental procedures were approved by the Animal Welfare Committee of Agricultural Research Organization, The Volcani Center, and the animals were maintained in accordance with the guidelines for care and use of laboratory animals.

**Cell cultures.** Chicken skeletal muscle satellite cells were cultured from the pectoral muscle of chicks as described by Halevy and Lerman (32). On all days (e.g., between days 2 and 8), cells were prepared under exactly the same conditions from 6 g of breast muscle that had been pooled from eight birds. An enriched population of myogenic cells was recovered with <5% of those cells being nonmyogenic. The coefficient of variation of cell preparations was ~5% (30). Cells

were counted using a hemocytometer, plated on 0.1% gelatin-coated plates at  $5 \times 10^4$  cells/cm<sup>2</sup> in DMEM supplemented with 10% horse serum and grown for 1 day. Cells were maintained at  $37^\circ\text{C}$  in a humidified atmosphere, 95% air and 5% CO<sub>2</sub>. Each cell preparation was repeated in three independent experiments.

**Thymidine incorporation.** Cells were incubated for 17 h in 24-well plates, and [<sup>3</sup>H]thymidine (Amersham Pharmacia Biotech, Uppsala, Sweden) was added (2 μCi/well) for an additional 2 h of incubation as previously described (32). The cells were then detached with 0.25% trypsin-EDTA and precipitated with 10% trichloroacetic acid. Radioactivity in the dissolved precipitates was counted in Ultima Gold scintillation fluid (Packard, Groningen, The Netherlands) using a Tri-Carb 1600CA scintillation counter (Packard). Equal plating efficiency was verified by measuring cell numbers in parallel wells.

**Plasma IGF-I assay.** Plasma IGF-I was measured by heterologous double-antibody RIA (38, 43), using recombinant chicken IGF-I (GropPep, Adelaide, Australia) for standards and human <sup>125</sup>I-labeled IGF-I (Amersham Pharmacia Biotech) as a tracer. Blood samples were extracted in acid-ethanol before the IGF-I RIA to minimize interference of binding proteins (38).

**Western blot analysis.** Western blot analysis was performed as described in Leshem et al. (37). In brief, cells were scraped off the dishes in lysis buffer, and muscle tissue was homogenized with a Kinematica homogenizer (Lucerne, Switzerland) for 30 s on ice in the same lysis buffer. All extracts were sonicated and normalized for protein content (BCA kit, Pierce, Rockford, IL), and equal amounts of protein were separated by SDS-PAGE and transferred to nitrocellulose filters (Schleicher and Schuell, Dassel, Germany). Membranes were incubated for 2 h at room temperature with the appropriate antibodies, then washed and incubated for 1 h with horseradish peroxidase-conjugated goat anti-mouse or goat anti-rabbit IgG (Zymed, San Francisco, CA). Proteins were visualized using enhanced chemiluminescence (Pierce). The following primary antibodies were used: anti-IGF-I monoclonal antibody (1:1,500; Upstate Biotechnology, Lake Placid, NY), polyclonal antibodies against chicken myogenin [1:5,000; a kind gift from Bruce Paterson, National Institutes of Health (NIH), Bethesda, MD], and MEF2 (1:250; Santa Cruz Biotechnology, Santa Cruz, CA). A polyclonal antibody against chicken HGF was prepared by immunizing rabbits with a recombinant peptide of the NH<sub>2</sub>-terminal region of the protein. This antibody is reactive against the α-chain of HGF, therefore detects a single band at ~60 kDa on an SDS-PAGE. Densitometric analysis was performed on bands using NIH software. Protein expression in breast muscle was examined individually for each chick, and therefore expression level on each day is presented as percentage of control.

