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**Evaluation and validation of the HTRF
insulin assay as a replacement for a
commercially available ELISA**

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Breakthrough Science of Aging & Metabolism



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Elixir Pharmaceuticals is a pharmaceutical company focused on the discovery, development and commercialization of novel pharmaceuticals for the treatment of metabolic diseases and obesity.

Significant Opportunity in Metabolic Disease

Type II Diabetes

- > 180M people worldwide have diabetes
- Type II accounts for 80-95% of all cases
- Frequently undiagnosed
- Sixth leading cause of death by disease
 - Leading cause of kidney disease
 - Leading cause of non-traumatic limb amputations
 - Leading cause blindness among young adults



Type II Diabetes

- Complicated metabolic disorder
- Characterized by:
 - Loss of sensitivity to insulin
 - Decrease in the body's ability to produce insulin
 - Overproduction of glucose by the liver
 - Uncontrolled diabetes leads to abnormally high blood sugar levels
 - a condition known as hyperglycemia
- Six classes of drugs approved for treatment
 - Still unmet medical need for diabetes / weight control drugs

Key to diabetes research is the ability to measure insulin effectively and accurately

Insulin

- Produced in the beta islet cells of the pancreas
- Stimulate uptake of glucose from the blood
- Critical in the control of glucose homeostasis
- Insulin deficiency is the hallmark of type I diabetes
- Hyperinsulinemia and insulin resistance characterize type II diabetes

- Diabetes research often involves rat and mouse models for *in vivo* studies
 - Diet induced obesity (DIO) model: animals fed high fat diet to induce an imbalance in blood glucose and insulin levels
 - Glucose tolerance test (GTT): where a bolus of glucose is administered and plasma insulin and glucose levels are measured over time

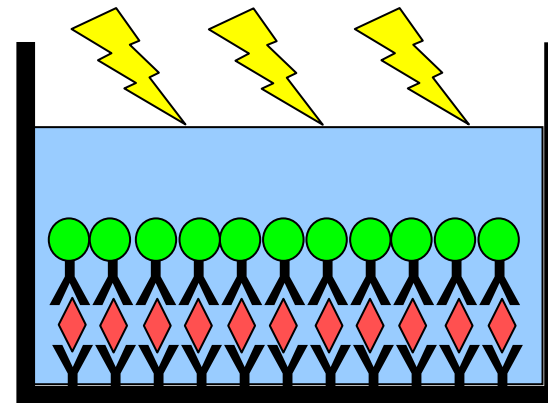
Key to diabetes research is the ability to measure insulin effectively and accurately

- Traditional assays for insulin
 - Radio-immunoassay (RIA)
 - Enzyme linked immunosorbant assay (ELISA)

- **Cisbio's HTRF insulin assay**

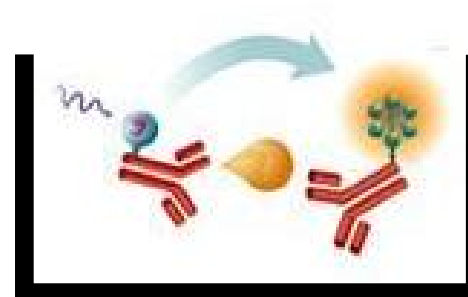
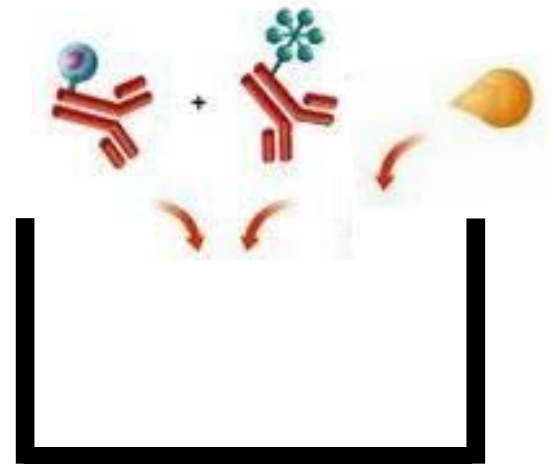
Insulin ELISA

- 96 well antibody coated plate
- Dispense 95 μ l sample diluent
- Pipette 5 μ l plasma sample or insulin standard
- Incubate 2 hours at 4C
- Wash 5 times with wash buffer
- Dispense 100 μ l anti-insulin conjugate
- Incubate 30 min at room temperature
- Wash 5 times with wash buffer
- Dispense 100 μ l enzyme substrate solution
- Incubate 40 min at room temperature
- Stop reaction by adding stop solution
- Measure A_{450} and subtract A_{630} values
- Calculate insulin concentrations using the standard curve



Cisbio's HTRF insulin assay

- Dispense 5 μ l sample or insulin standard
- Dispense 2.5 μ l each of anti-insulin Ab-cyptate and anti-insulin Ab-XL665
- Incubate 2 hours at room temperature
- Read on an HTRF compatible reader
- Calculate insulin concentration using the standard curve

