SAT-120

Development of Well Characterized ELISAs for Bound and Unbound Insulin-Like Growth Factors and Their Binding Proteins*

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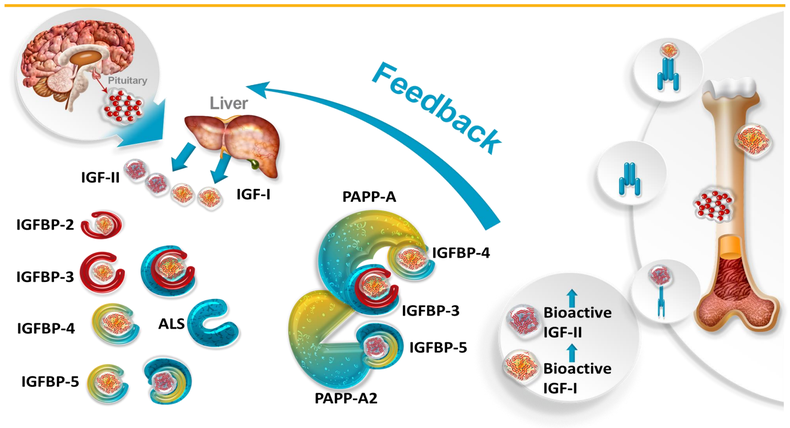
OBJECTIVE

Development of ELISAs specific for unbound and total IGF-I, total IGF-II, IGFBP-2, total and intact IGFBP-3, total and intact IGFBP-4 and total IGFBP-5 and measurement of these analytes in serum/plasma, saliva, urine and follicular fluid.

INTRODUCTION

The IGFBPs have profound effects on important biological processes such as modulation of bone cell proliferation and growth arrest of breast, ovarian and prostate cancer cells. The IGFBPs constitute a family of six circulating proteins that bind IGF-I and IGF-II with high affinity, thus controlling bioavailability of IGF-I, the major mediator of the anabolic- and growth-promoting effects of growth hormone. ELISAs to two of the IGFBPs protease, PAPP-A (a protease that targets IGFBP-4 and IGFBP-5) and PAPP-A2 (a protease that targets IGFBP-5 and IGFBP-3) are available and the measurement of IGF, IGFBPs and its proteases may find utility in various pathologies including acute coronary syndrome, cancer, hypertension, diabetes, cardiomyopathy, osteoarthritis, and diminished longevity.



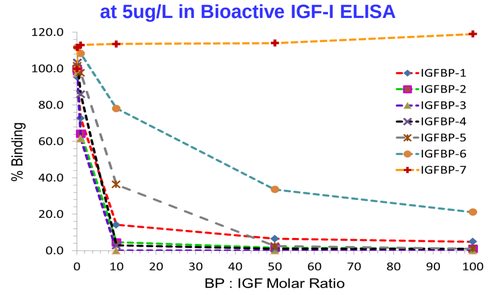
METHOD

ELISA Reagents	Antibody Binding Region	Dynamic Range (ng/mL)	Analytical Sensitivity	Imprecision % CV (Conc.)
Bioactive IGF-I AL-122	C-Terminal	0.03 – 32	0.025 ng/mL	6.3% (2.1 ng/mL) 6.0% (8.2 ng/mL)
Total IGF-I AL-121	C-Terminal (Acid & Neutralization)	0.8 - 800	0.625 ng/mL	6.3% (52.5 ng/mL) 6.0% (205 ng/mL)
Total IGF-II AL-131	N-(Capture) & C-(Detection)	2 - 1240	1.33 ng/mL	4.1% (107.1 ng/mL) 3.0% (261.9 ng/mL)
IGFBP-2 AL-140	Not Determined	0.45 - 16	0.08 ng/mL	3.4% (1.4 ng/mL) 5.8% (5.2 ng/mL)
Intact IGFBP-3 AL-149	C-(Capture) & N-(Detection)	5-130	1.37 ng/mL	6.0% (22.1 ng/mL) 4.2% (54.5 ng/mL)
Total IGFBP-3 AL-120	C-Terminal	5-130	0.3 ng/mL	4.2% (18.2 ng/mL) 4.2% (40.3 ng/mL)
Intact IGFBP-4 AL-128	N-(Capture) & C-(Detection)	1.5 - 96	0.67 ng/mL	2.7% (7.1 ng/mL) 4.6% (27.1 ng/mL)
Total IGFBP-4 AL-126	C-Terminal	50 - 700	4.7 ng/mL	3.8% (122.3 ng/mL) 3.9% (363.3 ng/mL)
Total IGFBP-5 AL-127	C-Terminal	37 - 1200	4.4 ng/mL	5.1% (55.6 ng/mL) 5.2% (283.5 ng/mL)

