German Mouse Clinic

Clinical Chemistry in Metabolic Phenotype assessment

Birgit Rathkolb

Head Clinical Chemistry and Hematology Screen
GERMAN MOUSE CLINIC

Clinical Chemistry of serum or plasma samples:

- Standard diagnostic tool of laboratory medicine

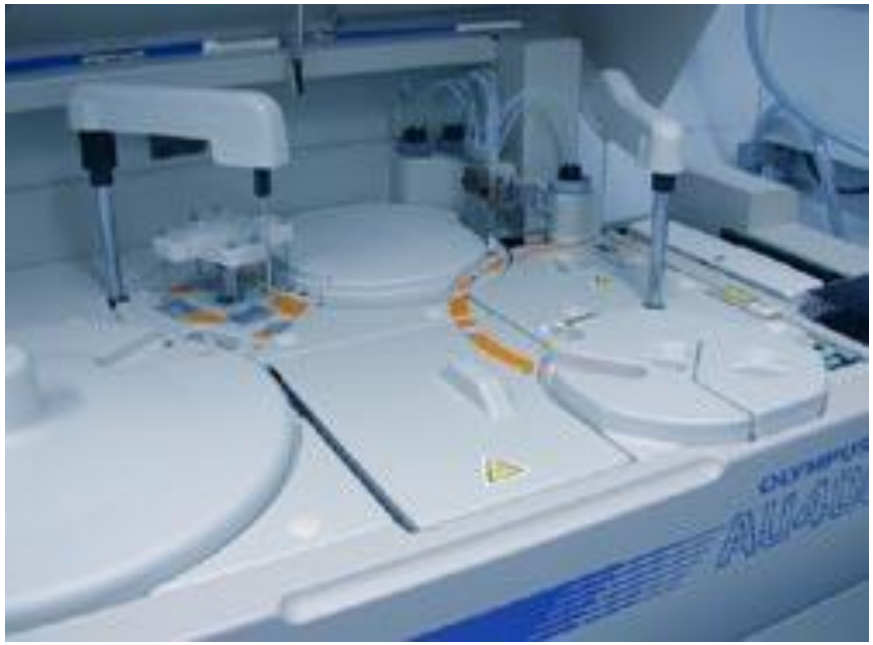
- Enables determination of a variety of clinically relevant parameters in a single sample

- Almost every parameter is influenced by a number of different factors
  >> deviations in one parameter can be due to several possible causes

- Many parameters influenced by method of sample collection (e.g. anti-coagulant used, anethesia), sample quality (e.g. hemolysis) and sample processing (e.g. storage temperature)

Olympus AU480

In the general profile we measured 6 fasting values from 80µl plasma and 18-20 parameters from 80-100 µl plasma (1:2 diluted)



Ad libitum fed mice
Proteins and plasma enzyme activities:
Albumin, Alanine-aminotransferase (ALT/GPT); Alkaline phosphatase; Alpha-amylase; Aspartate-aminotransferase (AST/GOT); Lactate Dehydrogenase (LDH); Lipase; Total protein

Plasma concentrations of specfic substrates:
Cholesterol; Creatinine; Glucose; Triglyceride; Urea, Bilirubin

Plasma concentrations of electrolytes:
Calcium; Chloride; Inorganic phosphate; iron; UIBC, Potassium; Sodium
Calculated: TIBC, Transf. saturation

Fasting values
Cholesterol, HDL-cholesterol, triglyceride, NEFA, Glycerol, Glucose;
calculated: non-HDL cholesterol

Grey: Only Bottom up Blue: Only IMPC

Clinical Chemistry of serum or plasma samples

Most specific parameters:

Creatine Kinase: Muscle damage
Lipase: Exocrine pancreas damage
Alanine Aminotransferase: Predominantly liver cells

Example for multiple influences on one parameter:

Cholesterol: Affected by
- regulation and biochemistry of lipid and steroid metabolism
- hepatopathia (lipoproteinsynthesis)
- nephropathia (proteinuria)

Aspartate Aminotransferase: Cellular damage of liver, mucle or red blood cells.

