**Introduction:** During endochondral bone formation, undifferentiated mesenchymal cells condense at a site where bone is newly formed, and these cells become chondrocytes. The proliferative chondrocytes actively secrete extracellular matrix, such as type II collagen and aggrecan, and then lose their ability to divide and terminally differentiate into hypertrophic chondrocytes. Mechanical stress has an important function in chondrocytes and plays a key role in endochondral bone formation. However, there have been few studies how mechanical stress regulates chondrocyte proliferation and differentiation in the process of endochondral bone formation.

**Materials and Methods:** ATDC5 cells, which undergo a reproducible multistep chondrogenic differentiation in response to insulin, were used in this study. Mechanical loading was applied to ATDC5 cells grown on the plastic plates in monolayer cultures with/without insulin by four-point bending of the plate using a Vitrodyne 1000 Universal Materials Tester. The cells were loaded at a magnitude of 2800 μstrain and a frequency of 0.5 Hz, and for a duration of 24 h at day 4, 7, 14, 21 and 28. The chondrogenic pathway in ATDC5 cells was studied by Alcian blue staining in order to search for the presence of cartilage-specific proteoglycans in the extracellular matrix, and analyzing stage-specific gene expression such as type II and X collagen, aggrecan, and histone H4 mRNA using northern blot analysis. Total RNA was extracted immediate after loading. Histone H4 synthesis occurs mainly in S phase and therefore we have used it as a marker for proliferation.

**Results:** We observed a progressive increase of Alcian blue positive extracellular matrix in the presence of insulin, suggesting the accumulation of cartilage-type proteoglycans, but not in control culture except for a few spotted areas at day 21 and 28. ATDC5 cells also responded to insulin with a significant increase in mRNA levels for histone H4 at day 4, type II collagen at day 21 and 28, and aggrecan at day 7 through 28 during a 28-day culture period. Applying mechanical stress in the presence of insulin further increased the mRNA levels for histone H4 at day 4, type II collagen at day 21 and 28, and aggrecan at day 7, and type X collagen at day 7 through 28. Just exposure to mechanical loading in the absence of insulin also increased slightly in these mRNA expressions.

**Discussion:** These results demonstrated adjunctive effects of mechanical loading on insulin-induced chondrogenesis, and peculiar effect to induce chondrogenesis in the absence of insulin although this effect was weak. Response of the cells to mechanical stress was differentiation dependent. Mechanical loading induced proliferation in immature ATDC5 cells, and increased the production of cartilage-type extracellular matrix in mature ATDC5 cells. In conclusion, our study provides clear evidence that mechanical stress stimulates proliferation and differentiation in ATDC5 cells in differentiation-dependent manner.