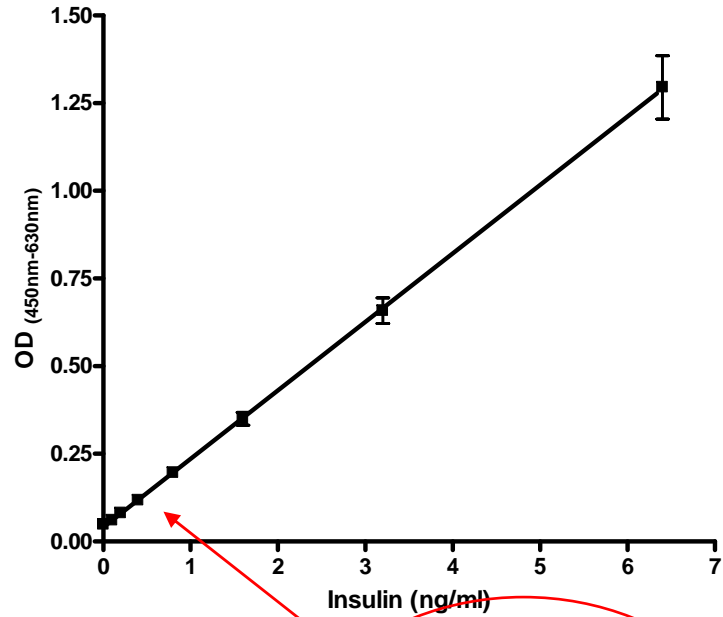


Assay comparison

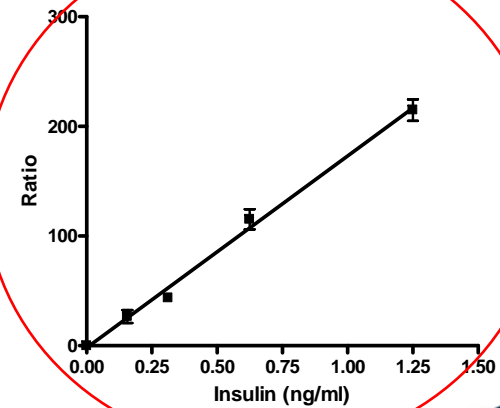
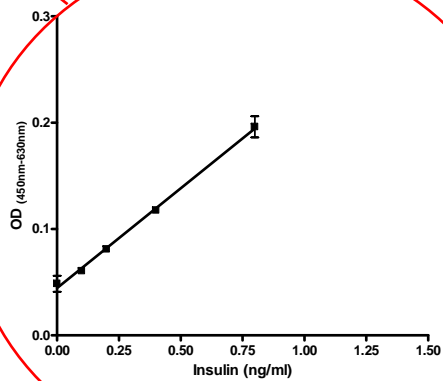
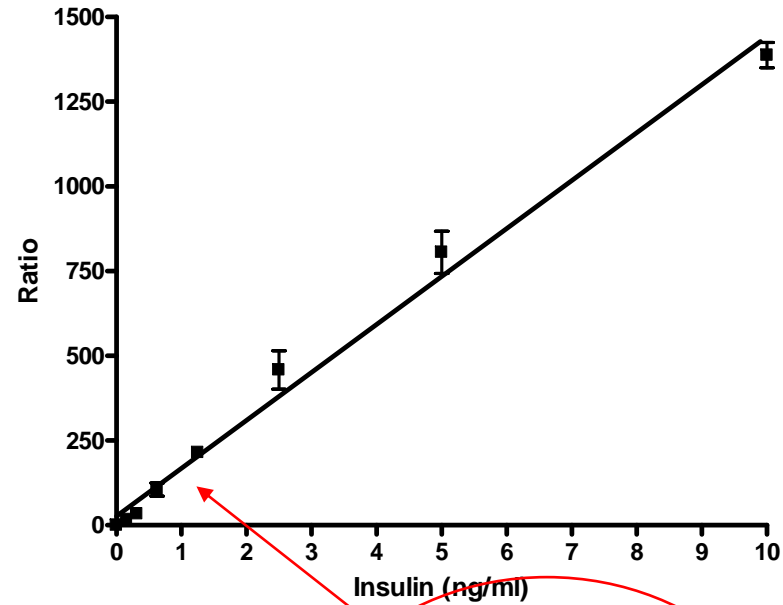
	<u>ELISA</u>	<u>HTRF</u>
Sample volume required	5 μ l	5 μ l
Standard curve range	0 - 6.4ng/ml	0 - 10ng/ml
Plate format	96 well	384 well
Miniaturizable	no	yes
Total # steps to perform assay	13	5
Total time to perform assay	4+hrs	~2hrs
Wash steps	10	zero
Cost per well (US\$)	\$3.46	\$0.13
Specificity	r, m	r, m, p, h