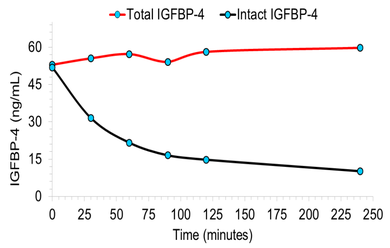
RESULTS

% Cross-Reactivity							
ELISA Reagents	IGF-1 1ug/mL	IGF-II 1ug/mL	IGFBP-2 1ug/mL	IGFBP-3 1ug/mL	IGFBP-4 1ug/mL	IGFBP-5 1ug/mL	IGF-IGFBP-3 1ug/mL
Bioactive IGF-I	100%	ND	ND	ND	ND	ND	<0.42%
Total IGF-I	100%	ND	ND	ND	ND	ND	100
Total IGF-II	ND	100%	ND	ND	ND	ND	ND
IGFBP-2	ND	ND	100 %	ND	ND	ND	ND
Intact IGFBP-3	ND	ND	ND	100%	ND	ND	100%
Total IGFBP-3	ND	ND	ND	100%	ND	ND	100%
Intact IGFBP-4	ND	ND	ND	ND	100%	ND	ND
Total IGFBP-4	ND	ND	ND	ND	100%	ND	ND
Total IGFBP-5	ND	ND	ND	ND	ND	100%	ND
ND = Non Detectable							

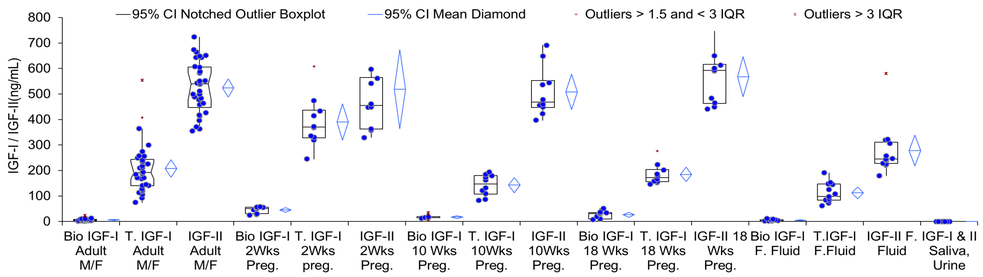
Effect of binding proteins addition to rhIGF-I at 5ug/L in Bioactive IGF-I ELISA



