Estradiol positively correlated to significant elevations of plasma PCSK9 in C57BL6 mice

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I. Introduction

Studies have suggested deviations in the risk of coronary artery disease (CAD) between males and females. Premenopausal woman have been found to have a 70% lower chance for CAD; however, this cardiovascular protection vanishes postmenopause[1]. This inconsistency has been partially attributed to differences in gender specific hormones, such as estrogens. Estrogens have been found to aid in prevention of atherosclerosis, in part by increasing levels of high density lipoprotein cholesterol, while decreasing levels of low-density lipoprotein cholesterol (LDLC)[2]. Beta estradiol (βE2), the major form of estrogen found in premenopausal women, has been linked to the reduction of LDLC through its regulation of the LDL receptor (LDLR)[7], the main receptor involved in LDLC clearance. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a secreted glycoprotein involved in plasma LDLC regulation through its involvement in LDLR regulation[8]. The effect of βE2 on PCSK9 transcription, expression, and secretion has not been thoroughly studied. Herein, we investigate the effects of βE2 on circulating PCSK9 levels in a mouse model. Our results suggest that βE2 increases circulating PCSK9 levels in mice.

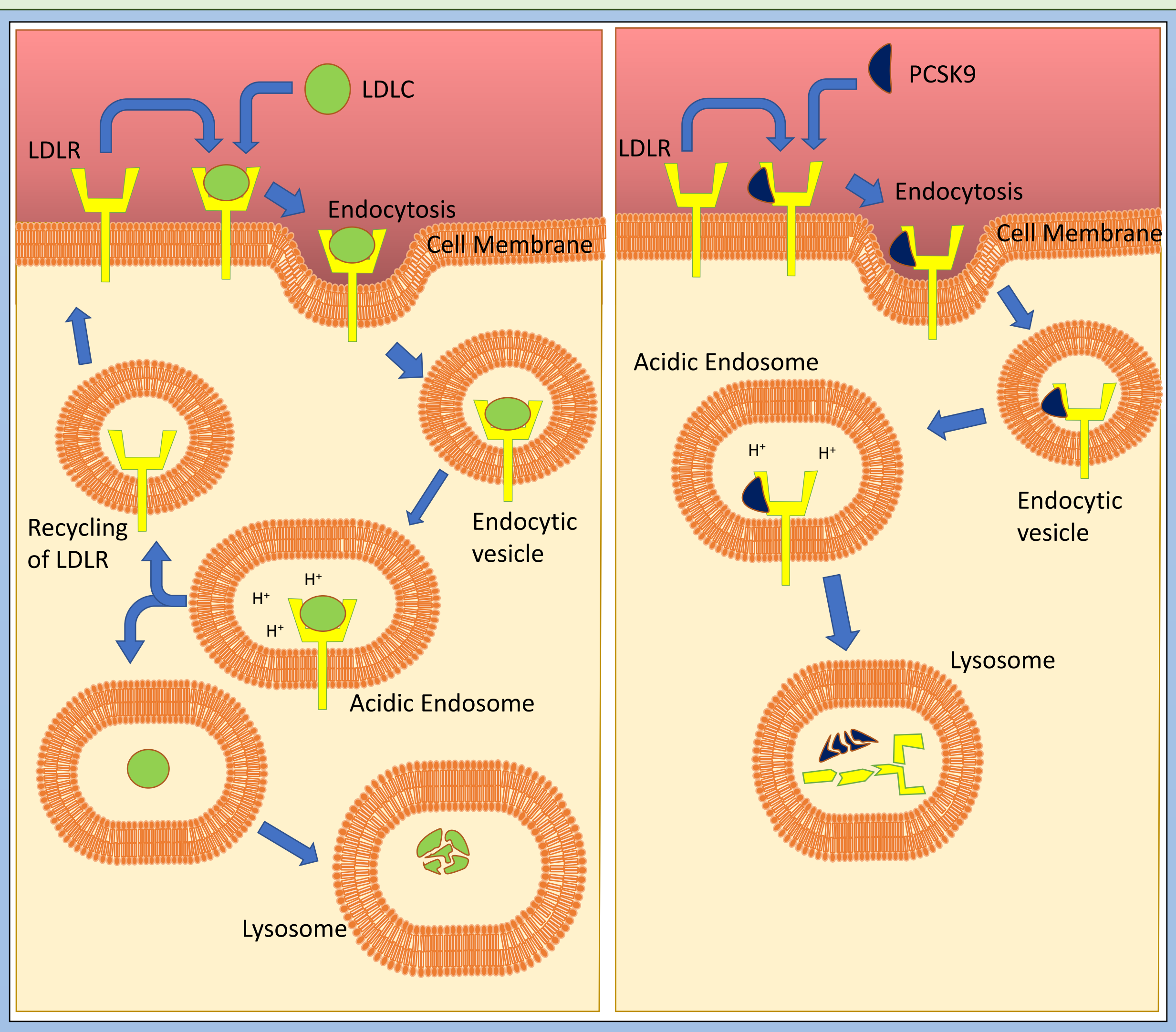


Figure 1: Hepatocyte cell. Left – Pathway for LDLC uptake and degradation by LDLR. Right – LDLR degradation with escort protein PCSK9. Exact mechanism unknown.

Polymorphism of PCSK9 has led to phenotypic expression for gain of function (GOF) PCSK9 variants, such as E32K, which raise circulating LDLC by 2.1 times[5], and loss of function (LOF) PCSK9 variants, such as Q152H, which lower circulating LDLC by 48%[6]. These contrasting polymorphisms lead to two extreme conditions of LDLC levels, hypercholesterolemia, and hypocholesterolemia. The regulation of LDLC by PCSK9 has resulted in PCSK9 antagonizing becoming a drug target in various clinical trials to manage autosomal dominant hypercholesterolemia (ADH)[4].

