EDI™ Human Intact FGF-21 ELISA

KT-879, KTR-879

CE

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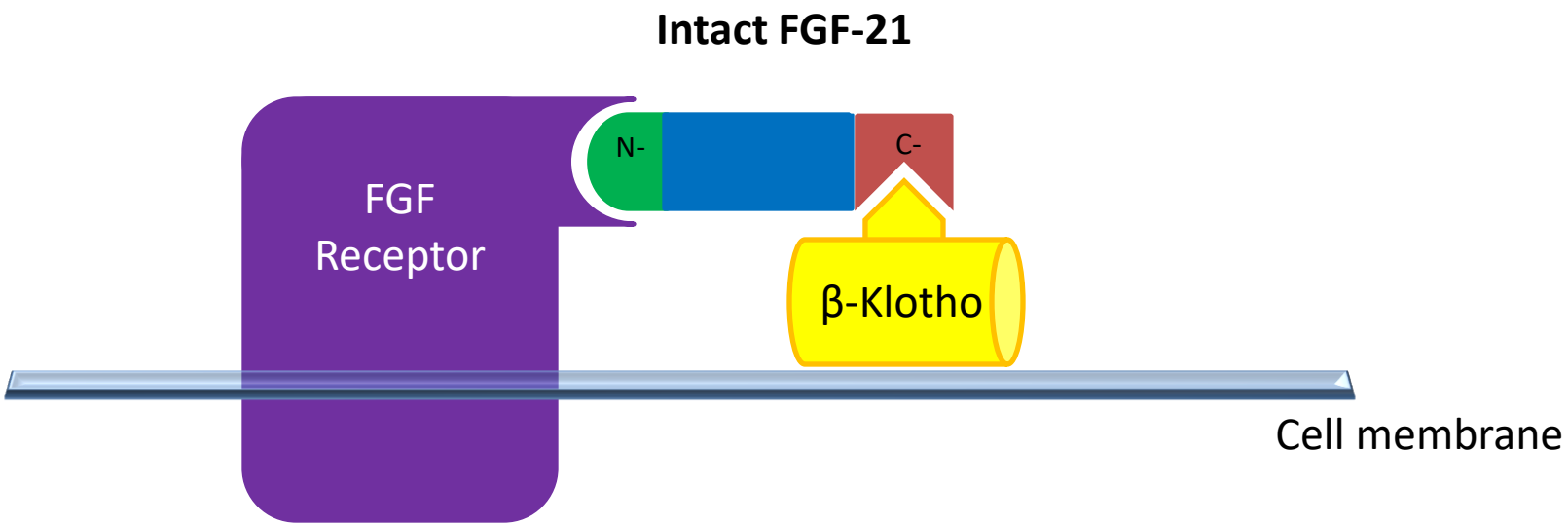
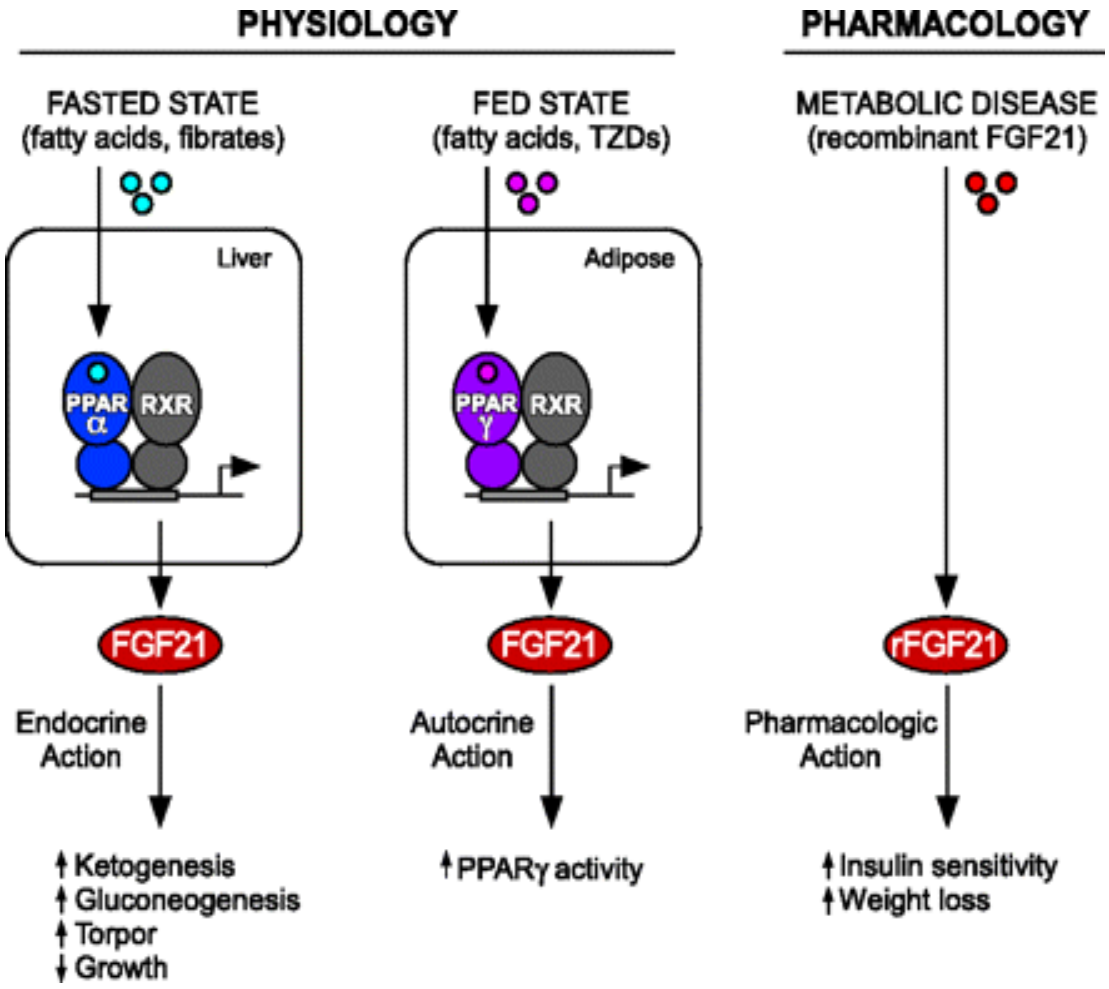
“FGFR-FGF21-β-Klotho” Complex



Form of “FGFR-FGF21-β-Klotho” complex is essential for FGF21 bioactive processing.

