

Controls metabolic syndrome

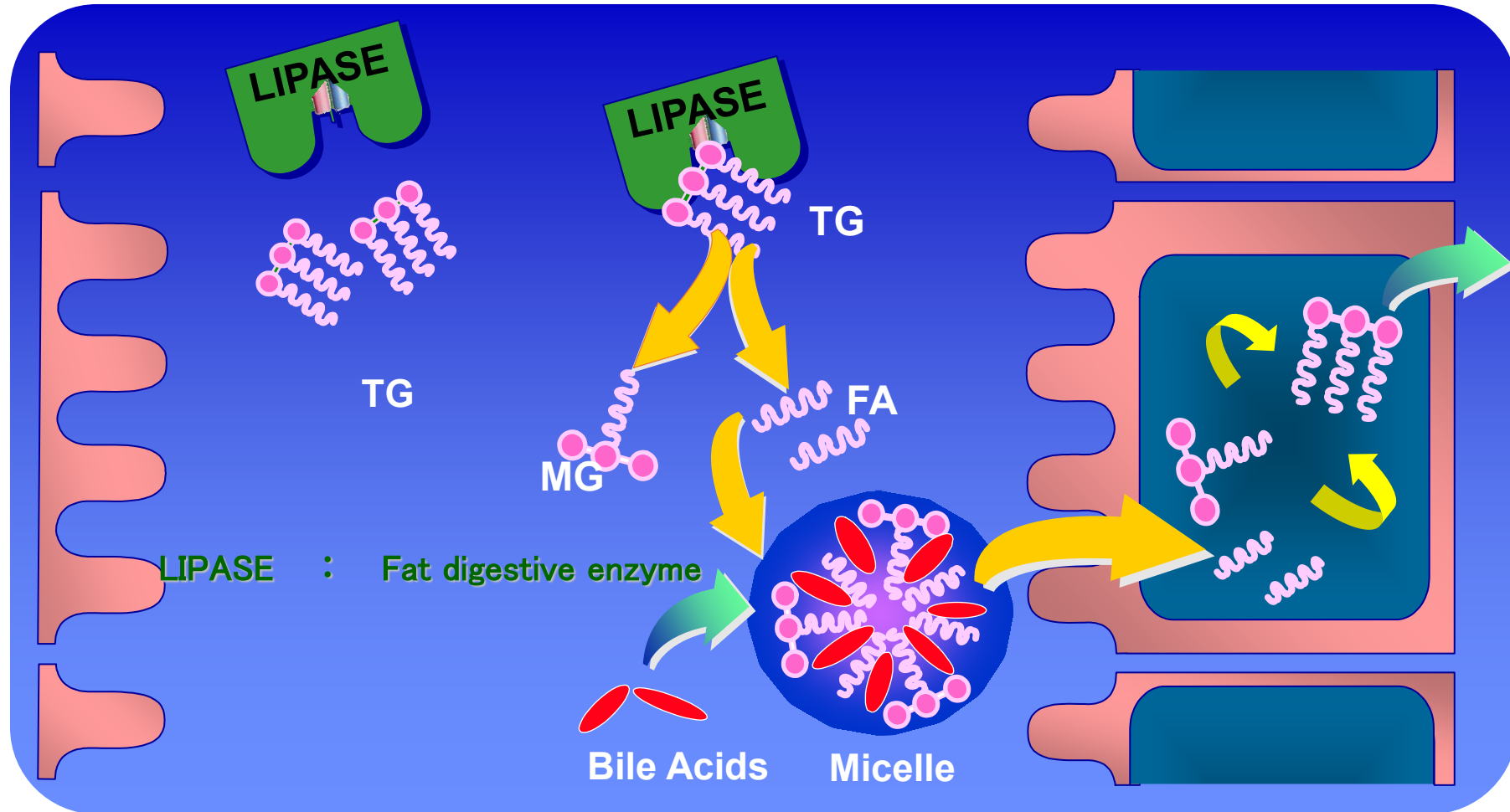
# Metabolic syndrome: The Problem

- Globally > 1 billion overweight adults. In the US: about 50 mln people with metabolic syndrome.
- Main characteristics of metabolic syndrome:
  - Abdominal obesity (excessive fat in abdomen)
  - Blood fat disorder: high triglycerides, high LDL cholesterol, low HDL cholesterol
  - Elevated blood pressure
  - Insulin resistance or glucose intolerance (the body can't properly use insulin or blood sugar)
- Parameters for metabolic syndrome
  - Waist size: >90 cm in men (US: 102 cm); >85 cm in women (US: 88cm)
  - Blood triglyceride (TG): 150 mg/dL or more
  - Blood glucose: 100 mg/dL or more

# How to prevent metabolic syndrome?

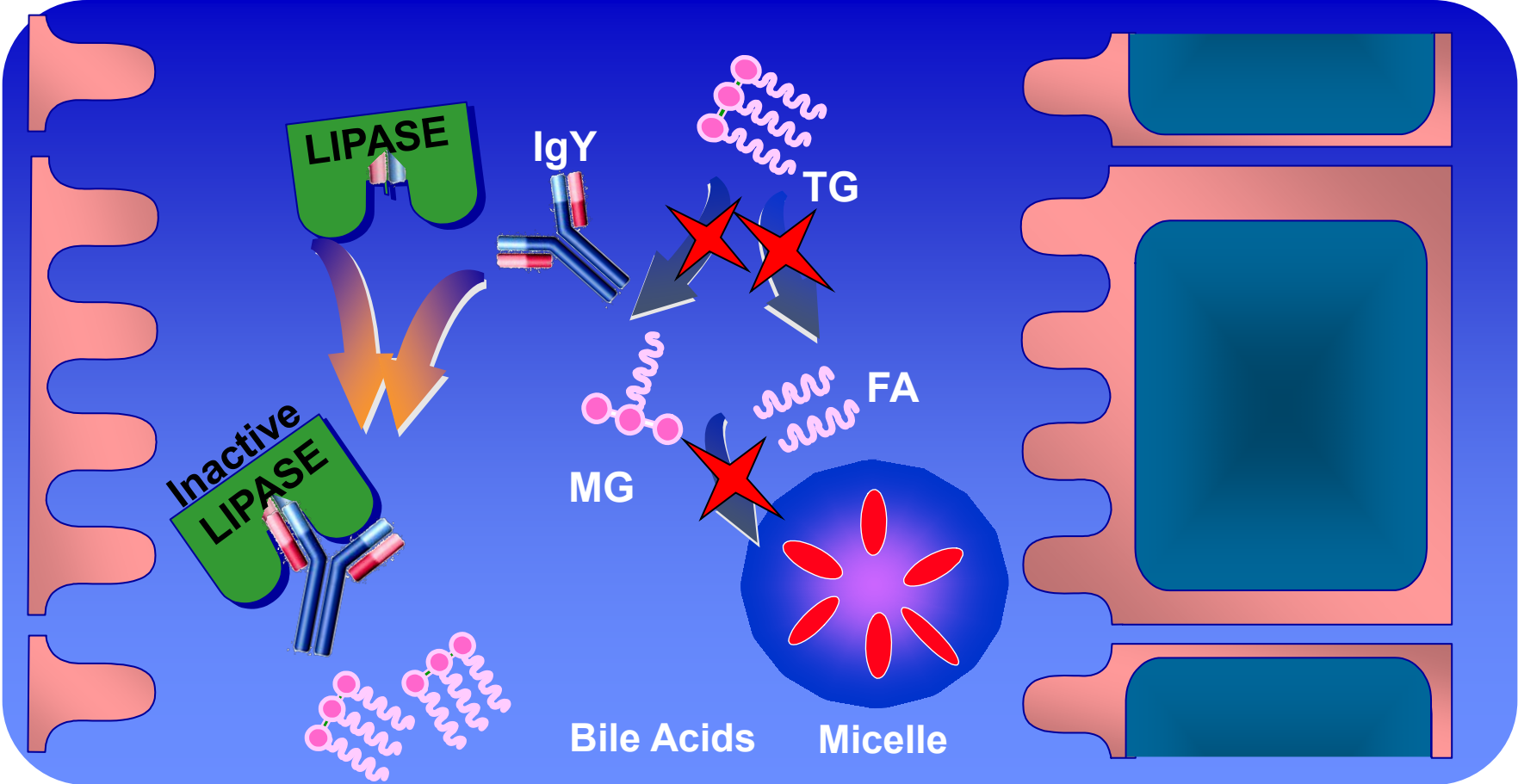
- Control overweight and obesity (weight loss)
- Increase physical activities
- Healthy eating plan (eating less fat and calories)
- Reduce blood TG by mean of drugs (most anti-obesity drugs have side effects)

# Fat Absorption Mechanism



FA = fatty acid; MG = monoglyceride; TG = triglyceride.

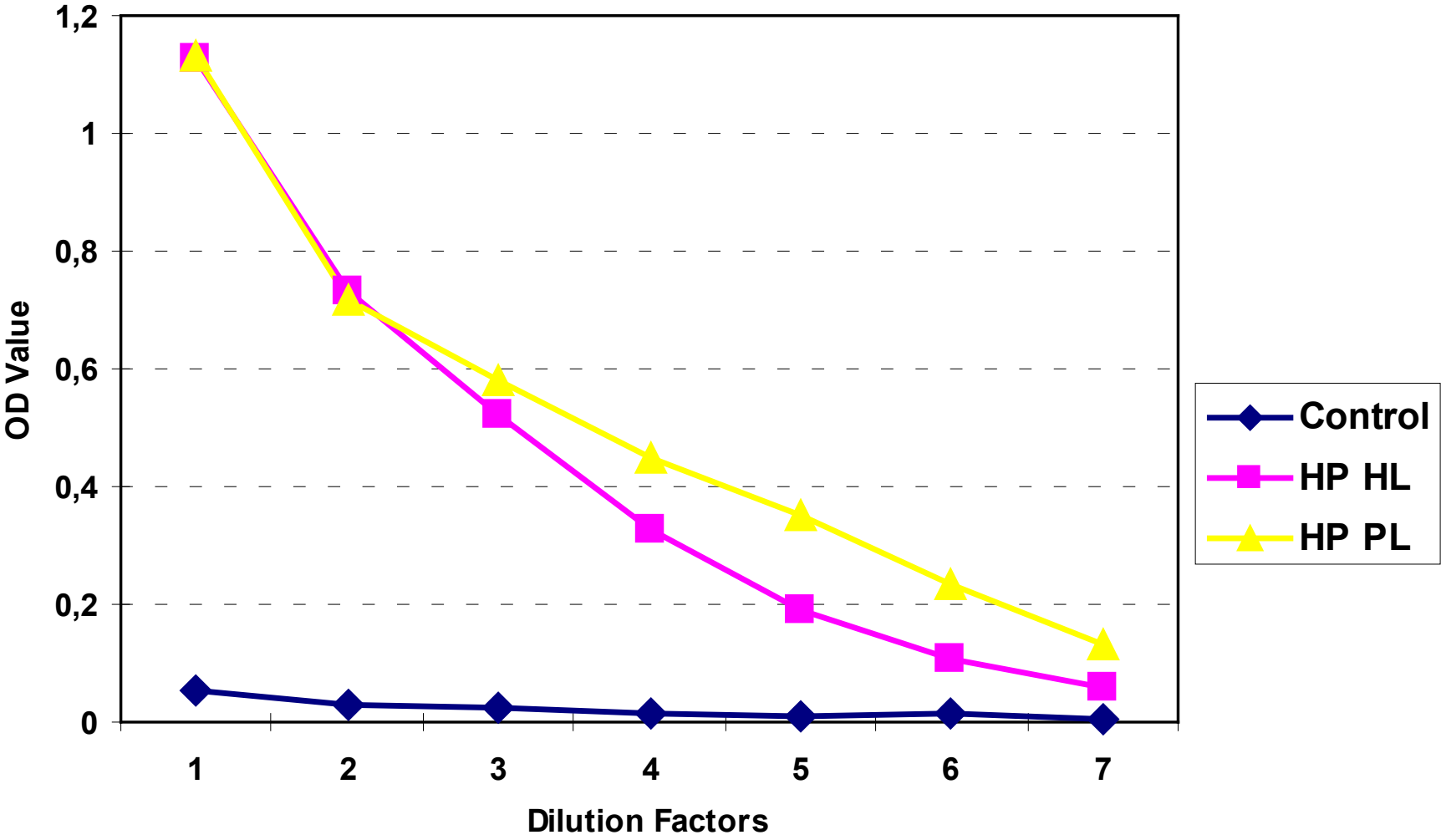
# Specific IgY (LP) contains IgY that inactivates lipase



FA = fatty acid; MG = monoglyceride; TG = triglyceride.