**Histological analysis.** Breast muscle samples were removed from the same longitudinal region and immediately fixed in fresh 4% paraformaldehyde, dehydrated, and embedded in paraffin. Sections were cut at 5 μm, placed on glass slides, deparaffinized, and rehydrated as previously described (30). Sections were immunostained with proliferating cell nuclear antigen (PCNA), a marker for dividing cells, using a commercial kit (Zymed) according to the manufacturer's protocol. After being rinsed for 1 h in PBS, sections were incubated for 2 h at room temperature in horseradish peroxidase-conjugated anti-mouse IgG diluted 1:200 in blocking buffer. A solution of 1 g/l diaminobenzidine hydrochloride (Sigma Chemicals, St. Louis, MO) was mixed (1:1; vol/vol) with 0.03% hydrogen peroxide. Sections were incubated with the peroxidase substrate for 10 min and rinsed with PBS. After immunostaining, sections were counterstained with hematoxylin, dehydrated, and mounted in Hist-

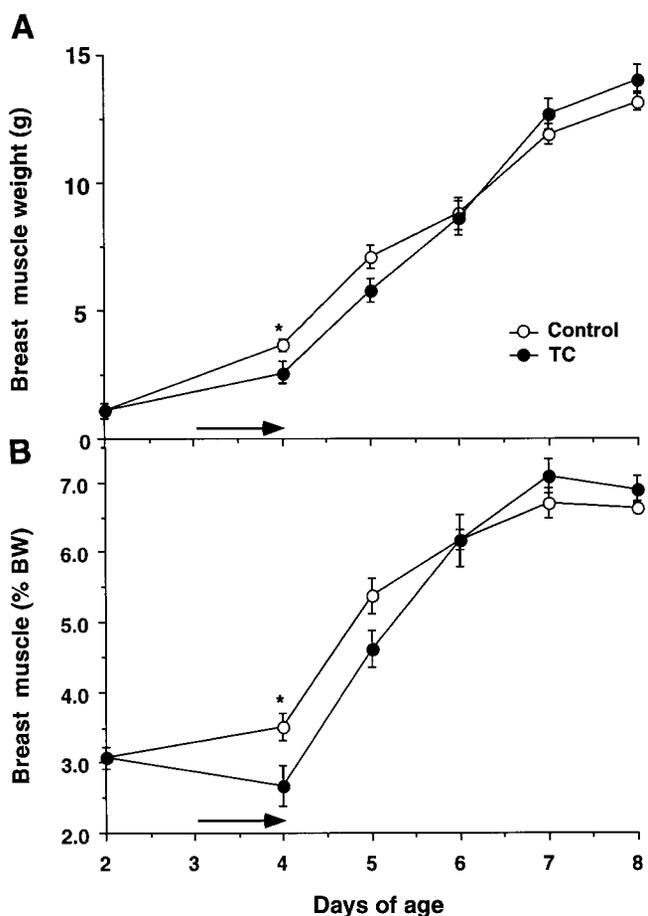


Fig. 1. Breast muscle as absolute weight (A) and as percentage of body weight (BW; B) of thermally conditioned (TC) and control chicks during the first 8 days of life. Results are means  $\pm$  SE;  $n = 8$ . \* $P < 0.05$  vs. control at the same age. The arrow indicates the TC period.

mount (Zymed). Negative control slides, without primary antibody, were examined in all cases. Digitized maps of the sections were analyzed using Image Pro Plus 3.0 software. Four or five random fields were analyzed in each section, and the proportion of stained nuclei was calculated as percentage of total nuclei for each of the fields.

**Statistical analysis.** All results were subjected to ANOVA (1 way) according to Snedecor and Cochran (47) and to Tukey's multiple-range test. Means were considered significantly different at  $P \leq 0.05$ .

## RESULTS

**Muscle growth at an early age.** At the end of the TC period, at 4 days of age, breast muscle weight as well as its percentage of body weight was reduced to levels significantly lower ( $P < 0.05$ ) than that of controls (Fig. 1). Thereafter, both breast muscle weight (Fig. 1A) and the percentage of breast muscle in the TC group began to rise (Fig. 1B), becoming equal on day 6 and then slightly higher than that of the control group. This trend continued (data not shown) until the age of 42 days, at which time the percentage of breast muscle weight in the TC chicks was reported to be significantly greater than that of controls ( $15.32 \pm 0.29$  and  $14.17 \pm 0.26\%$ , for control and TC chickens, respectively; Ref. 54). The similar pattern of the absolute weight and

percentage of breast muscle of body weight indicates that mild heat exposure indeed contributed to muscle growth. It should be noted that, during TC, the birds were not dehydrated nor were there significant differences of abdominal fat content (data not shown). Alterations in body weight were similar to those of the breast muscle throughout the entire experiment (data not shown; Ref. 54).

