# HOMOCYSTEINE LEVELS IN HIGH RISK AND APPARENTLY HEALTHY INDIVIDUALS THE CYPRUS EXPERIENCE

Charilaou Charis (1) MSc (FIBMS), Agathaggelou Petros (2) MD

Biomedical Labs
Tel: (+3572) 758858 Fax: (+3572) 755252
22 Eras 1061, Nicosia
Cyprus

Evangelistria Medical Centre
1 Prodromou & Giorgalla St.
1095, Nicosia
Cyprus

## INTRODUCTION:

Homocysteine (HCY) is a Thiol-containing aminoacid produced by the intracellular demethylation of methionine. Homocysteine is exported into plasma where it circulates, mostly in oxidized form bound to plasma proteins as a protein-HCY mixed disulfide with albumin [1-4]. Total homocysteine (tHCY) represents the sum of all HCY species found in plasma or serum (free plus protein bound).

Homocysteine is metabolized to either cysteine or methionine. In the vitamin B6 dependent transulfuration pathway, homocysteine is irreversibly catabolized to cysteine. A major part of homocysteine is remethylated to methionine. A methyl group is transferred from 5 - methyltetrahydrofolate (MTHF) to homocysteine utilizing vitamin B12-dependent methionine synthase. The creation of MTHF requires another B vitamin and the enzyme 5,10 – methylenetetrahydrofolate reductase (MTHFR). Molecular studies have shown that the most common genetic cause of hyperhomocysteinemia is an aminoacid substitution that converts 5,10 – methylenetetrahydrofolate to 5 – methylenehydrofolate, the methyl donor in the conversion of homocysteine to methionine[5]. The mutant enzyme exhibits reduced activity. Homozygous mutation in MTFHR is associated with a threefold increase risk for premature cardiovascular disease[6]. The frequency of this genetic variant in the homozygous state in Cypriot population is relatively high (17.8%), compared with French, Canadian, European, Middle Eastern and Japanese populations (12% - 15%) and significantly higher than the frequencies reported in Finnish (5.4%), Dutch (5.2%) and Black populations (1.4%)[7,8].

Observational data have shown that vitamin B12 and folic acid deficiencies enhance the metabolic and genetic predisposition to elevate plasma homocysteine levels.

A large number of clinical and epidemiological studies have shown that elevated serum or plasma concentration of homocysteine is an independent risk factor of cardiovascular disease (CVD).

In the present study, we tested, the homocysteine levels in a group of high-risk patients (CVD) and in the group of apparently healthy individuals attempting to determine the clinical correlation of high homocysteine levels in these two groups in the Greek-Cypriot population of Cyprus.

## DISCUSSION

A concentration-dependent correlation between homocysteine and the presence of atherothrombosis has been seen in coronary cerebral and peripheral vascular diseases. In addition to the prevalence of vascular disease, concentration has been associated with increased risk of acute events such as myocardial infarction and stroke. Hyperhomocysteinemia has been demonstrated to independently contribute in overall, all cause mortality in patients with non-coronary artery diseases. In our attempt to correlate hyperhomocysteinemia and presence of cardiovascular disease in Greek-Cypriot population, we randomly used 117 apparently healthy individuals 77 males and 40 females, ages between 30-65 years old. The mean HCY levels for whole of the population was $9.3 \pm 2.9$ nmol/l and the range between 5.3 – 15.1 nmol/l.

From the data seen in figure 3 one may depict that 21.4% of CVD patients who had more than 2 major risk factors for CAD had HCY > 15 nmol/l. A 28.5% of CVD patients has also demonstrated upper limit of normal HCY (13-14 nmol/l), in comparison with apparently healthy individuals, who demonstrated a much lower percentage (6.83%) in the upper limit of normal. Remarkably, a total 49.9% of the CVD studied patients have shown higher HCY plasma levels (see figure 3).

An interesting observation is that none of the 40 females involved in the study exceeded HCY levels more than 13nmol/l. Based on the above observation, further studies should be designed explain why CVD patients with increased HCY levels were seen in male population than the female group.

## CONCLUSION

The conclusive data of this study suggests a clinical correlation of the cardiovascular disease (CVD) with high Homocysteine levels. Considering the known risk factors for coronary artery disease, the measurement of HCY levels as an independent Biological marker could be useful adjuvant diagnostic tool for screening healthy and susceptible individuals for cardiovascular syndromes in the community.

