Greeting
Peace be upon you

Effect of Perinatal Low Protein Diet on the Expression of P-Glycoprotein and Organic Cation Transporter Novel-Type-2 in the Heart

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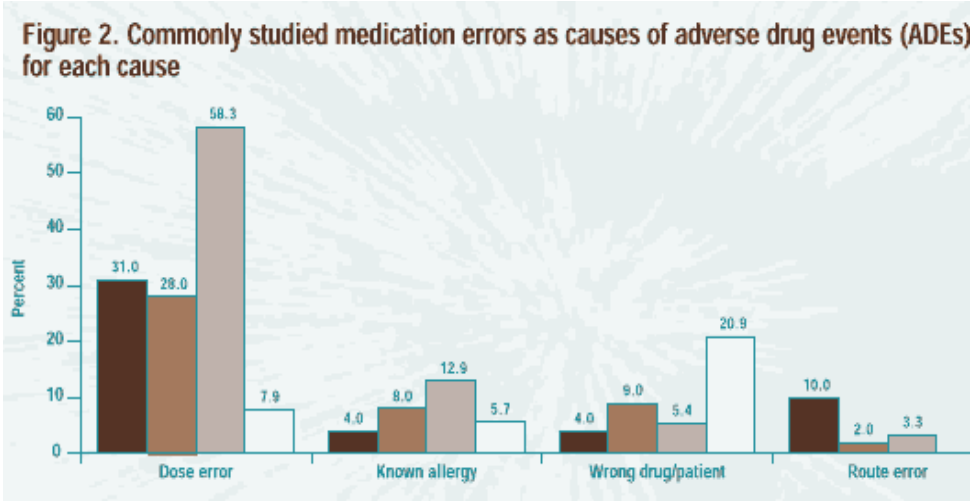
Significance of Optimizing Drug Dosage

- ~80% variability in drug response resides in dose-concentration relationships
- How much of a drug should be used and how often?
- Prevention of adverse drug reactions & more effective drug regimens
- May need to factor birth-weight into drug dosing decisions for optimal dose



Significance of Low Birth-Weight

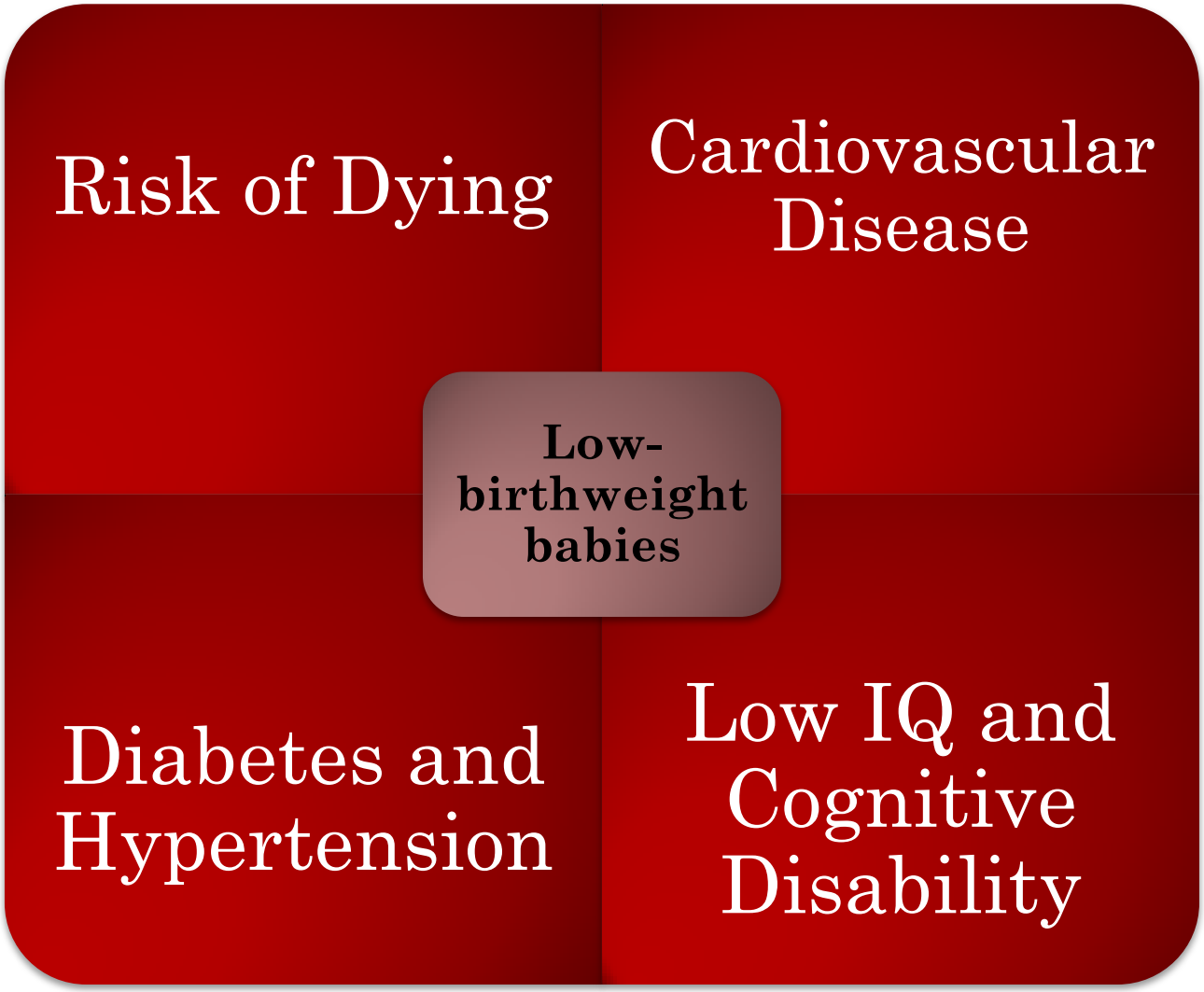
- ~1 in every 12 babies (8.33%) in the United States is born full-term with low birth weight (LBW)
- Global incidence of LBW is ~17%

