Growth Hormone and IGF-1 Measurements in Beagle Dogs by ELISA: Assay Implementation and Variations in Baseline Levels

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Scope:

Measurements of GH and IGF-1 in Beagle dogs are complicated by several technical and biological issues. In this poster, we present data from ELISA methods that were specially adapted and qualified at our facility for the quantification of GH & IGF-1 in canine serum. During assay implementation and routine use, several observations have been made with these hormone baseline levels and some biological & technical parameters.

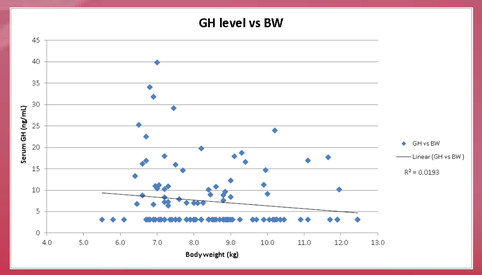
Experimental Procedures:

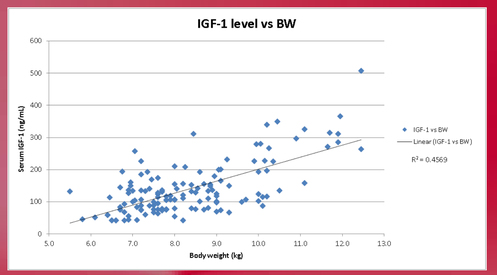
Beagle dog serum was obtained from males and females, originating from two different breeders; Marshall and Covance. Samples were tested from dogs housed at three different preclinical testing facilities; ITR Laboratories Canada Inc. ("ITR") and two other undisclosed facilities ("Lab B" & "Lab C"). A commercial GH ELISA kit designed for rat/mouse GH and another commercial human IGF-1 ELISA kit were adapted and qualified for use with dog serum samples.

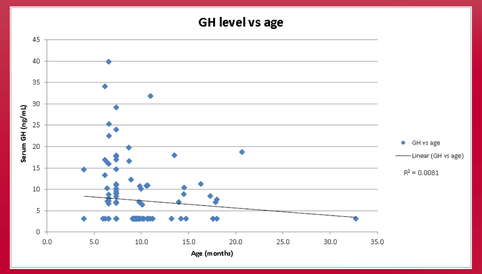
Results:

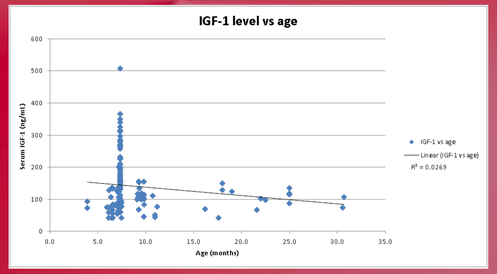
Typical basal GH levels in Beagle dogs vary from < 6.25 to 40 ng/mL. No significant differences were observed in the GH basal level between different genders, breeding source or test facility. However, GH levels generally increased with higher body weight and age. GH varied between individuals of the same study.

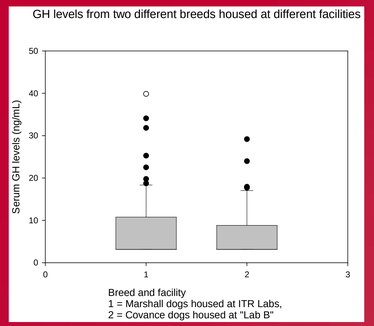
Typical basal IGF-1 levels in Beagle dogs varied greatly, from < 42 to 150 ng/mL for Marshall-bred dogs housed at ITR, while the normal range increased to 150-500 ng/mL with Covance-bred dogs housed at "Lab B". Age and body weight only had minor impact on the IGF-1 basal levels, while a gender difference was only seen within the Covance-bred dogs housed at "Lab B".

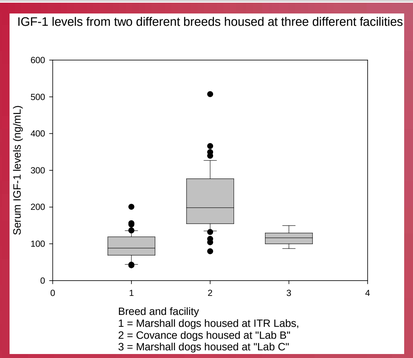




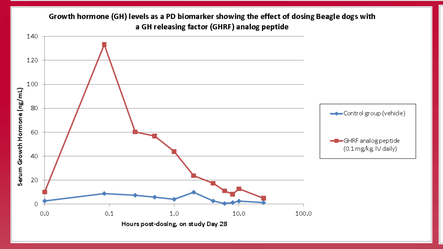


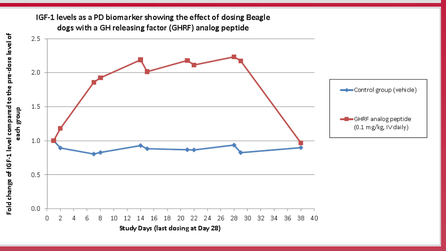






Preclinical study data, showing usefulness of GH and IGF-1 as biomarkers for pharmacodynamic (PD) analysis





Challenges with GH & IGF-1 measurements in Beagle Dogs	Solutions
- Unavailability of commercial kit for canine GH, and unavailability of recombinant canine GH protein. - Low cross-reactivity rodent or human GH kits on canine GH.	- Use a recombinant porcine GH as the calibrator, in a rodent GH ELISA kit. Porcine and canine GH have the same amino acid sequence.
- Endogenous GH levels vary naturally in every animal in a cyclic manner (circadian cycle).	- Perform sample collections at the same time of day, and in a larger number of animals per group. (ex. n = 6 minimum). - Perform multiple pre-dose collections, with the last timepoint as close as possible to the dosing with the test item.
- Strong binding of IGF-1 by IGF-binding proteins (IGFBP's).	- A human IGF-1 ELISA kit can be used when it employs an acid dissociation treatment of sampled prior to loading in ELISA plate.
- Endogenous IGF-1 levels are closely linked to body weight.	- Obtain Beagle dogs with similar body weights. - Report data as IGF-1/BW ratio, or normalize data (to pre-dose levels, or control group levels).
- Endogenous IGF-1 levels **vary** with Beagle dogs from different suppliers.	- Ensure the same supplier is used throughout the preclinical toxicology testing process.

Conclusions:

The GH/IGF-1 data gathered internally from several preclinical studies with Beagle dogs have shown that basal IGF-1 levels can vary significantly depending on the dog breeding source in addition to age and body weight. It was also found that due to the natural cyclic activity of GH, multiple pre-dose samplings are useful, with the last pre-dose sample being ideally taken as close as possible to dosing with the test item.

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