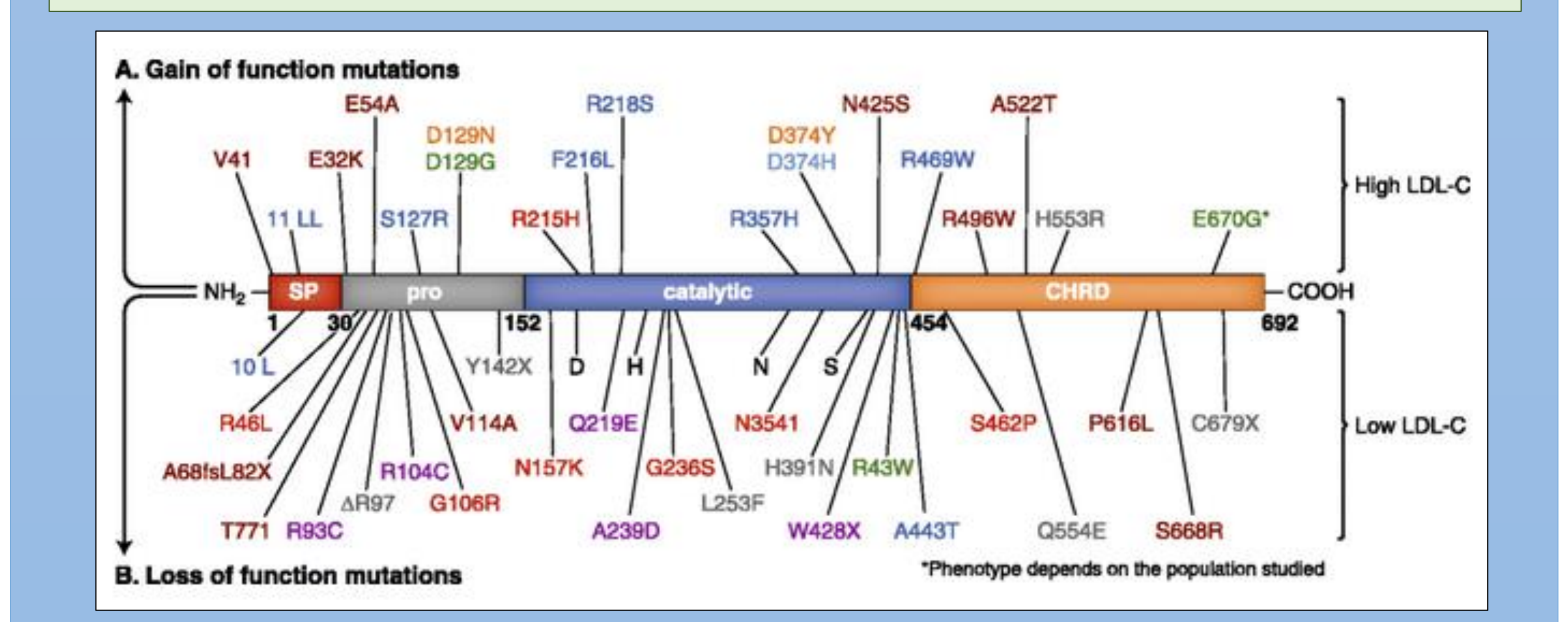