Comparison of ELISA and HTRF insulin standard curves

ELISA insulin standard curve

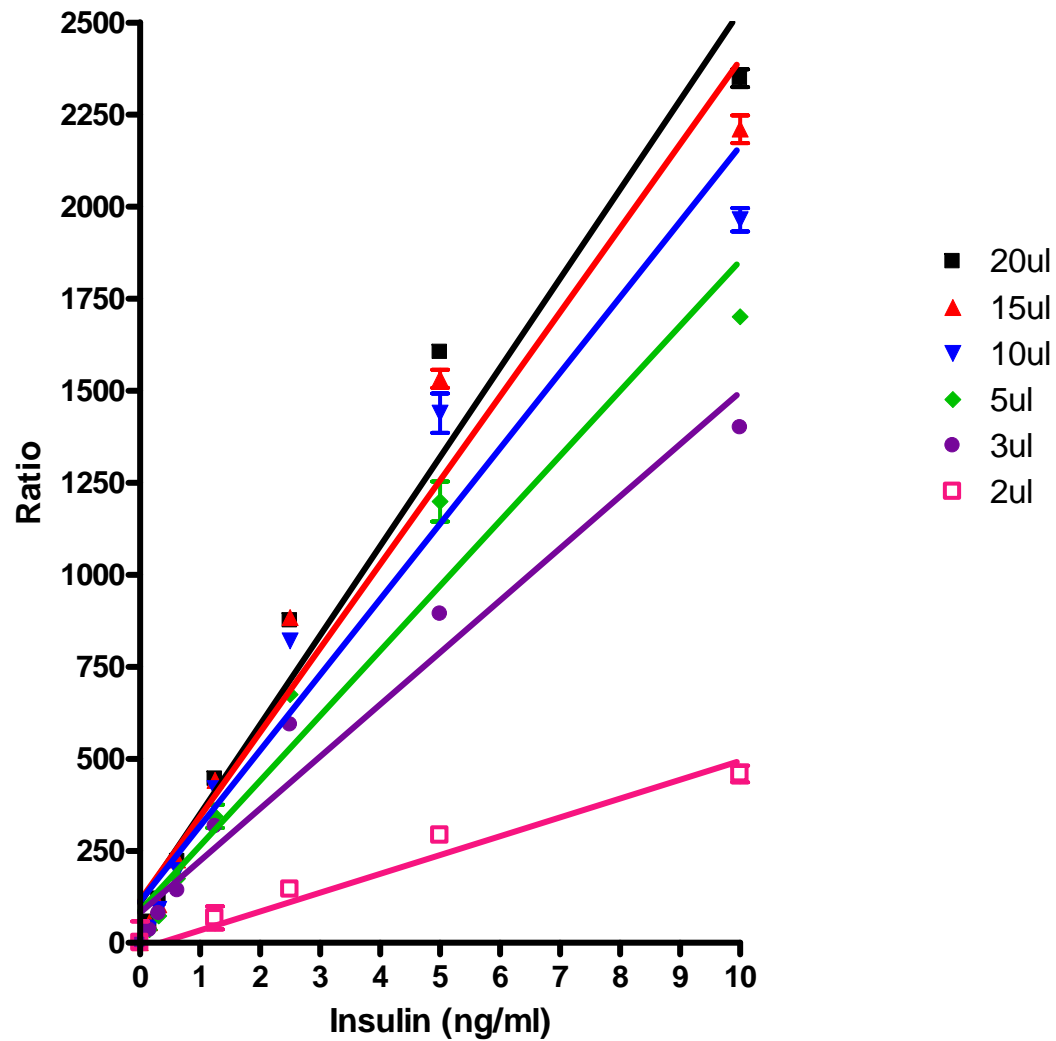


HTRF insulin standard curve



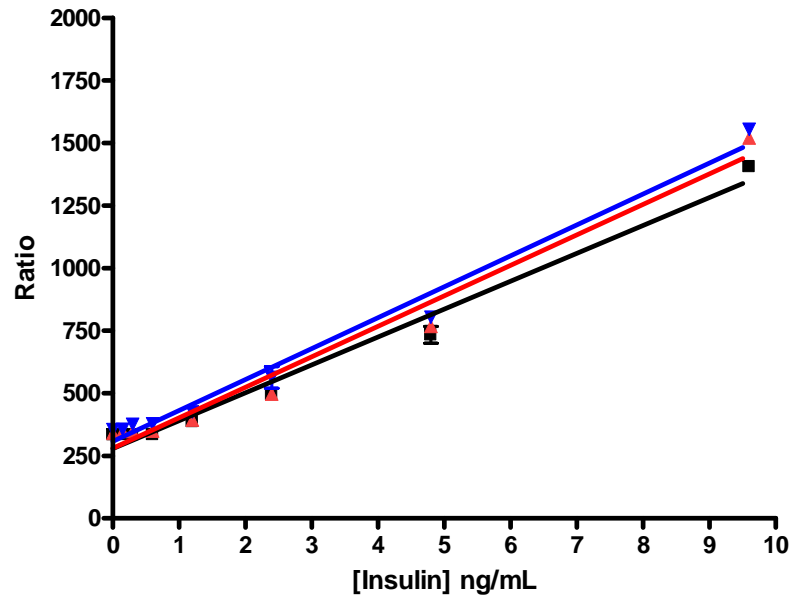
Miniaturization assessment

Insulin standard curves
Scaling assay volume