Alkaline Phosphatase: Hepatopathia (mainly cholestasis), disorders of bone metabolism

Clinical Chemistry of serum or plasma samples

Rathkolb et al. 2013,
Clinical chemistry and other laboratory tests on mouse plasma or serum,
Curr. Protoc. Mouse Biol. 3:69-100

Effects of sample quality:

Hemolysis: Effects on K, ASAT, LDH, iron, bilirubin and others
Lipemia and icterus

Effects of sample processing:

- anticoagulant: EDTA impairs measurements of Ca, Mg, iron, ALP, Amylase and others
- Time between sample collection and separation of plasma and cells: Glucose levels decrease, K levels increase with time
- Time and storage temperature of blood and plasma before analysis: NEFA and glycerol values increase with time, especially if samples are not cooled

Clinical Chemistry of serum or plasma samples

Parameter [SI units]	Test-kit [1)]	Calibrator	Control	Sample volume	Specimen	notes
Sodium [mmol/l]	ISE*	OE66313 OE66314 OE66319	OE66316 (low) OE66317 (high)	20 µl (for ISE 1-3 parameters)	Serum or heparin-plasma	Do not use Na-salts as anticoagulant.
Potassium [mmol/l]	ISE*	OE66313 OE66314 OE66319	OE66316 (low) OE66317 (high)	20 µl (for ISE 1-3 parameters)	Serum or heparin-plasma	Do not use K-salts as anticoagulant.
Chloride [mmol/l]	ISE*	OE66313O E66314 OE66319	OE66316 (low) OE66317 (high)	20 µl (for ISE 1-3 parameters)	Serum or heparin-plasma	
Calcium [mmol/l]	OSR60117 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	3,5 µl	Serum or heparin-plasma	EDTA-, Citrate-, or Oxalate-plasma cannot be used
Magnesium [mmol/l]	OSR 6189 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	1,6 µl	Serum or heparin-plasma	EDTA-, Citrate- or Oxalate-plasma cannot be used; hemolysis will falsify results.
Iron [µmol/l]	OSR6186 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	12 µl	Serum or heparin-plasma	EDTA-, Citrate-, or Oxalate-plasma cannot be used; hemolysis and lipemia falsify results
UIBC** [µmol/l]	OSR61205 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	8 µl	Serum or heparin-plasma	EDTA-, Citrate-, or Oxalate-plasma cannot be used; hemolysis will falsify results.
Inorganic Phosphorus [mmol/l]	OSR6122 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	2,5 µl	Serum or heparin-plasma	

Parameters of electrolyte, mineral and iron homeostasis

>> chloride (sodium) indicator of acid-base balance

>> iron + UIBC = TIBC: surrogate for transferrin level – can be indicator of hepatic function

Clinical Chemistry of serum or plasma samples

Parameter [SI units]	Test-kit [1]	Calibrator	Control	Sample volume	Sample preparation	notes
Cholesterol [mmol/l]	OSR6116 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	1,6 µl	Serum EDTA-or heparin-plasma	Oxalat- or citrate-plasma should be avoided.
HDL-Cholesterol [mmol/l]	OSR6187 (BC)	ODC0011	ODC0005	1,6 µl	Serum or heparin-plasma	Uses specific antibodies directed against human β-lipoprotein [2]
LDL-Cholesterol [mmol/l]	OSR6183 (BC)	ODC0012	ODC0005	1,6 µl	Serum or heparin-plasma	
Triglycerides [mmol/l]	OSR61118 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	1,6 µl	Serum, EDTA- or heparin-plasma	Oxalat- or citrate-plasma should be avoided.
NEFA* [mmol/l]	NEFA HR (Wako) REF434-91795 (HR-1) REF436-91995 (HR-2)	NEFA Standard REF91096	REF410-00102 (Control I) REF416-00202 (Control II)	6 µl	Serum, EDTA-plasma	Heparin[3] has been found to activate lipases in plasma, samples should be cooled.
Glycerol [mmol/l]	GY105 (Randox)	151 GY	GY 1369	6 µl	Serum, EDTA- or heparin-plasma	Samples should be cooled after collection.
Glucose [mmol/l]	OSR6121 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	1,6 µl	Serum, EDTA- or heparin-plasma	Values decrease during storage without separation from blood cells.
Urea [mmol/l]	OSR6134 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	2 µl	Serum, EDTA- or heparin-plasma	Do not use NH_4-heparin
Uric Acid [mmol/l]	OSR 6198 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	5,6 ml	Serum, EDTA- or heparin-plasma	
Creatinine[3] [µmol/l]	OSR61204 (BC) (enzymatic method)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	5,6 µl	Serum or heparin-plasma	
Bilirubin total [µmol/l]	OSR6112 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	10 µl	Serum, EDTA- or heparin-plasma	Hemolysis will falsify results, samples should be protected from light.
Bilirubin direct [µmol/l]	OSR6111 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	2,5 µl	Serum or heparin-plasma	Hemolysis will falsify results, samples should be protected from light.