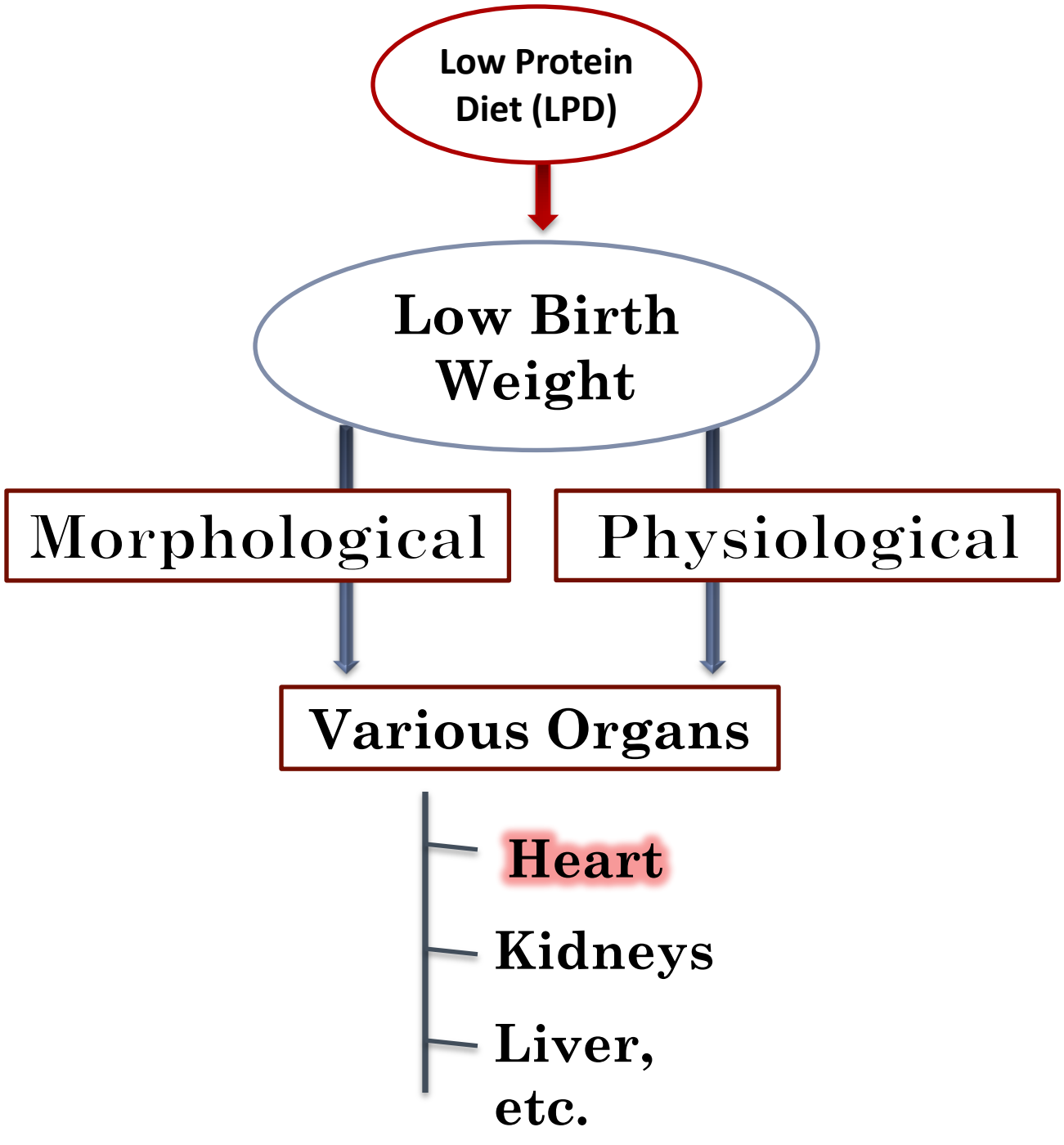
Overview

- Fetal Programming
- Poor conditions in the womb
- Low Birth Weight (LBW)

Developmental Origins of Health and Disease

- Perinatal malnutrition
  - Malnourished fetuses make functional, structural, or morphological alterations to organs
  - Cardiovascular disease or diabetes originate from such adaptations
- Strong link between low birth weight and adult onset of metabolic syndrome diseases