# REFERENCES


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# MATERIALS AND METHODS

Serum samples from 117 apparently healthy individuals and 14 CVDs patients were tested for homocysteine levels.

The IMx homocysteine assay is based on the Fluorescence Polarization Immunoassay (FPIA) technology.

Bound homocysteine (oxidized form) is reduced to free homocysteine and the free HCY is enzymatically converted to S-adenosyl-L-homocysteine (SAH) as outlined below:

**Reduction:** Homocysteine and mixed disulfide and protein-bound forms of HCY in the sample are reduced to form free HCY by the use of dithiothreitol (DTT).

HCY-SS-HCY (Homocysteine) DTT
R1-SS-HCY (R1 = thiol residue) → HCY
Protein-SS-HCY

**Enzymatic Conversion:** Total free HCY is converted to S-adenosyl-L-homocysteine (SAH) by the use of SAH hydrolase and excess adenosine.

SAH-Hydrolase
HCY + Adenosine → SAH

Under physiological condition, SAH Hydrolase converts SAH to homocysteine. Excess adrenosine in the Pretreatment Solution drives the conversion of HCY to SAH Hydrolase.

# RESULTS

Homocysteine levels were measured in a total of 117 apparently healthy individuals, 77 males and 40 females. The normal levels for homocysteine for our study was 5-15 nmol/l. Our findings show that 5 healthy male adults had HCY >15nmol/l (4.27%), 8 male adults had HCY levels, between 13-14 nmol/l (6.83%) and 104 adults, 40 females and 64 males had HCY levels <13 nmol/l (88.9%) Fig.1.

The homocysteine levels in the group of CVD patients, all males were as follow: Three patients had HCY levels >15 nmol/l (21.4%). Four had HCY levels between 13-14 nmol/l (28.5%) and seven had HCY levels <13 nmol/l (50%) Fig.2. One patient with a known chronic renal problem had HCY level > 15 nmol/l. The mean for HCY concentration in the CVD patients was significantly higher than the mean of the apparently healthy individuals ($p < 0.005$) Fig.3.

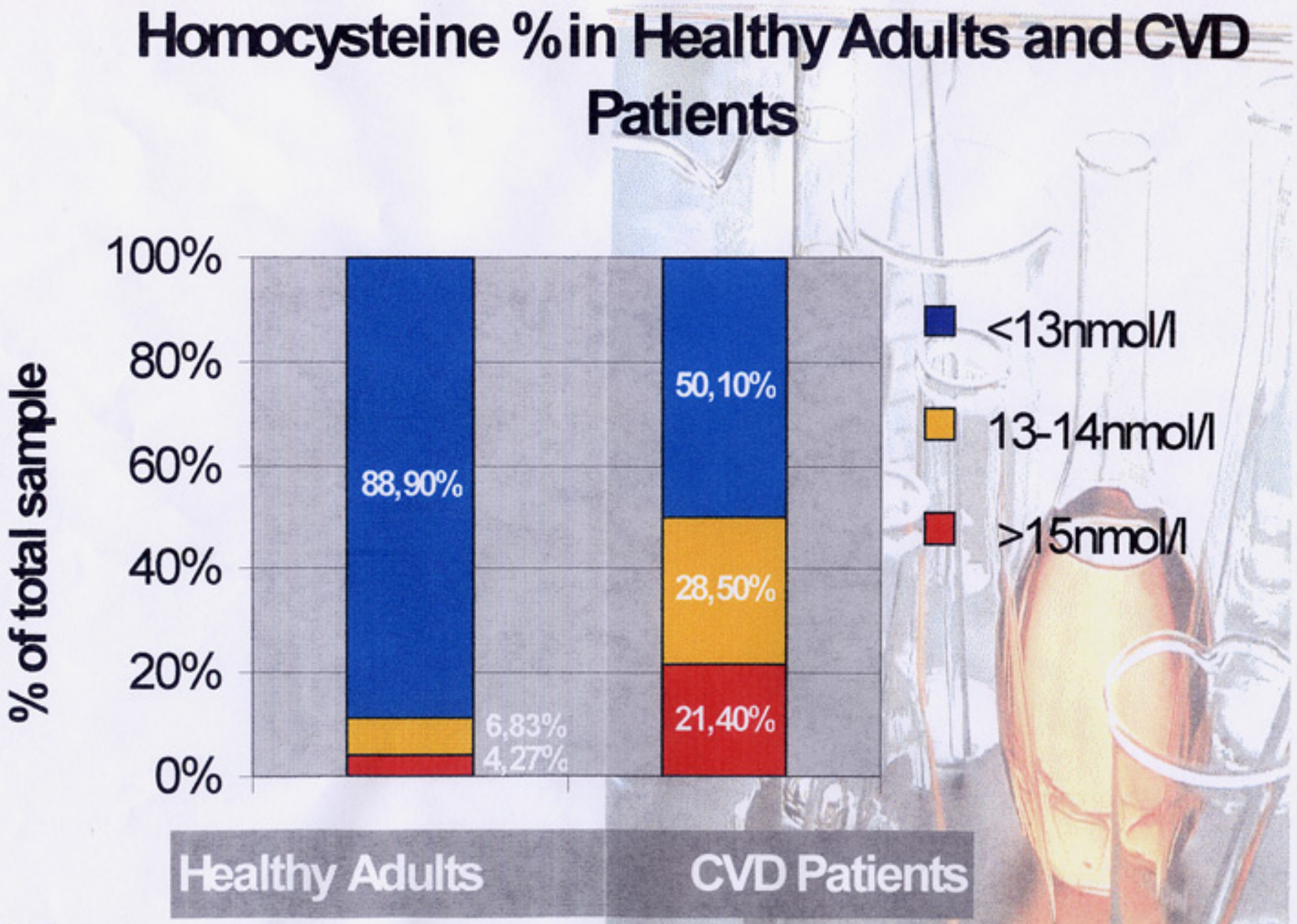
Homocysteine % in Healthy Adults and CVD Patients
% of total sample
100%
80%
60%
40%
20%
0%
88,90%
6,83%
4,27%
50,10%
28,50%
21,40%
<13nmol/l
13-14nmol/l
>15nmol/l
Healthy Adults
CVD Patients