Plasma lipid and glucose levels - directly associated with relvant pathways of energy metabolism; cholesterol levels also indicator of hepatic lipoprotein production.
Bilirubin – indicator of hepatic function; urea – product of protein katabolism

Clinical Chemistry of serum or plasma samples

Parameter [SI units]	Test-kit [1)]	Calibrator	Control	Sample volume	Sample preparation	notes
Total Protein [g/l]	OSR6132 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	5 µl	Serum, EDTA- or heparin-plasma	
Albumin [g/l]	OSR6102 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	1,6 µl	Serum, EDTA- or heparin-plasma	Macroglobulinemia can falsify results.
Ferritin [pmol/l]	OSR61203 (BC)	ODR3021	ODC0014 ODC0015 ODC0016	6 µl	Serum or heparin-plasma	Uses specific antibodies against human ferritin[2)]
Transferrin [µmol/l]	OSR6152 (BC)	ODR3021	ODC0014 ODC0015 ODC0016	1,6 µl	Serum, EDTA- or heparin-plasma	Uses specific antibodies against human transferrin[2)]
Alpha-Amylase[3)] [U/l]	OSR6106 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	1,6 µl	Serum or heparin-plasma	No EDTA-, Citrate- or Oxalate-plasma, strongly hemolyzed or icteric samples should be avoided
ALAT* [U/l]	OSR6107 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	5 µl	Serum, EDTA- or heparin-plasma	Muscle injury during blood collection can falsify results.
ASAT** [U/l]	OSR6109 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	5 µl	Serum or heparin-plasma	Hemolysis and muscle injury during blood collection will falsify results.
ALP*** [U/l]	OSR6104 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	1,8 µl	Serum or heparin-plasma	No EDTA-, Citrate- or Oxalate-plasma, strong hemolysis will falsify results.
LDH**** [U/l]	OSR6128 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	1,8 µl	Serum, EDTA- or heparin-plasma	Hemolysis and muscle injury during blood collection will falsify results, avoid plasma contamination with platelets.
Creatine kinase [U/l]	OSR6179 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	2,5 µl	Serum or Heparin-plasma	No EDTA-, Citrate- or Oxalate-plasma, hemolysis and muscle injury during blood collection will falsify results.
Lipase [U/l]	OSR6230 (BC)	OE66300	ODC0003 (Level 1) ODC0004 (Level 2)	1,6 µl	Serum, EDTA- or heparin-plasma	Strongly icteric or lipemic samples should be avoided.