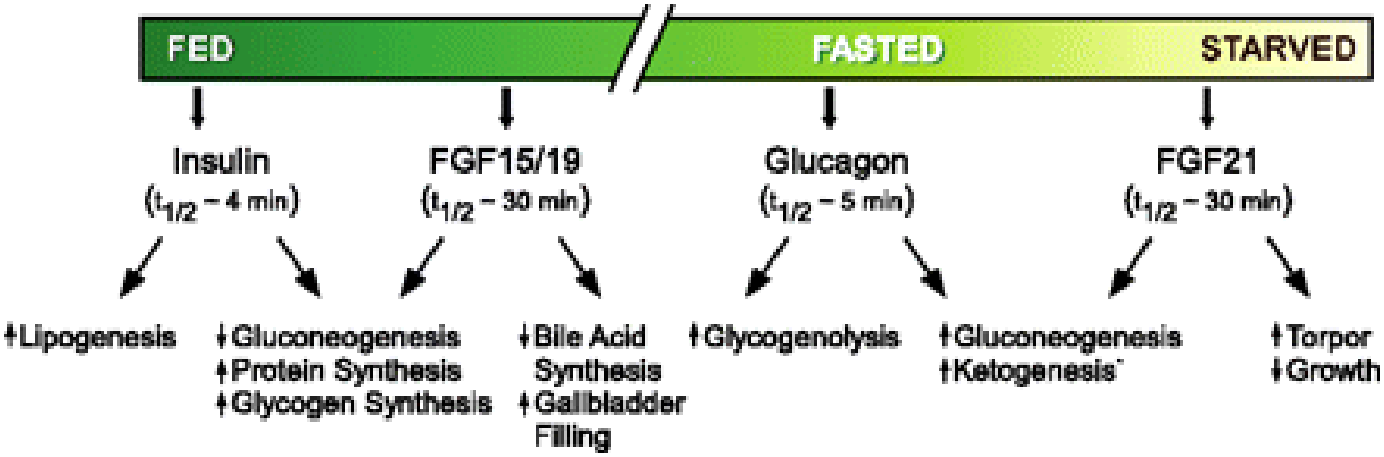
Physiology and Pharmacology Action



Endocrine, autocrine, and harmacological actions of FGF21. (*Left*panel) In response to fasting or fibrate drugs, FGF21 expression is induced in the liver by the PPARα/RXR heterodimer. Secreted FGF21 acts as an endocrine hormone to induce ketogenesis, gluconeogenesis, and torpor and to inhibit somatic growth. (*Middle* panel) In response to feeding or thiazolidinedione drugs (TZDs), FGF21 expression is induced by the PPARγ/RXR heterodimer in WAT, where FGF21 acts through an autocrine mechanism to stimulate PPARγ activity. (*Right*panel) Pharmacological administration of recombinant FGF21 (rFGF21) affects multiple tissues and has beneficial effects in metabolic disease.

M. J. Potthoff, et al. Gene & Dav 2012;26:312-24

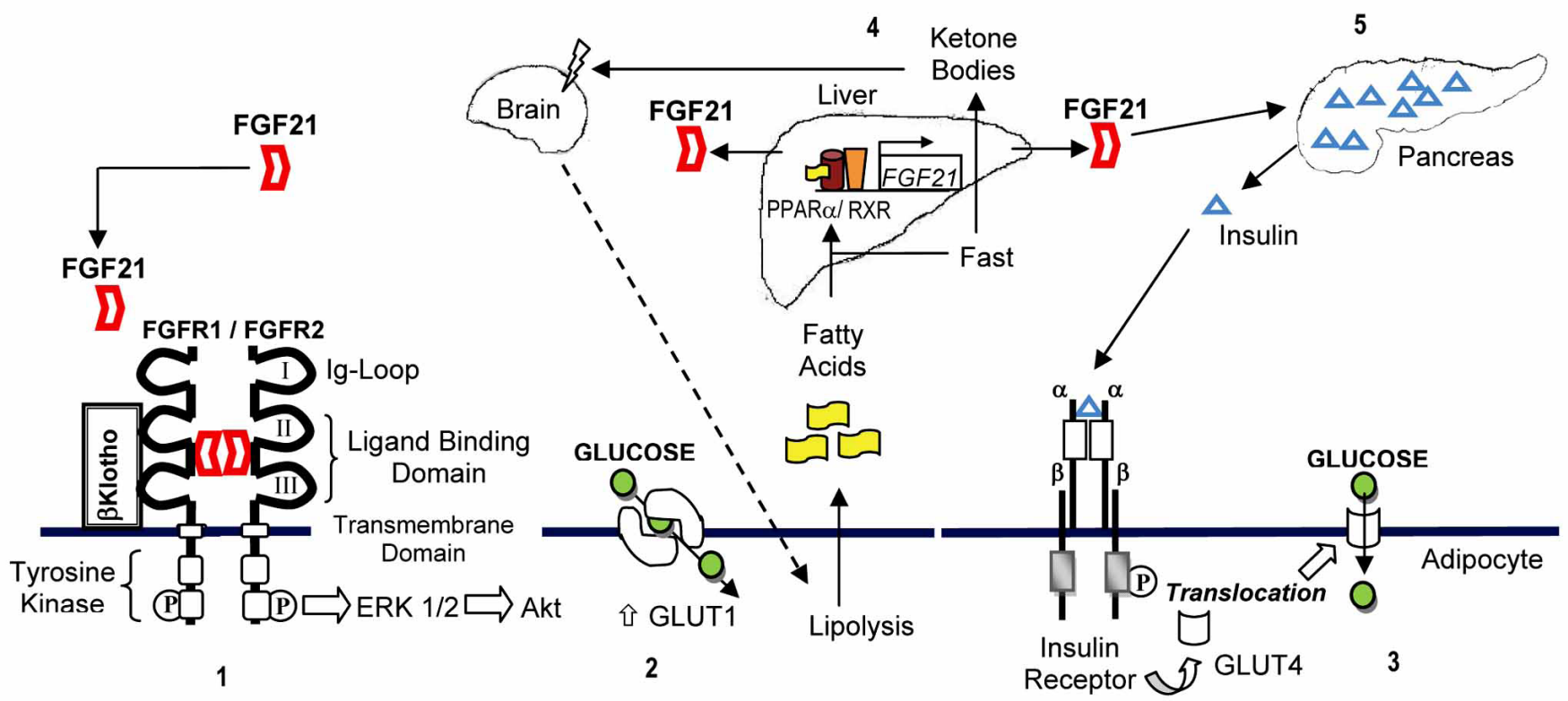
FGF15/19 and FGF21 function in a temporal cascade of hormones to regulate responses to nutritional stress