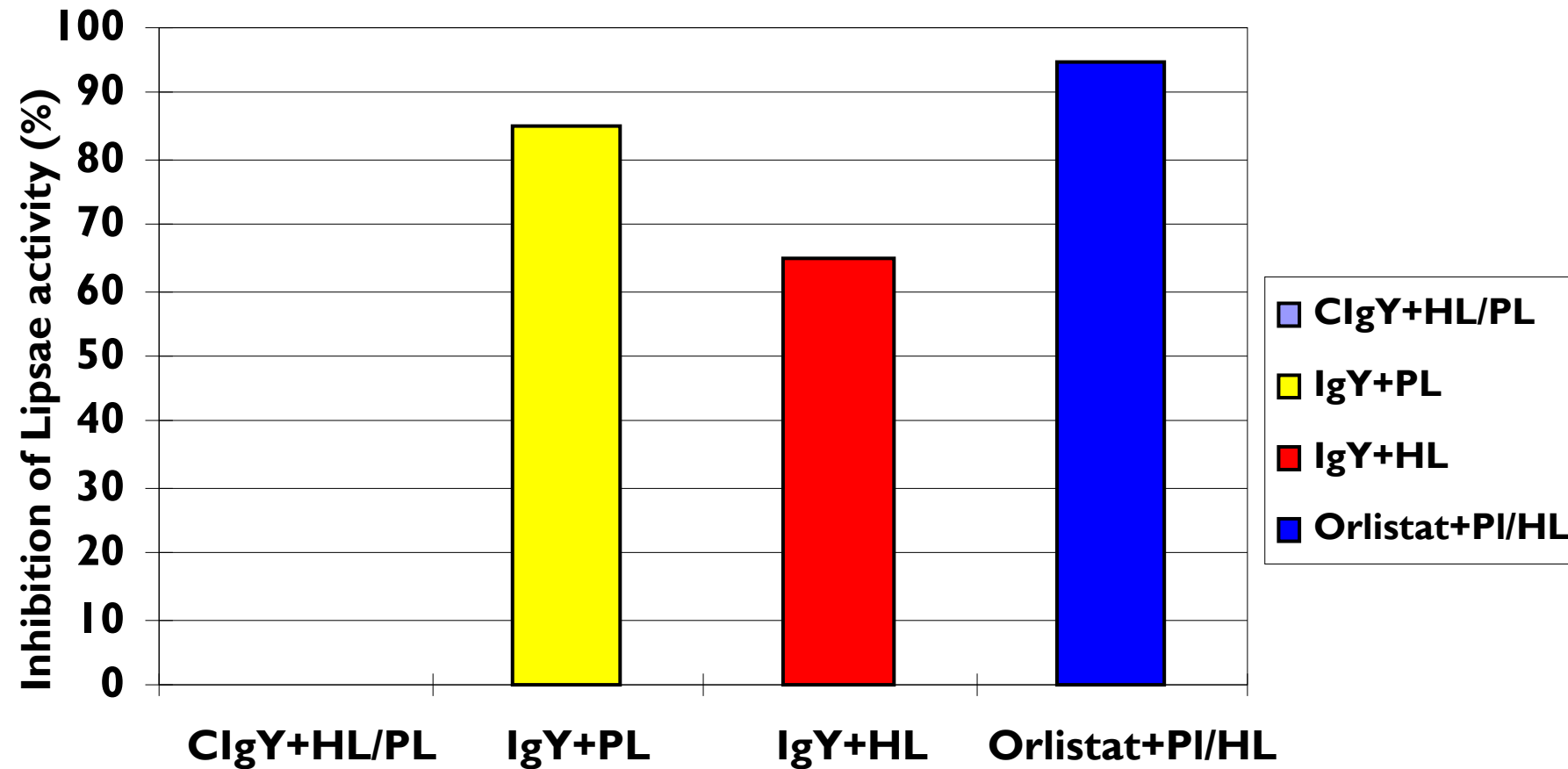
In-vitro tests: reactivity and inhibiting effect of Specific IgY (LP) against lipase from human and animals

# Reactivity of Specific IgY (LP) against lipase: ELISA



HL: human lipase; PL: porcine lipase

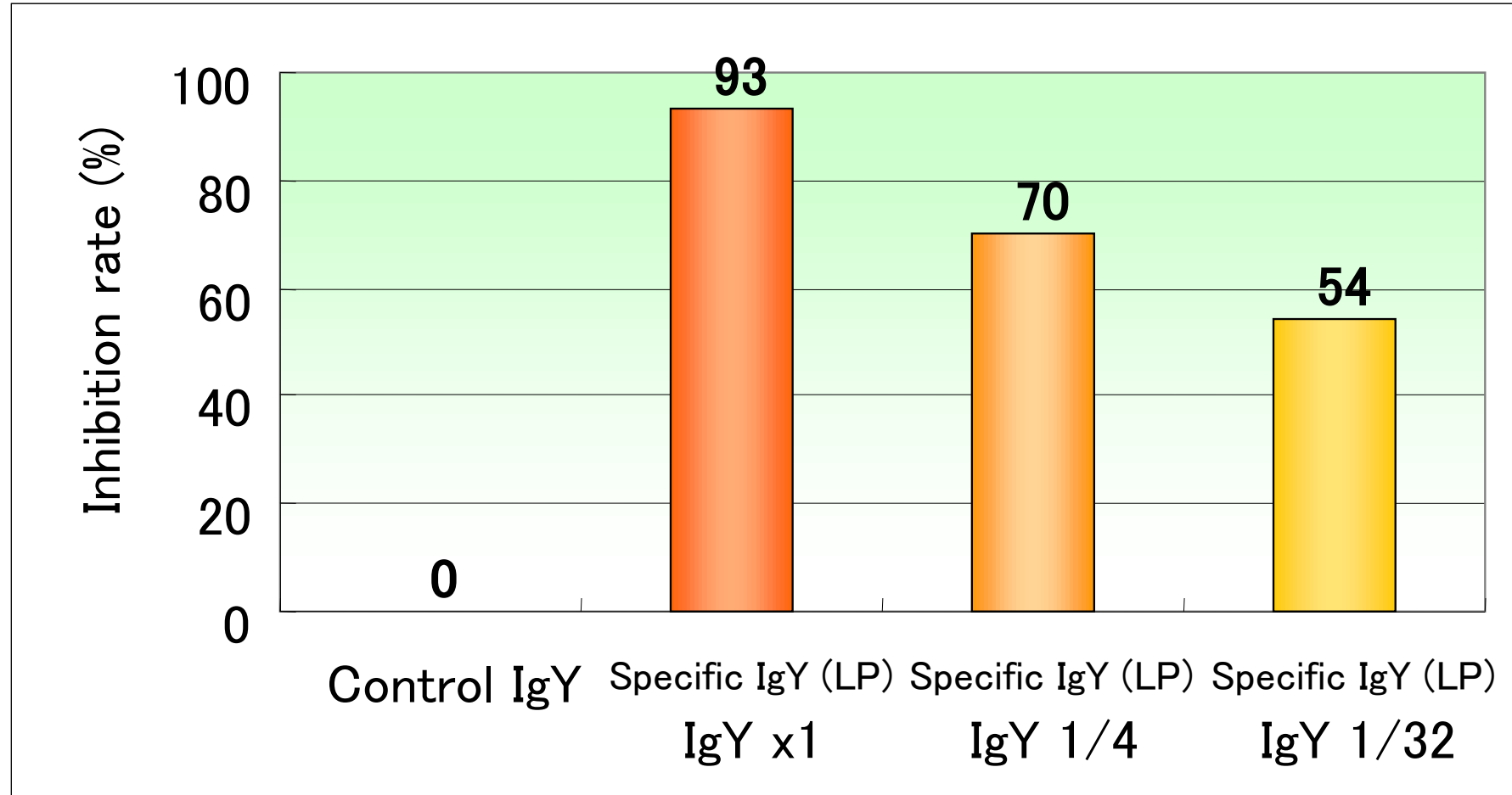
# In-vitro Lipase inhibition: compare with Orlistat



*PL: Pig lipase; HL: human lipase*



## Reactivity of Specific IgY (LP) against dog lipase



# In-vitro test summary

- Specific IgY (LP) has broad reactivity against lipase from different sources.
- Like anti-obesity Orlistat, Specific IgY (LP) has specific inhibition activity against lipase from human and animals. By this inhibition activity, Specific IgY (LP) reduces adsorption of fat in foods into blood.

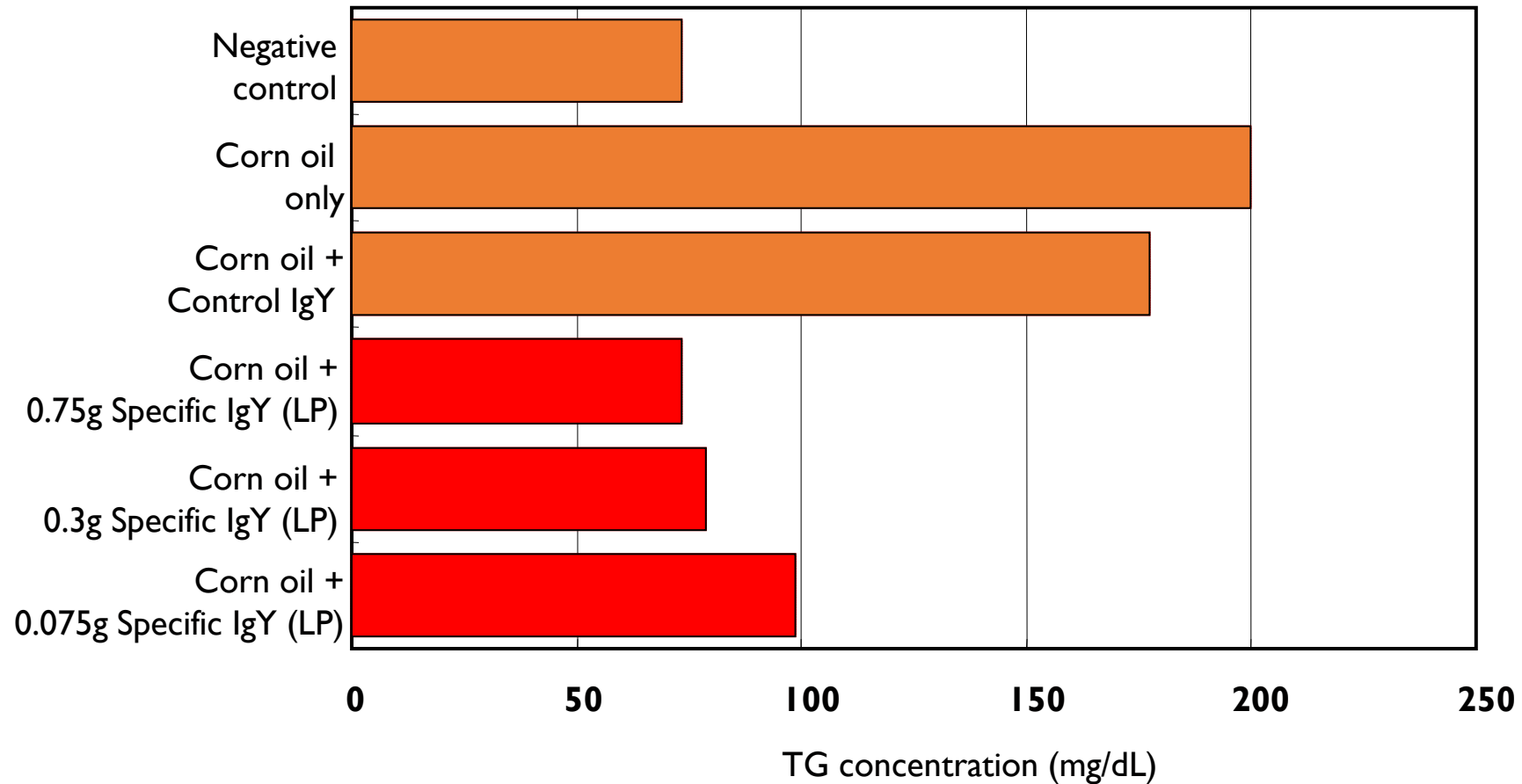
In-vivo tests on experimental  
animals

# Rat study: effect of Specific IgY (LP) on blood TG adsorption

- Animal: **Wister rat** male, 6 weeks old; 10 rats/group
- Feed corn oil together with various doses of Specific IgY (LP) to each rat, collect blood after 4 hours.
- Measure TG level in blood