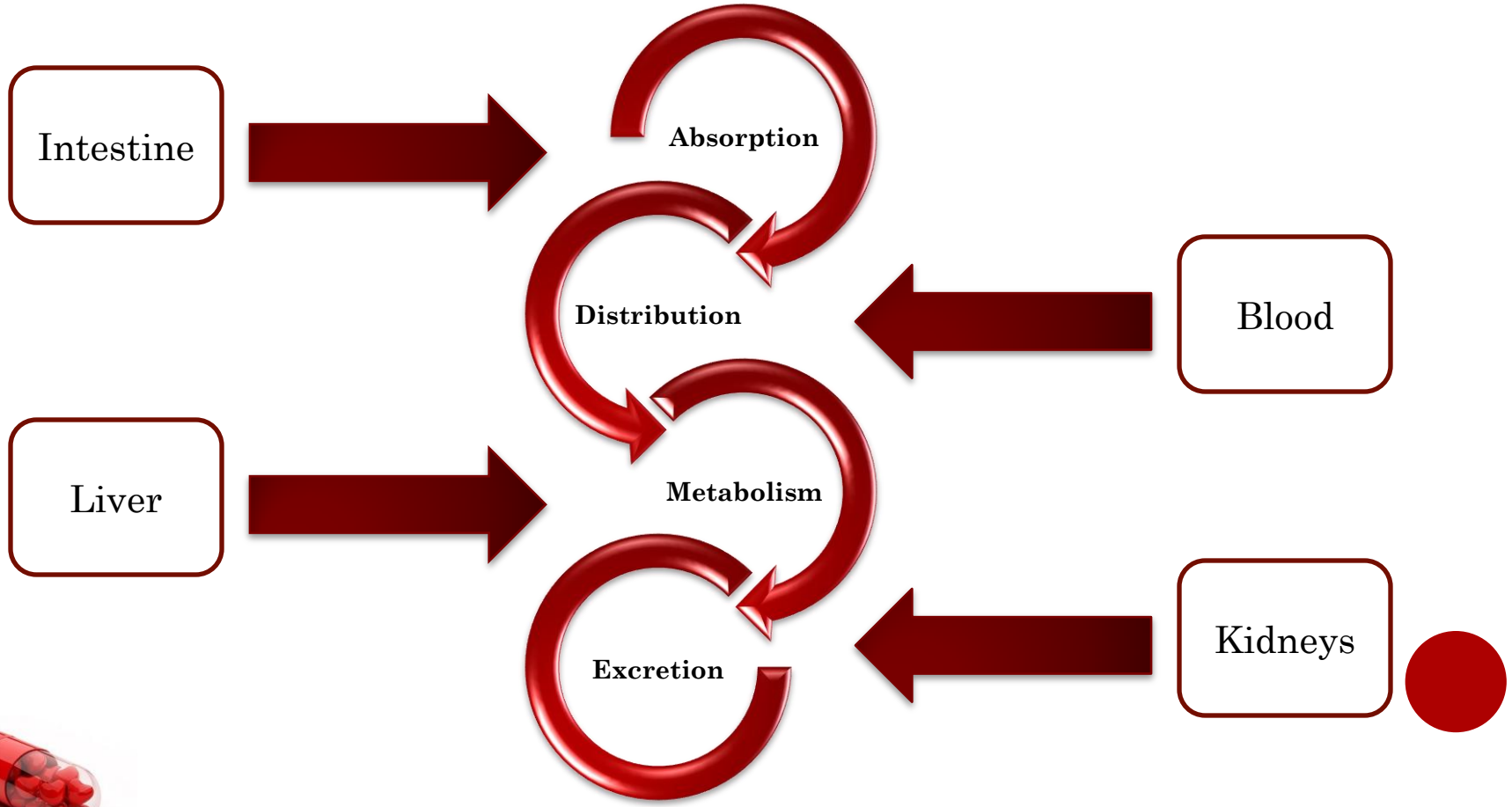

Risk of Dying
Cardiovascular Disease
Low-birthweight babies
Diabetes and Hypertension
Low IQ and Cognitive Disability


Low Protein Diet (LPD)
Low Birth Weight
Morphological
Physiological
Various Organs
Heart
Kidneys
Liver,
etc.

Drug Transport & Pharmacokinetics

Pharmacokinetics (PK)

Study of how drug travels through the body



Study Focus

Heart

- Major organ, maintains blood supply to the entire body
- Drug transportation in cardiomyocytes → optimizing treatment

P-gp

- ATP-Binding Cassette (ABC) transporter proteins
- In the heart, it effluxes drugs out of cardiac myocytes →minimizing cardiotoxicity

Octn2

- Organic Cation Transporter protein-family, serves as Na+ coupled carnitine transporter
- Deficit expression of OCTN2 → cardiomyopathy → cardiotoxicity

Drugs Transported

P-gp

- Quinidine
- Doxorubicin
- Vinblastine
- Digoxin

Octn2

- Spironolactone
- Mildronate
- Verapamil

Hypothesis

- Maternal exposure to low protein diet during gestation and lactation will alter the expression of cardiac drug transporters