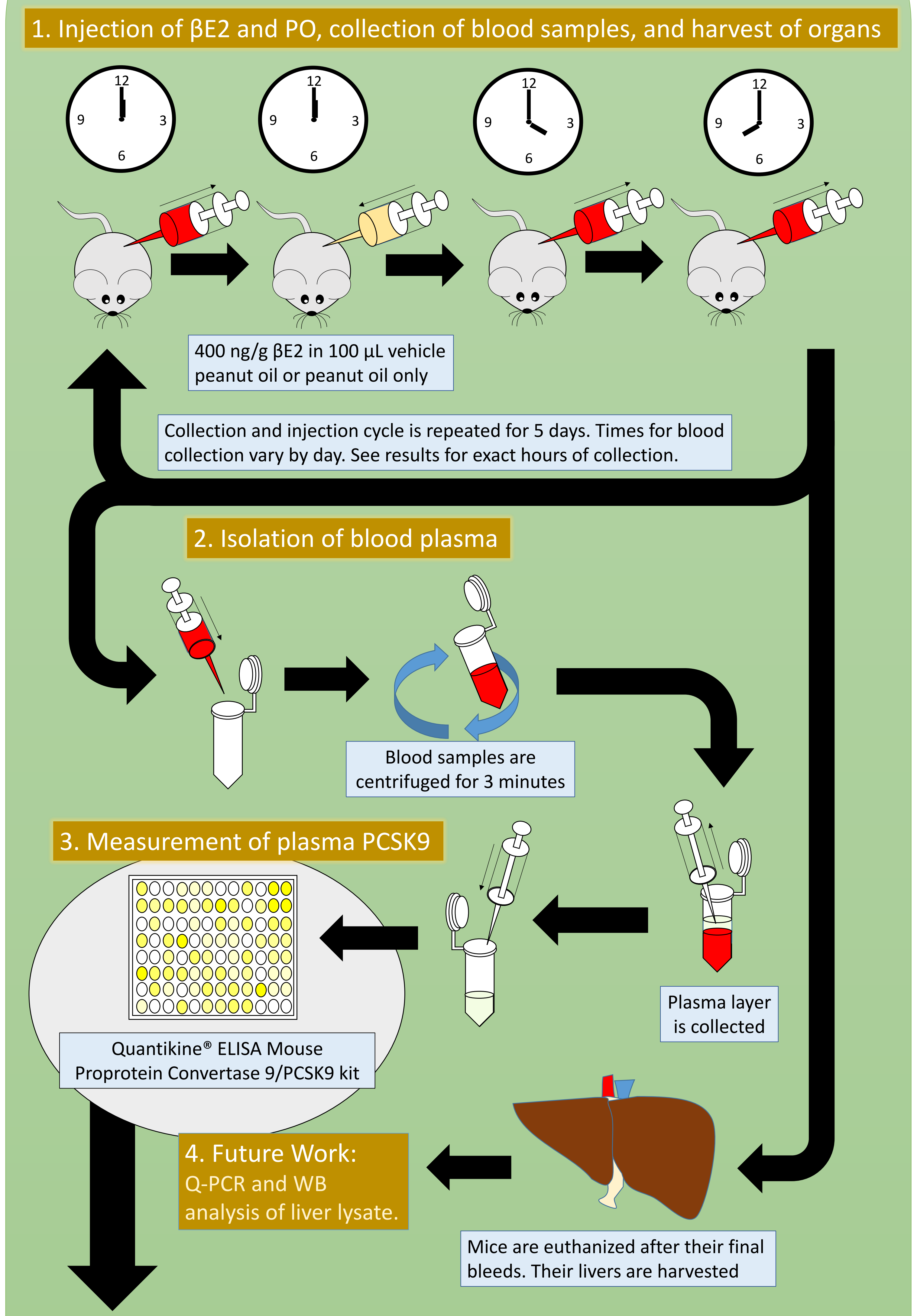