The temporal relationship among insulin, FGF15/19, glucagon, and FGF21 is shown along with hormone half-lives and biological actions.

M. J. Potthoff, et al. Gene & Dav 2012;26:312-24

Mechanisms of action and metabolic activities of FGF21 in different tissues

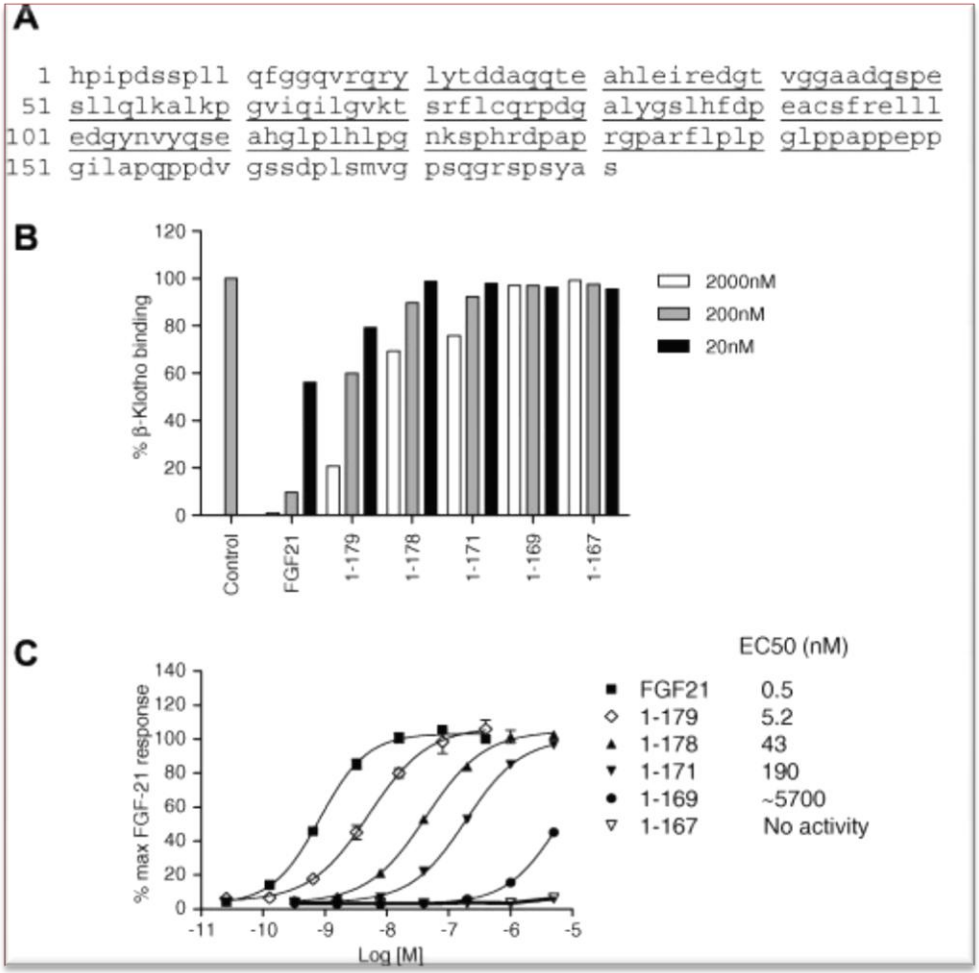


Daniel Cuevas-Ramos, et al. Current Diabetes Reviews, 2009, 5, 216-220

Measures Intact FGF-21, not FGF-21 Fragments

- Human Intact FGF-21 (1-181) binds to β-Klotho and FGFR.
- N-truncated and C-truncated FGF-21 is not biologically active.
- Current commercial FGF-21 assays show extremely high normal cut-off, ~ 800 pg/ml - 1100 pg/ml.
- Current commercial FGF-21 assays don't different well between normal subjects and patients with diabetes and obesity.

Intact C-terminal FGF-21 is essential for binding β-Klotho



C-terminal FGF21 is critical for β-Klotho binding. The binding to β-Klotho determines FGF21's potency in the reporter assay.

(A) Shown is the amino acid sequence of human FGF21. Signal peptide sequence is omitted. Underlined is the predicted β-trefoil core domain of FGF21.

(B) Biacore data with FGF21 C-terminal deletion mutants. Different amounts of FGF21 deletion mutants were incubated with 10 nM β-Klotho for 1 h before the mixture was injected over the biotin–FGF21 surface. Free β-Klotho was used as a control to demonstrate maximal binding. Concentrations of FGF21 constructs used are: white bar, 2 μM; gray bar, 200 nM; black bar, 20 nM.

(C) The luciferase reporter assay of the selected FGF21 C-terminal deletion mutants in the 293T stable cell line. This data has been repeated multiple times with triplicate in each experiment.