**Analysis of satellite cells.** The changes in breast muscle weight on heat exposure raised the possibility of an immediate effect of TC on satellite cell proliferation. Satellite cells were prepared from breast muscle derived from the chicks before and at selected intervals after TC. Cells were counted, and their ability to proliferate in vitro was evaluated after 1 day in culture by thymidine incorporation assay. In both control and TC groups, thymidine incorporation levels were elevated on days 4 and 5, but the increase was significantly

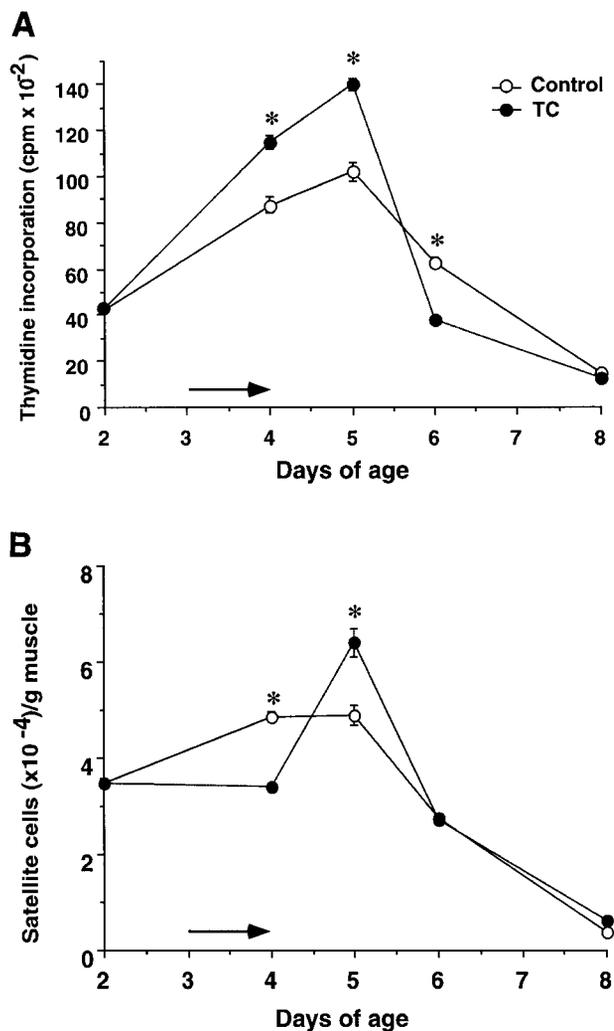


Fig. 2. Labeled thymidine incorporation into DNA in satellite cells (A) and number of satellite cells per gram of breast muscle (B) of TC and control chicks at various ages. Breast muscle was removed from the experimental chicks, pooled within each group, and weighed. Satellite cells were prepared under similar conditions and counted. Cells were then seeded for 17 h, and [<sup>3</sup>H]thymidine was added for an additional 2 h. Results are means  $\pm$  SE ( $n = 6$ ) of a representative 3 independent experiments. \* $P < 0.05$  vs. control at the same age. The arrow indicates the TC period.