# Concentrations of triglyceride in blood



# Mouse trial 1: Effect of Specific IgY (LP) after one-time application

- **Method:**

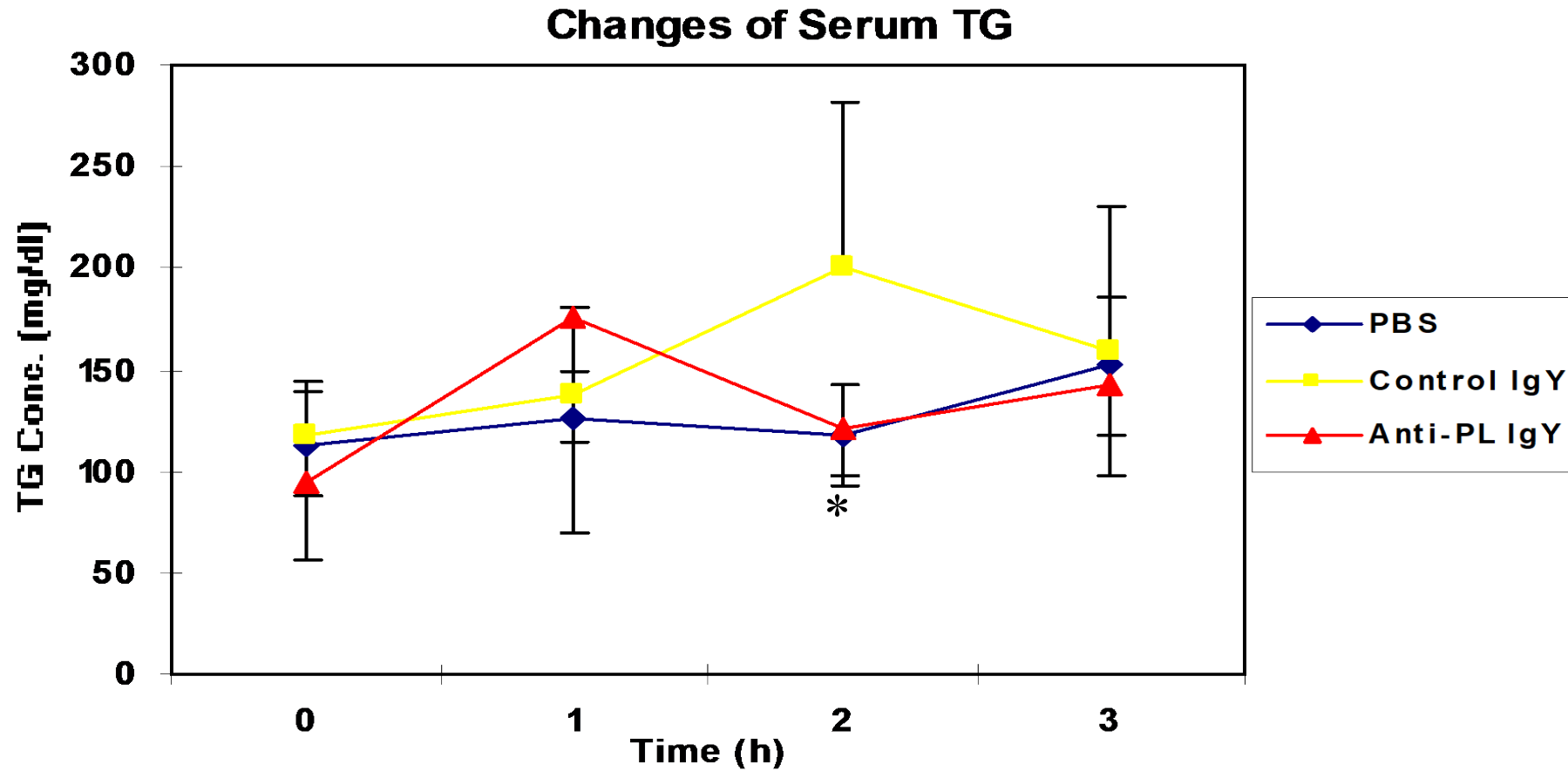
- Mice: **Male C57BL/6J mice, 6 w (22g BW)**
- No. of mice: **15 (5/group)**
- Administration: **15 mg Specific IgY (LP) + 200 ul lipid by long needle**
- Test period: **Blood samples at 0, 1, 2 & 3 h**
- Parameters: **Measurement of Serum concentration of:  
i) Triglycerol, ii) Cholesterol & iii) Glucose.**

- **Groups:**

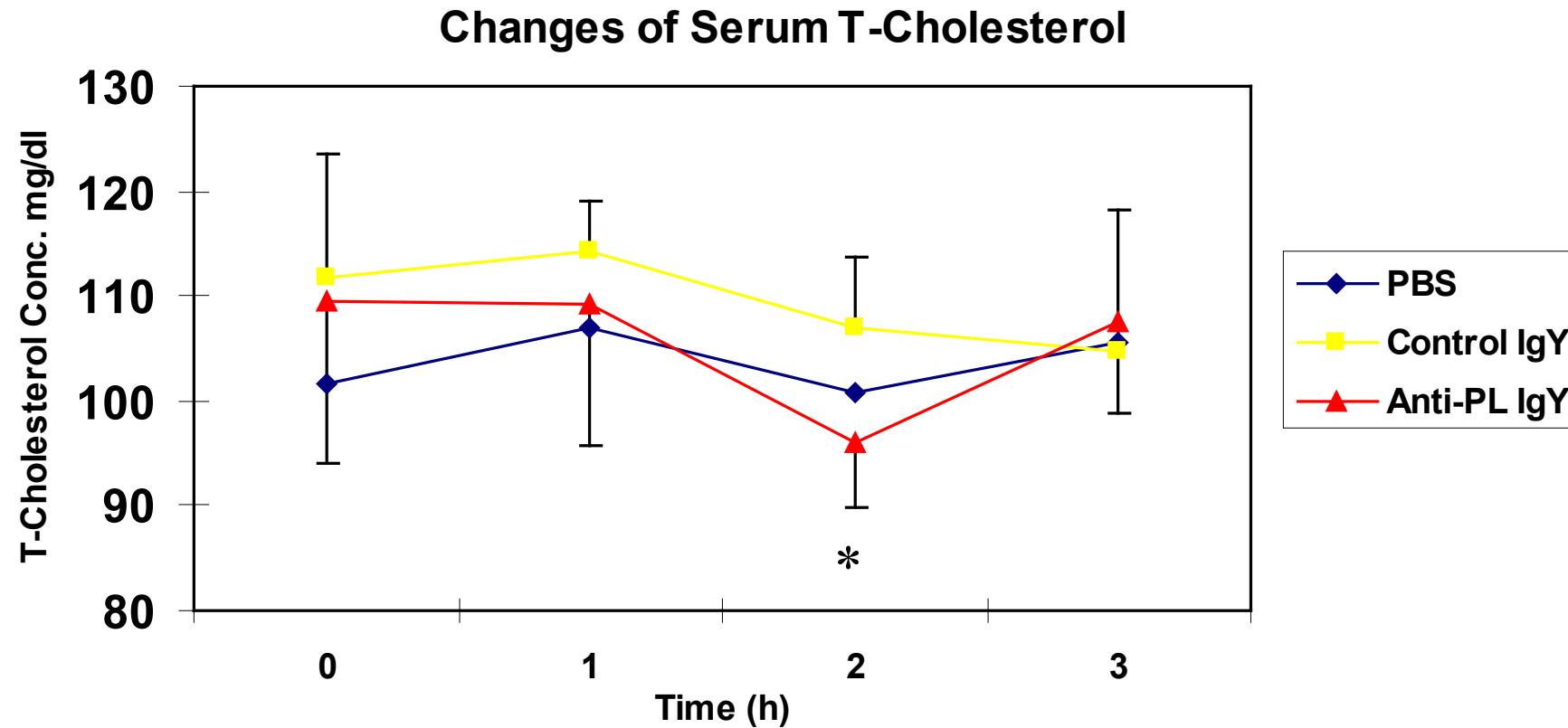
- a) Negative control (PBS only)
- b) Control IgY + Lipid emulsion
- c) Specific IgY (LP) + Lipid emulsion