J Yie, et al. FEBS Letters 583 (2009) 19–24

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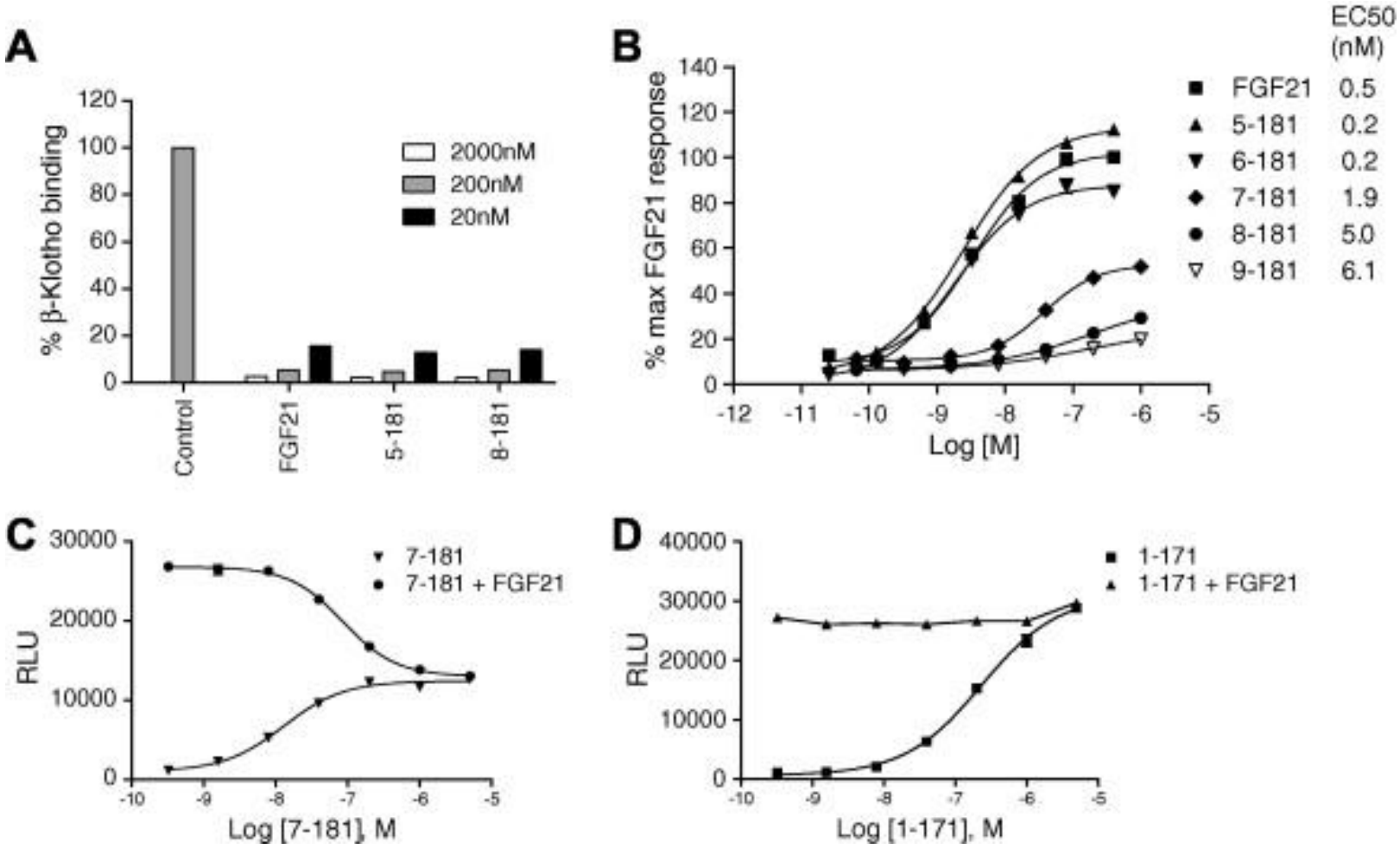
N-terminal FGF21 deletions resulted in partial agonist effect and have no impact on β-Klotho interaction



J Yie, et al. FEBS Letters 583 (2009) 19–24

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EDI™ Human Intact FGF-21 ELISA

Catalog No. KTR-879 & KT-879

- This is the first immunoassay kit that exclusively measure human intact FGF-21.
- “Sandwich” ELISA with one antibody specific to the most N-terminal portion and the other antibody to the most C-terminal portion of the FGF21.
- No cross reaction to FGF21 fragment
- No cross reaction to other FGFs
- US Patent pending

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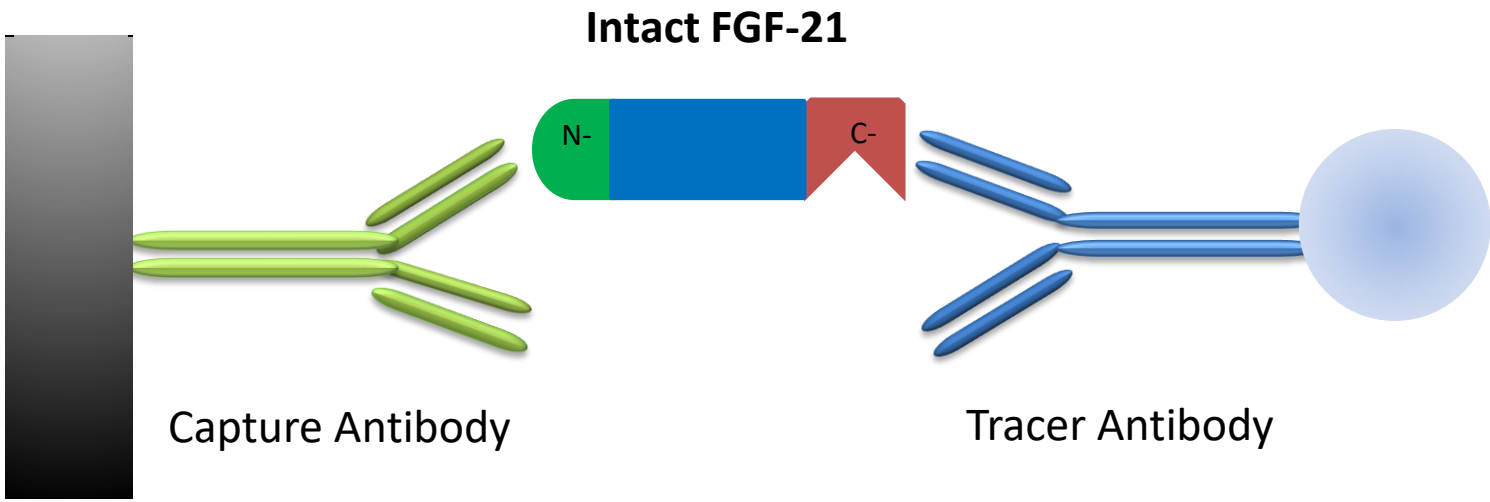
EDI™ Human Intact FGF-21 ELISA Assay Scheme