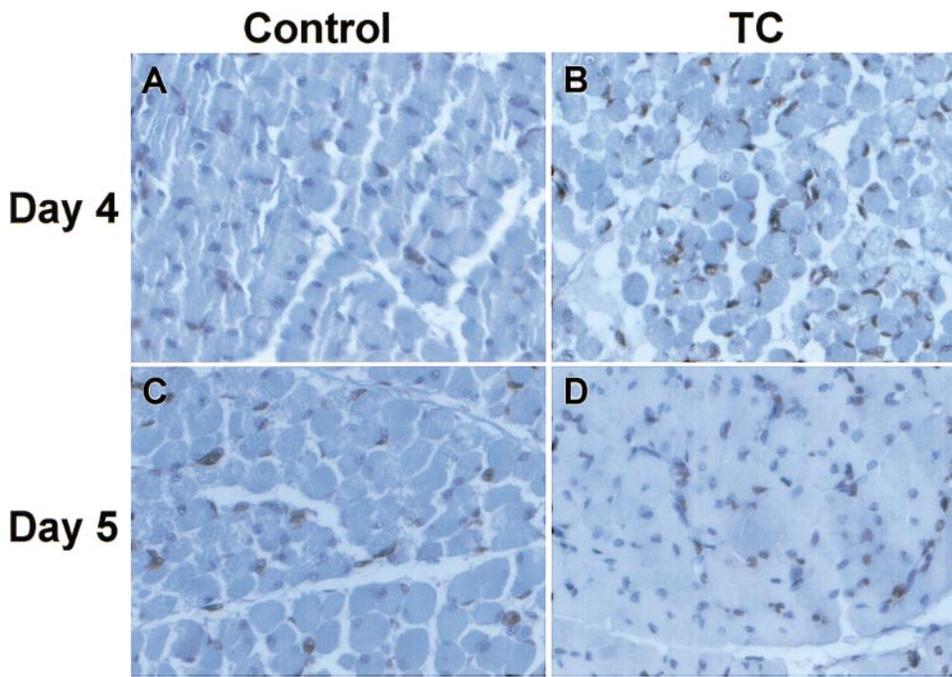


Fig. 3. Representative proliferating cell nuclear antigen (PCNA) staining of breast muscle cross sections prepared from control (A, C) and TC (B, D) chicks at various days of age. Sections were immunostained for PCNA (dark brown nuclei) and counterstained with hematoxylin (blue nuclei). Magnification  $\times 400$ .

higher in cells prepared from the TC group (Fig. 2A). On subsequent days, the activity of satellite cells derived from both groups declined. Interestingly, the decrease in both thymidine incorporation and cell number was more pronounced on *day 6* in the cells derived from the TC group than controls. On *day 8*, both cultures reached the same low level of thymidine incorporation.

The number of satellite cells per gram of muscle in the control group increased for the first few days, peaked on *days 4* and *5*, and then declined (Fig. 2B). In contrast, the number of cells in the TC group remained the same before and after the heat exposure and was significantly lower than controls on *day 4*. However, on *day 5*, the number of cells in the TC group increased nearly twofold, becoming significantly higher than that in the control group. The number of satellite cells in both groups then decreased to a very low level.

The immediate stimulatory effect of TC on the entrance of satellite cells into the cell cycle was also observed in vivo by immunohistochemical staining for PCNA in muscle sections (Fig. 3). The number of PCNA-expressing cells increased in both control and TC groups from *day 4* to *day 5*; however, it was significantly higher in muscle from the TC group (Fig. 3, compare A to B and C to D; Table 1).

Table 1. Effect of TC on PCNA-expressing cells in chick breast muscle

Age	Control	TC
Day 4	29.2 $\pm$ 0.5	35.0 $\pm$ 0.2*
Day 5	31.0 $\pm$ 0.6	41.6 $\pm$ 0.5*

Values are means  $\pm$  SE of 4 or 5 fields of 2 independent muscle samples in % of total cells. TC, thermal conditioning; PCNA, proliferating cell nuclear antigen. \* $P < 0.05$  vs. control at the same age.

*Muscle regulatory proteins.* The rapid and pronounced decline in the proliferation of satellite cells derived from the TC-treated chicks on *day 6* implied that some of these cells had undergone differentiation. Therefore, expression of the muscle regulatory factors myogenin and MEF2, markers for myogenic cell differentiation (29, 31, 53), was analyzed in whole muscle extracts. In general, expression kinetics were similar for both proteins; on *day 5*, both myogenin and MEF2 protein levels in the TC chick muscles were significantly higher than in the control chicks, reaching two-fold and 1.65-fold higher levels for myogenin and MEF2, respectively (Fig. 4A). On subsequent days, the difference between the control and TC groups in terms of both myogenin and MEF2 levels was reduced, and on *day 8*, no significant differences were observed between the two groups. The reduction of myogenin in the TC chicks' muscles was more rapid and pronounced than that of MEF2 (Fig. 4A). Note that on *day 4*, whereas myogenin was expressed at equal levels in the control and TC muscles, MEF2 levels in the latter group were significantly lower.