Figure 2: Various GOF and LOF mutations located on PCSK9. PCSK9 has 4 domains: signal sequence, prodomain, subtilisin-like catalytic domain, and C-terminal cysteine- and histidine-rich domain [8]. Retrieved from Davignon, J., et al. [3]

II. Objective

The goal of this research project is to: 1) determine whether there is a change in circulating levels of PCSK9 due to βE2, 2) propose a mechanism by which this change occurs, and 3) study the effect on liver LDLR levels transcriptionally by quantitative PCR (Q-PCR) and at the protein level via western blot (WB).

III. Experimental Design



1. Injection of βE2 and PO, collection of blood samples, and harvest of organs

400 ng/g βE2 in 100 µL vehicle peanut oil or peanut oil only

Collection and injection cycle is repeated for 5 days. Times for blood collection vary by day. See results for exact hours of collection.

2. Isolation of blood plasma

Blood samples are centrifuged for 3 minutes

Plasma layer is collected

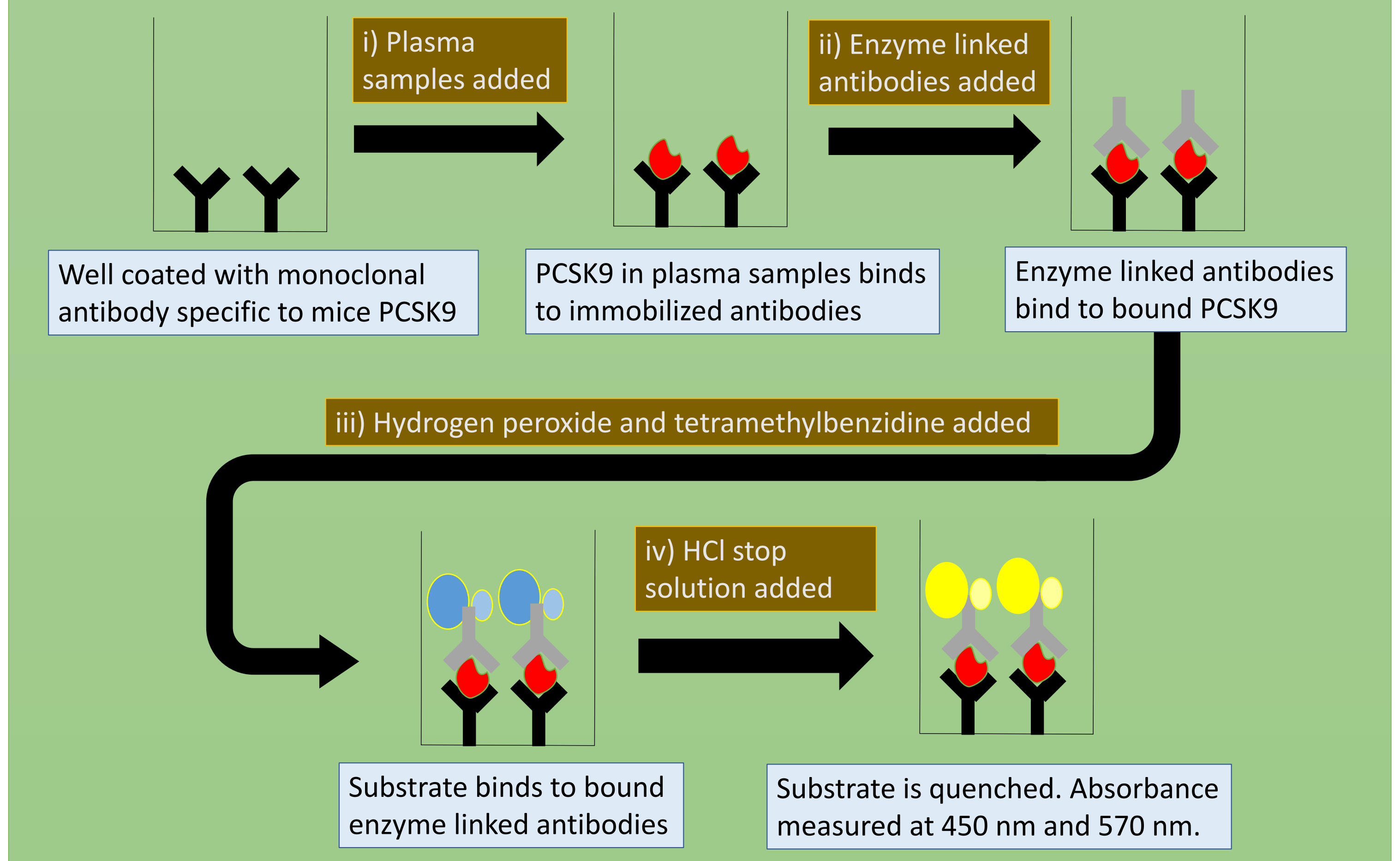
3. Measurement of plasma PCSK9

Quantikine® ELISA Mouse Proprotein Convertase 9/PCSK9 kit

4. Future Work:
Q-PCR and WB analysis of liver lysate.

Mice are euthanized after their final bleeds. Their livers are harvested

Mouse plasma PCSK9 concentrations were determined by Quantikine® ELISA Mouse Proprotein Convertase 9/PCSK9 kit



i) Plasma samples added

ii) Enzyme linked antibodies added

Well coated with monoclonal antibody specific to mice PCSK9

PCSK9 in plasma samples binds to immobilized antibodies

Enzyme linked antibodies bind to bound PCSK9

iii) Hydrogen peroxide and tetramethylbenzidine added

iv) HCl stop solution added

Substrate binds to bound enzyme linked antibodies

Substrate is quenched. Absorbance measured at 450 nm and 570 nm.

C57Bl6 mice at 7-8 weeks of age were used for hormone injection studies. Mice were treated according to the Canadian Council on Animal Care Guidelines and under protocols approved by the University of Ottawa/Ottawa Hospital Research Institute Animal Care committee.