# Effect of Specific IgY (LP) on Serum Triglycerides (TG)

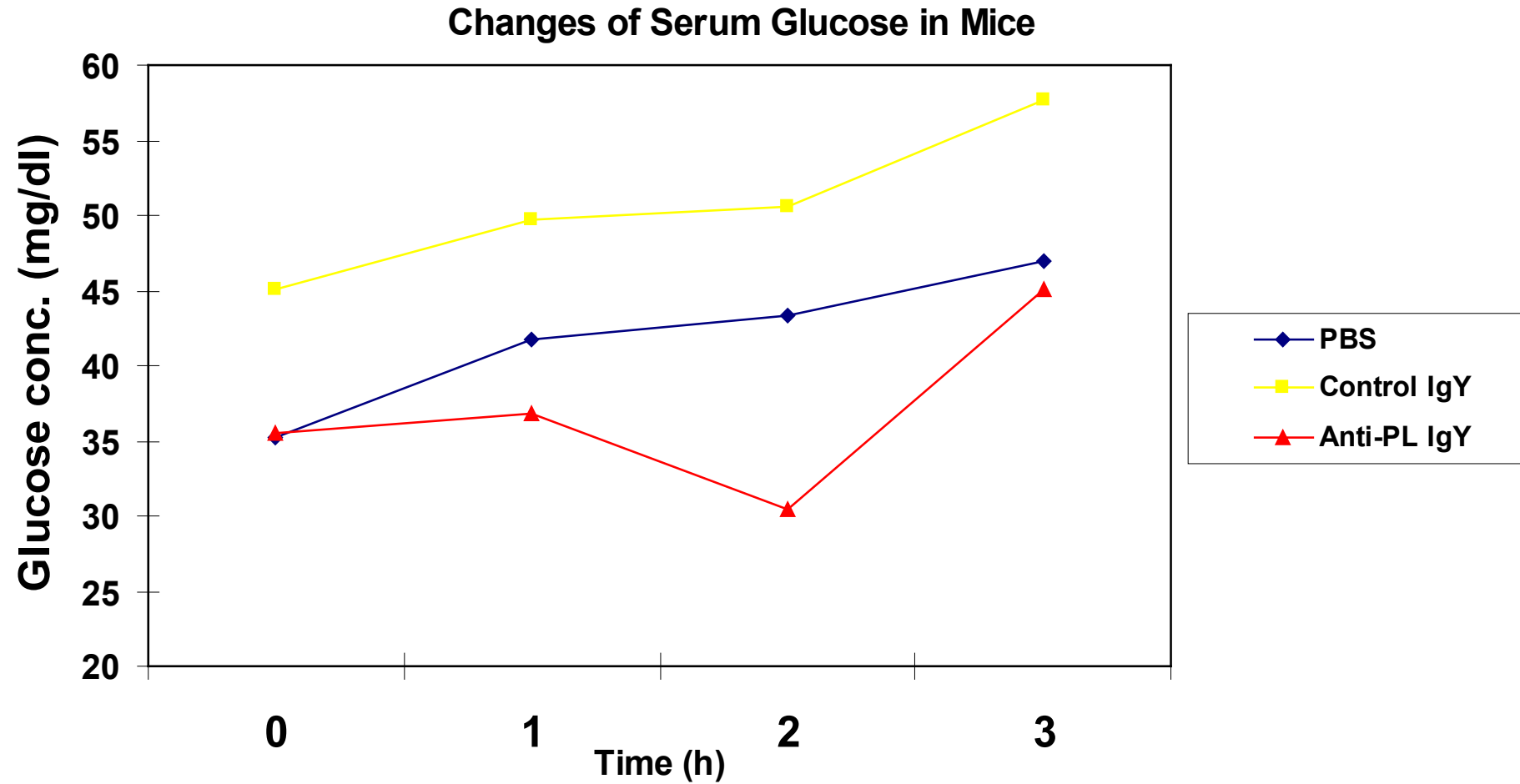


# Effect of Specific IgY (LP) on total serum cholesterol





# Effect of Specific IgY (LP) on Serum Glucose



## Mouse trial 2: Mid-term application

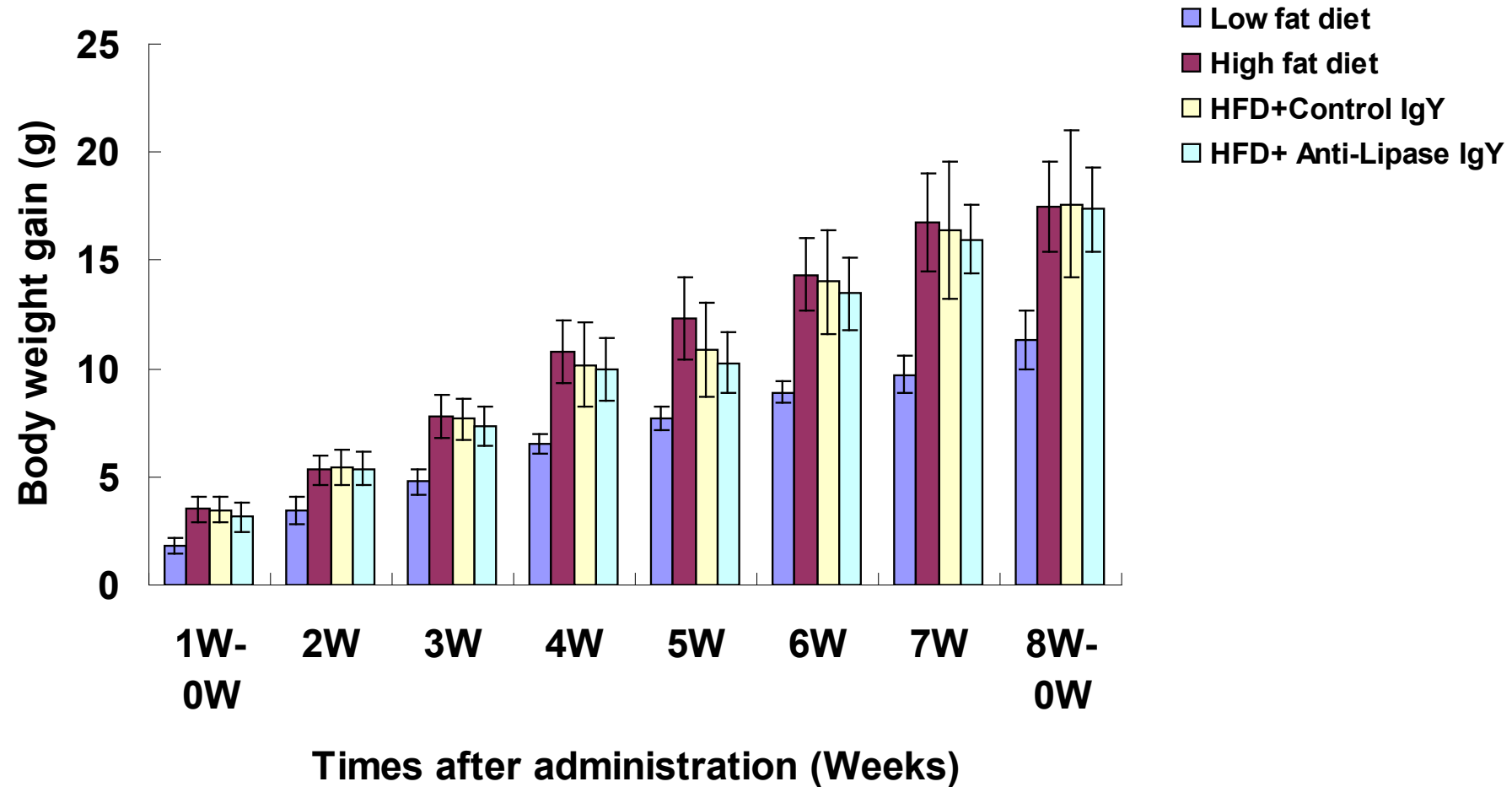
### Details:

- Mice strain: Male C57BL/6J mice
- Age: 6 weeks old (approx. weight: 22 g/mouse)
- IgY Administration: Mixed with experimental high-fat diet
- Dose of Specific IgY (LP): 2% in feed
- Test period: 8 weeks
- Mice groups: 4 groups × 6
  - Low fat diet
  - High fat diet
  - High fat diet + Control IgY
  - High fat diet + Specific IgY (LP)

# Composition of experimental diets

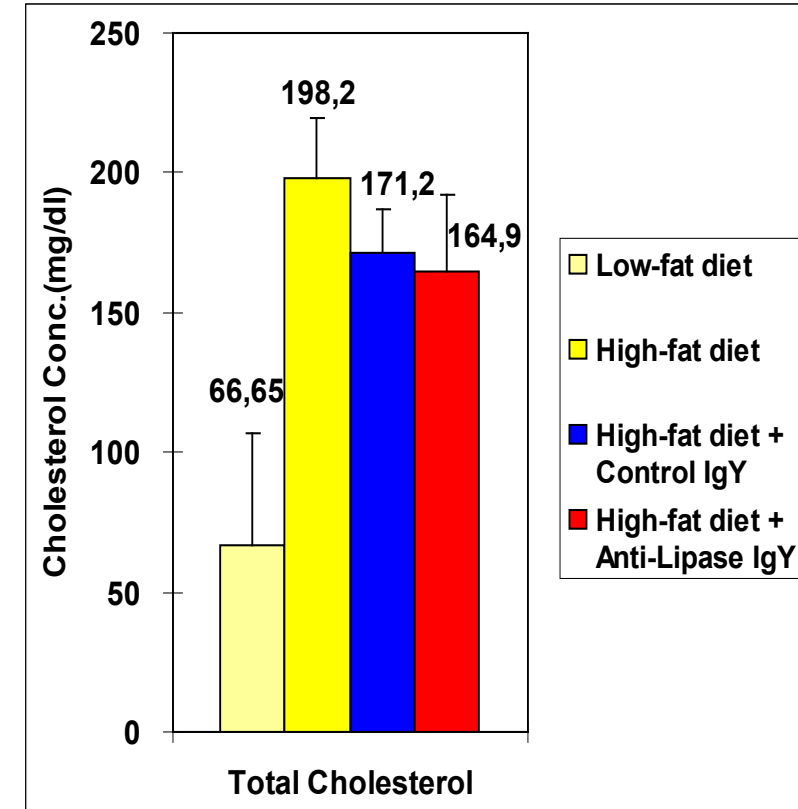
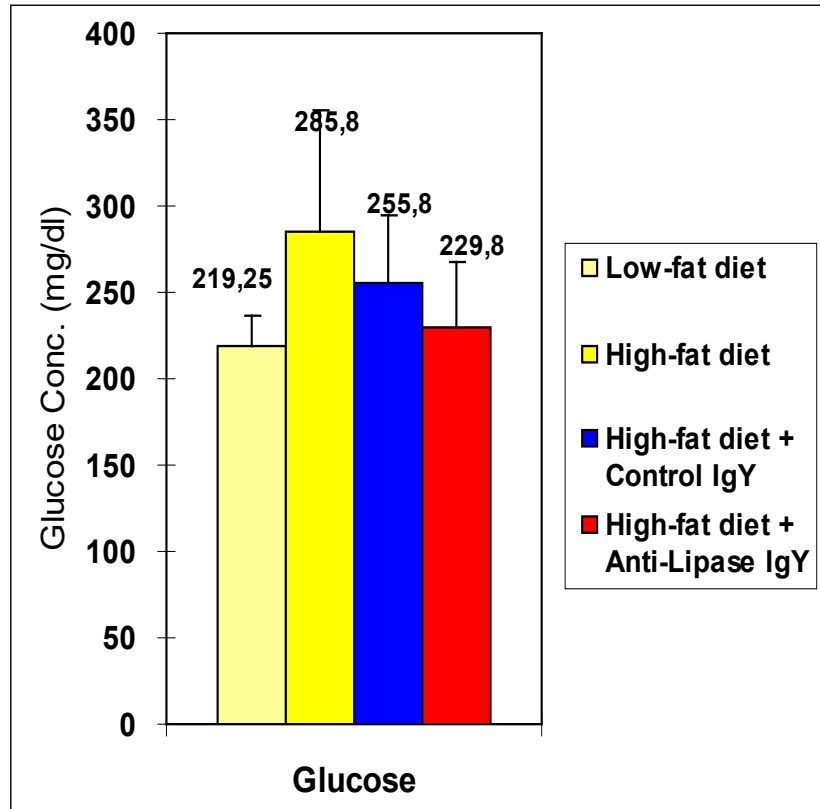
Component	Low-Fat Diet	High-Fat Diet	High-fat diet + Control IgY	High-fat diet + Specific IgY (LP)
Moisture (%)	6.70	6.90	6.79	6.79
Protein (%)	25.60	25.00	26.50	26.50
<b>Fat (%)</b>	<b>4.00</b>	<b>32.04</b>	<b>32.03</b>	<b>32.03</b>
Fiber (%)	3.80	2.90	2.90	2.90
Ash (%)	4.90	4.00	3.99	3.99
Carbohydrates	50.50	28.80	28.70	28.70
<b>Energy (kcal/100g)</b>	<b>340.40</b>	<b>506.80</b>	<b>506.07</b>	<b>506.07</b>