Total protein and albumin >> decreased in severe energy deficiency

Transferrin, albumin, alpha-amylase >> indicators of hepatic function

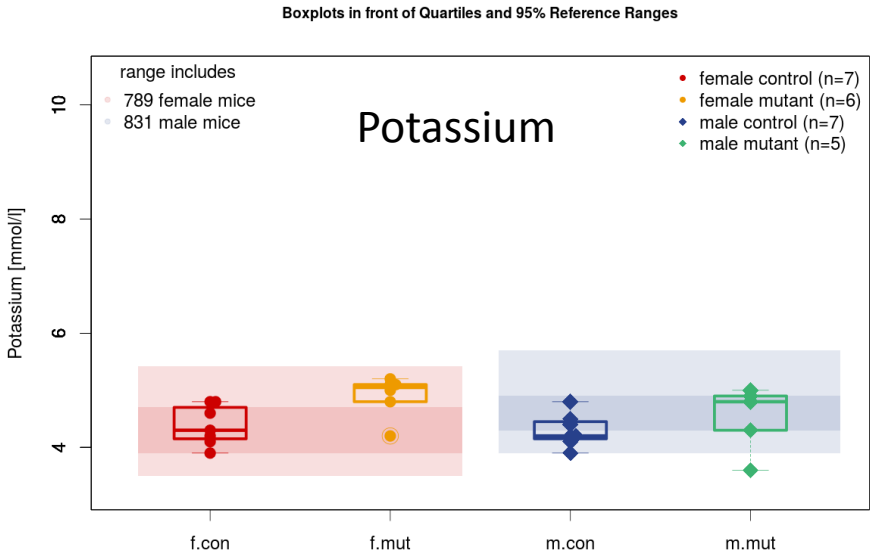
ALAT, ASAT, LDH >> indicators of hepatocyte damage (ASAT, LDH – unspecific!)

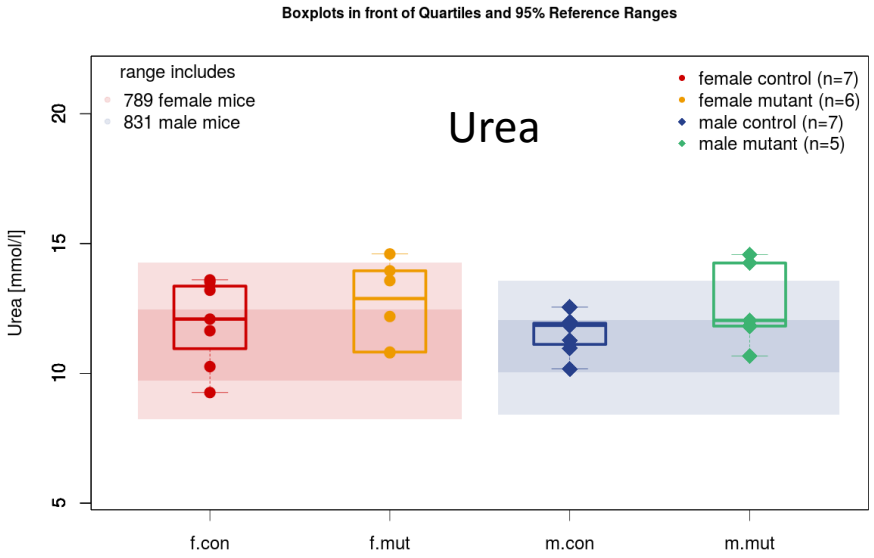
Alpha-Amylase, Lipase >> indicators of damage of exocrine pancreas

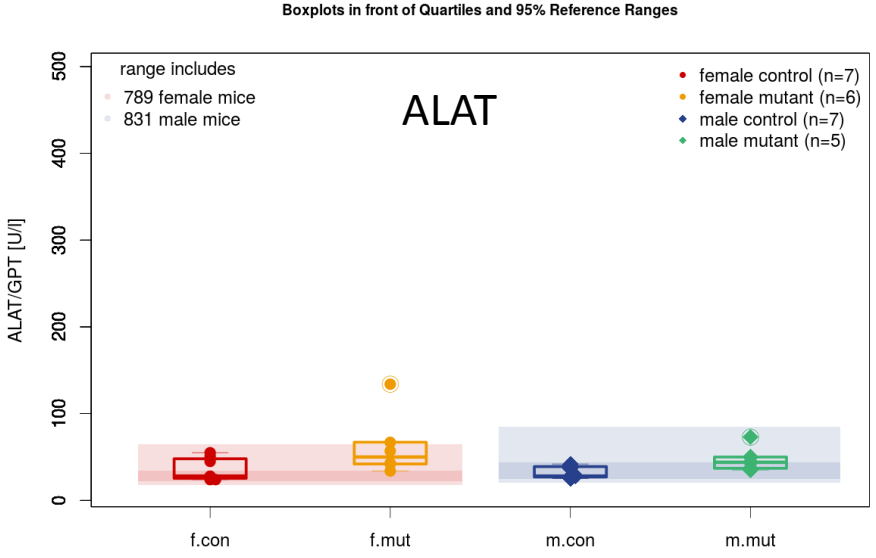
ALP >> indicator of hepatic cholestasis (but also bone metabolism)