Specific Aim

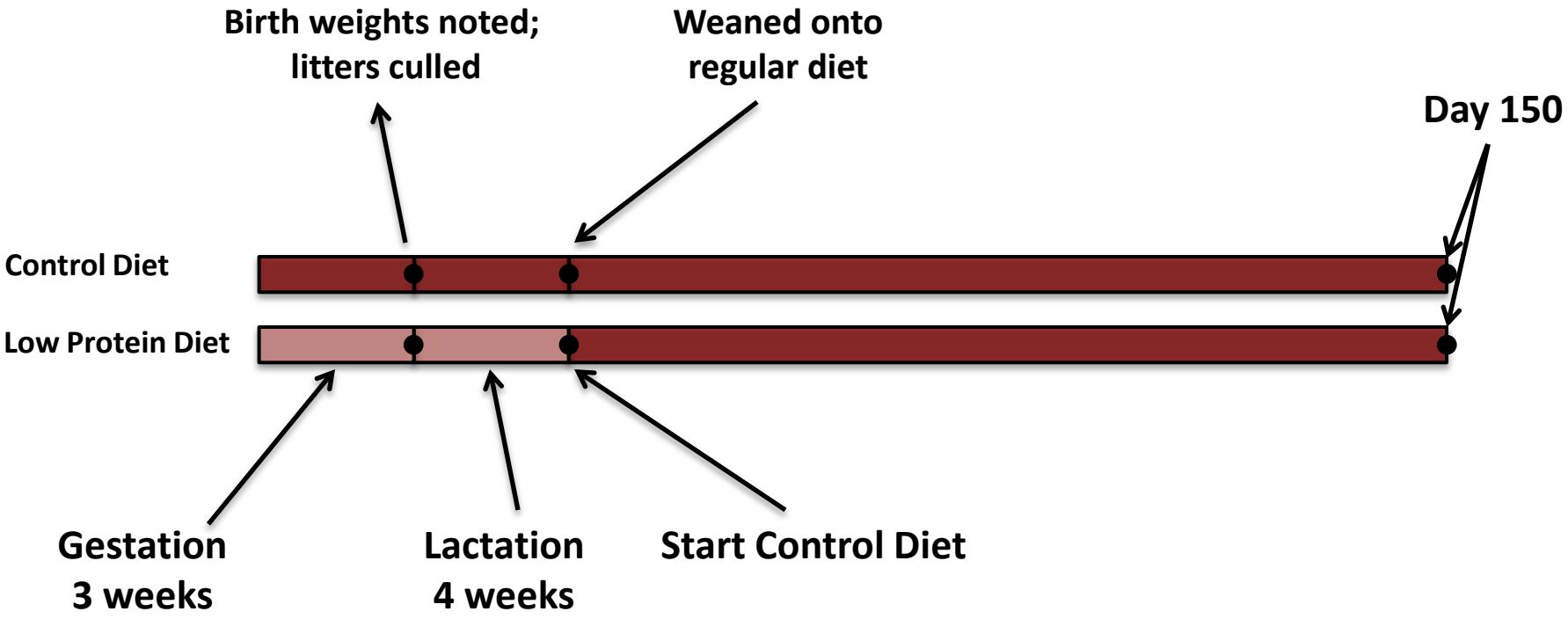
- To examine the effect of maternal low protein diet during gestation and lactation on the expression of cardiac P-gp and Octn2 in offspring