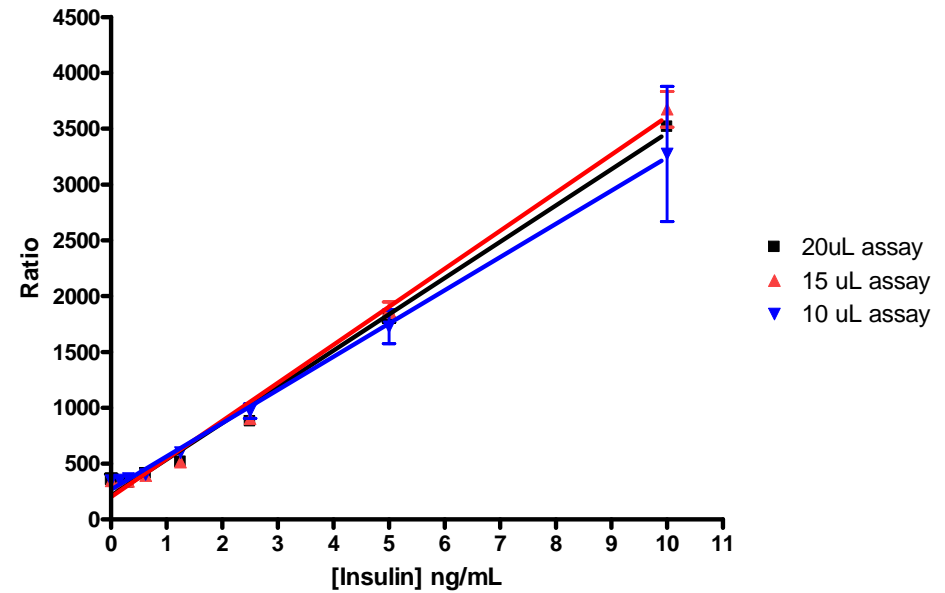


Rodent and Human insulin standard curves

Rat/mouse insulin standard curve



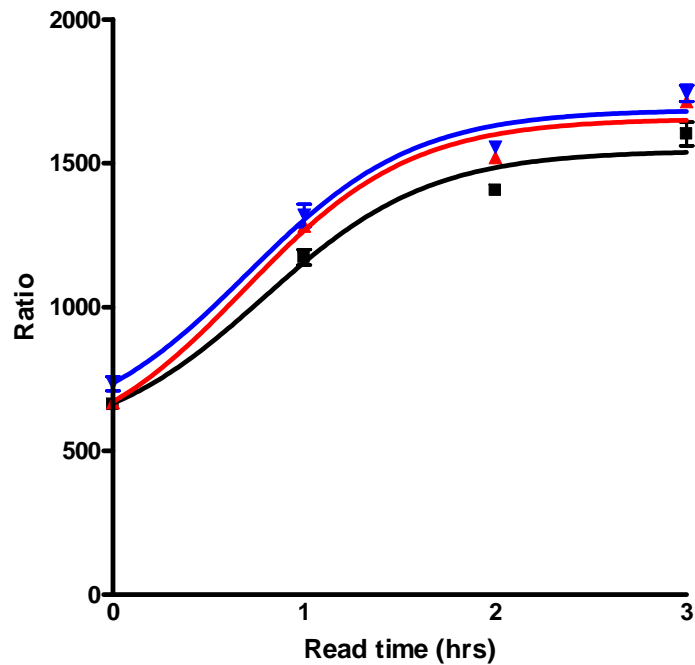
Human insulin standard curve



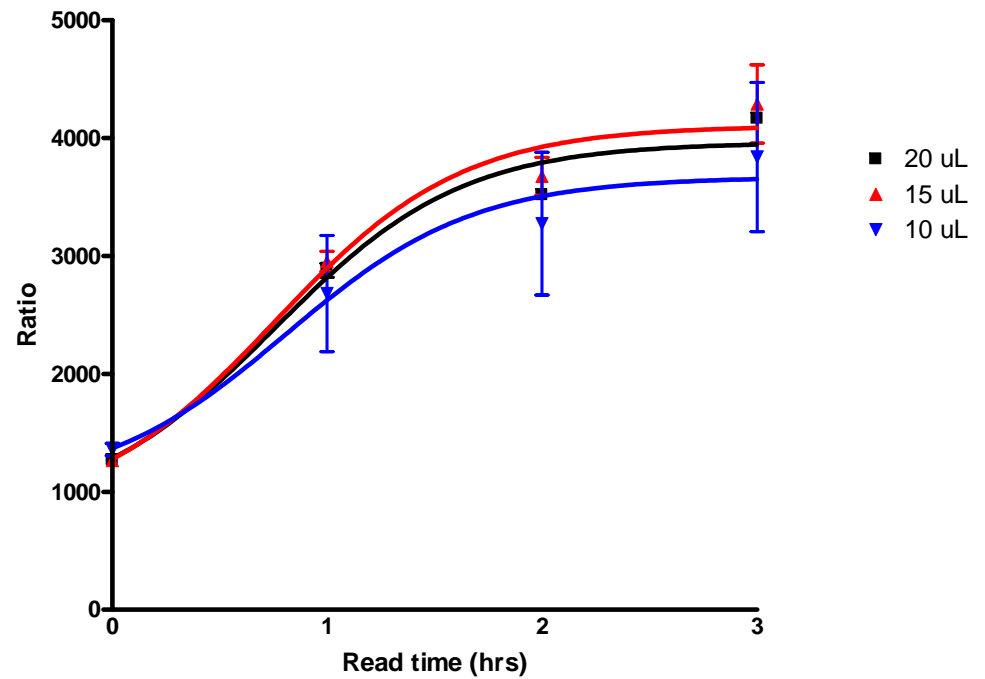
- 20uL assay
- ▲ 15 uL assay
- ▼ 10 uL assay