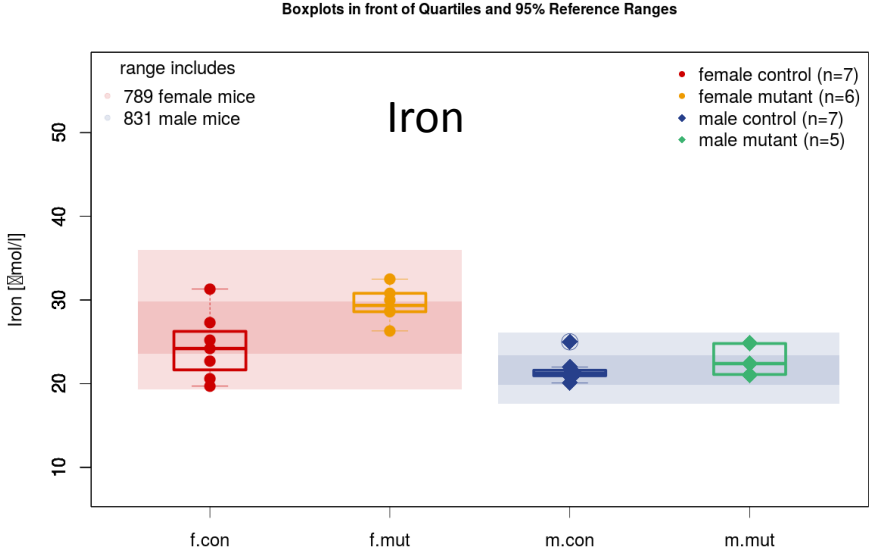
Clinical Chemistry – *result evaluation and interpretation*

Wsb2







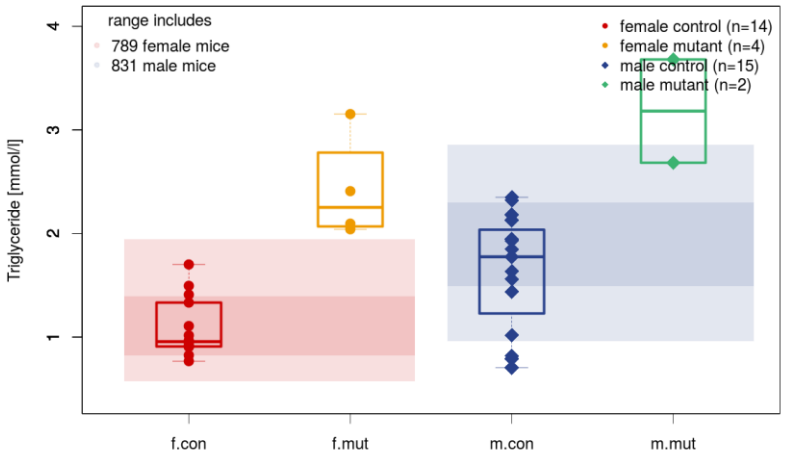


Further findings: Elevated albumin, clearly increased ALP and decreased body mass