Study Design

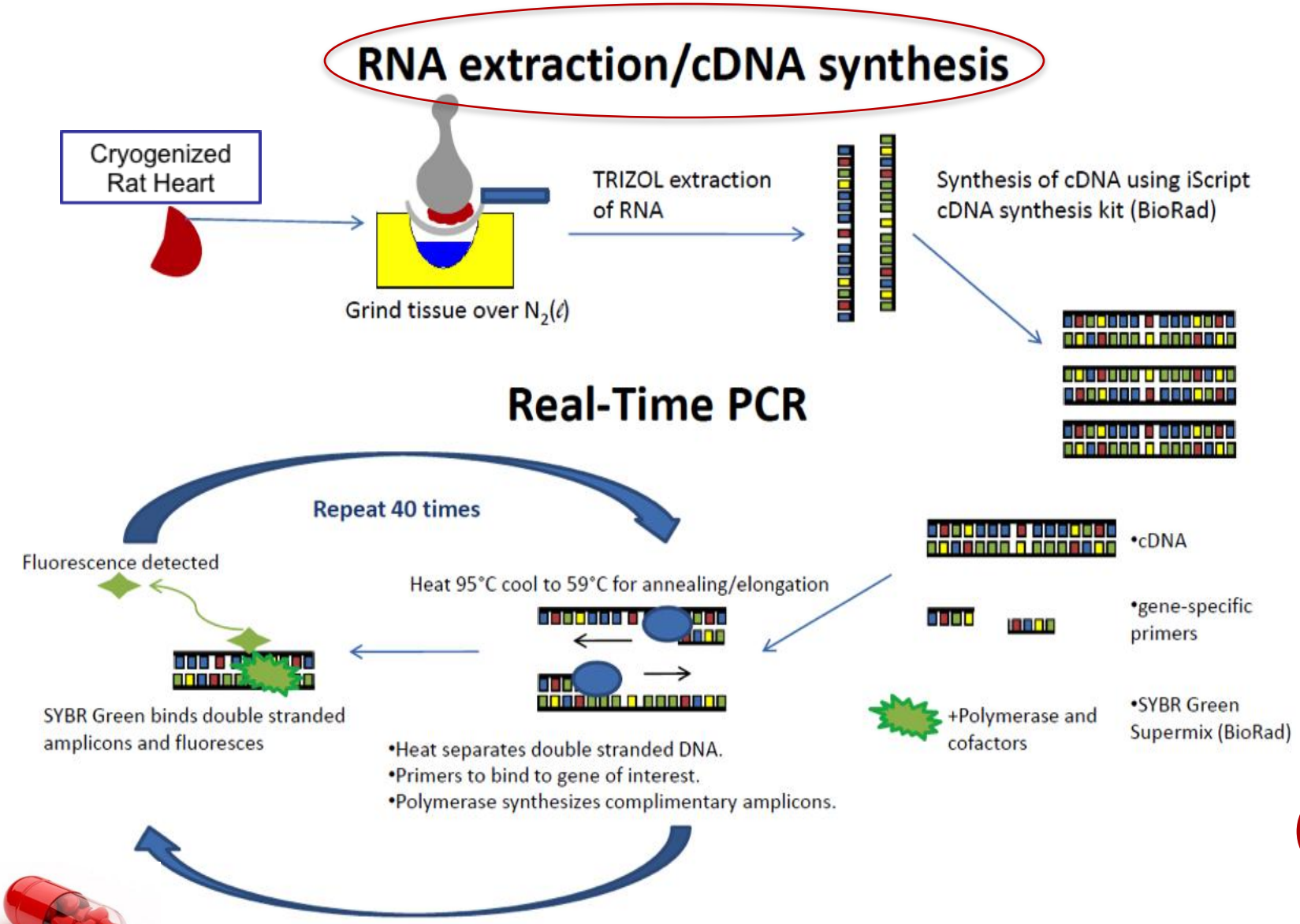
Animal model

- Maternal low protein diet (LPD)
  - Rat animal model
- Treatments
  - Control – fed laboratory chow (18% protein)
  - Low Protein – fed modified diet (8% protein)

