Leptin Synergizes with Thyroid Hormone Signaling in Promoting Growth Plate Chondrocyte Proliferation and Terminal Differentiation

Wang, L; Shao, Y Y.; Ballock, R T
Departments of Biomedical Engineering and Orthopaedic Surgery, Cleveland Clinic, Cleveland, OH

INTRODUCTION
Leptin is a cytokine-like hormone which is primarily produced by adipocytes and regulates food intake and energy balance through its central signaling in the hypothalamus. Lack of leptin protein causes obesity in mice. Growth plate chondrocytes are capable of leptin synthesis and secretion, and possess leptin receptors (OB-Rs). Studies in leptin-deficient (ob/ob) obese mice show disturbed columnar structure, decreased type X collagen, increased apoptosis and premature mineralization in the mouse growth plates. Thyroid hormone is a systemic nuclear hormone that stimulates resting energy expenditure and basal metabolic rate through the hypothalamic-pituitary-thyroid axis. Thyroid hormone has also been described as a positive regulator of growth plate chondrocyte proliferation and terminal differentiation. These maturation effects are partly modulated by both IGF1-mediated PI3K/AKT signaling and Wnt/β-catenin signaling pathways. The objective of this study was to investigate the role of leptin in modulating thyroid hormone signaling in growth plate chondrocytes.

METHODS
Monolayer or pellet cultures of two-day-old rat distal femoral growth plate chondrocytes were maintained in serum-free medium supplemented with ITS+1. Leptin and T3 (triiodothyronine) were used at concentrations of 0.5 µg/ml and 100 ng/ml, respectively. The PI3K inhibitor LY294002 was used at a concentration of 10 µM. Real-time RT-PCR was used to study the mRNA expression of cyclin D1, Col10a1, IGF1R, Wnt-4, Runx2, leptin receptor (OB-Rb) and the leptin post-membrane inhibitor SOCS3. Protein levels of IGF1R, pAkt and active β-catenin were examined by immunoblotting.

RESULTS
Leptin enhanced both T3-induced increases in cyclin D1 (Figure 1A) and Col10a1 (Figure 1B) mRNA expression in growth plate chondrocytes. Leptin also increased IGF1R expression at both the mRNA and protein levels (Figures 2A and 2B). IGF-1 downstream pathways involving PI3K/AKT were activated by leptin through increasing the phosphorylation of Akt (Figure 2C). Inhibition of the PI3K/AKT pathway by LY294002 resulted in decreases in leptin-upregulated Col10a1 mRNA expression (Figure 2D). Leptin also activated Wnt/β-catenin signaling by increasing the cellular accumulation of active β-catenin protein (Figure 3A), increasing TCF/LEF transcriptional activity (Figure 3B), upregulating Wnt-4 mRNA expression (Figure 3C) and increasing expression of the Wnt target gene Runx2 (Figure 3D). Treatment of the growth plate chondrocytes with T3 for one day led to the increases in both leptin ligand and receptor expression (Figure 4). The leptin inhibitor SOCS3 was downregulated by T3 treatment.

DISCUSSION
This study demonstrates that leptin activates both the IGF-1-mediated PI3K/AKT signaling pathway and the Wnt/β-catenin signaling pathway, which are also downstream pathways of thyroid hormone signaling in growth plate. Thyroid hormone also activates leptin signaling by increasing both ligand and receptor expression, and by decreasing expression of the leptin inhibitor SOCS3. We therefore conclude that leptin synergistically functions with thyroid hormone in promoting growth plate cell proliferation and terminal differentiation. In addition to its systemic effects in modulating food intake and energy expenditure, leptin may also locally regulate growth plate development. These findings may have clinical implications regarding longitudinal bone growth in obese children, whose serum leptin levels are significantly elevated.

Figure 1. Leptin enhances thyroid hormone effects on growth plate cell proliferation and terminal differentiation. Cyclin D1 (A) and Col10a1 (B) mRNA expression in chondrocytes treated with T3 and/or leptin for 5 days (* p<0.05).

Figure 2. (A, B) Leptin upregulates IGF1R mRNA expression (A) and protein levels (B) in growth plate chondrocytes after five days of treatment. (C) Leptin activates PI3K signaling by increasing phosphorylation of Akt. (D) PI3K inhibitor LY294002 inhibits leptin-upregulated Col10a1 mRNA expression after five days of treatment.

Figure 3. Leptin activates Wnt/β-catenin signaling. Leptin increases the cellular accumulation of active β-catenin (A), TCF/LEF (TOPFlash) transcriptional activity (B), Wnt-4 gene expression (C) and Runx2 gene expression (D) in growth plate chondrocytes after five days of treatment.

Figure 4. T3 upregulates leptin and its receptor OB-Rb mRNA expression and decreases the expression of the leptin post-membrane inhibitor SOCS3 after one day of treatment (* p<0.05).