Figure 3

# Homocysteine in Healthy Adults

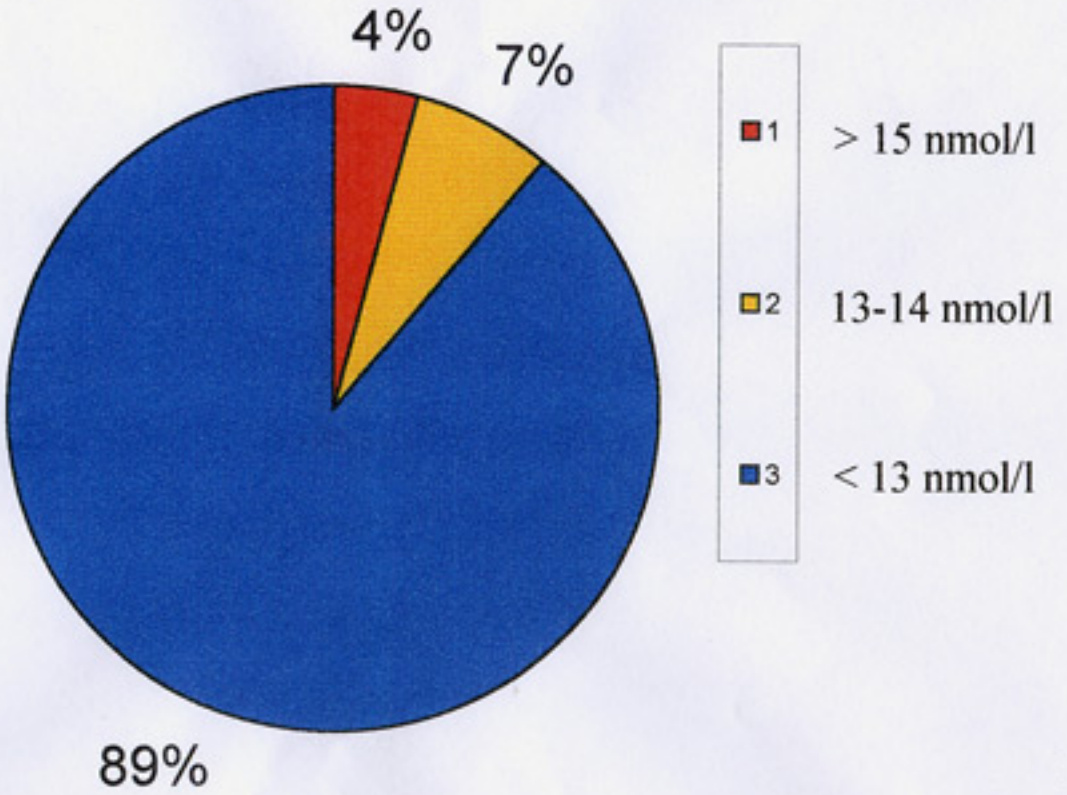


Figure 1

