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FINAL REPORT

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**Developing Nutritional-Management Protocols
which prevent Tibial Dyschondroplasia**

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**Developing nutritional-management protocols which
prevent tibial dyschondroplasia**

BARD Project No. US-2627-95C

Final Report

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ABSTRACT

The objectives of this proposal were (1) to develop early age short-term restrict feeding protocols which eliminate the incidence of TD without compromising market weight performance and (2) monitor the components of the PTH/PTHrP cascade in conjunction with the development of the protocols in Objective 1.

In this investigation it was established that changes in gene expression associated with TD occur as early as 13 days of age. This means that management strategies for the control of this disease must be established during the initial two weeks of rearing. In order to determine a focus for these management strategies, attempts were made to identify the metabolic defect responsible for tibial dyschondroplasia. Therefore, the parathyroid hormone/parathyroid related peptide (PTH/PTHrP) cascade of events was investigated. This emphasis was based on the fact that many nutritional factors that influence TD could be operating through this system. Secondly, the receptor for these peptides acts as the gatekeeper of chondrocyte differentiation. Examination of many aspects of this cascade led to the conclusion that TD is not the direct result of perturbation of this PTH/PTHrP receptor but is likely to develop from an interruption of a pathway downstream from this receptor.

ACHIEVEMENTS

Skeletal deformities are significant financial and animal welfare problems for the world poultry industry. Under normal conditions, between two and seven percent of all broilers and turkeys are afflicted with skeletal deformities that result in mortality, morbidity or condemnation at the processing plant. However, under less than ideal conditions the losses may exceed 30%. In addition, birds with severe lesions are more susceptible to fractures during handling at the processing plant.

Tibial dyschondroplasia (TD) is the most prevalent skeletal abnormality found in young and growing broilers, turkeys and ducks. TD results from a perturbation of chondrocyte differentiation in the epiphyseal growth plate, located at the ends of long bones and responsible for longitudinal bone growth. To date traditional approaches, especially nutritional ones have not resulted in feasible protocols that substantially reduce the occurrence of this debilitating condition. Lack of cell and animal models of TD on one hand and lack of sufficient knowledge on the cellular and molecular sequence of events leading to TD on the other, are the main obstacles in designing future strategies to address the problem of skeletal deformities in poultry. As a first step towards the goal of reducing the incidence of TD, we established an *in vitro* system in which chondrocytes proliferate and differentiate in culture, mimicking precisely the *in vivo* processes. These cultures are used as a rapid and reliable screening tool for searching TD therapies. Any success in the *in vitro* system can immediately be tested and verified in two animal models of TD that we established. The first is based on genetic selection for strains susceptible and resistant to the disease and the second based on changing the balance of critical elements in the diet. These animal models provided a way (that was almost impossible before), to test new nutritional approaches

such as early age short-term feed restriction to normalize growth plate morphology without compromising market weight. From the results of this research, it is becoming clear that TD is not a nutritional disease. Although the incidence can be modified by dietary manipulation, no nutrient has been identified that will reduce the incidence to negligible levels. Using the *in vivo* and *in vitro* models we studied the expression of genes known to be key regulators of chondrocyte differentiation. This includes matrix proteins, growth factors, and hormone receptors. Moreover, using our animal models, the critical time period for the initiation of the TD lesion was established. This approach provides targets and window of opportunity for future intervention in chondrocyte differentiation as a modality for developing therapies for TD. These targets will be used also as markers in the genetic selection program for reducing TD. This research project was not limited to local effects within the growth plate but also provides critical information on the regulation of the synthesis, secretion and biological activity of major systemic hormones involved in calcium homeostasis. Cloning of specific avian genes was part of this effort. The project was a true collaboration between the US and IS groups as reflected by numerous joint publications. The publications resulting from this research confirm its significant contribution to the understanding of normal chondrocyte biology in general and provide insight into the processes in the development of TD in poultry. BARD funding has made a major contribution to these research activities.

BACKGROUND

The purpose of this research was to design nutritional-management strategies that will eliminate tibial dyschondroplasia (TD); which is the most prevalent skeletal deformity in broilers and turkeys. Tibial dyschondroplasia results from a perturbation in the sequence of events of the epiphyseal growth plate, the tissue responsible for

longitudinal bone growth. It is our hypothesis that tibial dyschondroplasia is the result of inappropriate activity of a key regulatory system, the parathyroid hormone/parathyroid hormone related peptide cascade. Therefore, measurements of selected markers of this regulatory system were utilized in the development of the feeding-management protocols.

OBJECTIVES AND RESULTS

Objective 1. Develop early age short-term restrict feeding protocols which eliminate the incidence of TD without compromising market weight performance.

Results. In order to investigate specific management protocols, it has been essential to establish the critical age for the development of tibial dyschondroplasia. Therefore, an experiment was conducted whereby broiler chicks were killed daily from 1 day of age until 21 days of age (Pines *et al.* 1998). Lesions were detected as early as 8 days of age. The lesions enlarged with age and were characterized by a decline in the expression of osteopontin, bone sialoprotein and type X collagen. Most of the reductions in these markers of chondrocyte hypertrophy were apparent by 13 days of age.

Objective 2. Monitor the components of the PTH/PTHrP cascade in conjunction with the development of the protocols in Objective 1.