In cultured satellite cells prepared from the TC muscles, myogenin protein levels were lower than in controls on *day 4* (Fig. 4B). However, as had been observed in vivo (Fig. 4A), these levels increased on *day 5* in the TC-derived cells to twice that in controls. The difference in myogenin levels between TC and control-derived cells declined on *day 6*. A similar pattern of expression was seen in a single-cell immunofluorescence assay for myogenin in parallel cultures (data not shown). Due to our culturing procedure, very low numbers of satellite cells were obtained on *day 8*, and it was not possible to determine the myogenin level in these cultures (see MATERIALS AND METHODS).

*Growth factor expression.* Densitometric analysis of the breast muscle samples derived from chicks at var-

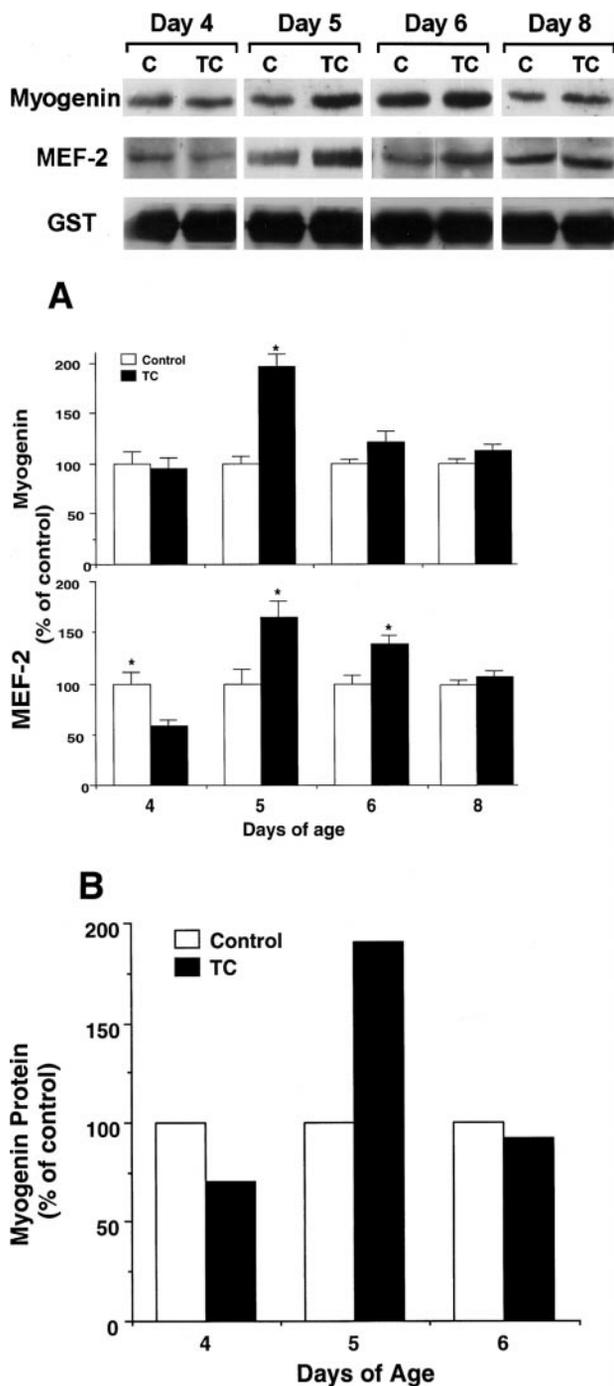


Fig. 4. Muscle regulatory factor expression is upregulated in response to TC. *Top*: representative Western blot analysis for myogenin and myocyte enhancer factor-2 (MEF2) at various days of the experiment. Equal quantities of protein were loaded as evidenced by the glutathione-S-transferase (GST) bands in the lower panel. C, control. A: densitometric analysis of myogenin and MEF2 expression relative to loaded protein levels in breast muscle samples derived from control and TC chicks on various days of age. Results are means  $\pm$  SE and presented as percentage of control;  $n = 8$ . \* $P < 0.05$  vs. control at the same age. B: densitometric analysis of myogenin expression levels relative to loaded protein levels in cultured satellite cells derived from the experimental chicks at various days of age. Results are averages of 2 independent repeats.