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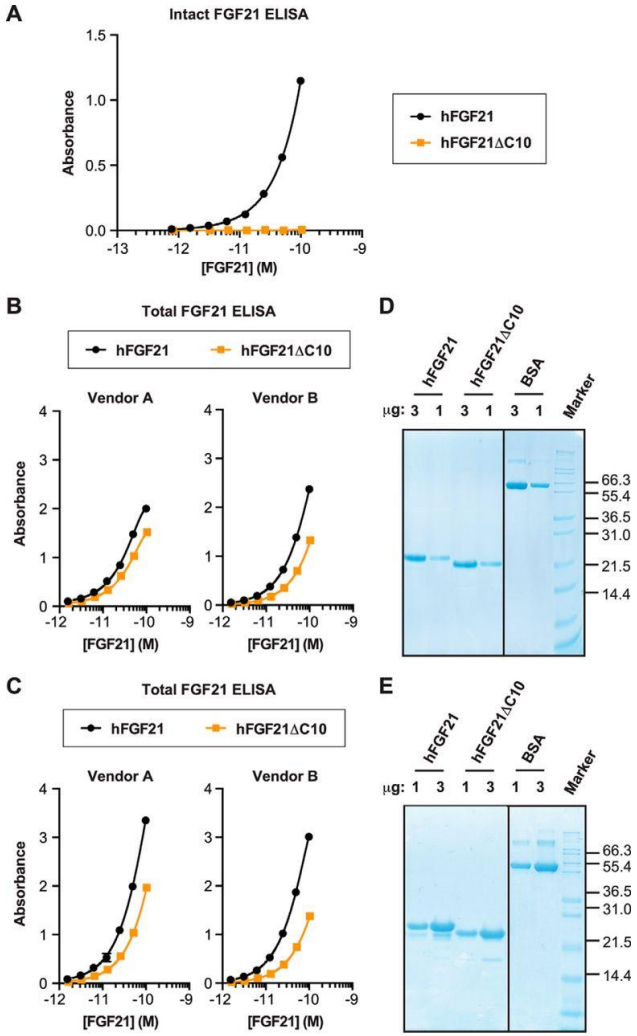
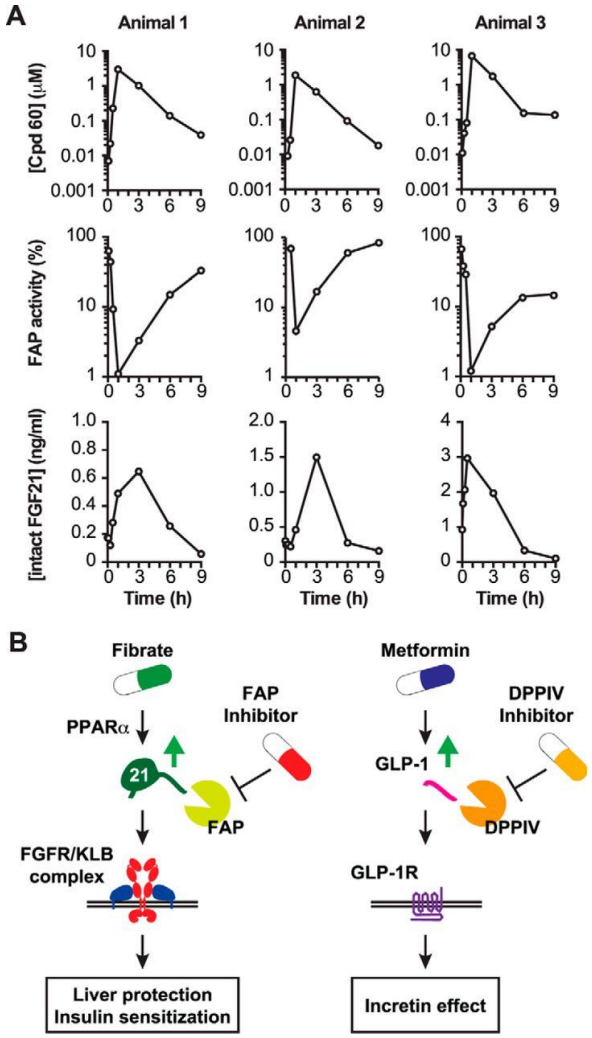
Evaluation of FGF21 ELISA. A and B, wild-type hFGF21 and the FAP-digested derivative (hFGF21ΔC10) were tested by using intact hFGF21 ELISA (A) or total hFGF21 ELISA from two separate sources (B).



Diana Ronai Dunshee et al. J. Biol. Chem. 2016;291:5986-5996

The effect of FAP inhibition on endogenous intact FGF21 levels in non-human primates.



Diana Ronai Dunshee et al. J. Biol. Chem. 2016;291:5986-5996

EDI™ Human Intact FGF-21 ELISA

Catalog No. KTR-879 & KT-879

Intended Use:

- This “sandwich” ELISA is intended for the quantitative determination of **β-Klotho & FGF Receptor Active Human Intact FGF-21** level in EDTA-plasma or serum.
- This assay measures human intact FGF-21, not FGF-21 fragments.