# Homocysteine Content
in
Healthy Adults and CVD patients

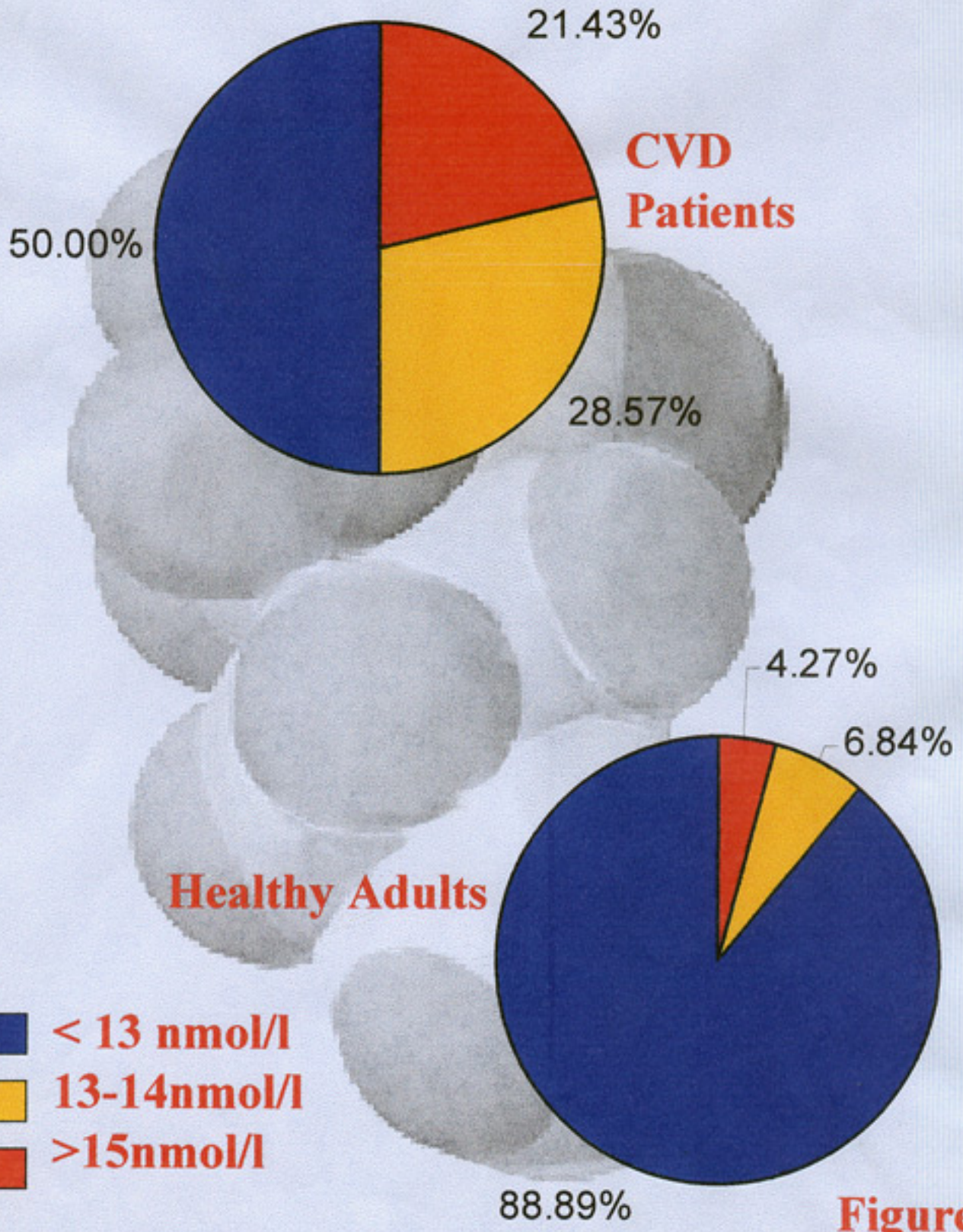


Figure 2