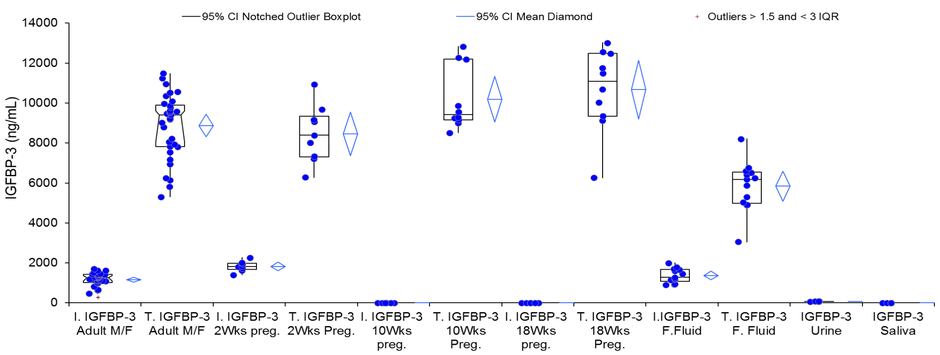
Measurement of active PAPP-A cleaved IGFBP-4 fragments in IGFBP-4 ELISAs



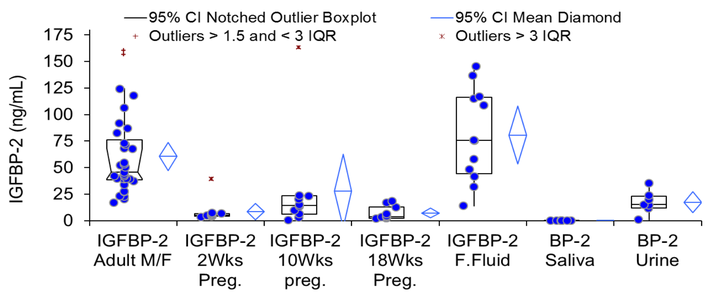
Measurement of Bioactive IGF-I, Total IGF-1 and IGF-II in Biological Fluids



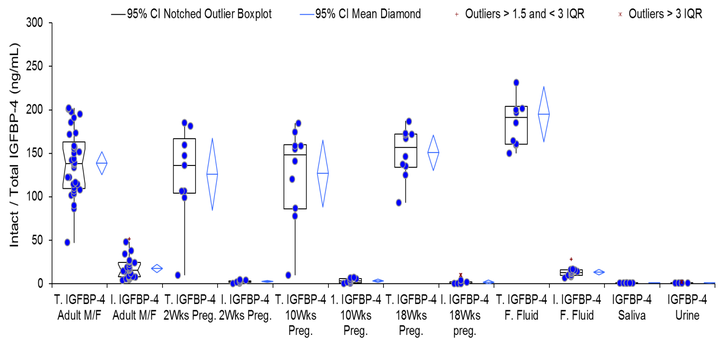
Measurement of Intact and Total IGFBP-3 in Biological Fluids



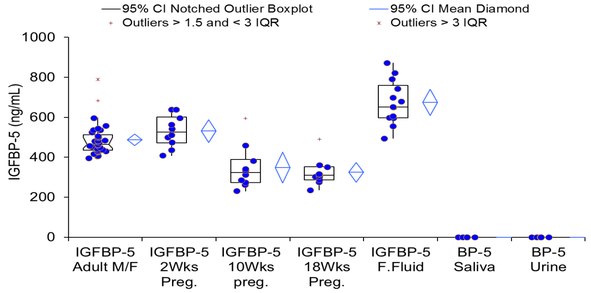
Measurement of IGFBP-2 in Biological Fluids



Measurement of Intact and Total IGFBP-4 in Biological Fluids



Measurement of Total IGFBP-5 in Biological Fluids



CONCLUSIONS

1. Accurate measurements of both bioactive and total IGF-I has been demonstrated for the first time using same calibration and antibody pair.
2. Accurate measurements of both intact and total IGFBP-3 has been demonstrated for the first time using same calibration and acidification and neutralization methods.
3. Accurate measurements of both intact and total IGFBP-4 has been demonstrated using PAPP-A cleaved IGFBP-4 fragments.
4. Accurate measurements of total IGFBP-5 and its proteases PAPP-A-2 will help better understand their physiological roles.
5. These well characterized IGF and IGFBP ELISAs will serve as important tools and help researchers revisit and quantitate these important growth factors in physiological and pathophysiological studies.

***Research Use Only**.

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