Time course in variable volume assay

Rat / mouse insulin HTRF assay
10 ng/ml insulin

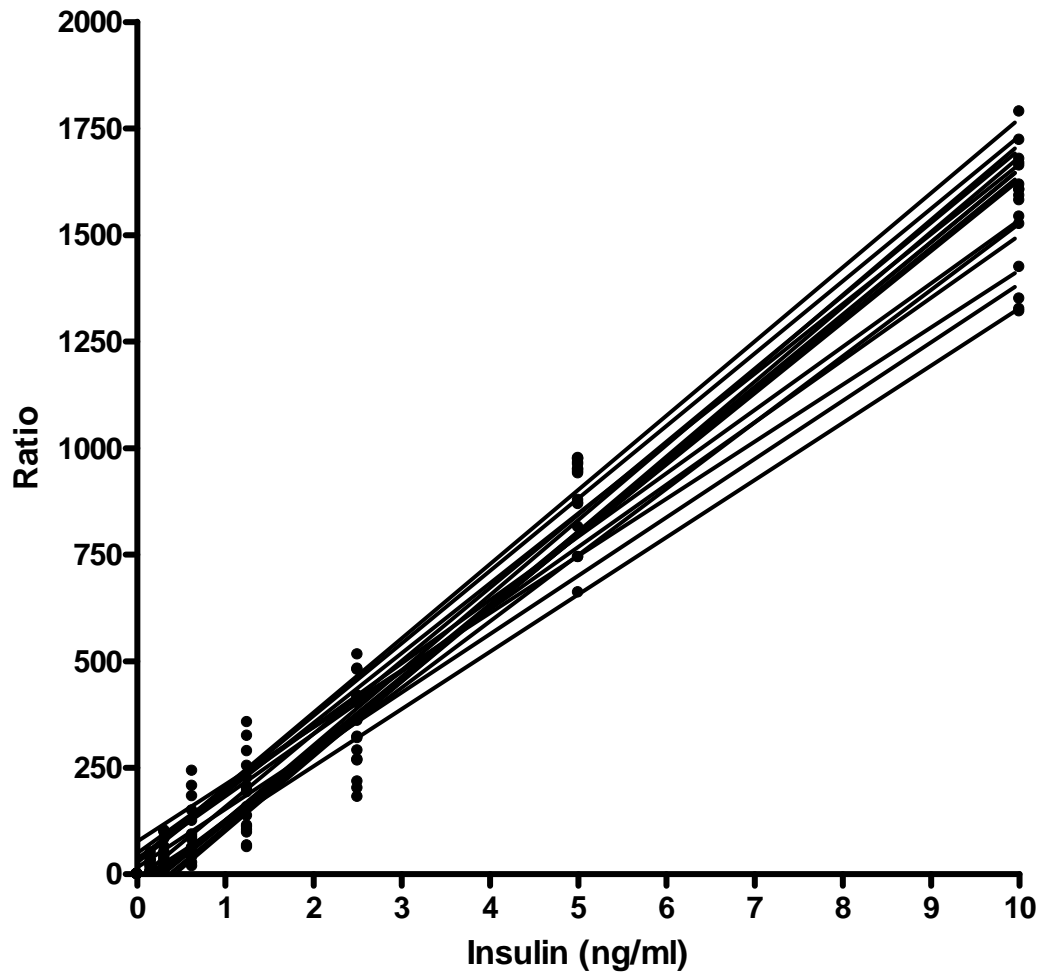


Human insulin HTRF Assay
10 ng/ml insulin



Replicate standard curves

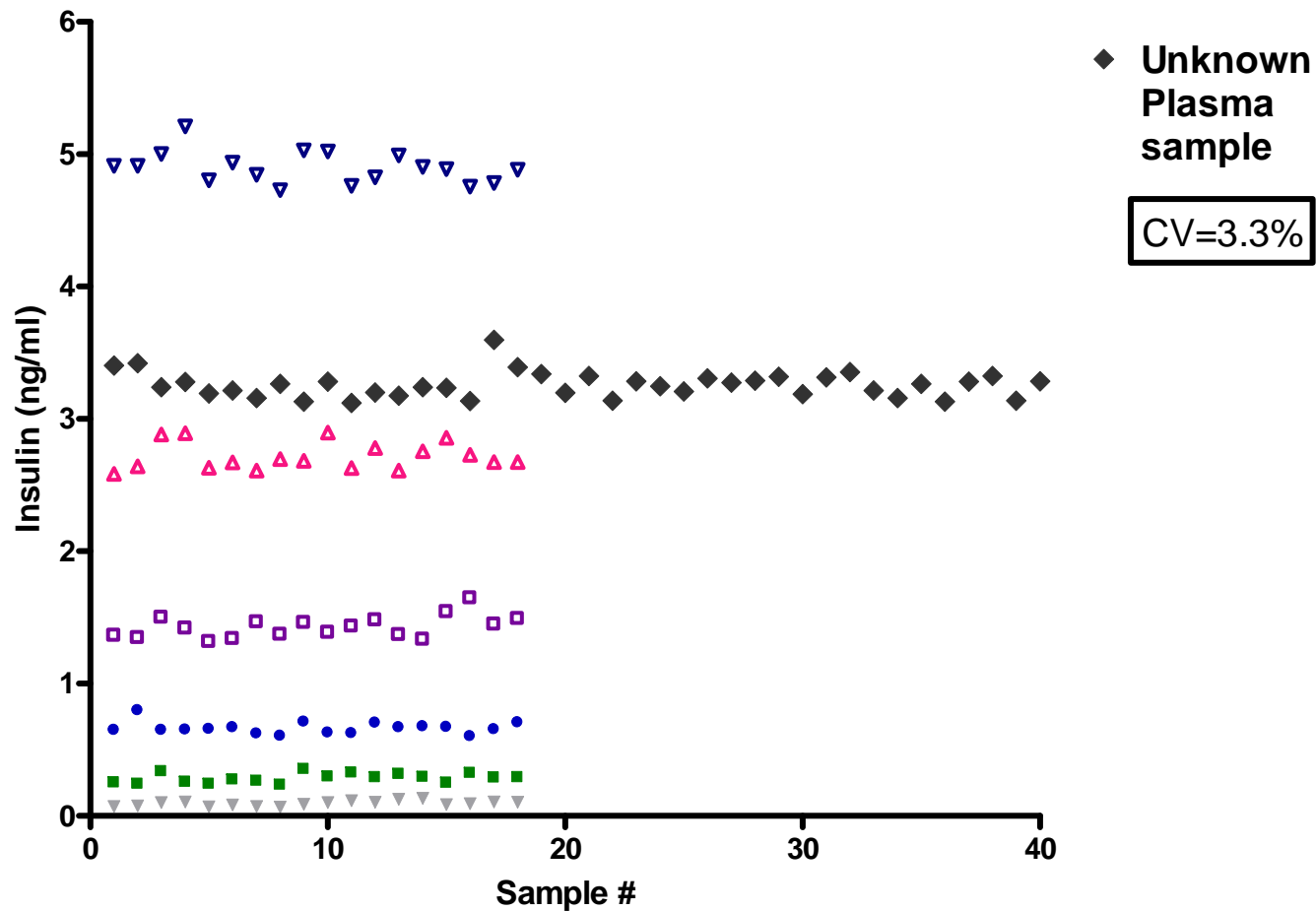
Rat / mouse insulin standard curves



Insulin standard concentration (ng/ml)	CV (%)
0	4.4
0.15625	3.6
0.3125	4.7
0.625	4.4
1.25	4.7
2.5	2.8
5	1.7
10	1.8