Study Design



Study Design



Purity and Concentration of RNA/DNA Products Using Nanodrop

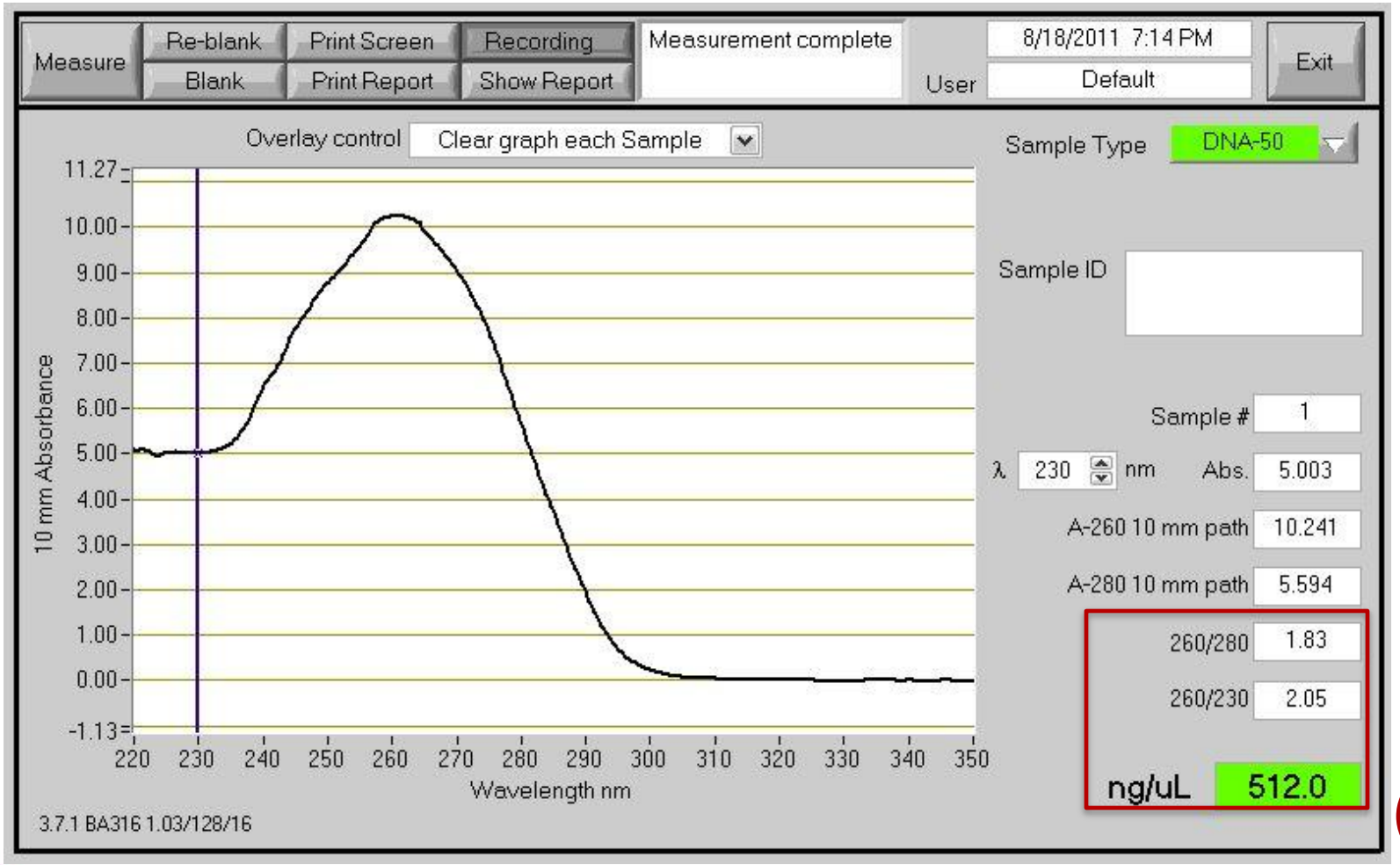


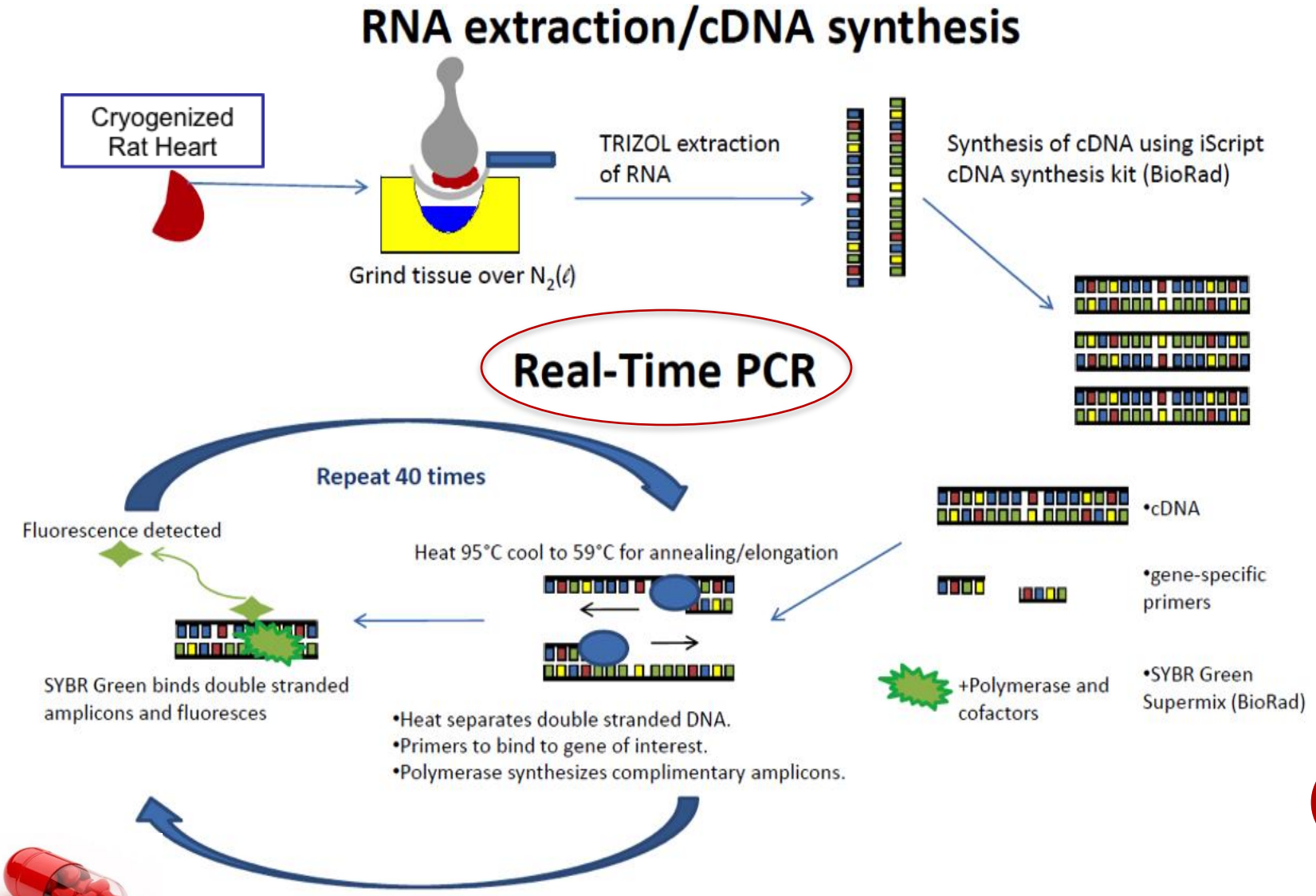
Table 1. RNA mean concentrations in (ng/μL), and RNA absorbance measurement data summary for 260/230 ratios, and 260/280 ratios.

	260/230 (Mean±S.D.)	260/280 (Mean±S.D.)	Conc.(ng/μL) (Mean±S.D.)
LPD male	1.77±0.17	2.18±0.10	316.06±163.16
Control male	2.03±0.23	2.16±0.10	329.07±165.54
LPD female	2.09±0.28	2.13±0.09	522.84±218.60
Control female	1.86±0.22	2.17±0.11	432.88±137.61

Table 2. cDNA mean concentrations in (ng/µL), and DNA absorbance measurement data summary for 260/280 ratios.

	260/280 (Mean±S.D.)	Conc.(ng/µL) (Mean±S.D.)
LPD male	1.82±0.01	512.98±8.60
Control male	1.82±0.01	512.28±10.31
LPD female	1.82±0.01	514.86±8.80
Control female	1.82±0.01	512.85±15.70

Study Design



PCR Plate Layout

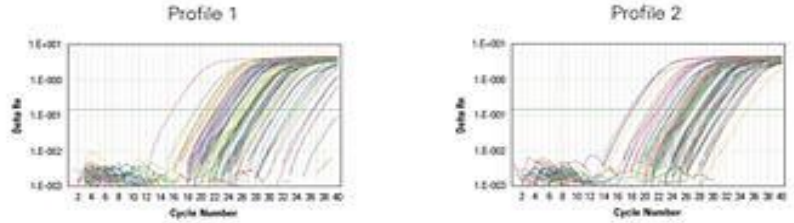
- Samples from both LP and control groups:
  - Day 150 males
  - Day 150 females
  - ß-actin
- Positive control
  - Same sample used on all plates
- Negative Control
  - Nuclease-free H2O

Study Design

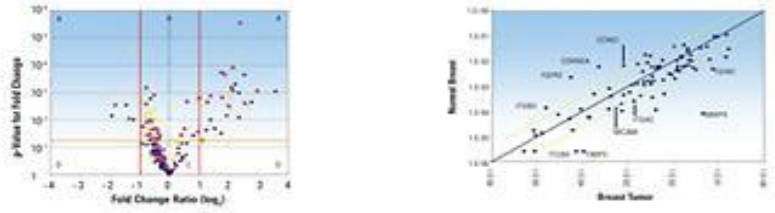
1. Convert Total RNA to cDNA.

