THE IMPORTANCE OF CHOLESTEROL SYNTHESIS AND ABSORPTION MARKERS DETERMINATION IN HEALTHY SUBJECTS AND PATIENTS WITH ISCHEMIC HEART DISEASE

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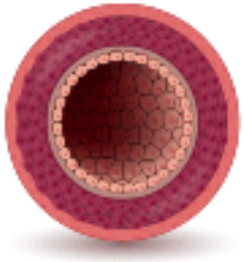
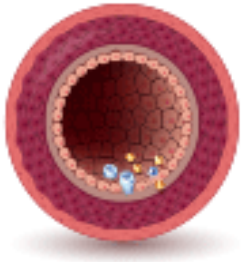
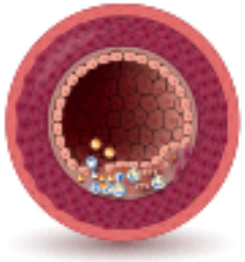
Introduction



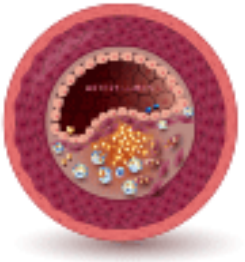
normal



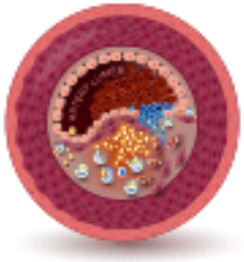
fat deposition



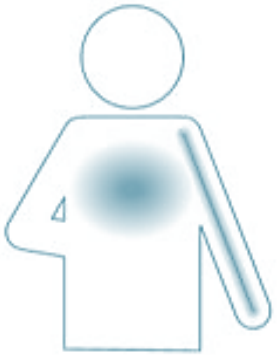
plaque buildup with macrophage infiltration



damage to plaque attracting clotting machinery

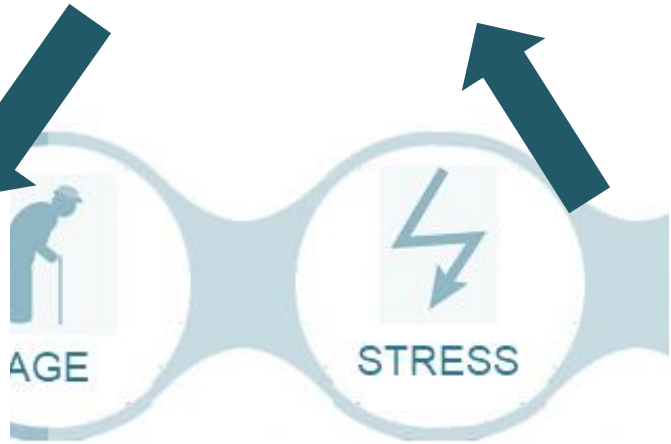


complete or near-complete arterial blockage

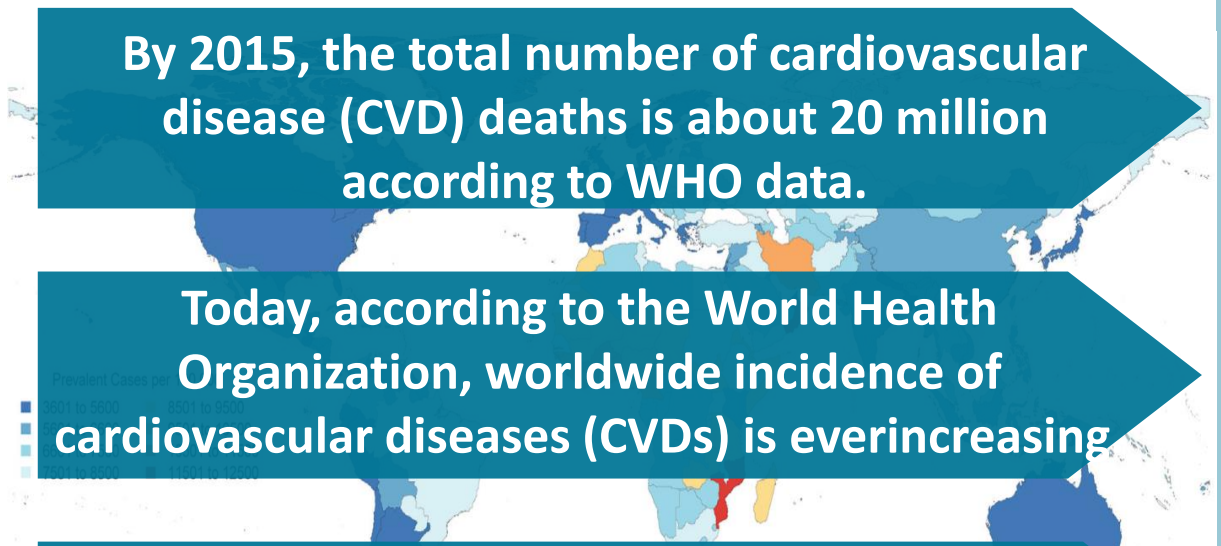


HEART ATTACK

CEREBROVASCULAR INSULT



Epidemiology of coronary artery disease (CAD)



By 2015, the total number of cardiovascular disease (CVD) deaths is about 20 million according to WHO data.

Today, according to the World Health Organization, worldwide incidence of cardiovascular diseases (CVDs) is everincreasing