# Effect of Specific IgY (LP) on weekly body weight gain in mice

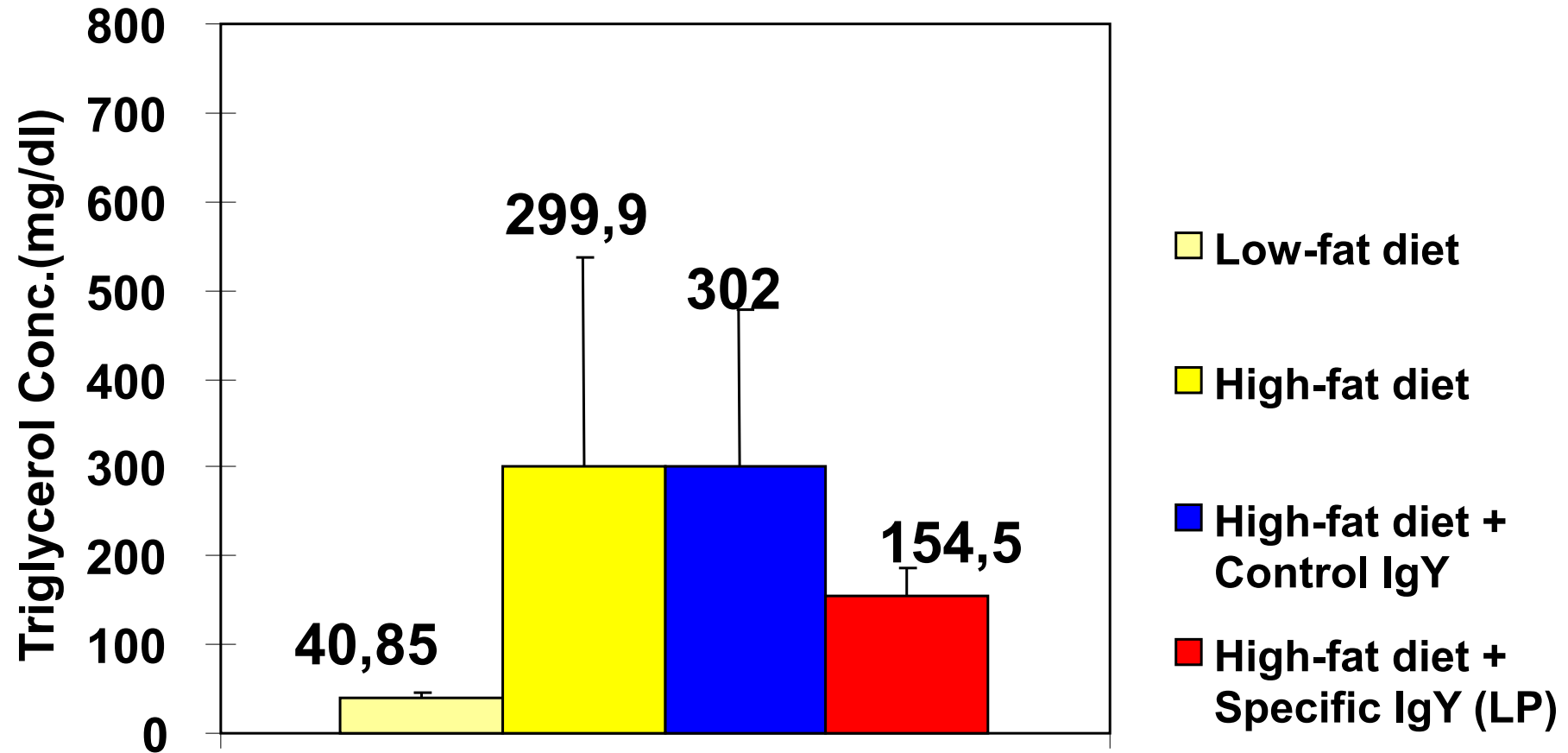


Significant difference seen from 4<sup>th</sup> to 6<sup>th</sup> weeks

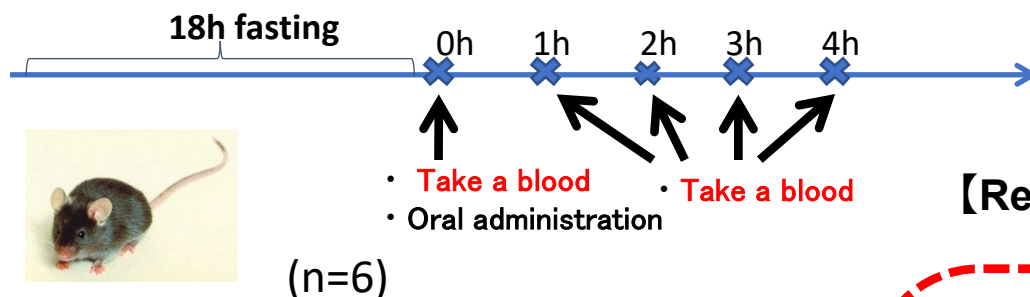
# Effect of Specific IgY (LP) on blood glucose and total cholesterol



# Effect of Specific IgY (LP) on blood TG in mice



【 Mouse trial 3】 Efficacy of Specific IgY (LP) on mouse challenged with lipid emulsion administering orally



【

Mice strain Male C57BL/6J  
 Age 6 weeks (Approx. 20~25g)  
 Test period Blood sampling at (0,1, 2, 3, 4h)  
 Parameters Serum triglyceride

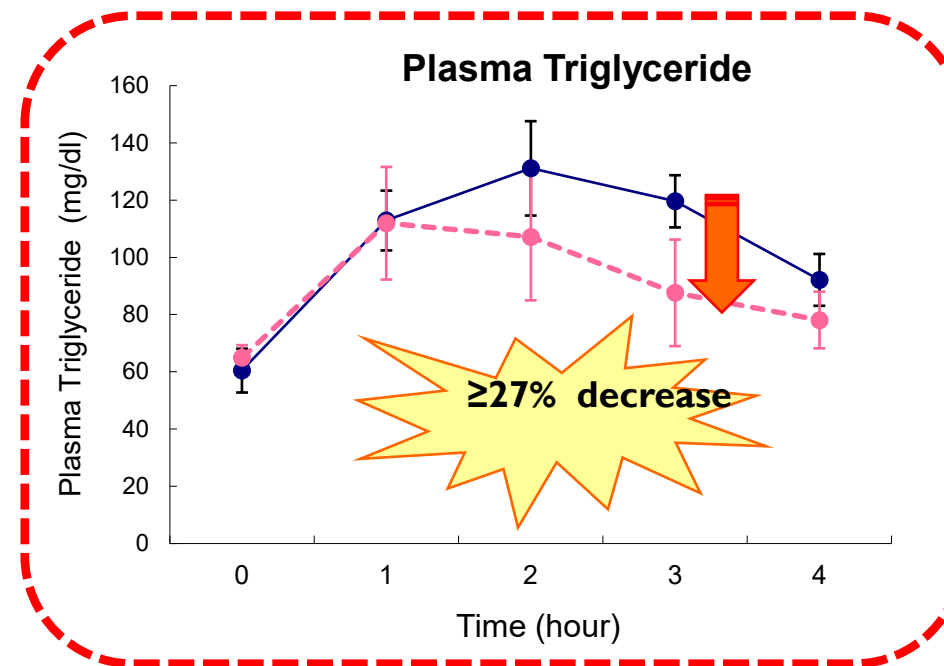
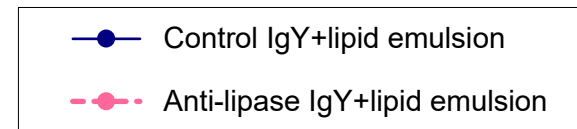
Groups

- Control IgY + Lipid emulsion (Control)
- Specific IgY (LP) + Lipid emulsion [0.7g IgY/10mL emulsion/ kg B.W.]

《Lipid emulsion》

6mL	Corn oil	} Sonicated for 5min	} mixed for 5min
80mg	Cholic acid		
2mg	Cholesteryl oleate		
6ml	D.D.W.		
	+ IgY sample (Control IgY or Specific IgY (LP))		

【Results】



# Mouse trial 4: Long-term application of Specific IgY (LP)

《Mice strain》 C57BL/6J Male mouse  
《Age》 6 weeks (Initial body weight, 20~25g)  
《Test period》 35 days  
《Group》 3 groups (n = 8)  
C) Casein high fat diet: control group  
CY) Control IgY(0.2%) mixed in casein high fat diet  
AY) Specific IgY (LP) (0.2%) mixed in casein high fat diet



《Feed Intake》 Everyday  
《Fast period》 22 hr (10 AM - next 8 AM) fasting before blood collecting  
《Blood collection》 28<sup>th</sup> day (plasma triglyceride value measurement)  
《Autopsy》 35<sup>th</sup> days  
《Test Parameters》  
BWG, Feed intake, Liver weight, Liver lipid  
Total WAT (Mesenteric, Epididymal, Perirenal, Retroperitoneal)  
Plasma triglyceride, cholesterol, blood sugar and insulin values

WAT = [White adipose tissue](#)

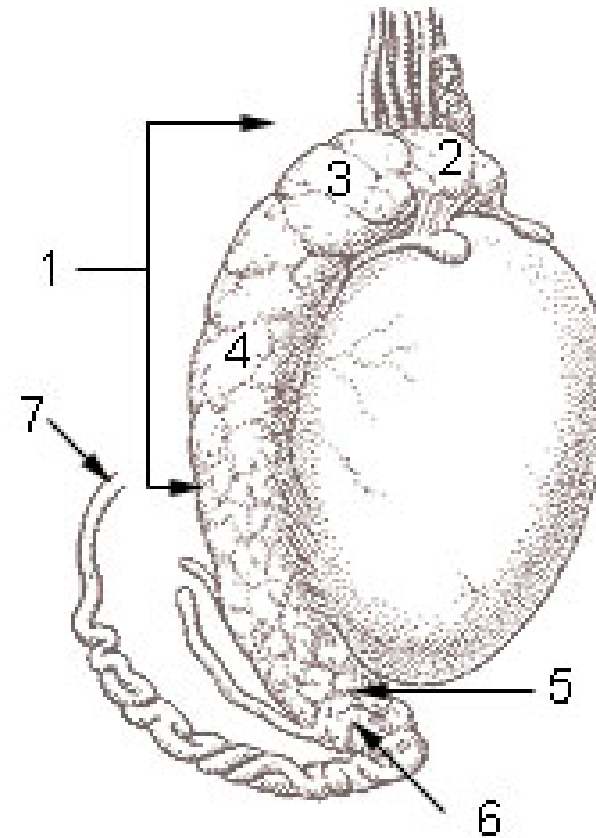


## Epididymis:

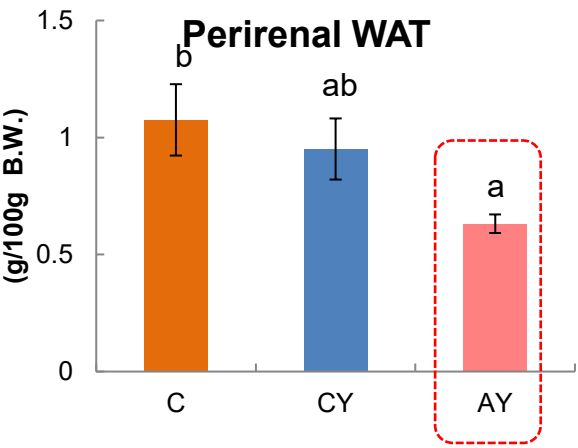
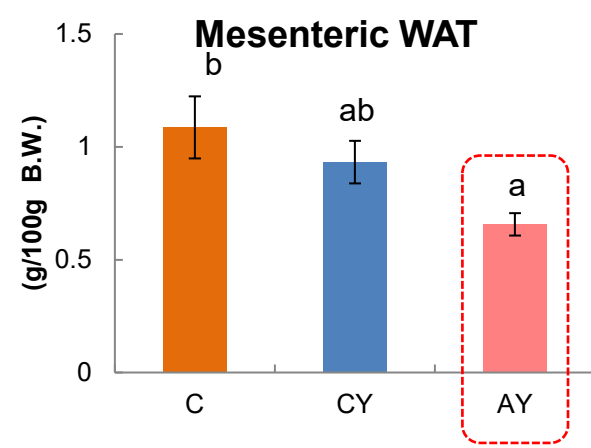
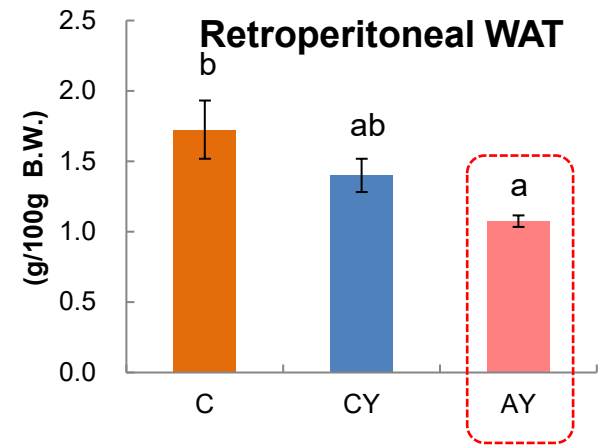
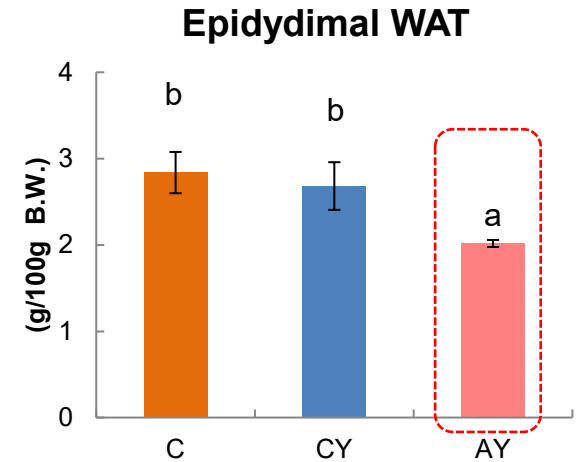
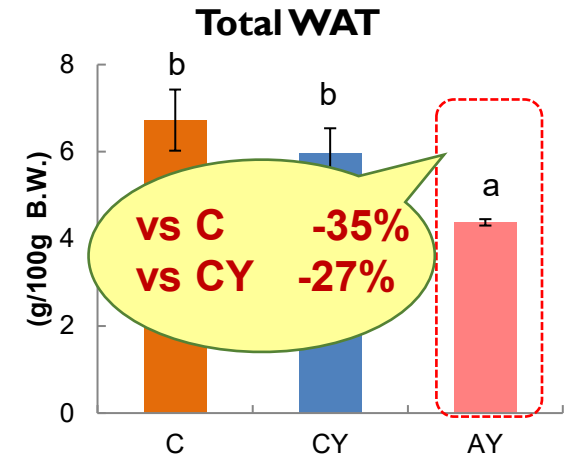
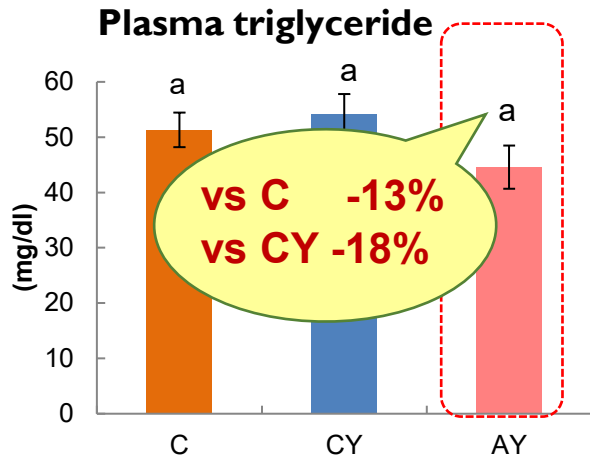
The epididymis is part of the [male reproductive system](#) and is present in all male [amniotes](#). It is a narrow, tightly-coiled tube connecting the [efferent ducts](#) from the rear of each [testicle](#) to its [vas deferens](#).