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EDI™ Human Intact FGF-21 ELISA

Catalog No. KTR-879 & KT-879

Indication of Use:

- **Diagnosis of primary muscle-manifesting respiratory chain deficiencies**
- **Nonalcoholic fatty liver disease**
- **Other conditions related to type 2 diabetes**
- **Gestational diabetes**
- **Obesity**

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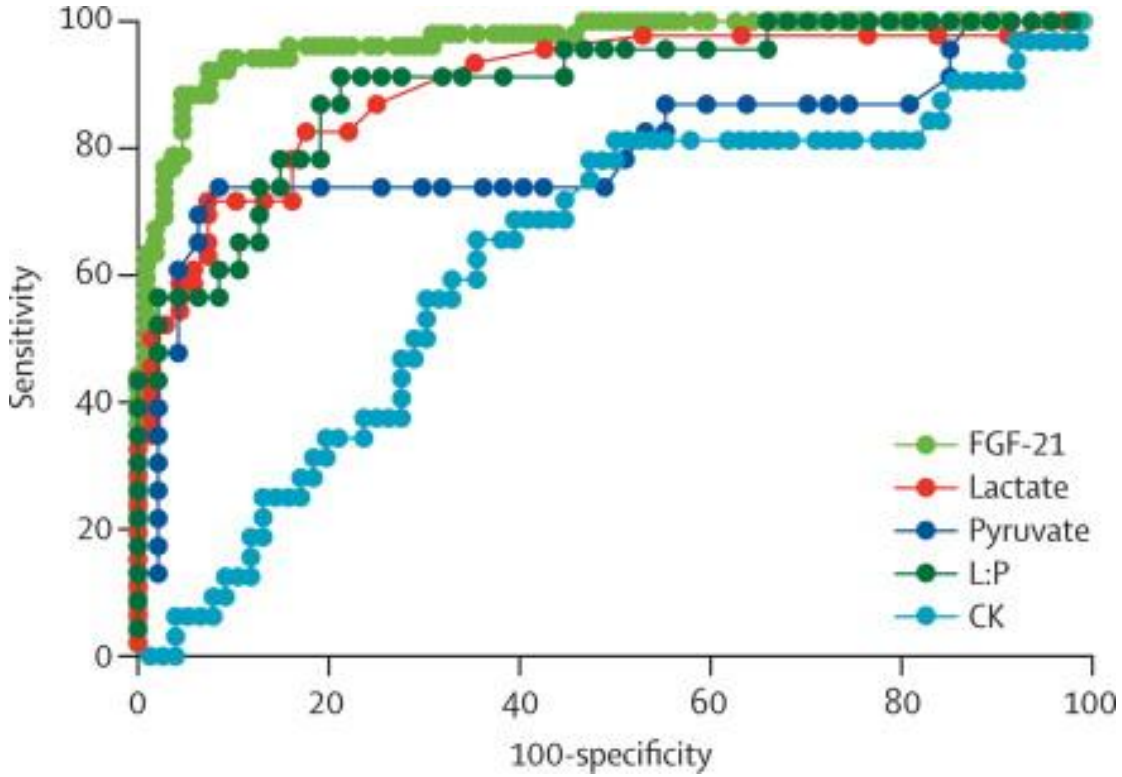
FGF-21 as a biomarker for muscle-manifesting mitochondrial respiratory chain deficiencies



Receiver-operating-characteristic curves for different biomarkers (continuous values) of muscle-manifesting respiratory chain deficiencies in adults and childrenAreas under the curves are: 0·97 (95% CI 0·94–0·99) for FGF-21 in serum; 0·90 (0·84–0·96) for lactate; 0·80 (0·70–0·93) for pyruvate; 0·90 (0·82–0·98) for L:P; and 0·63 (0·51–0·74) for CK. L:P=ratio of lactate to pyruvate. CK=creatine kinase.

Anu Suomalainen, et al. **FGF-21 as a biomarker for muscle-manifesting mitochondrial respiratory chain deficiencies: a diagnostic study. The Lancet Neurology 2011;10:806-818**

Simple Assay Procedure

- A One-Step Antibody Binding Procedure
- Only 50 µL sample/well

- 2 hrs – 20 min
- Room temperature

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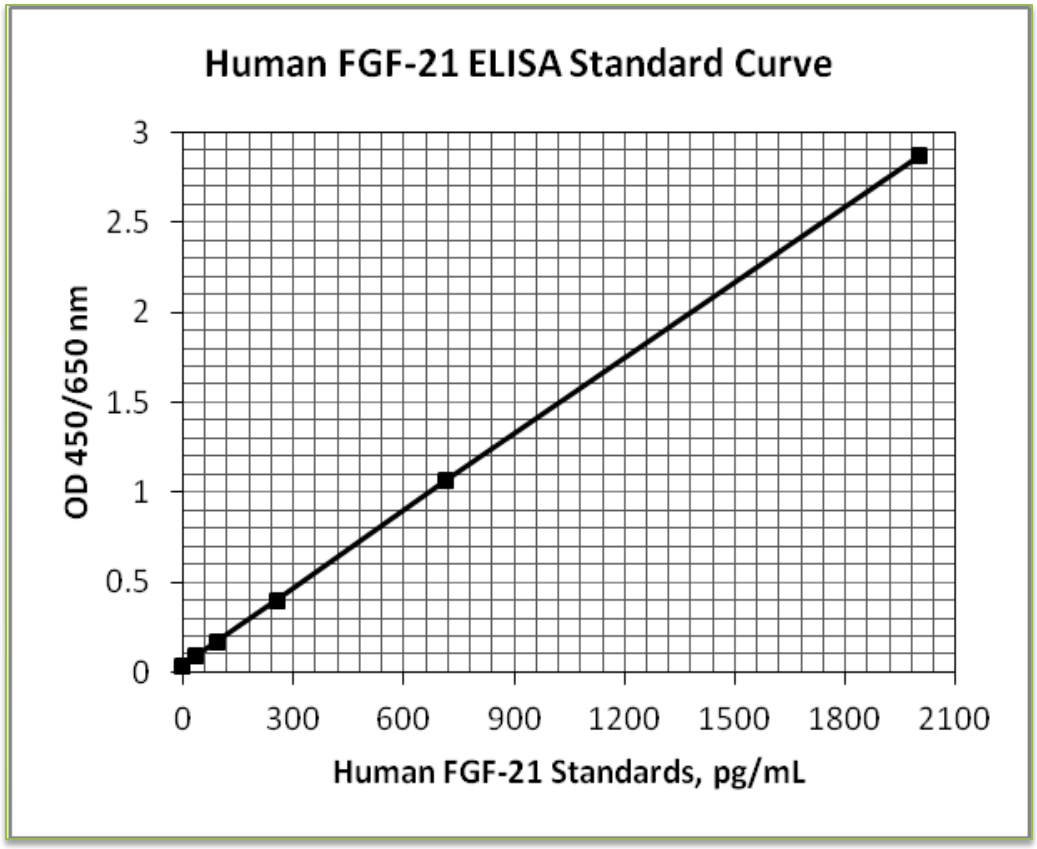
Analytical Sensitivity (LLOD): 1.7 pg/mL