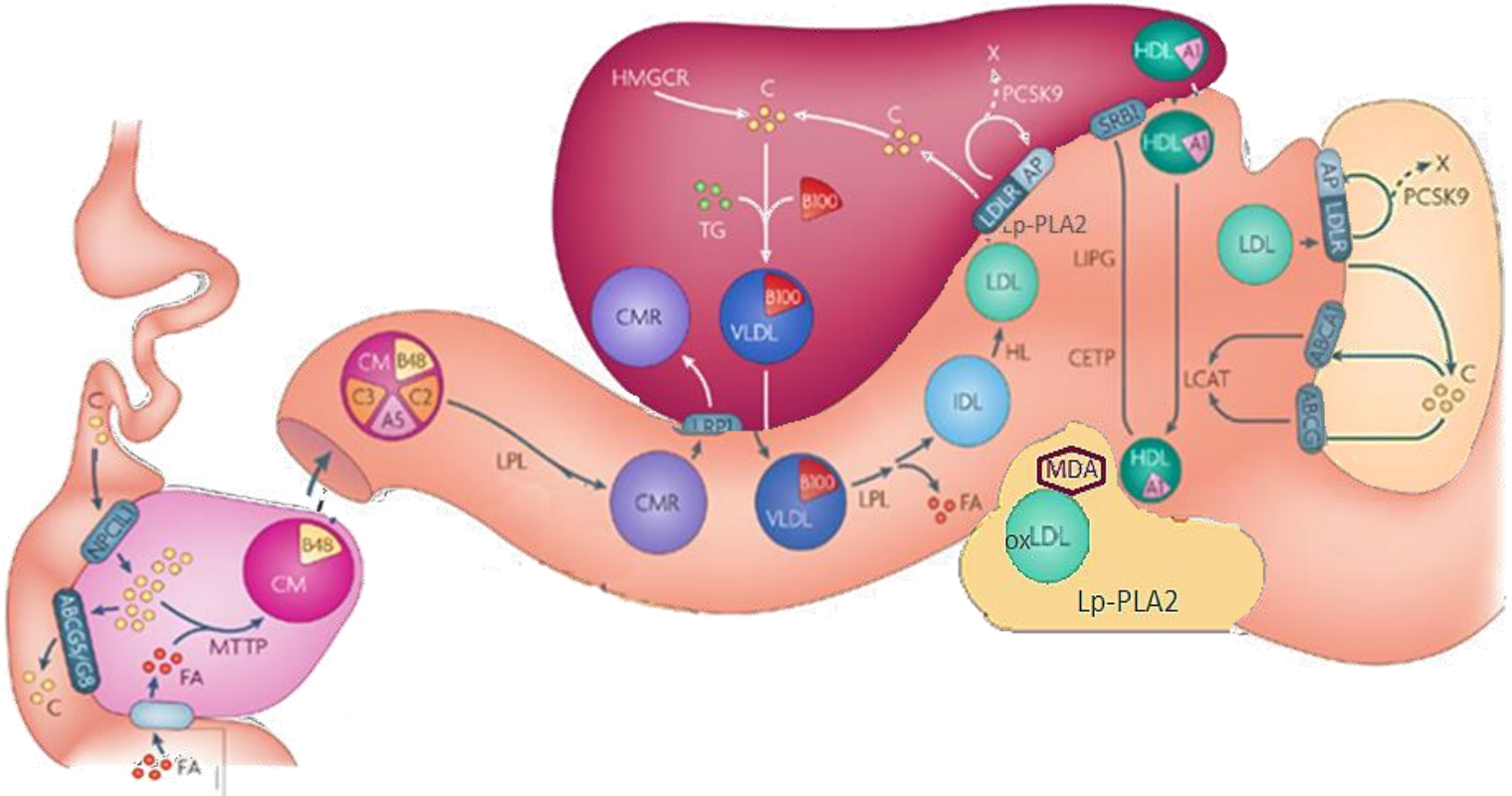
American Heart Association estimates that in 2020 approx. every 42 seconds, one American will have an MI.

Akira Endo - Statins

4S – Merck & Co.

Cholesterol homeostasis

Cholesterol homeostasis represents the balance between cholesterol absorption and synthesis, and also its distribution among lipoprotein particles.



Cholesterol homeostasis

HMG CoA

HMG-CoA reductase

Mevalonate

Farnesyl pyrophos phate

Squalene synthase

Squalene

Lanosterol

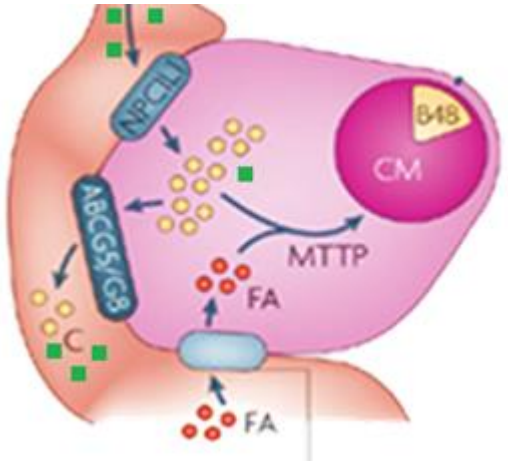
Kandutsch-Russel pathway

Lathosterol

Desmosterol

Bloch pathway

Cholesterol



- Campesterol
- Stigmasterol
- β-sithosterol

The importance of analizying cholesterol synthesis and absorption markers

- Key to understanding cholesterol homeostasis lies in monitoring the efficiency of cholesterol synthesis and absorption.
- Different physiological and pathological conditions can lead to a change in cholesterol homeostasis, and subsequently the development of dyslipidemia and atherosclerosis.
- Evaluation of noncholesterol sterols (NCSs) as synthesis and absorption markers combined with lipoprotein particles quality may indicate the dyslipidemia early development.
- Prediction of statin therapy response.

Aim

- The aim of this study was firstly to examine if different patterns of cholesterol metabolism exist in three groups of participants:
  - healthy individuals
  - CAD patients on statin treatment
  - CAD patients receiving no statin treatment

- Secondly, the goal was to estimate the efficiency of cholesterol synthesis and absorption by determining the synthesis/absorption markers ratio, as well as its association with traditional lipid status parameters.

- Finaly, we investigated the potential use of aforementioned markers for optimizing the treatment of CVD.