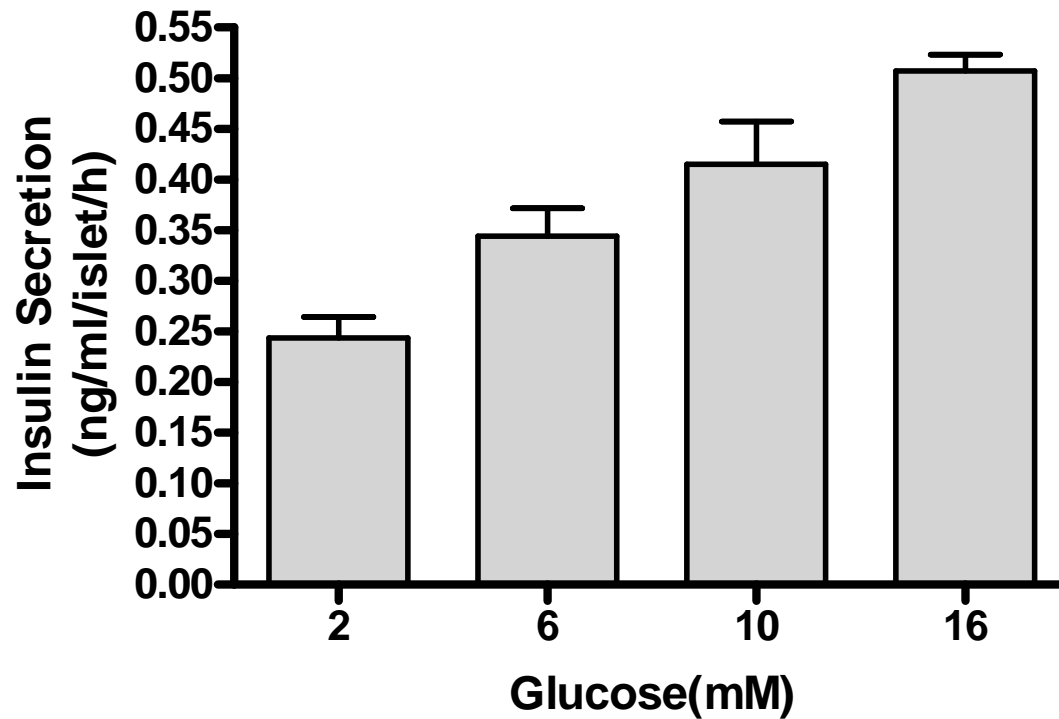
Variability testing

Variability testing on unknown plasma sample

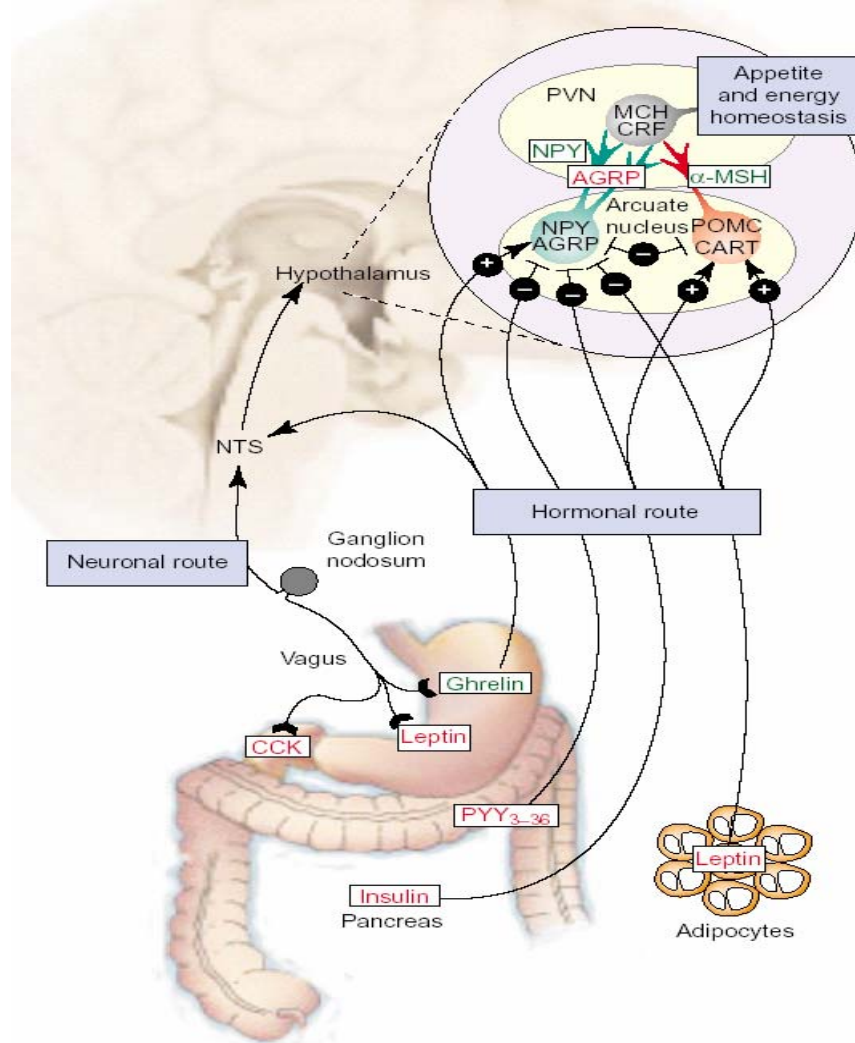


Insulin measurement in pancreatic islets

Glucose Dose Response of Insulin Secretion in Pancreatic Islets isolated from mice (n=3)