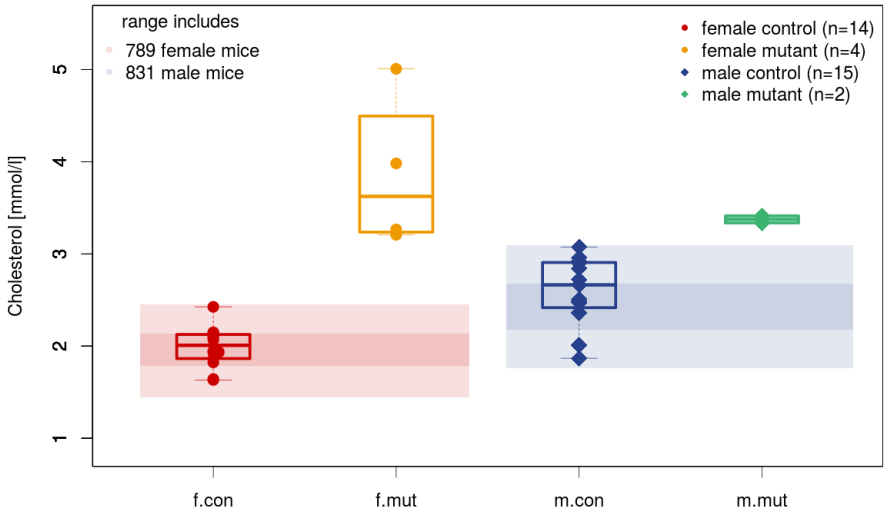
Clinical Chemistry: Examples from IMPC screen

Cpe-KO mice:
Only 4f and 2m homozygous mutants tested.

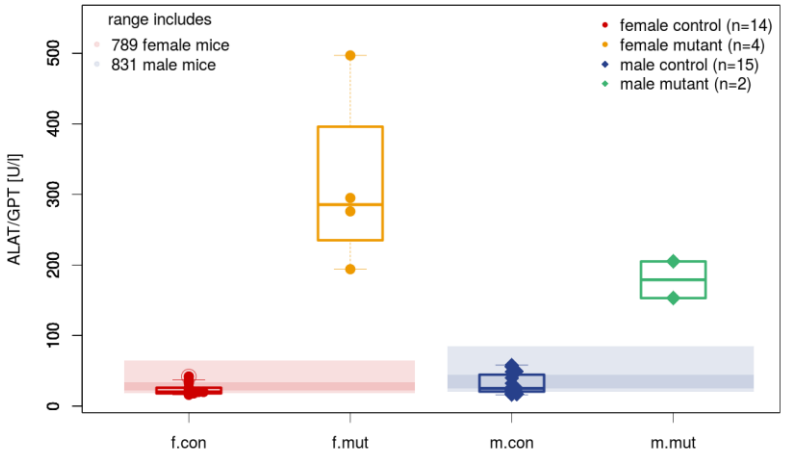
Triglyceride



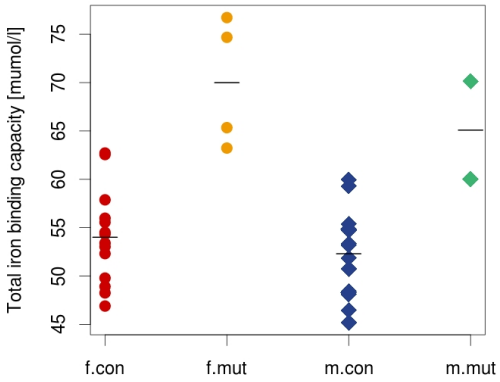
Cholesterol



ALAT



TIBC



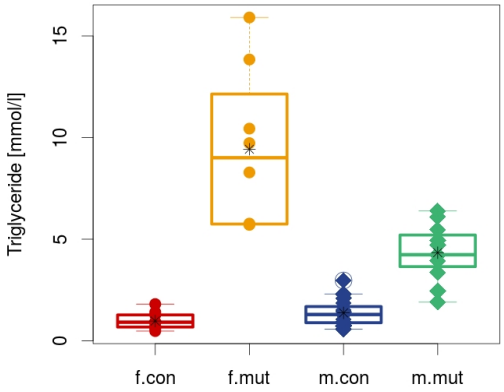
High TIBC (transferrin) >> increased hepatic protein production.