Material and method

Material

79 CAD patients

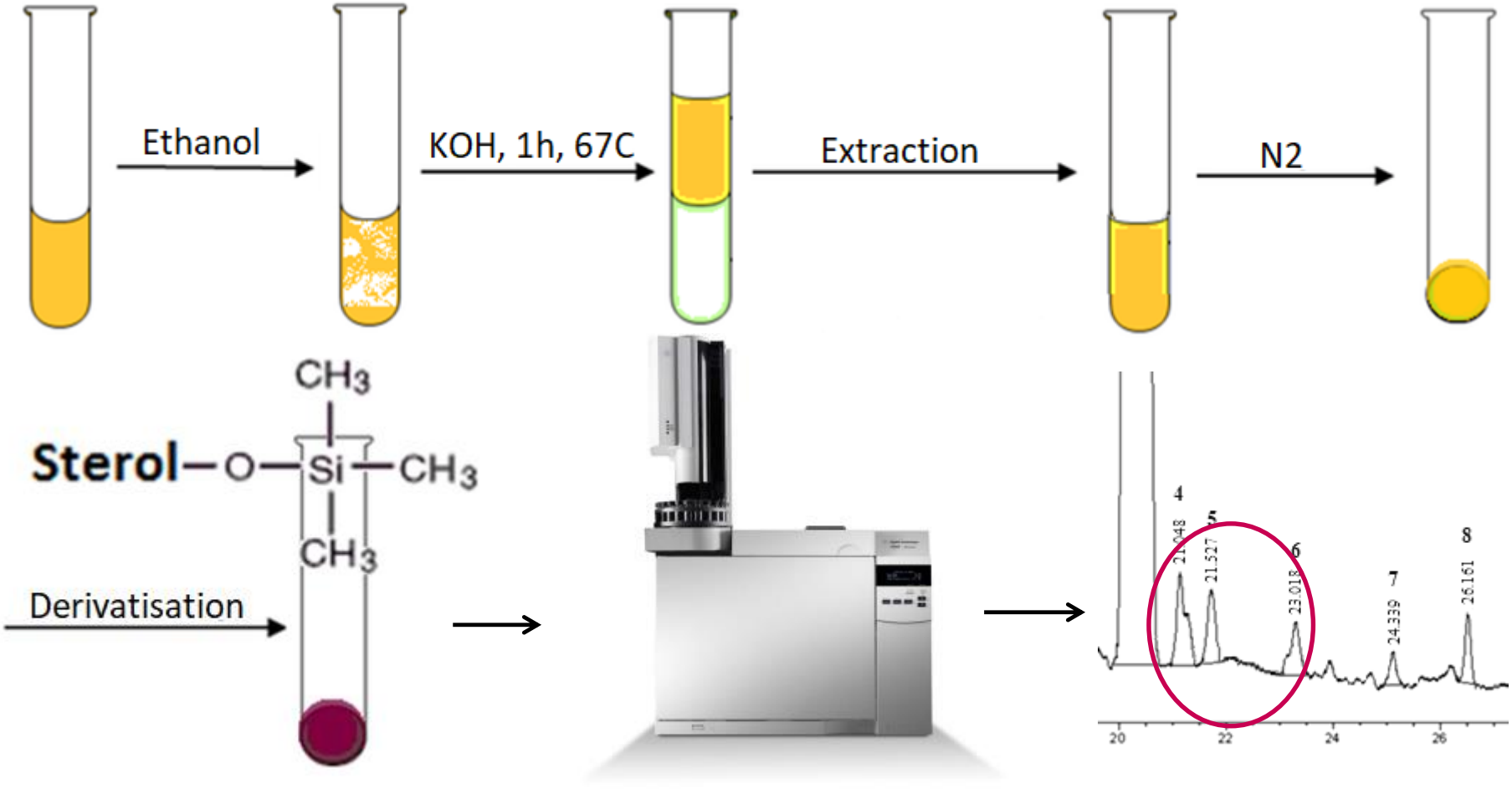
32 patients on statin therapy (CAD Th+)

47 CAD statin-naive patients (CAD Th+)

31 healthy subjects

IMT a. carotis < 1mm
DTA ≤ 90mm Hg i/ili
STA ≤ 140mm Hg
UTC< 5.16 mmol/L
LDL-C < 3.35 mmol/L
HDL-C > 1.03 mmol/L
TG < 1.70 mmol/L
glucose ≤ 6.1 mmol/L
Without statin
Without antihypertensive drugs

Method for NCSs determination



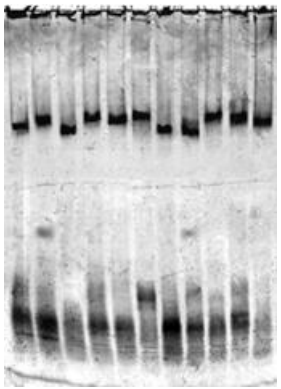
Method

Biochemical parameters

spectrophotometric

LDL subclasses

GGE



Results

- Values for demographic, anthropometric data and lipid status parameters

Parameters	CG (N=31)	CAD Th+ (N=31)	CAD Th- (N=47)	p value
Age, years*	43.2±10.34	59.5±9.96	60.6±12.39	**<0.001[a]** **<0.001[b]** 0.910[c]
Gender, (m/f)*	13/18	15/16	22/25	0.610[a] 0.672[b] 0.891[c]
BMI, kg/m^2	24.2±4.05	27.3±3.80	25.9±3.67	**<0.05[a]** 0.145[b] 0.137[c]
Waist-to-hip ratio	0.82±0.078	0.92±0.106	0.93±0.091	**<0.001[a]** **<0.001[b]** 0.933[c]
TC, mmol/L	4.6±0.63	5.0±1.08	4.9±1.59	0.217[a] 0.230[b] 0.894[c]
TG mmol/L[§]	0.97 (0.83-1.14)	1.52 (1.32-1.75)	1.45 (1.26-1.68)	**<0.001[a]** **<0.001[b]** 0.702[c]
LDL-C, mmol/L	2.7±0.49	3.0±0.96	3.1±1.25	0.260[a] 0.129[b] 0.696[c]
HDL-C, mmol/L	1.7±0.46	1.2±0.31	1.1±0.33	**<0.001[a]** **<0.001[b]** 0.150[c]