Results. The components of the PTH/PTHrP cascade are outlined in Figure 1. We have examined individual components of this cascade in relation to the development of tibial dyschondroplasia. The results are as follows.

1. Calcium-sensing gene is found in the parathyroid gland and was found to be sensitive to serum ionic calcium. Expression of this gene was directly related to the serum calcium concentration – low when serum Ca^{++} was low and high when serum

Ca^{++} was high. In contrast, parathyroid gland PTH content was inversely related to serum Ca^{++} content (Yarden *et al.*, 2000).

2. The relationship between serum PTH and the occurrence of tibial dyschondroplasia was also investigated. A low calcium TD-inducing diet was used in this investigation.

This resulted in a spectrum of growth plate lesions including normal, tibial dyschondroplasia and rickets. These data are presented in Table 1 (Praul *et al.*, 2000).

Only those chicks exhibiting rickets had a significantly elevated serum PTH level.

These results led to the conclusion that systemic PTH was not a key factor in perturbing growth plate PTH/PTHrP receptor activity in chicks developing tibial dyschondroplasia.

3. The gene expression of the PTH/PTHrP receptor was also investigated in growth plate tissue from normal, rachitic and dyschondroplastic chickens. The receptor was expressed in the pre-hypertrophic and early hypertrophic zone of the tissue from normal and chicks exhibiting TD. Receptor expression was absent in the rachitic tissue. It was concluded that direct perturbation of PTH/PTHrP signaling by systemic PTH is not involved in the development of TD (Ben-Bassat *et al.*, 1999).

4. PTHrP endogenous to the growth plate was also investigated (Medill *et al.*, 2000).

In normal tissue, the greatest immunostaining intensity was observed in hypertrophic chondrocytes. Similar staining intensity was observed within small mostly non-apoptotic TD lesions. The reduced staining observed with large TD lesions could have been compromised by the large amount of apoptosis associated with chondrocytes in this tissue (Praul *et al.*, 1997).

CONCLUSIONS

This proposal was developed to form management strategies to control tibial dyschondroplasia (TD). The specific strategies would be dictated by an understanding

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	4	2	1	7
Submitted, in review, in preparation				
Invited review papers	1			1
Book chapters	1			1
Books				
Master theses				
Ph.D. theses		1	1	2
Abstracts				
Not refereed (proceedings, reports, etc.)				

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Visits/Meetings	1		1	2
Sabbaticals				
Postdoctorates				

- **Cooperation**, briefly explain whether synergistic, complementary or supportive.

Synergistic and complementary

of the mechanisms involved. Since several studies had indicated that vitamin D metabolites calcium, and phosphorus would influence the incidence of TD, we focused on the most likely metabolic pathway to be influenced by these nutrients. This system is the PTH/PTHrP cascade of events that when perturbed produces lesions similar to that observed with TD. After extensive studies on this pathway we conclude that TD is not the direct result of perturbation of this pathway but is likely to develop from interruption of a pathway downstream from the PTH/PTHrP receptor.

DESCRIPTION OF COOPERATION

The US investigators contributed expertise on tibial dyschondroplasia. This included techniques for inducing TD through the use of thiram, Ca/P imbalanced diets and genetic strains. The Israeli group contributed expertise in molecular biology techniques and supplied many of the reagents used for immunohistochemistry and *in situ* hybridization in these studies.

PUBLICATIONS

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Pines, M., N. Yarden, S. Ben-Bassat, I. Lavelin, and R. M. Leach. 1999. Regulation of the calcium-sensing and parathyroid hormone receptor genes in the chick. In: Calcium Metabolism: Comparative Endocrinology. pp. 67-73.

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Yarden, N., I. Lavelin, O. Genina, S. Hurwitz, R. Diaz, E. M. Brown, and M. Pines. 2000. Expression of calcium-sensing receptor gene by avian parathyroid gland *in vivo*: Relationship to plasma calcium. General and Comparative Endocrinology 117:173-181.

Accepted:

Medill, N. J., C. A. Praul, B. C. Ford, and R. M. Leach, Jr. 2000. Parathyroid hormone-related peptide expression in the epiphyseal growth plate of the juvenile chicken: evidence for the origin of the parathyroid hormone-related peptide found in the epiphyseal growth plate. Journal of Cellular Biochemistry

Figure 1. PTH/PTHrP Cascade of Events

- AC – Articular cartilage
- GP – Growth Plate
- P – Proliferative Zone
- PH – Pre-hypertrophic Zone
- H – Hypertrophic Zone
- PTH – Parathyroid Hormone
- PTHrP – Parathyroid Hormone Related Peptide

TABLE 1. The effect of diet on body weight, incidence of tibial dyschondroplasia (TD), and plasma parathyroid hormone (PTH) bioactivity in chickens

Diet	Body weight (g)	Incidence of		Plasma PTH bioactivity ²		
		Incidence of TD ----- ----- (%)-----	Incidence of Rickets ----- ----- (%)-----	Growth plate lesions		
				Normal	TD	Rickets
Marginal calcium	518 (45)	58 (45)	24 (45)	1,757 ^b (4)	2,297 ^b (10)	2,957 ^a (3)
Normal calcium	553 (50)	18 (50)	0 (50)	1,758 (12)	1,689 (5)	