ious days of age revealed virtually no IGF-I protein expression in muscles derived from the control group, its levels rising only on *day 8* (Fig. 5, A and B, *top*). In contrast, IGF-I protein was induced in the muscles

derived from TC chicks as early as *day 4* and stayed at high levels until *day 6* and then declined but remained significantly higher than levels in the control group (Fig. 5B, *top*). Plasma IGF-I levels did not differ between treatments, either immediately after TC or at any sampling period (Fig. 5C).

In contrast to the IGF-I expression pattern, HGF was expressed on all sampled days in the breast muscle derived from control and TC chicks (Fig. 5A). In general, HGF was expressed at comparable levels in both groups (Fig. 5B, *bottom*).

## DISCUSSION

Previous studies have shown that TC of chicks at an early age results in transient growth arrest, followed by immediate compensatory growth. This leads to higher body and breast muscle weights of the TC chicks vs. their untreated counterparts at later ages (54–56). The study presented here focuses on the early events of postnatal skeletal muscle development that lead to enhanced hypertrophy at later ages. The results show for the first time that mild heat exposure, at least at an early age, has a stimulatory effect on skeletal muscle growth due to an immediate increase in satellite cell proliferation followed by accelerated differentiation.

We monitored satellite cell proliferation in culture using thymidine incorporation and in breast muscle sections using immunohistochemistry for PCNA. Although the latter technique enabled us to locate proliferating cells of various types (e.g., fiber nuclei, fibroblasts, endothelial cells), most of the PCNA-positive cells are suggested to be satellite cells. Results from us and others have demonstrated that satellite cells account for  $\sim 30\%$  of total nuclei during the first days of life (12, 24; Table 1). Moreover, in a recent study we demonstrated that the myogenic state of primary cultures of satellite cells derived from chick skeletal muscle reflects their *in vivo* state (30), suggesting that satellite cell cultures are a reliable tool for studying postnatal muscle growth.

Satellite cells responded to TC rapidly: immediately after the heat treatment (i.e., *day 4*), the number of cycling cells was significantly higher in the TC groups than in controls, both in satellite cell cultures and in breast muscle sections derived from the experimental chicks. This cell activity was followed by a twofold rise in cell number on the following day. It is worth noting that the maximal effect on satellite cell proliferation was achieved when TC was performed on the third day of life (Halevy and Yahav, unpublished results). This is in agreement with the effect observed for body weight and breast muscle percentage of body weight (54). Taken together, these results suggest that the timing of TC is crucial for maximal satellite cell response.

Although the number of cycling satellite cells in the TC group continued to rise on *day 5*, the total number of cells declined rapidly the following day (Fig. 2A), suggesting that TC accelerated their differentiation. Indeed, a marked rise in myogenin level was observed on *day 5* in the cultured satellite cells derived from the

TC chicks, similar to its and MEF2's elevation in the muscle of the TC chicks, reflecting the differentiation of satellite cell population *in vivo*. Increased muscle regulatory factor levels have been attributed to satellite cells and not to myofibers in mammals (1, 29, 53) and chicks at an early age (31). We have no explanation for the lower MEF2 levels in the TC chicks relative to controls on *day 4*, yet an immediate and specific inhibitory effect of TC on this protein cannot be ruled out. On the other hand, its slower decline after *day 5* relative to that of myogenin (Fig. 4A) could be due to its role as a later differentiation regulatory factor (41).

What can cause the acceleration of satellite cell myogenesis as a result of heat exposure? One possibility could be the induction of HSPs. In most instances, acute heat exposure or muscle exercise, resulting in hyperthermia, is followed by the upregulation of HSPs, mainly HSP70 (45, 46). This is a physiological response that may lead, in some cases, to muscle damage (14). However, no HSP70 expression was observed in breast muscle (data not shown) nor were this or other HSPs expressed in other tissues (58) of young TC chicks. Therefore, the immediate response of satellite cells to TC is unlikely to be modulated by HSPs.