Validation of methods for NCSs determination

Desmosterol
1,394-22,305 µmol/L

Lathosterol
1,587-19,049 µmol/L

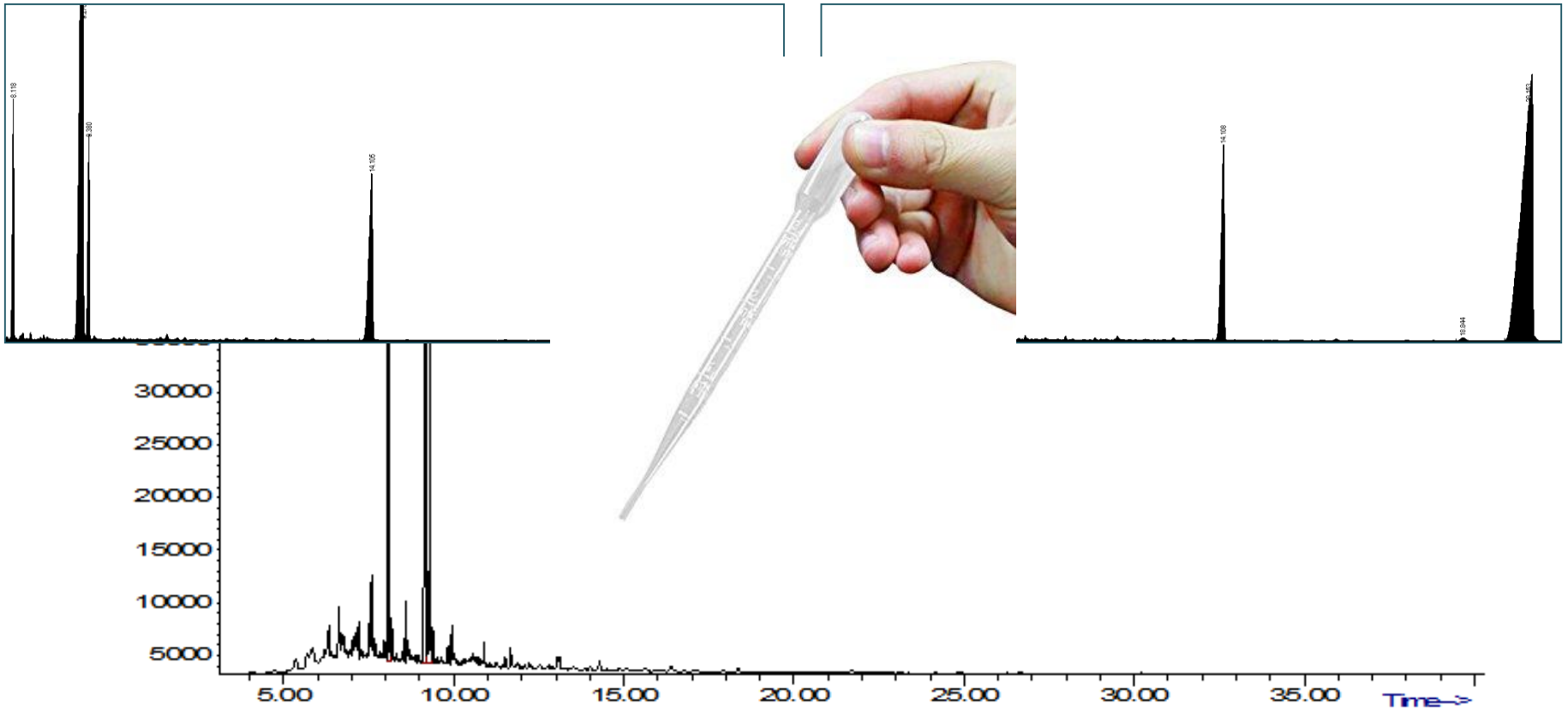
Campesterol
1,846-44,309 µmol/L

Stigmasterol
0,981-15,694 µmol/L

β-sitosterol
1,674-26,790 µmol/L

Linearity	LOD i LOQ	Accuracy	Precision
	STANDARDS 0,185-0,370 µmol/L 0,744-1,209 µmol/L	**PLASMA** *Recovery* 93-108%	**PLASMA** KV_{us}=2,75-9,55 KV_{is}=5,80-7,75%
	MATRIX 0,401-0,591 µmol/L 1,243-1,952 µmol/L	**SERUM** *Recovery* 83-127%	**SERUM** KV_{us}=3,10-5,72 % KV_{is}=3,05-10,97%.
	S/N=3 i S/N=10 ✓	**25%** ⏻	**15%** ✓

Validation of NCSs quanfication method



Gojkovic T. et al. Preanalytical and analytical challenges in gas chromatographic determination of cholesterol synthesis and absorption markers. Clin Chim Acta. 2018; 1; 478: 74-81.

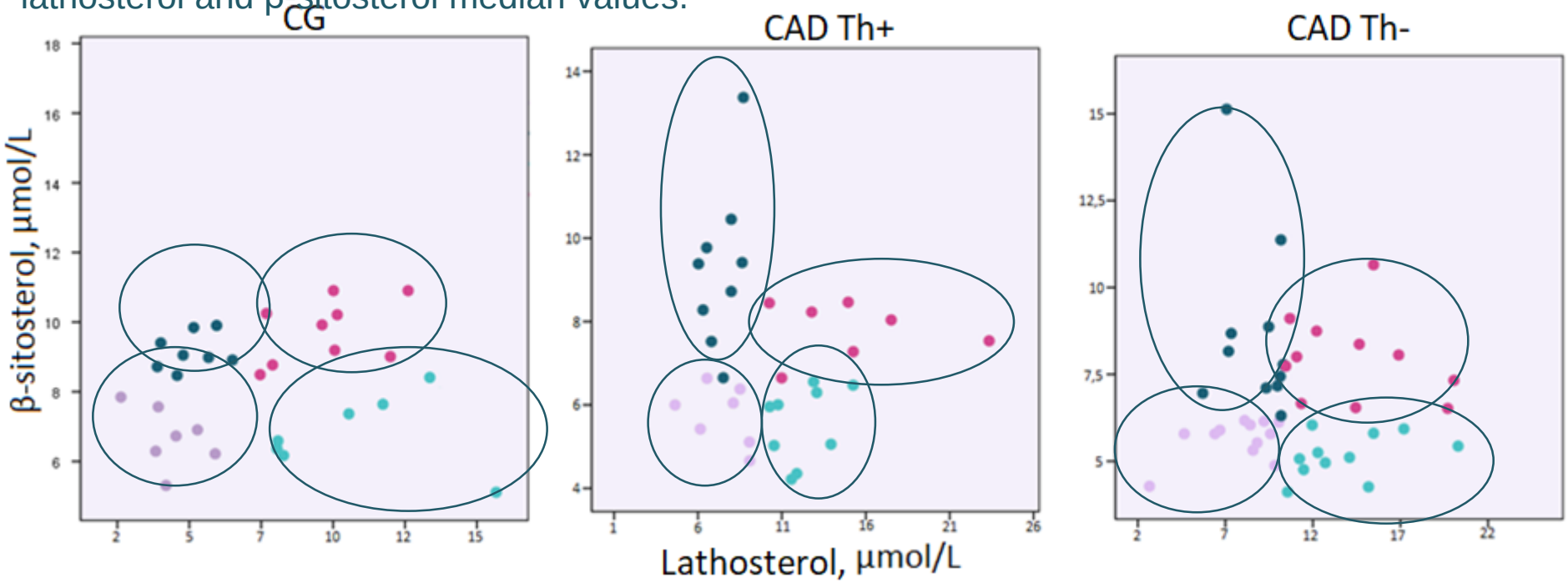
NCSs concentration

In order to assess the efficiency of cholesterol homeostasis - desmosterol/β-sitosterol and lathosterol/β-sitosterol ratios