Epididymal WAT = [Epididymal white adipose tissue](#)

- 1: Epididymis
- 2: Head of epididymis
- 3: Lobules of epididymis
- 4: Body of epididymis
- 5: Tail of epididymis
- 6: Duct of epididymis
- 7: Deferent duct (ductus deferens or [vas deferens](#))



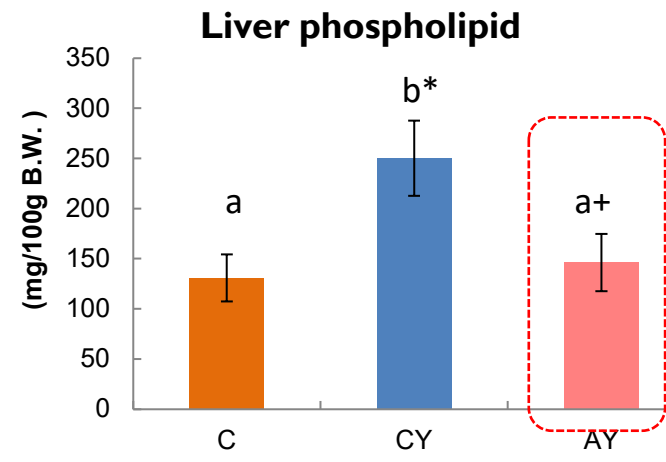
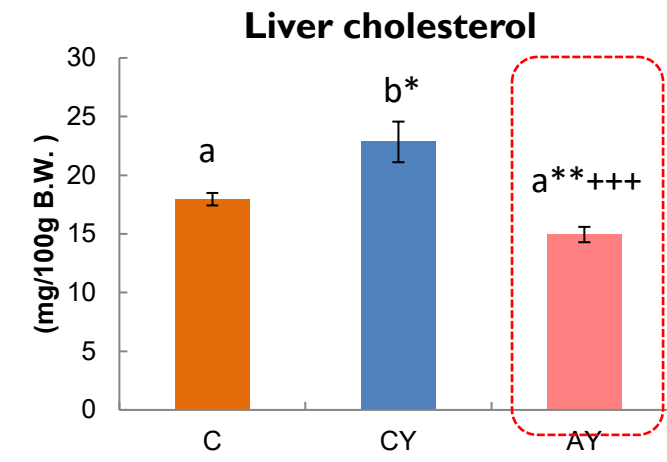
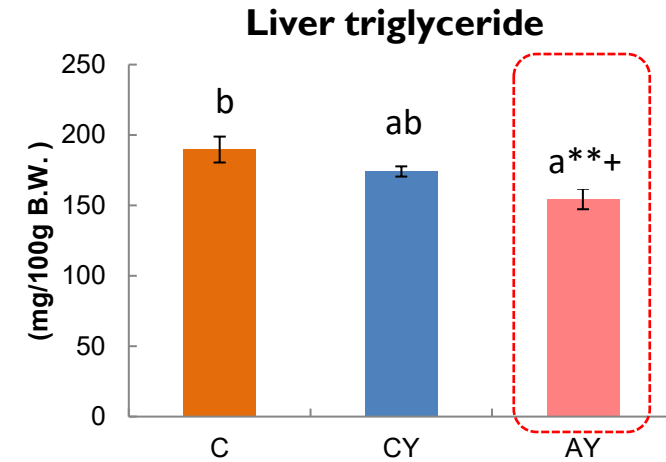
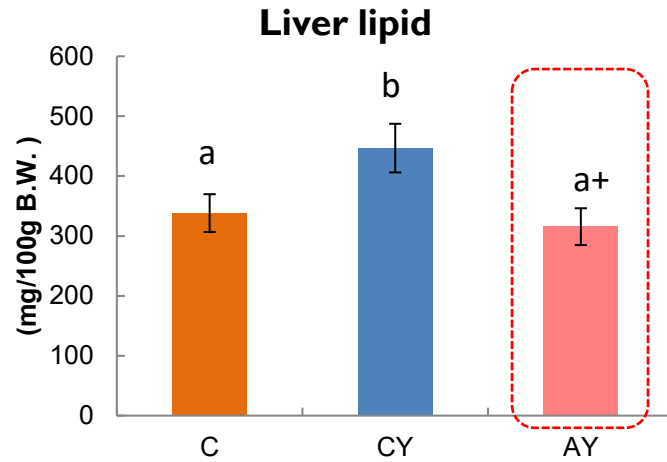
# Mouse trial 4 Results: Long-term application of Specific IgY (LP)



Note: P < 0.05 for different letters

- C: Casein high fat diet: control group
- CY: Control IgY(0.2%) mixing casein high fat diet
- AY: Specific IgY (LP) (0.2%) mixing casein high diet

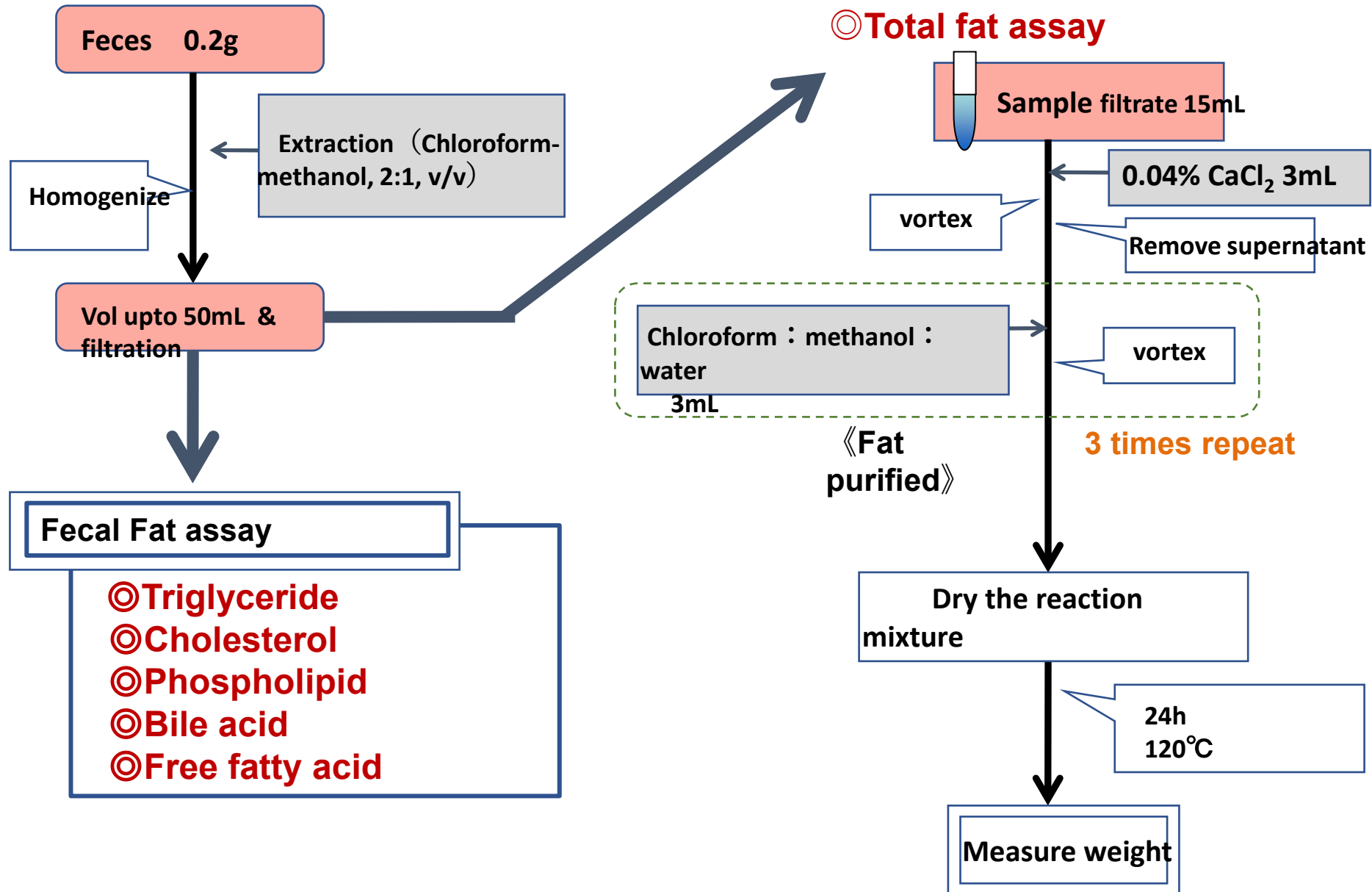
# Mouse trial 4 Results: Long-term application of Specific IgY (LP)