Good candidates for mediating the satellite cell response to heat exposure could be locally produced growth factors (i.e., within the muscle). Indeed, TC caused the rapid induction of IGF-I protein expression in breast muscle derived from the TC chicks concomitantly with the rise in satellite cell proliferation. IGF-I has been shown to stimulate primary satellite cell proliferation in rats (3) and chickens (19, 34). Moreover, the increase in IGF-I expression preceded that of myogenin, suggesting that the latter is induced by the former, in agreement with the proposed role for IGF-I in the regulation of myogenin expression (25). It has been hypothesized that IGF-I first stimulates proliferation and subsequently muscle-specific factors that are involved in differentiation (23, 25). The rapid elevation in IGF-I parallel to increased cell proliferation, followed by increases in myogenin and MEF2, supports this hypothesis. Moreover, the finding that muscle IGF-I levels in TC chicks remained significantly higher than in controls, even when satellite cell number had dropped to low levels in both groups (*day 8*), implies that IGF-I also stimulates muscle hypertrophy. In a previous study, we reported greater breast muscle weight in TC chicks at later ages (54). Consistent with that, IGF-I has been reported to increase hypertrophy of skeletal muscle in tissue culture (51) and *in vivo* (8, 9, 15). Although some of the IGF-I found in the muscle

could be inactive due to its binding to IGF-I-binding proteins (33), the induction of myogenin suggests that even a fraction of active IGF-I is sufficient for its biological activity.