Parameter	*CG (N=31)*	*CAD Th+ (N=32)*	*CAD Th- (N=47)*	*p*
Relative values (plasma sterol markers/cholesterol) mmol/mol				
Desmosterol/UH	2,03 (1,84-2,25)	2,36 (2,07-2,71)	2,95 (2,56-3,39)	0,138[a] **<0,001[b] <0,05[c]**
Lathosterol/UH	1,53 (1,30-1,80)	2,06 (1,74-2,43)	2,18 (1,97-2,42)	**<0,01[a] <0,01[b]** 0,540[c]
Campesterol/UH	3,95 (3,47-4,50)	3,12 (2,74-3,54)	3,39 (3,02-3,80)	**<0,05[a]** 0,138[b] 0,328[c]
Stigmasterol/UH	0,89 (0,57-1,39)	0,46 (0,38-0,56)	0,47 (0,41-0,54)	**<0,001[a] <0,001[b]** 0,907[c]
β-sitosterol/UH	1,78 (1,61-1,97)	1,41 (1,27-1,55)	1,40 (1,26-1,56)	**<0,05[a] <0,05[b]** 0,940[c]
Odnos markera sinteze i apsorpcije				
Dezmosterol/ β-sitosterol	1,14 (1,01-1,28)	1,64 (1,38-1,96)	2,09 (1,79-2,44)	**<0,05[a] <0,001[a] <0,05[c]**
Latosterol/ β-sitosterol	0,86 (0,68-1,07)	1,46 (1,21-1,75)	1,58 (1,37-1,81)	**<0,001[a] <0,001[b]** 0,514[c]

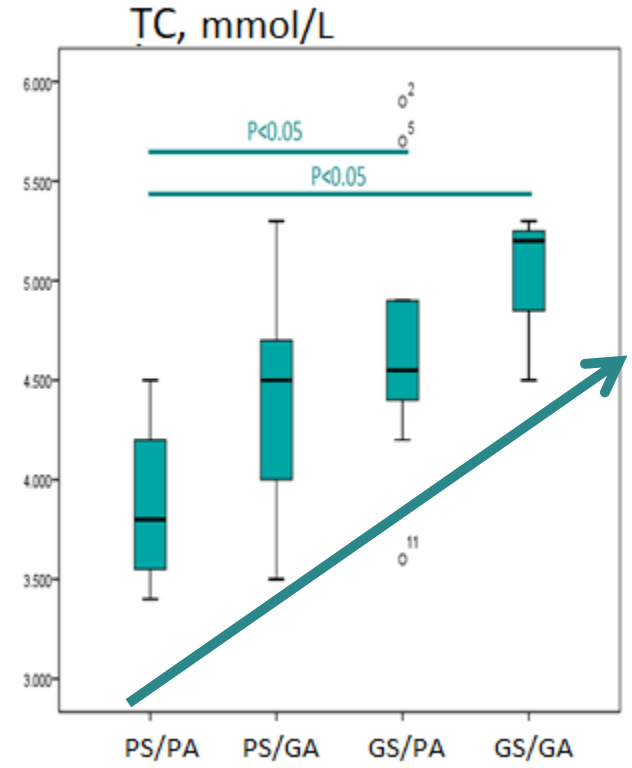
Patterns of cholesterol homeostasis

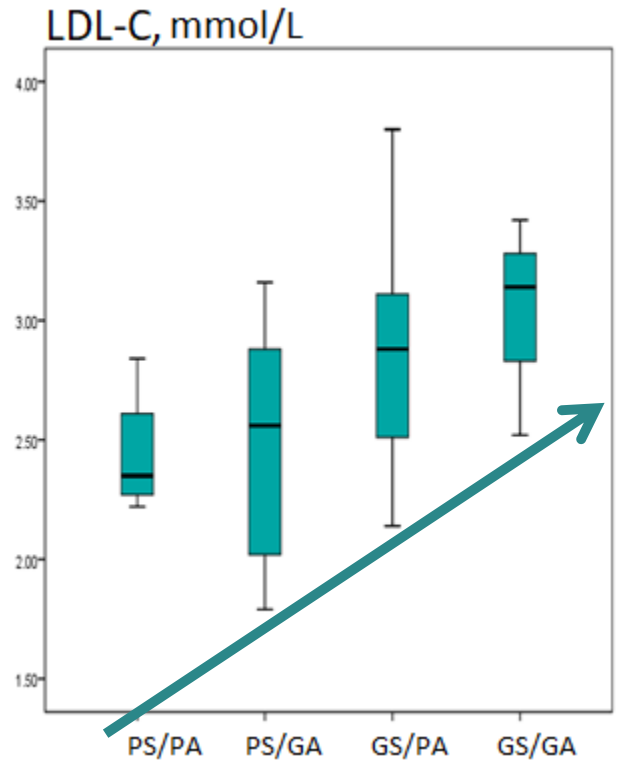
In order to further explore the relationship of cholesterol synthesis and absorption with lipid profile parameters, we have divided each group of participants into four subgroups, according to lathosterol and β-sitosterol median values.

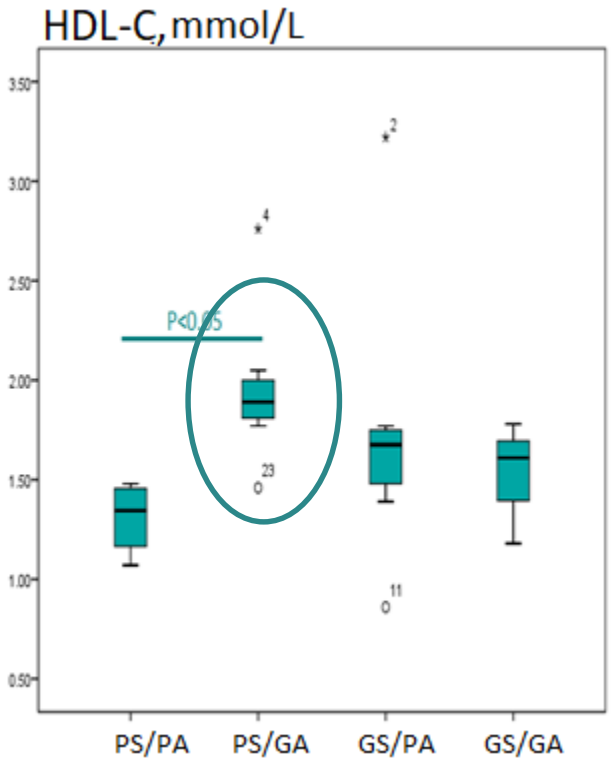


Gojkovic T. et al. Can non-cholesterol sterols and lipoprotein subclasses distribution predict different patterns of cholesterol metabolism and statin therapy response?. Clin Chem Lab Med. 2017; 55: 447-57.