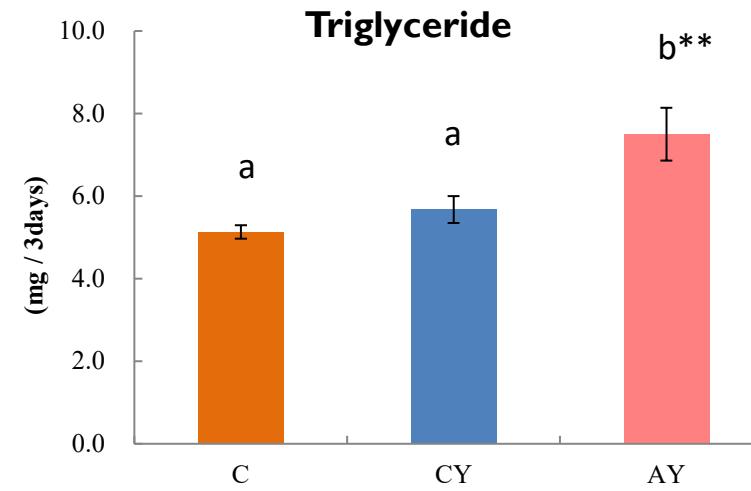
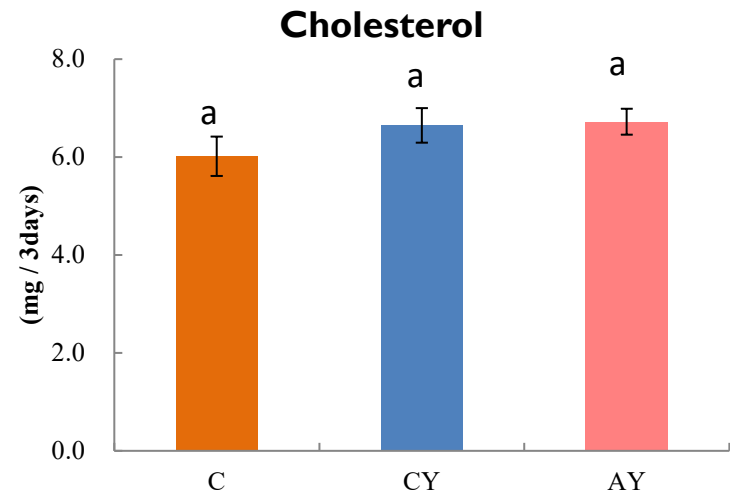
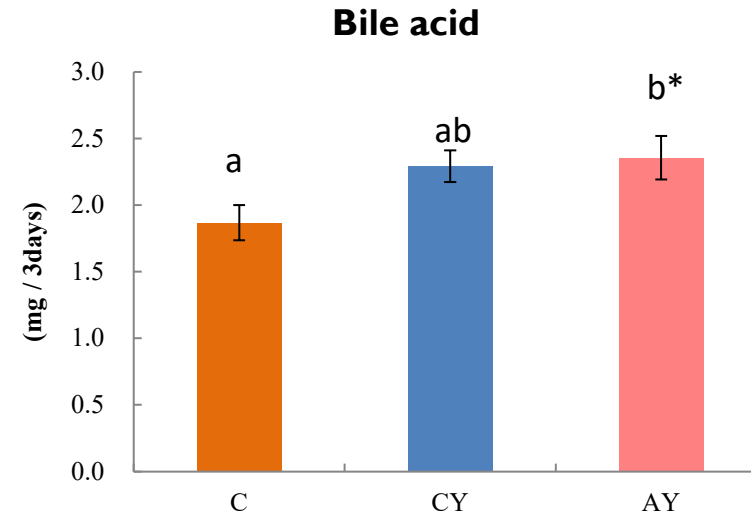
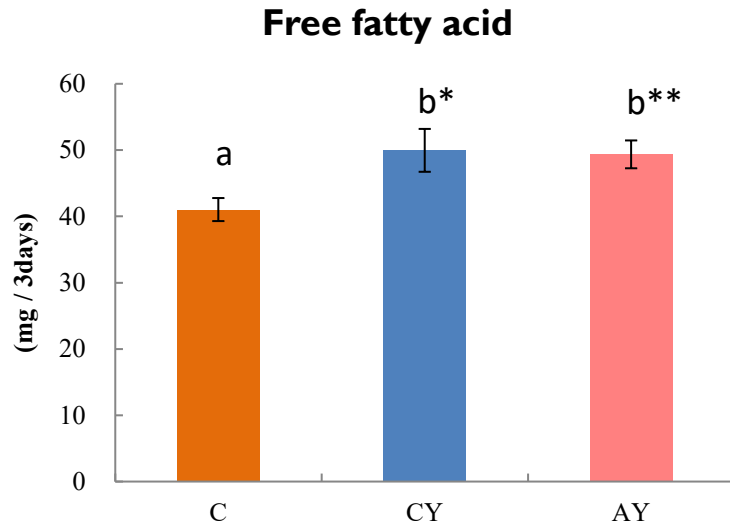
■ C: Casein high fat diet: control group  
■ CY: Control IgY(0.2%) mixing casein high fat diet  
■ AY: Specific IgY (LP) (0.2%) mixing casein high diet

Student's *t*-test: \*:P<0.05, \*\*:P<0.01, \*\*\*:P<0.001

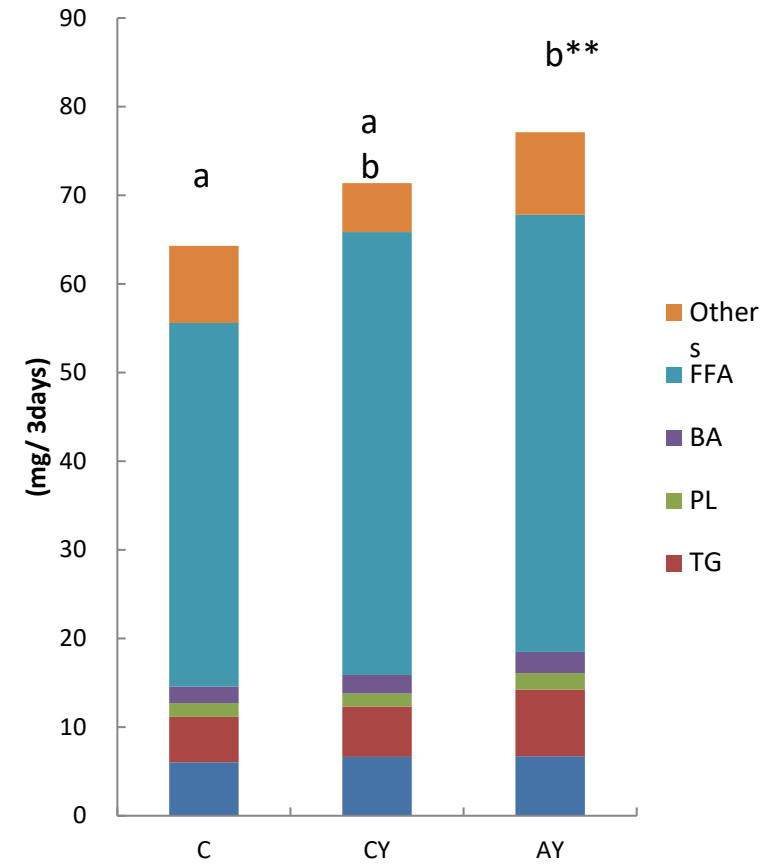
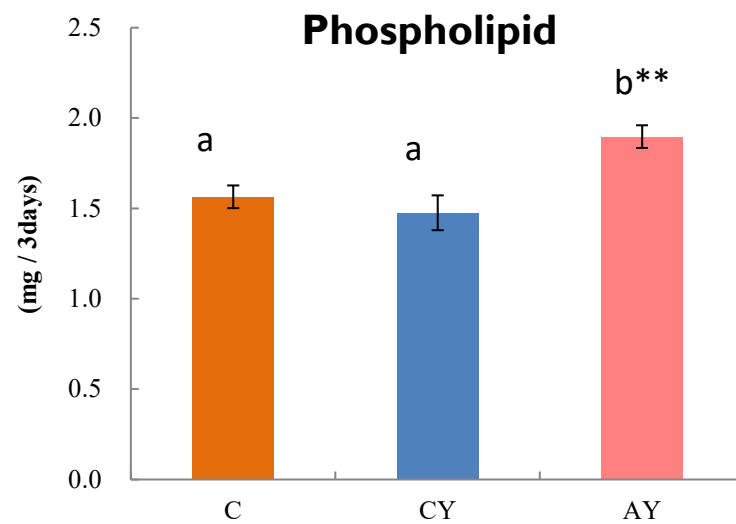
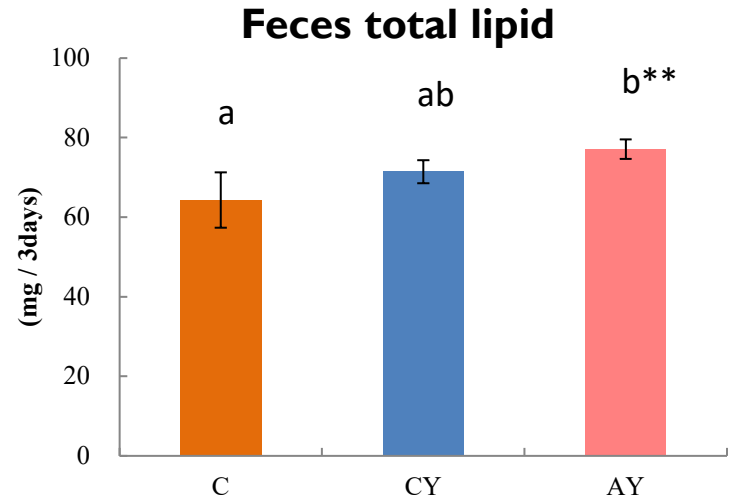
# In vivo : Lipid extraction from the mouse feces



# Mouse feces lipid analysis



# Mouse feces lipid analysis



# In-vivo mouse studies summary

- Specific IgY (LP) reduced TG, in blood of mice.
- Specific IgY (LP) reduced total epidymal white adipose tissue (WAT) of mice.
- Concentration of all lipid components in feces increased.

Clinical trial on human volunteers



# Clinical trial on volunteers

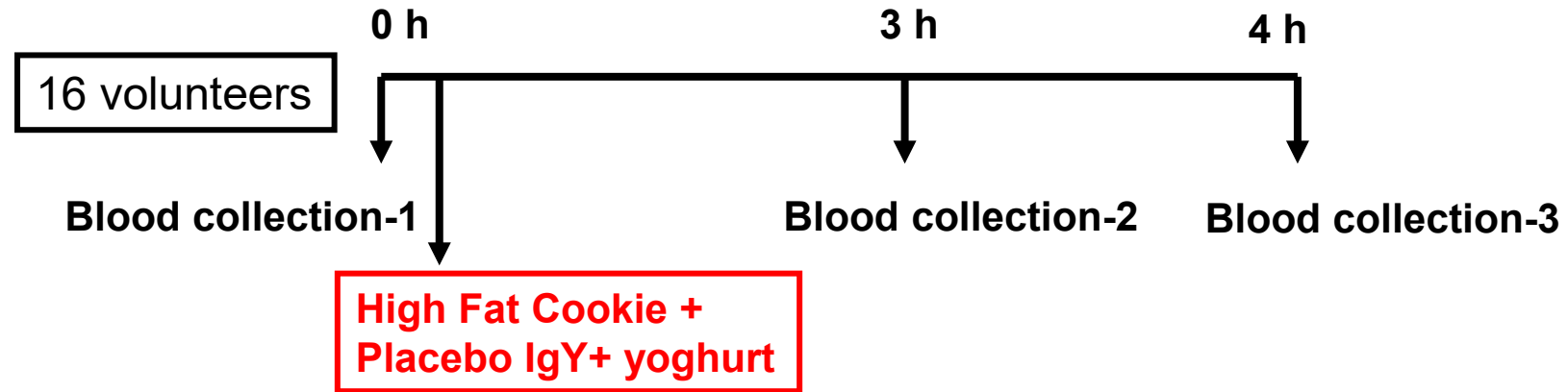
## Study Outline

Model:	Cross-over trial on the same volunteers
Subjects :	16 Volunteers (Male and Female)
Ages :	30 ~ 55 Years
Groups:	First Phase Placebo (n=16) and Second Phase Test (n=16)
Way of Administration :	High Fat Cookie+ Yoghurt+ IgY
Dosage:	0.5 g control and Specific IgY (LP)
Test Parameters:	Blood TG