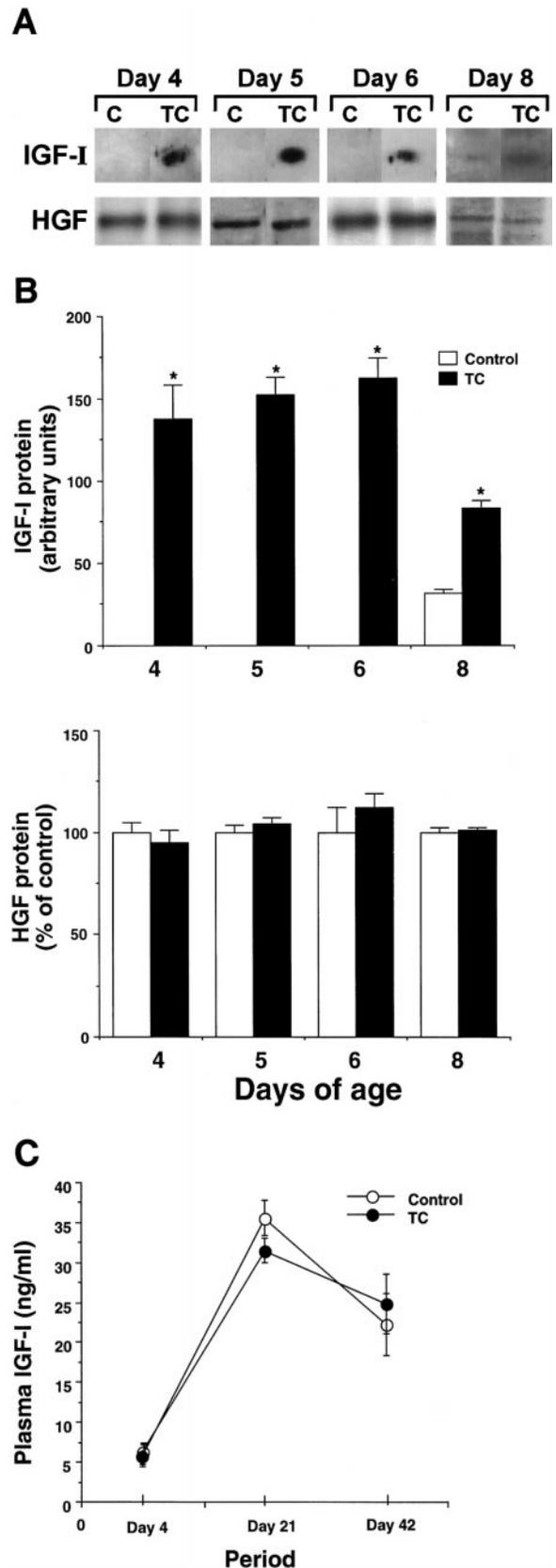


Fig. 5. Insulin-like growth factor (IGF)-I, but not hepatocyte growth factor (HGF) protein expression is induced in breast muscle in response to TC. A: representative Western blot analysis for IGF-I and HGF at various days of the experiment. B: densitometric analysis of IGF-I and HGF expression relative to loaded protein levels in breast muscle samples derived from control and TC chicks at various days of age. Results are means  $\pm$  SE presented as arbitrary units for IGF-I and as percentage of control for HGF ( $n = 8$ ,  $*P < 0.05$ ). C: plasma IGF-I concentrations do not significantly differ at various days of age. Values are means  $\pm$  SE ( $n = 8$ ).

The induction of IGF-I in muscle in response to TC could be due to overproduction of growth hormone-dependent hepatic IGF-I, thus indirectly increasing circulating IGF-I, or to locally produced IGF-I that acts via autocrine/paracrine pathways. We propose that the latter possibility is more likely. First, previous studies have found that increased circulating levels of IGF-I have no effect on the degree of muscle hypertrophy (48), whereas localized infusion of IGF-I has (2). Second, in normal chicks, or those given chicken growth hormone, IGF-I mRNA is expressed independently of the hormone levels in extrahepatic tissues, including muscle (43, 49). Third, IGF-I mRNA expression has been found in satellite cells in regenerating muscle (21), and fourth, there is no significant difference in circulating IGF-I levels up to *day 42* in chicks that had undergone TC at 3 days of age (Fig. 5C).

In contrast to IGF-I, protein expression of other growth factors known to be mitogenic for satellite cells was not altered in response to TC. This was particularly true in the case of HGF (Fig. 5A, *bottom*), because its levels in TC chick muscles were similar to those in controls during the entire experimental period. In the case of basic FGF (bFGF), some increased expression was observed on *day 4* in the TC chicks relative to controls but it was insignificant (data not shown). Taken together, these results support a central role for IGF-I in the modulation of satellite cell proliferation and differentiation in TC chicks immediately after heat exposure, most likely accounting for hypertrophy at later stages. Nevertheless, because HGF and bFGF have also been reported to inhibit muscle cell differentiation (6, 27, 37, 42), it cannot be excluded that the ratio between IGF-I and HGF and/or bFGF expression in the muscle of the TC chicks affects satellite cell myogenesis in response to heat exposure.

An increase in IGF-I has been reported under various stress conditions, such as muscle overload and injury, suggesting its involvement in regulating the skeletal muscle's compensatory hypertrophy response to muscle damage, which requires stimulation of satellite cell proliferation (1, 9, 18). Because HGF is responsible for quiescent satellite cell activation (5, 27) and has been detected in crushed muscle extracts (50), it is conceivable that in these types of stress, HGF also increases. Indeed, both HGF and IGF-I have been shown to be upregulated after muscle injury (21, 36). In view of these data and our results, we believe that in acute stress conditions there is a need for increases in various growth factors for maximal effect on satellite cell proliferation and muscle regeneration. However, under mildly stressful conditions, IGF-I appears to be the major growth factor playing a role in regulating satellite cell proliferation and differentiation, in this case in response to TC at an early age.

### Perspectives

Mild heat exposure of chicks at an early age has a stimulatory effect on the early events of postnatal skeletal muscle growth due to an immediate increase in satellite cell proliferation and accelerated differen-

tiation. It is well known that under various stress conditions such as muscle overload and injury, satellite cells are being activated. However, our finding that heat exposure of young chicks can stimulate satellite cell proliferation is unique, because exposure of these birds to similar temperature at 6 wk of age leads to muscle damage. Temperature regulation in young chicks is in an immature stage, therefore it may well be that at this age the muscle responds to heat exposure with an increase of growth factors, which in turn affect satellite cell myogenesis. Indeed, we found that IGF-I but not HGF appears to be the major growth factor playing a role in this process. It is conceivable that in mammals, mild heat stress would have a similar promoting effect on muscle growth as in birds; however, this question should be addressed.

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