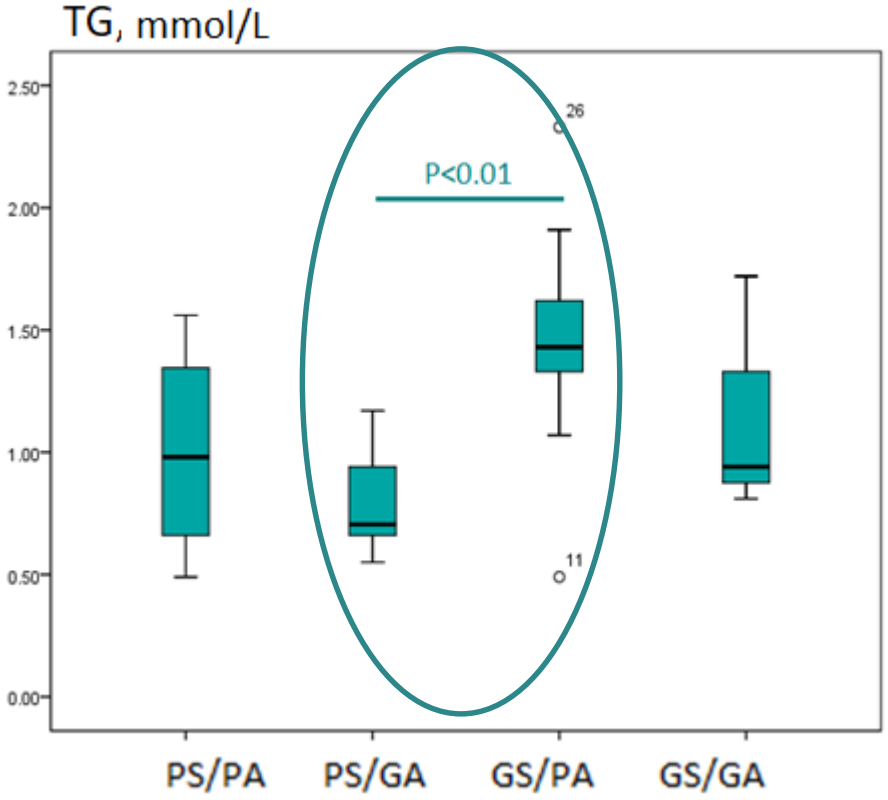
Patterns of cholesterol homeostasis in CG

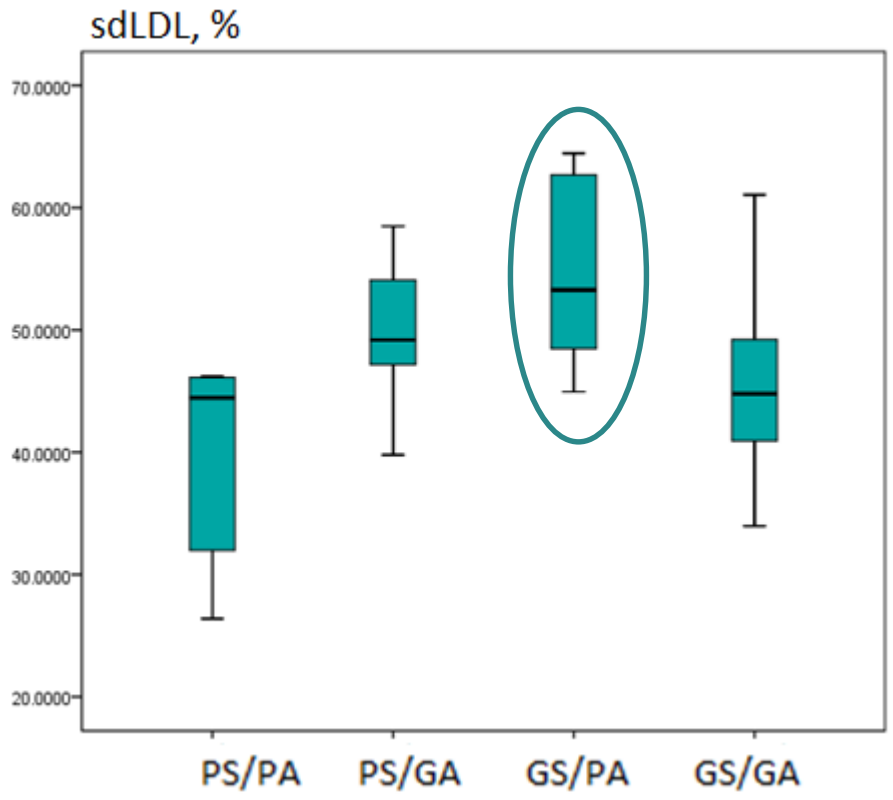






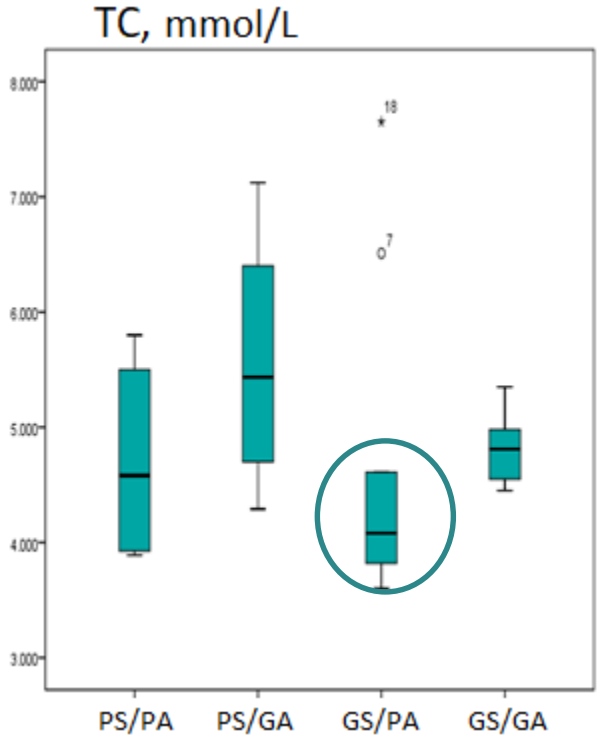
Patterns of cholesterol homeostasis in CG