IV. Results

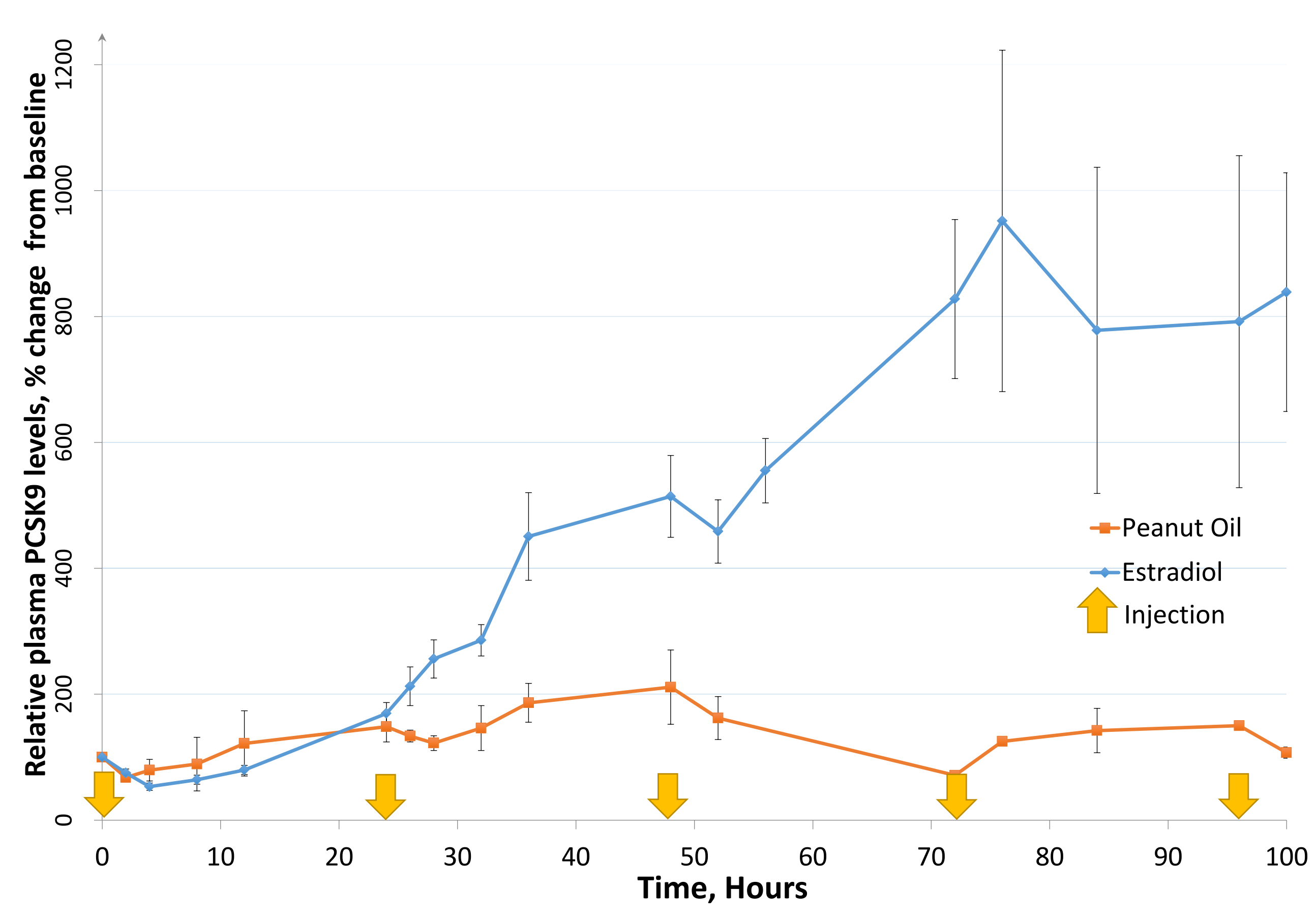


Figure 3: The levels of PCSK9 found in male C57BL6 mice at specific hours after initial injection. PCSK9 levels were calculated as a percent change from baseline blood withdrawals (pre-0h). βE2 injections were given at time 0h, 24h, 48h, 72h, and 96h. Blood withdrawals were cycled through mice so that <10% of total blood was taken from each mouse. Sample size for data points were n>3 with total sample size n=24. A complementary study was done on female C57BL6 mice; however, results are pending.

V. Discussion

βE2 was determined to significantly increase the levels of circulating PCSK9 from 28 hours onward. Future studies will determine if this up-regulation is due to:

(1) increase in PCSK9 mRNA expression,
(2) increase in secretion of PCSK9,
(3) no change in PCSK9 expression or secretion but a decrease in the clearance of circulating PCSK9 in the presence of βE2, or
(4) a combination of (1)-(3).

Future studies will also determine the effect of this up-regulation of circulating PCSK9 on liver LDLR levels. Q-PCR will be used to test mRNA levels and WB to test protein changes in liver for PCSK9 and LDLR.

VI. Acknowledgements

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Additional Questions? Feel free to reach me at Dling016@uottawa.ca