Ghrelin is a key metabolic regulator



- Peptide hormone
- Secreted from stomach
- Interacts with receptors in the brain and periphery
- Controls and integrates a variety of metabolic functions
- Part of an intricate neuroendocrine system

Ghrelin signaling as a validated target in metabolic disease

- Ghrelin KO and ghrelin receptor KO mice resist diet-induced obesity (DIO)
- KO mice resist decline in metabolic parameters when placed on a high fat diet
- Block of ghrelin inhibits body wt gain, food intake and fat mass content in rodents
- Vaccination against ghrelin causes lack of weight gain and increased relative fat free mass in rodents
- A small molecule ghrelin antagonist inhibits body weight gain and insulin levels in DIO mice

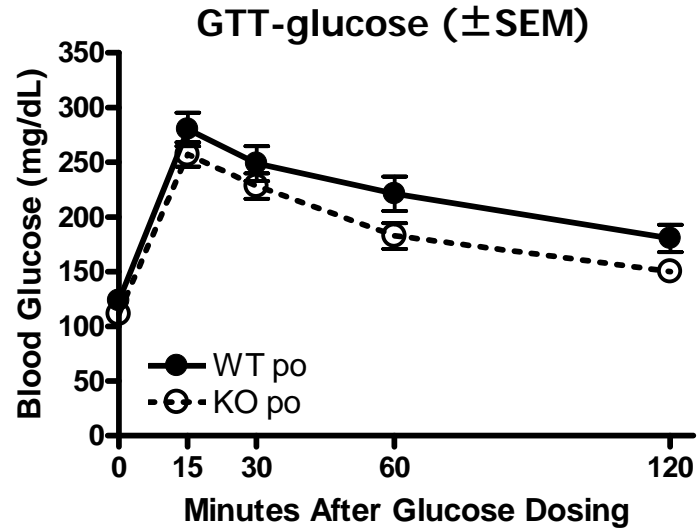
Favorable metabolic profile in ghrelin receptor KO mice

Parameter	<i>GhrR</i> +/+	<i>GhrR</i> -/-	<i>p</i> value
Body Weight (g)	46.2 ± .8	38.9 ± 1.3	<0.0001
Glucose (mg/dl)	118.6 ± 4.7	98.6 ± 4.3	<0.01
Insulin (ng/ml)	.97 ± .10	.53 ± .05	<0.001
HOMA-IR	7.38 ± .92	3.34 ± .43	<0.001
CLAMP (GIR, mg/Kg/min)	26.7 ± 2.4	37.3 ± 3.6	<0.05
% HbA1c	4.20 ± .10	3.93 ± .08	<0.05
TG (mg/dl)	101.1 ± 3.7	103.2 ± 5.0	n.s.
TC/HDL-C	1.22 ± .03	1.14 ± .02	<0.05

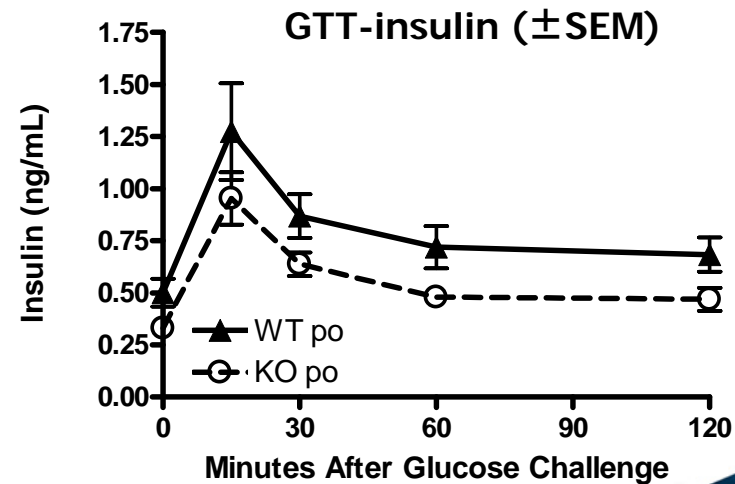
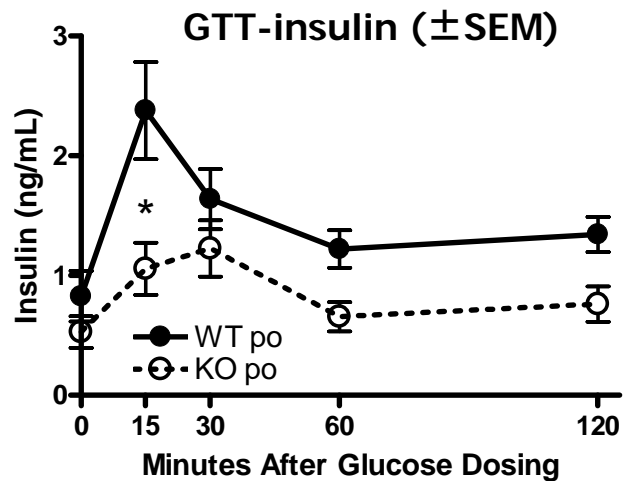
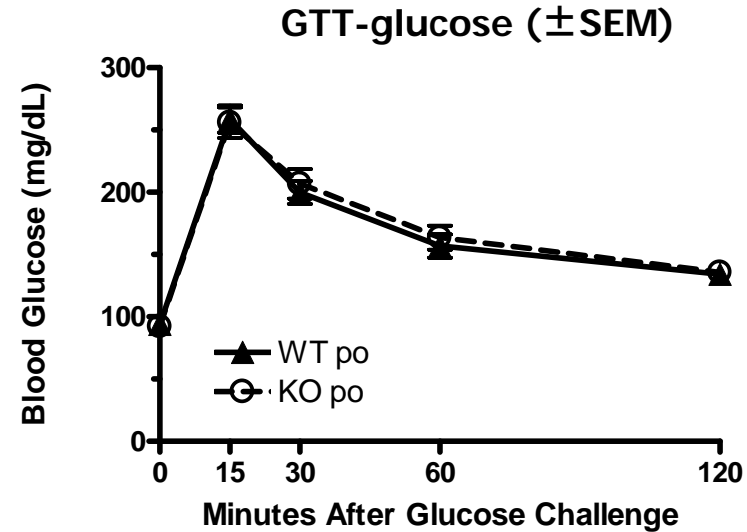
Improved insulin sensitivity