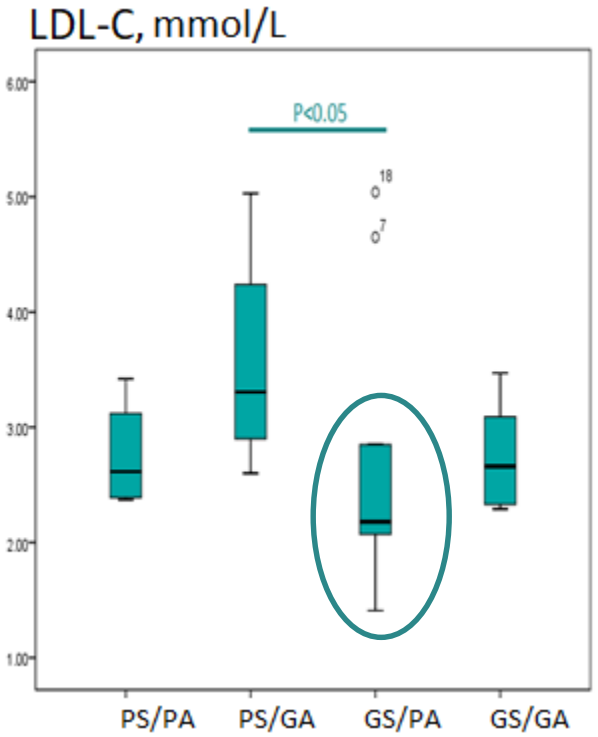


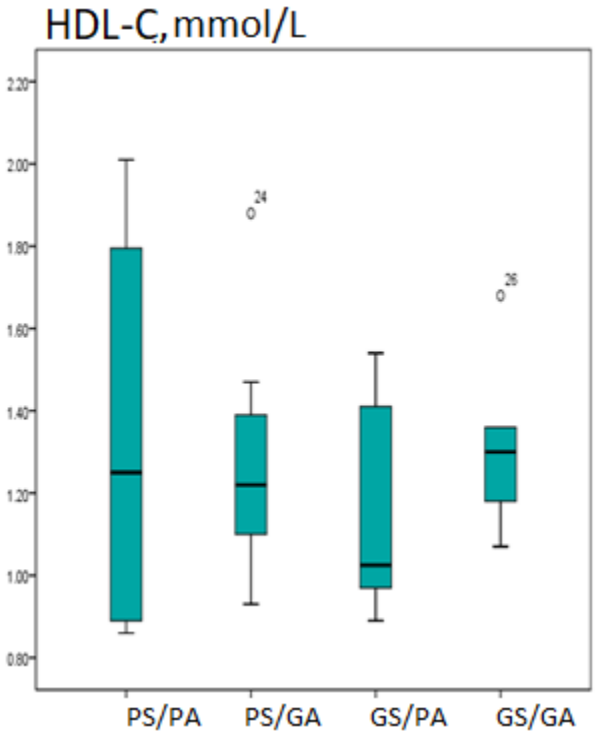


Dezmosterol/β-sitosterol

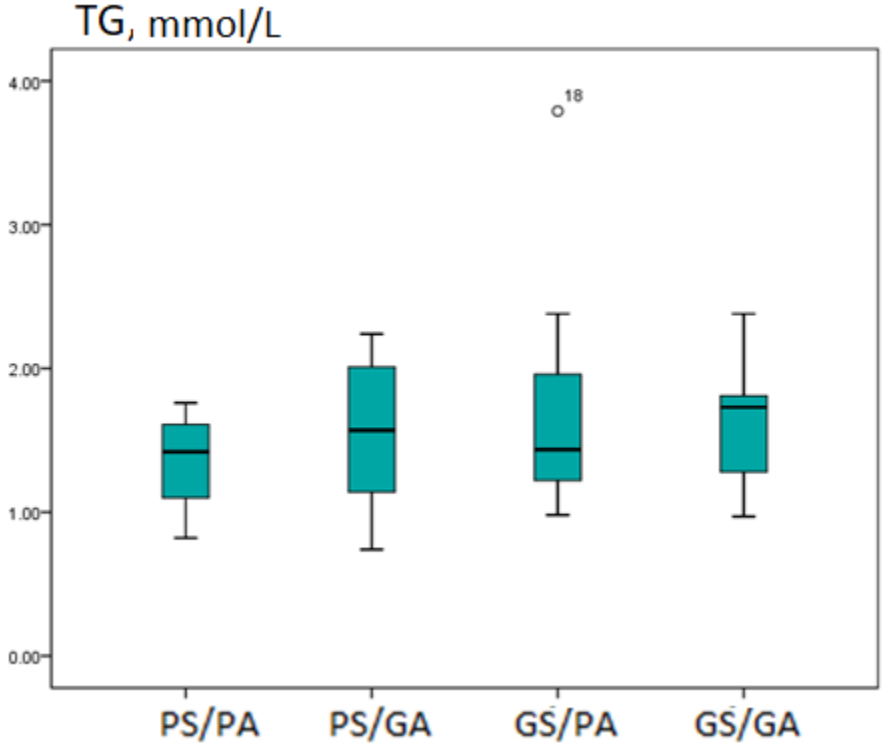
Patterns of cholesterol homeostasis in CAD Th+ group