High liver enzyme activities >> damage of liver cells.

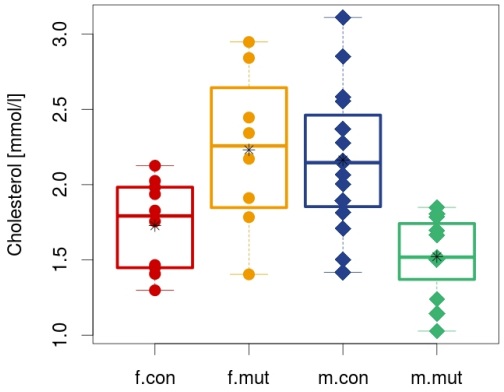
Clinical Chemistry : Example from IMPC-Screen

Apoc4-KO

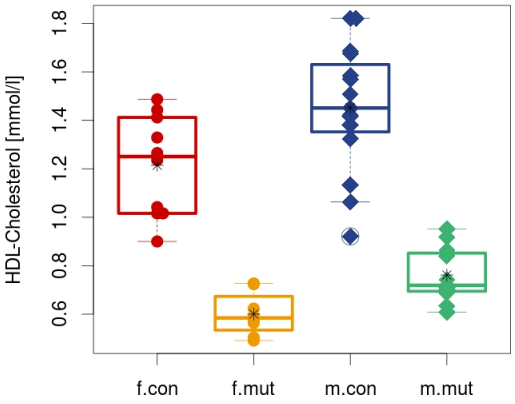
Triglyceride



Cholesterol

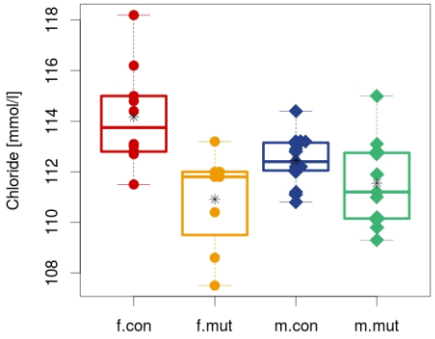


HDL-Cholesterol

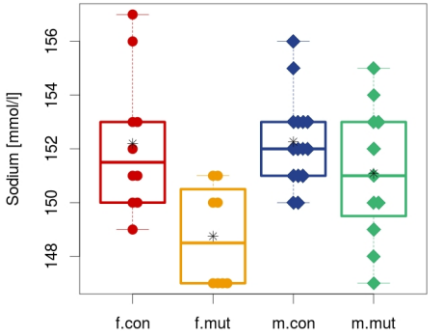


Cholesterol bound in lipoproteins containing beta-lipoprotein