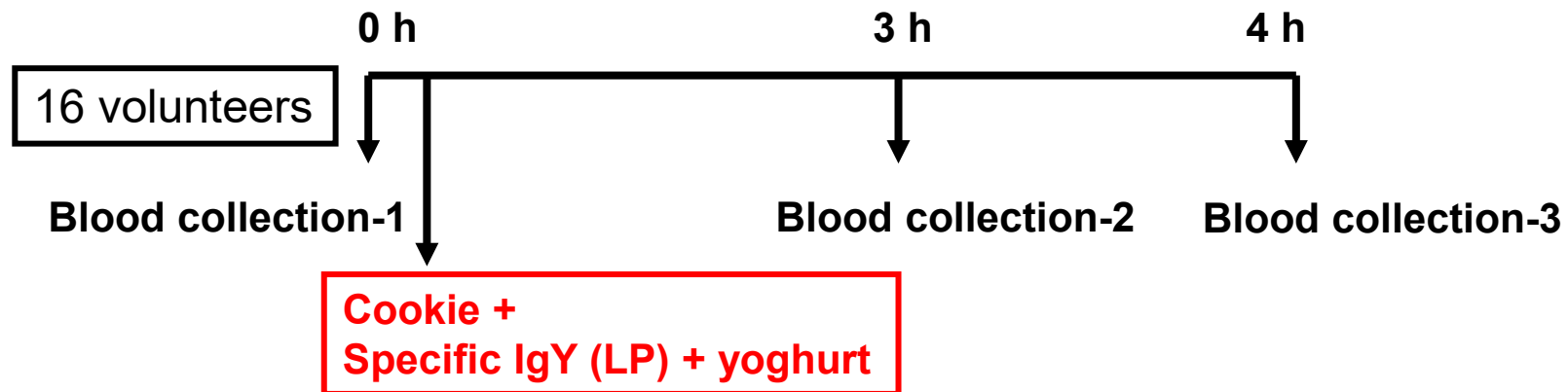
**QUESTIONNAIRES were provided to all volunteers**

# Clinical Trial Protocol

## 1. First Phase Placebo group



## 2. Second Phase Test group



## QUESTIONNAIRES – Sample Copy

試験についてのアンケート	QUESTIONNAIRE- Lipase Clinical Trial
第1回目 試験後	First Phase
A. 被験食品摂取にあたって	A. About the test food samples
Q1. 卵の臭いは気になりましたか？	Q1. Was egg smell unpleasant to you?
a. すごく気になる b. 少し気になる c. 気にならない d. 全く気にならない	a. terribly ; b. a little; c. Not; d. absolutely not
Q2. 被験食品についてQ1以外に何か気になったことがあったら自由に書いて下さい。	Q2 . Please write if you have other comments about test food samples besides Q1
Q3. 摂取直後、何か症状がありましたか？	Q3 . Were there any symptoms immediately after the intake?
a. 特に無し b. 気分が悪くなった c. 膨満感を感じた d. 食欲が増した e. 食欲がなくなった f. その他	a. None; b. unpleasant; c. sense of distension ; d. Increased appetite; e. loss of appetite lost. f. Others ( )
B. 被験食品摂取後から採血までについて	B. Time from eating until blood collecting
Q1. 採血までの時間に、何か症状がありましたか？	Q1 . Were there any symptoms during this time?
a. 特に無し b. 気分が悪くなった c. 膨満感を感じた d. 空腹感があった e. 食欲がなくなった f. お腹が痛くなった g. 便意があった h. 下痢をした i. イライラした j. その他 ( )	a. None; B. unpleasant . C. sense of distension . e feel hungry; F. loss of appetite; g. stomach ache;. H. Bowel movement . i . Diarrhea; j . irritated . k .Others ( )

## High Fat food : High Fat cookie



栄養成分1食(115g)当り	
エネルギー	592kcal
たんぱく質	8.0g
脂質	28.5g
糖質	75.0g
食物繊維	0.9~2.3g
ナトリウム	125mg

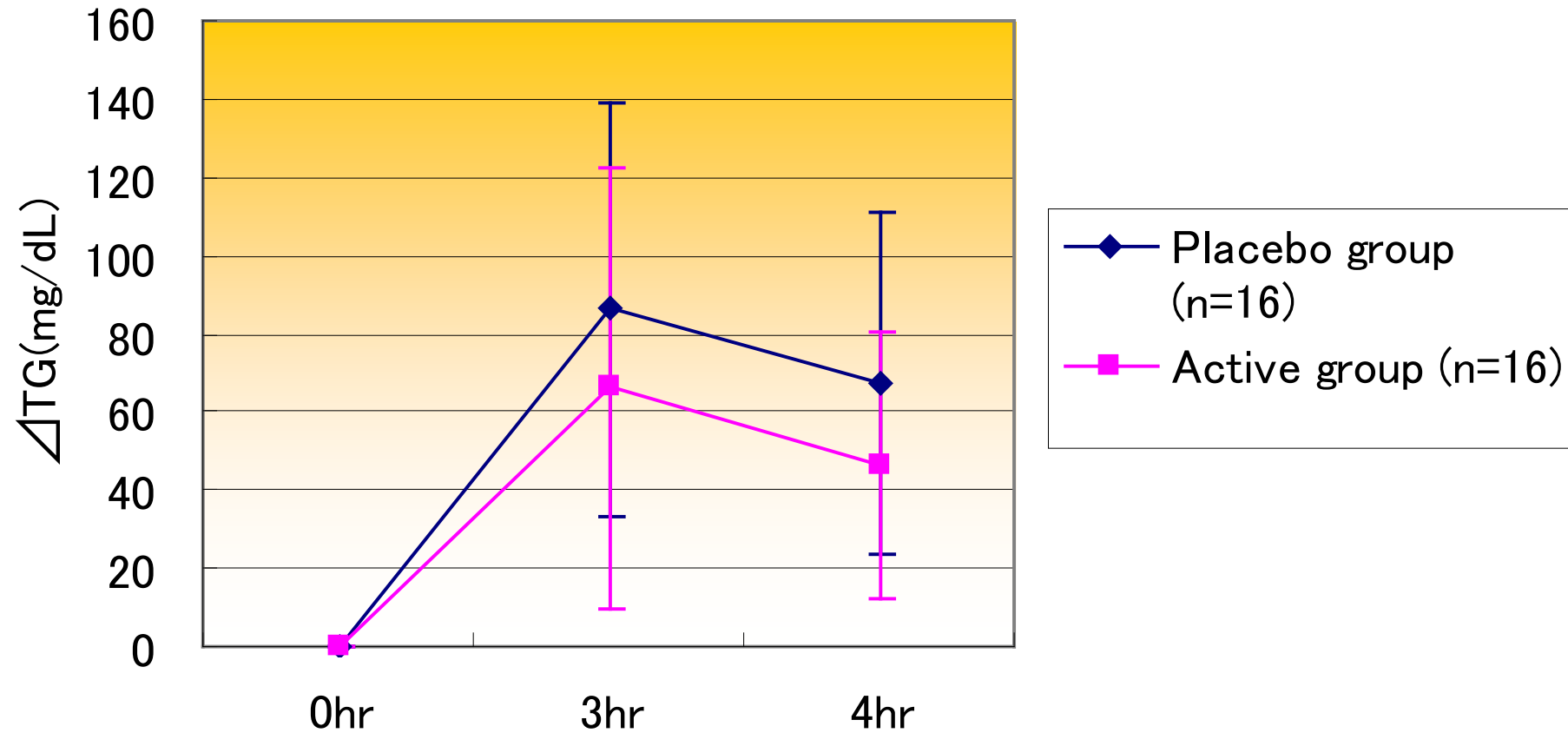
脂質の測定法は酸分解法

**30 Pieces cookie**





# Effect of Specific IgY (LP) on Blood Triglyceride (TG) in Human



# Results of questionnaire

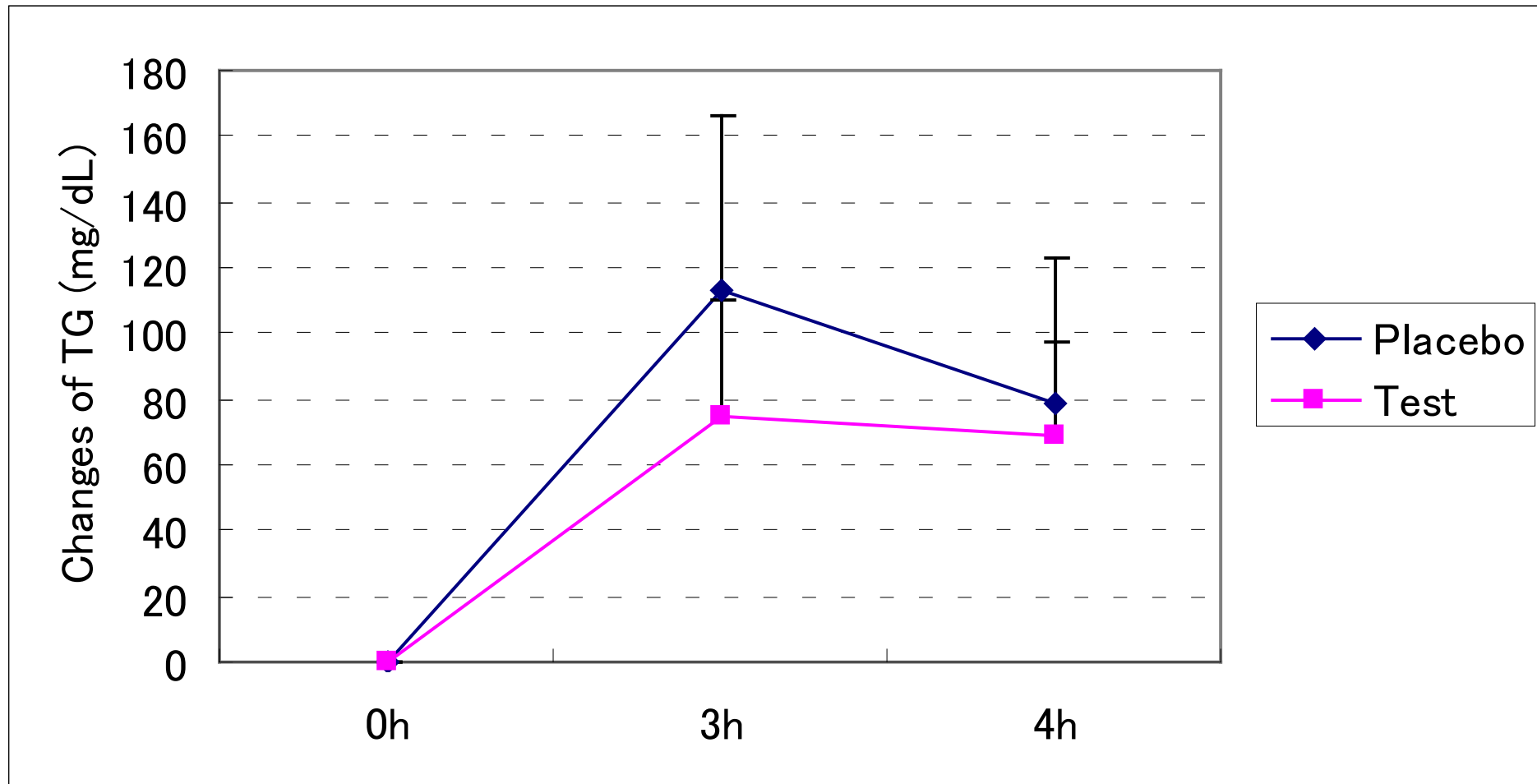
- No adverse reaction during or after Specific IgY (LP) administration
- No diarrhea or change in stool physical appearance during and after the trial



## 2<sup>nd</sup> volunteer trial

- Subject: 10 volunteers (male and female)
  - Phases: 1<sup>st</sup> phase: placebo (n = 10); 2<sup>nd</sup> phase: test (n = 7)
  - Administration: High fat cookie + Yogurt + IgY
  - Dosage: 1g Specific IgY (LP)
  - Examined parameter: Blood TG
- Questionnaire provided to each volunteer

# Result: changes of TG level in blood



# Safety of Specific IgY (LP)

## Data available

1) Single oral dose toxicity study in Rats (Saitama Lab. Drug Safety Testing Center Co. Ltd. Study No.08316) . Specific IgY (LP) at dose 2000mg/kg did not show any adverse reactions.

2) Oral toxicity study in mouse for 28 days (Japan Bioresearch center- Study No.040623) . Specific IgY (LP) 500 mg/kg/day did not show any adverse reactions.

3) Single oral dose efficacy trial in Human volunteers done 2 times. Specific IgY (LP) 500 mg and 1 ml yolk liquid administered. No adverse effect was observed.