2. Add cDNA to RT^2 qPCR Master Mix & Aliquot Mixture Across PCR Array.

3. Run in Your Real-Time PCR Instrument.

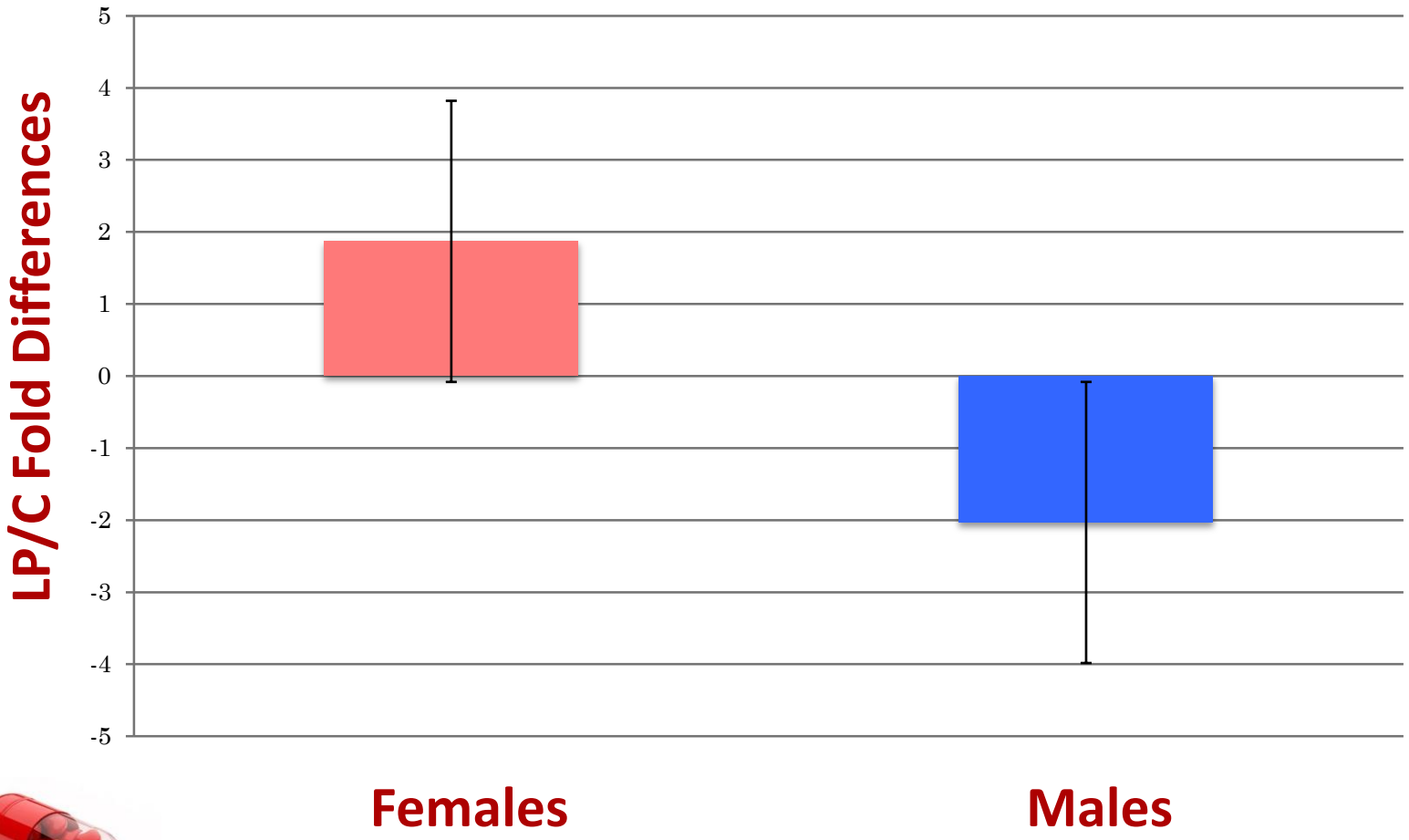


4. Data Analysis.

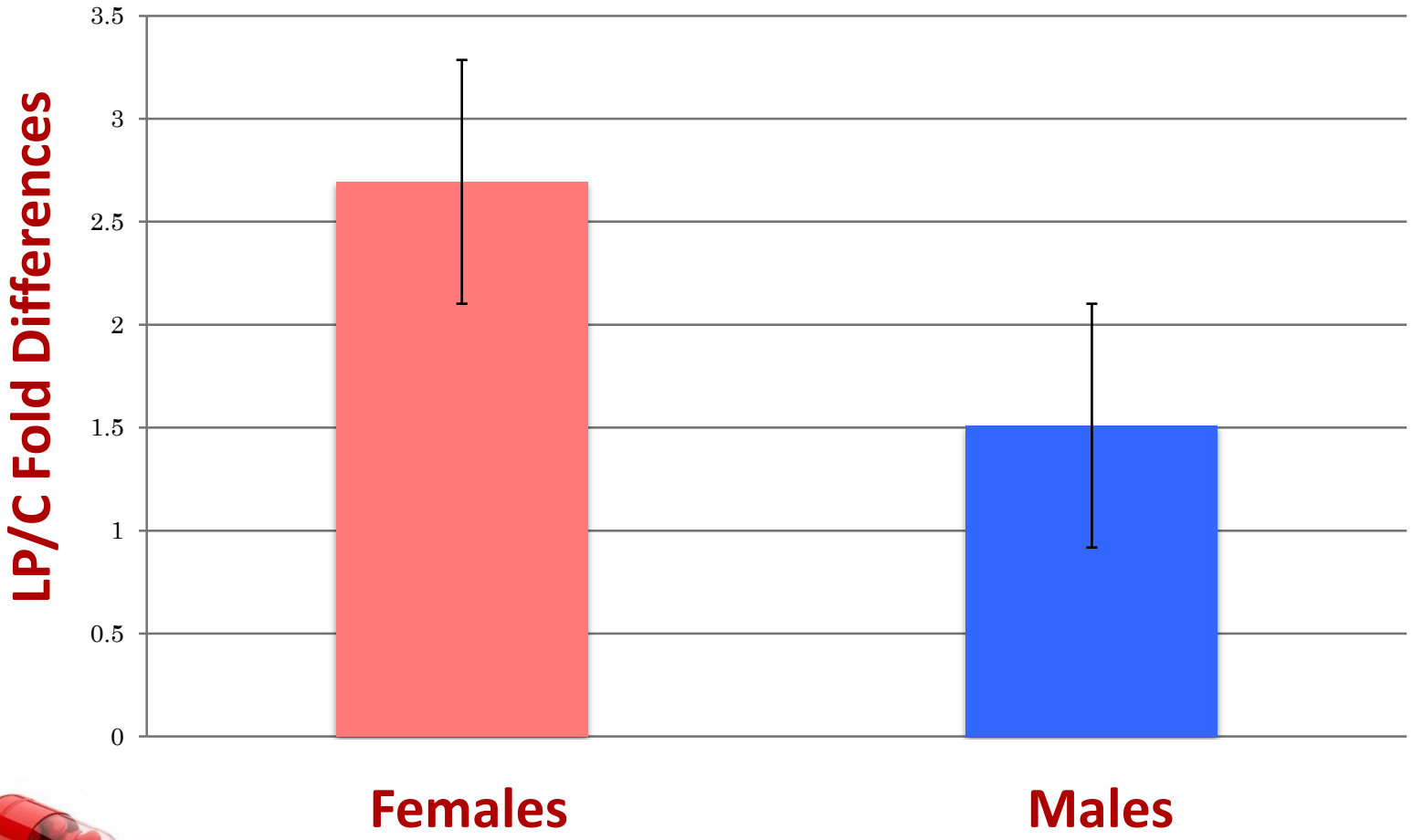


RT-PCR RESULTS

P-GP MRNA DIFFERENCES



OCTN2 mRNA DIFFERENCES



DISCUSSION

Conclusions

P-gp Expression

- ~2 fold increase in Day 150 LP/Control females
- ~2 fold decrease in Day 150 LP/Control males

Alterations in P-gp Expression Resulting in Gender-Dependent Outcomes

- Females eliminate drug quicker → lower drug exposure in cardiac tissue → sub-optimal therapeutic outcomes
- Males accumulate drugs → cardiotoxicity

Conclusions

Octn2 Expression