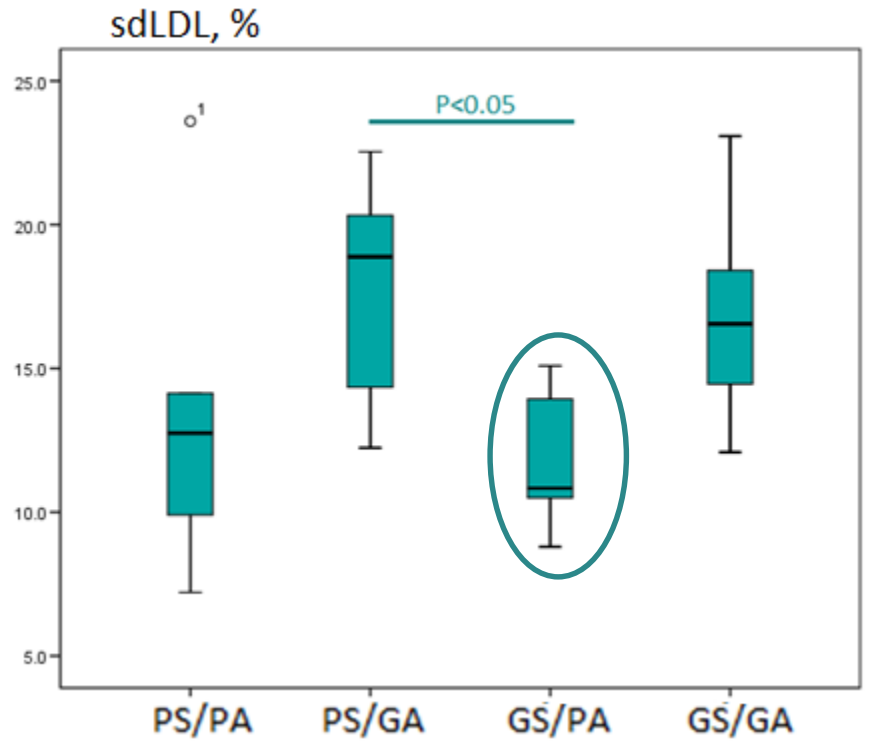






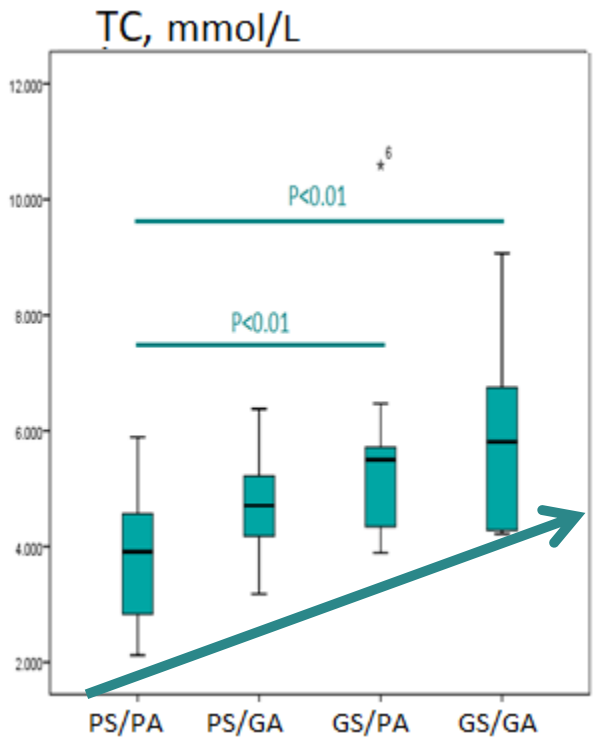
Patterns of cholesterol homeostasis in CAD Th+ group

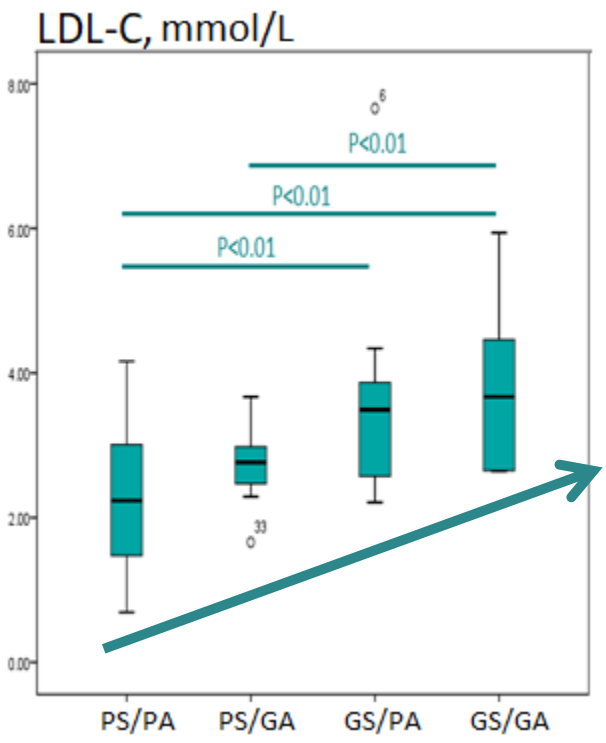


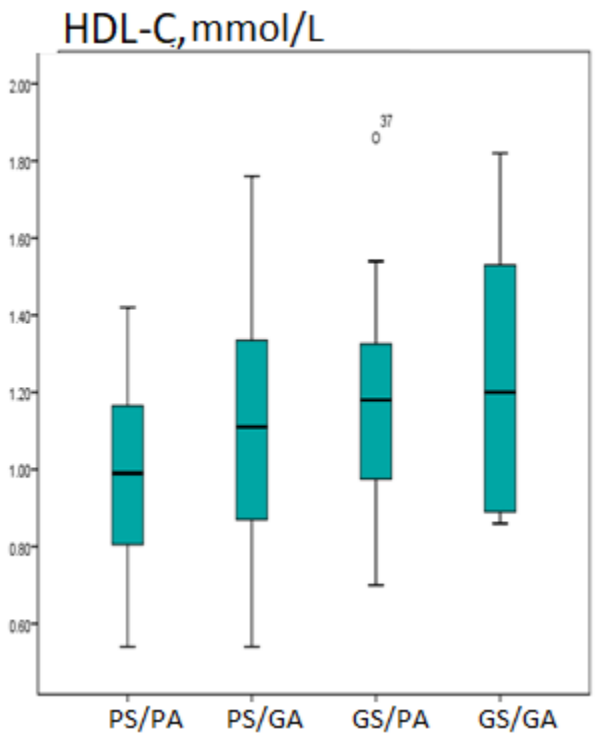


Dezmosterol/β-sitosterol

Patterns of cholesterol homeostasis in CAD Th- group







Conclusion

All results of the *in-house* validation procedures proved to be useful for minimizing the preanalytical and analytical variations, as shown in the validation results. These results promise the future transferability of the aforementioned method between different laboratories as well as the reproducibility of the results.

Based on NCSs concentrations, it is possible to determinate cholesterol synthesis and absorption patterns and identify individuals with high risk for the CVD development and progression. In addition, determinations of cholesterol homeostasis patterns are potentially useful tool for predicting the individual propensity towards hypolipidemic therapy response.

THANK YOU FOR YOUR ATTENTION