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Assay Performances

- **No High Dose "hook" effect up to 20,000 pg/mL.**

- **Precision**

Intra-assay

Mean Human Intact FGF-21 (pg/mL)	CV (%)
63.2	5.7
171	4.2
480	5.4

Inter-assay

Mean Human Intact FGF-21 (pg/mL)	CV (%)
69.8	6.9
181	3.0
486	1.9

Assay Performance

- Linearity

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	Neat	286	-	-
	1:2	138	143	96
	1:4	75	72	104
	1:8	37.9	36	105
	1:16	19.5	18	108
2	Neat	61.8	-	-
	1:2	32.1	30.9	104
	1:4	15.9	15.5	103
	1:8	7.2	7.7	94

- Spike Recovery

#	Orig. Value	Amount Spiked	Observed Value	Expected Value	Recovery %
1	45.9	91	64.9	68.5	95
	(serum)	255	150	151	100
		714	388	380	102
2	40.4	91	71.2	65.7	108
	(plasma)	255	148	148	100
		714	406	377	108

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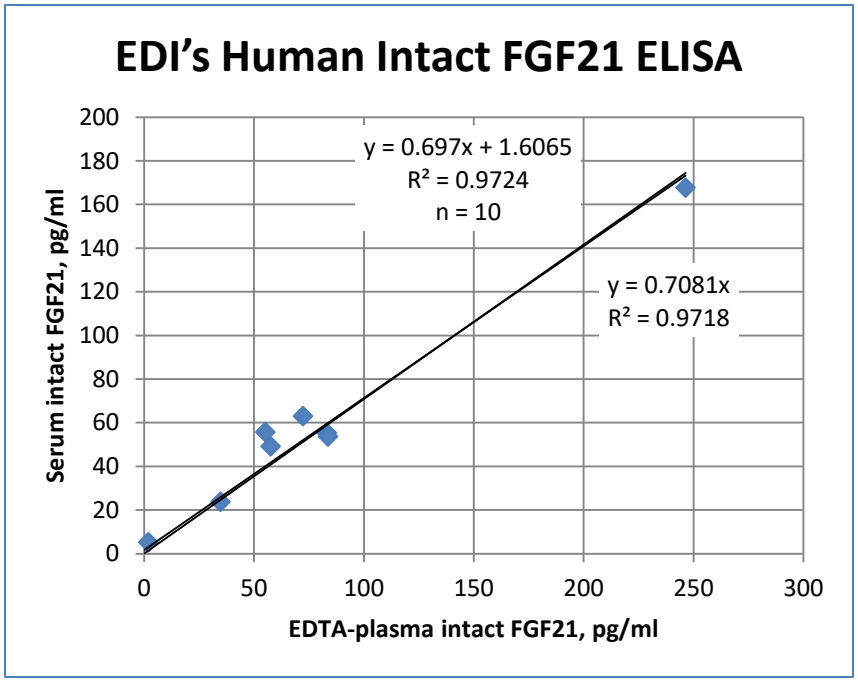
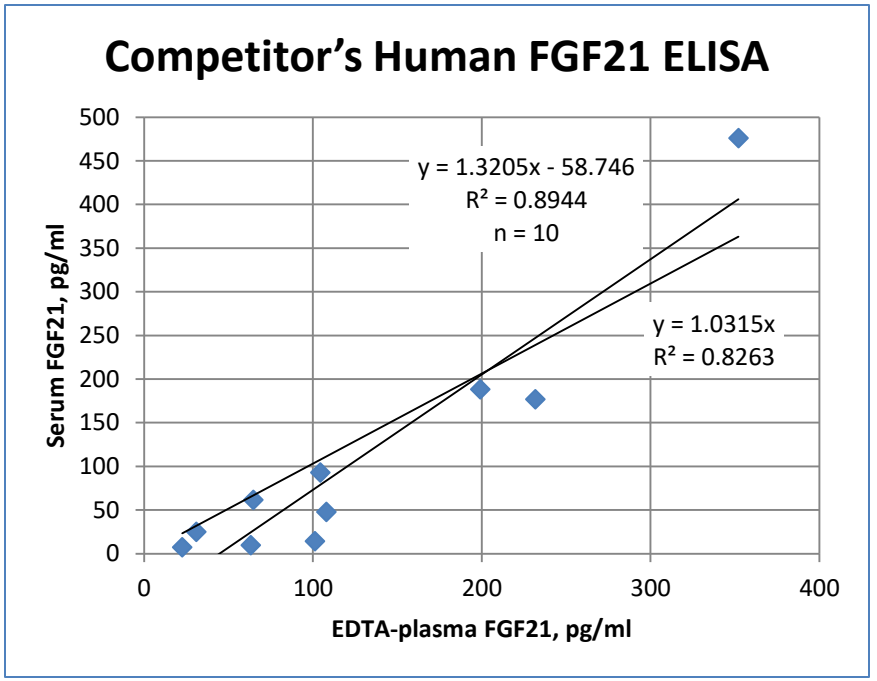
Normal Cut-Off

200 pg/mL

This intact FGF-21 assay shows a much lower normal cut-off than other previous FGF-21 assays (*usually 800 – 1100 pg/ml*), which may lead to a better differentiation between normal subjects and patients with diabetes and obesity.