Dramatic improvement in insulin sensitivity in both male and female DIO GhrR KO mice

Male DIO GhrR KO vs WT mice

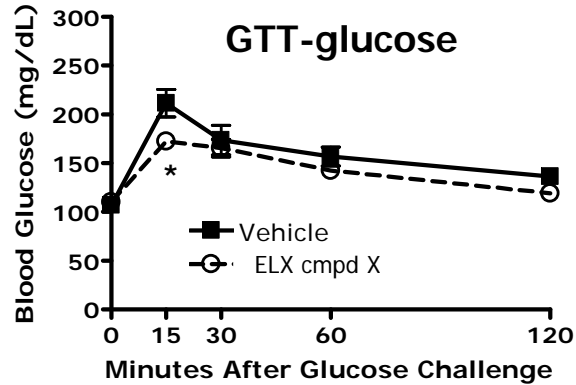


Female DIO GhrR KO vs WT mice

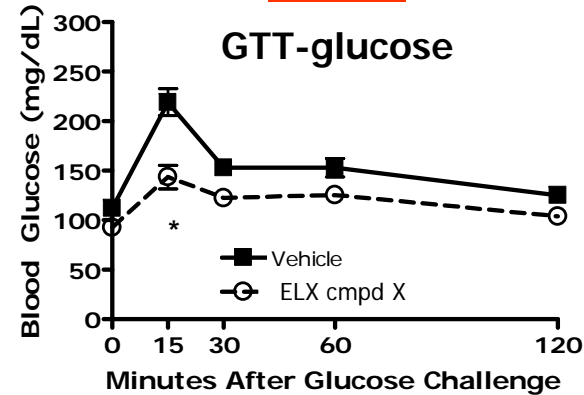


GhrR antagonism recapitulates the insulin sensitivity of HFD fed GhrR KO mice

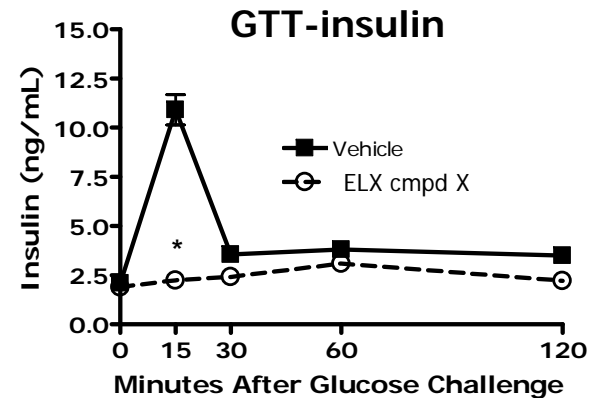
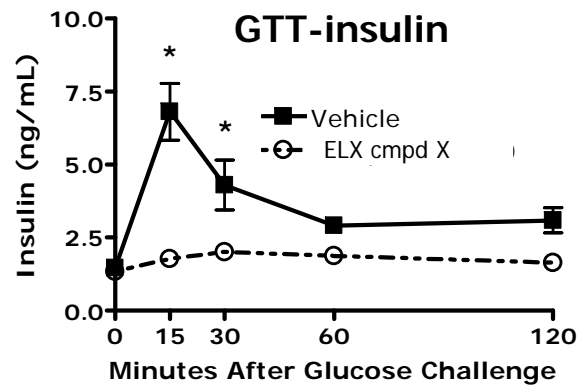
DAY 28



DAY 56



Decreased plasma glucose excursion



Dramatically decreased insulin requirement

Conclusion

- The HTRF insulin assay allows:
 - Seamless migration from ELISA to HTRF format
 - Extremely cost effective assay compared to ELISA
 - ~4% of the cost
 - Comparable sample requirements
 - Time savings over the ELISA
 - >2 hours savings
 - Assay volumes scalable to screening levels
 - Easy measurement of insulin levels across multiple systems
 - *In vivo*: rodent and human plasma or serum samples
 - *In vitro*: pancreatic β cells

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