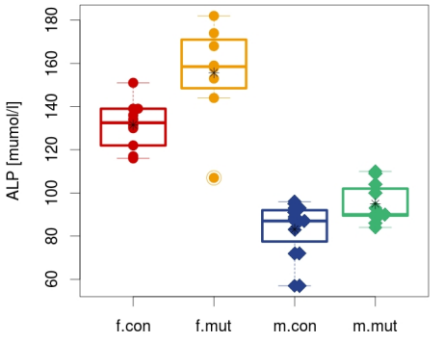
Chloride



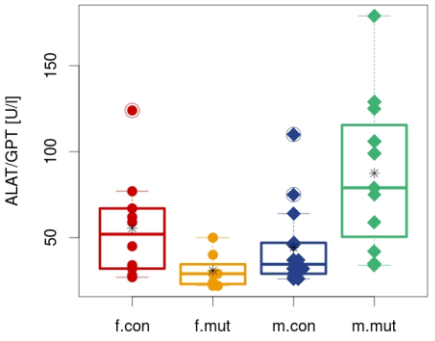
Sodium



ALP



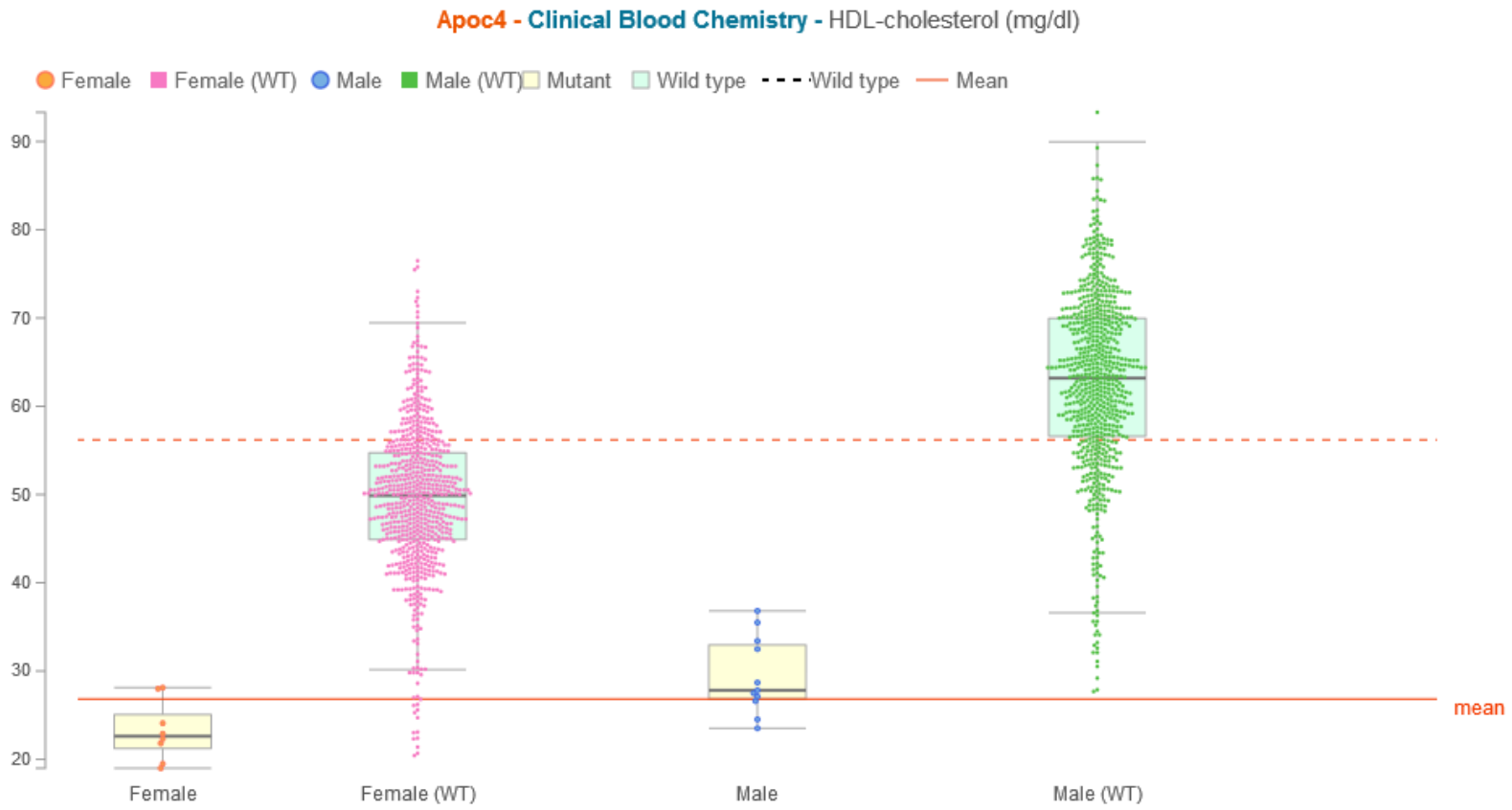
ALAT



Measurements affected by lipemia in female mutants.

Clinical Chemistry – *Example from IMPC*

Apoc4-KO



Result presentation on IMPC website: www. Mousephenotype.org/phenoview/