FGF21 in paired donor EDTA-plasma and serum: a side-by-side study





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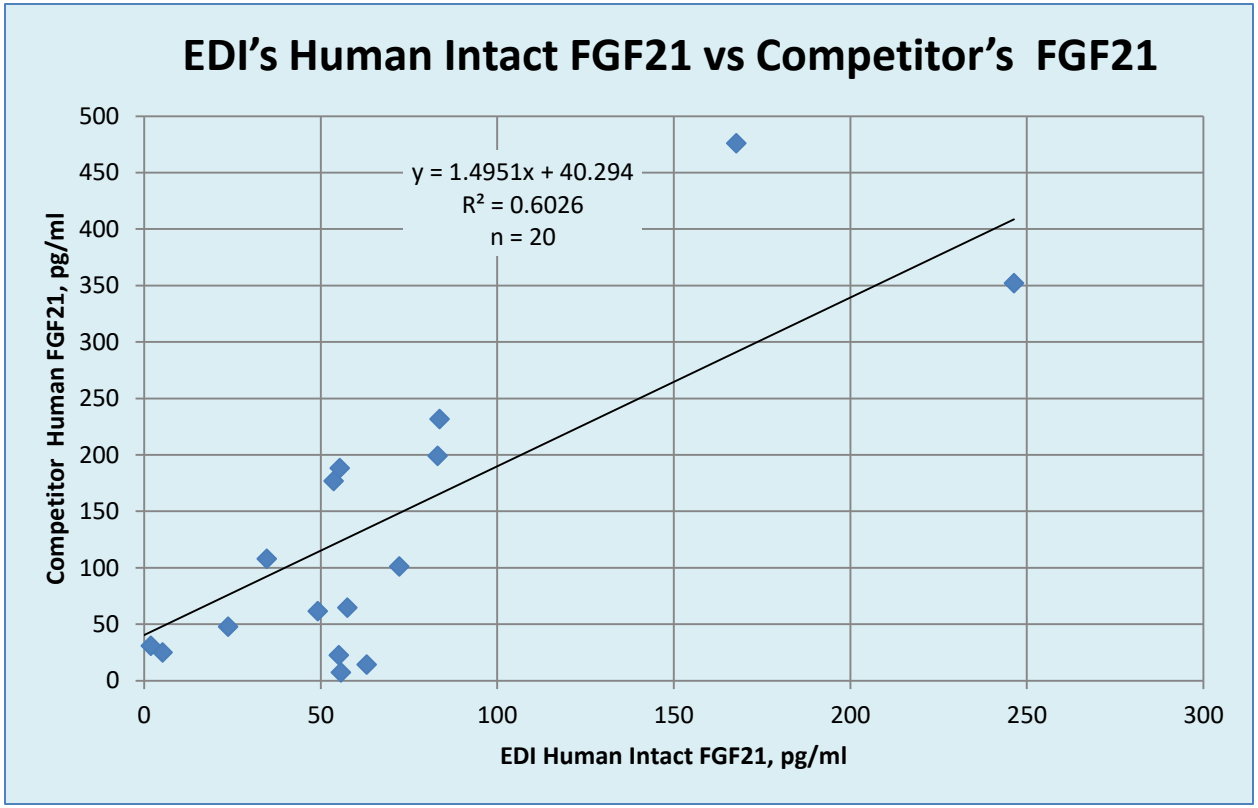
Intact vs. non-intact FGF21 Assays: what do we measure and what does it tell us?

Normal Donors



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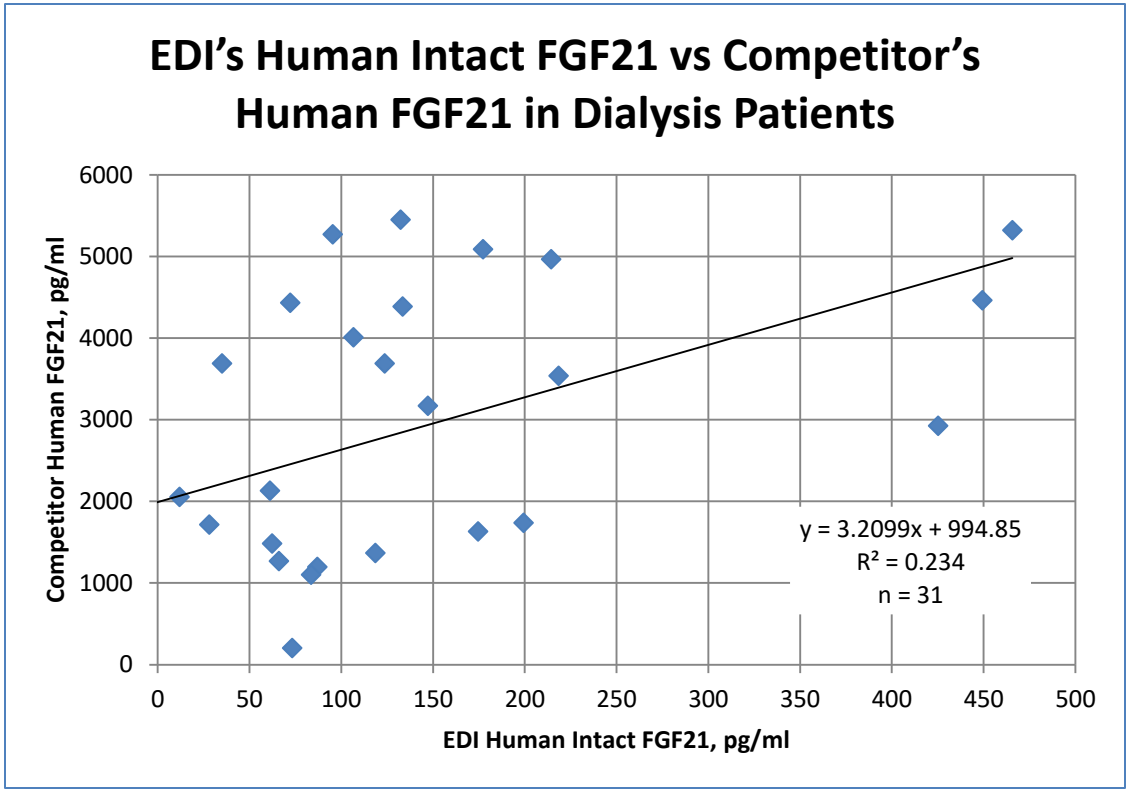
Dialysis Patients



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Additional ELISA Kits for Diabetes and Obesity

- Active GLP-1 (7-36) ELISA Kit, KT-871
- Total GLP-1 ELISA Kit, KT-876
- GLP-1 Sample Extraction Kit, KT-910

Proudly developed and manufactured in USA

Epitope Diagnostics,Inc.
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www.epitopediagnostics.com