INTRODUCTION Although hypothyroidism is a known risk factor for children to develop slipped capital femoral epiphysis (SCFE), the largest group of patients with SCFE are obese children with normal thyroid hormone levels. PPAR-γ is a member of a new family of DNA-binding steroid hormone receptors termed the peroxisome proliferator activated receptors (PPARs), and may be upregulated in individuals with high fat diets (1). PPARs compete with thyroid hormone receptors for binding to the heterodimerization partner RXR, thereby interfering with normal thyroid hormone regulation of gene transcription (2). Given the central role of thyroid hormone in regulating terminal differentiation of growth plate chondrocytes into hypertrophic cells, it is possible that the biological dysfunction of the growth plate in obese children with SCFE may be due, in part, to interference with normal thyroid hormone signaling via upregulation of PPAR expression in growth plate chondrocytes. The objectives of this study were 1) to determine if PPAR-γ is expressed in growth plate chondrocytes, and 2) to determine if thyroid hormone signaling in growth plate chondrocytes could be inhibited by PPAR-γ.

METHODS Levels of PPAR-γ mRNA were assayed in neonatal rat growth plate cartilage by northern blotting and RT-PCR. The primer DNA sequences for PPAR-γ amplification were TCCAGCATTTCTGCTCCACAC (nucleotides 121-141) and TTTCTGTGCAAGATCGCCCTGG (nucleotides 713-692). To determine if activation of endogenous PPAR-γ would inhibit thyroid hormone-induced terminal differentiation, the PPAR-γ agonist ciglitazone (10 µM) was added to pellet cultures of growth plate chondrocytes isolated from the neonatal rat distal femur and maintained in serum-free, chemically-defined medium for 7 days. Growth plate chondrocytes were also transfected with an expression vector containing a full-length PPAR-γ cDNA construct under the control of the CMV promoter. Transfected cells were maintained for 7 days as three-dimensional pellet cultures in the presence or absence of thyroid hormone (100 ng/ml T3). In all cell culture experiments, measurements of alkaline phosphatase enzymatic activity were used as a marker of terminal differentiation.

RESULTS Levels of PPAR-γ mRNA were initially assayed in neonatal rat growth plate cartilage and found to be below the limits of detection by northern blotting. RT-PCR of cDNA synthesized from growth plate cartilage mRNA using isoform-specific PPAR-γ primers was successful in documenting the presence of PPAR-γ mRNA in growth plate cartilage. The identity of this PCR product was confirmed by DNA sequencing. Addition of the PPAR-γ agonist ciglitazone to pellet cultures of rat growth plate chondrocytes maintained under serum-free, chemically-defined conditions inhibited T3-induced increases in alkaline phosphatase activity at both day 4 and day 7 (Figure 1). Ciglitazone also induced expression of PPAR-γ mRNA in the cultured cells as measured by RT-PCR. Forced overexpression of PPAR-γ cDNA in pellet cultures markedly inhibited T3-induced increases in alkaline phosphatase activity by day 4 and day 7 compared to transfection of the empty vector (Figure 2). This inhibition was not altered by the addition of the PPAR-γ agonist ciglitazone, or by co-transfection of full-length cDNA constructs encoding the thyroid hormone receptor isoforms TRα1, TRα2, or TRβ1.

CONCLUSIONS These data show for the first time that PPAR-γ is not only expressed in the growth plate and is inducible by the PPAR-γ agonist ciglitazone, but also is able to interfere with thyroid hormone regulation of terminal differentiation in growth plate chondrocytes. These observations lend strong support to the hypothesis that the pathophysiology of SCFE in obese children with normal thyroid hormone levels may be due in part to the inhibitory action of PPAR-γ in the growth plate.