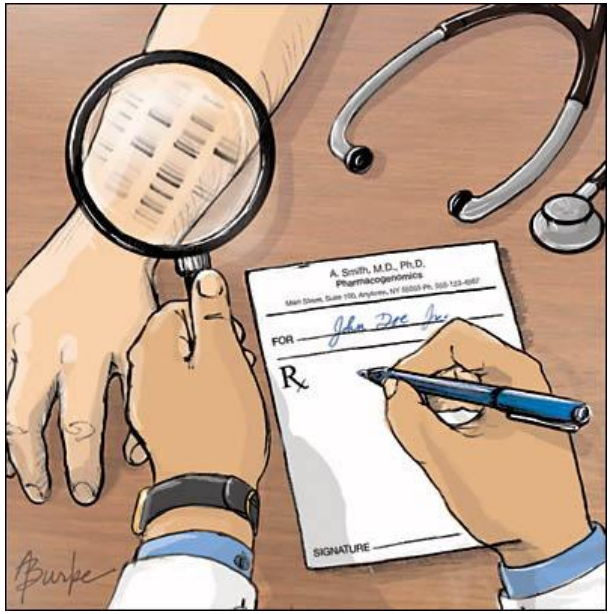
- ~2.5 fold increase in Day 150 LP/Control females
- ~1.5 fold increase in Day 150 LP/Control males

Alterations in Octn2 Expression Resulting in a Similar Trend in Both Genders

- Increased transport of carnitine by Octn2 → greater utilization of fatty acids
- If true, this adaptation is advantageous for the survival of low birth weight offspring
- However, the transport of drugs via Octn2 → cardiac accumulation → cardiomyopathy

Benefits of knowing the Consequences

- Doctors and pharmacists can:
  - Optimize dosages
  - Personalize dosing regimens
  - Prevent adverse drug reactions



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Thank You

QUESTIONS?