Omocisteina e malattie reumatiche

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L’omocisteina è un aminoacido solforato che si forma in seguito alla perdita di un gruppo metilico da parte della metionina, aminoacido essenziale, che deve quindi essere introdotto con la dieta.

![Chemical structures of methionine, homocysteine, and cysteine.](image)

**Fig. 1.** Chemical structures of methionine, homocysteine, and cysteine.
Homocysteine metabolism

in carenza di metionina, rimetilata a metionina stessa

donatore di gruppi metili ad una serie di accettori tra cui la creatina, gli ormoni steroidei, le basi puriniche di DNA e RNA

trans-sulfurata irreversibilmente a cistationina e quindi a cisteina

5-metiltetraidrofolato come donatore di metili, a sua volta rigenerato dalla metilene tetraidrofolato reduttasi (MTHFR), reazione catalizzata poi dalla metionina sintetasi (MS) che necessita della vitamina B12 come cofattore.

Una via alternativa di rimetilazione coinvolge la betaina come donatore di metili e l’enzima betaina-omocisteina metiltransferasi (BOM)

cistationina β-sintetasi
Lieve iperomocisteinemia

Unlike severe hyperhomocysteinemia, which is encountered mainly in patients with rare genetic disorders, **mild hyperhomocysteinemia** (plasma tHcy 12–50 µmol/L) is quite prevalent in the general population.

It can be caused by **nutritional deficiencies** of folate or vitamin B12, certain **medications** (e.g. niacin, fibrates, **methotrexate**, isoniazid, L-dopa, theophylline, phenytoin, nitrous oxide, and trimethoprim), a **common polymorphism in the methylene tetrahydrofolate reductase (MTHFR) gene**, or **renal disease**

Meccanismi del danno vascolare mediati dall’iperomocisteinemia

• L’omocisteina e i composti tiolici, specie chimiche molto reattive, tendono nel plasma ad auto-ossidarsi rapidamente, **producendo radicali liberi dell’ossigeno, come il superossido e perossido di idrogeno.**

• Quest’ultimi non **danneggiano solamente le membrane delle cellule endoteliali, ma anche le lipoproteine circolanti con formazione di LDL ossidate**, molecole che rivestono un ruolo cruciale nell’innescare dell’aterosclerosi

• le medesime LDL, inoltre, sono in grado di determinare **attivazione piastrinica e produzione di trombossano**

| danno endoteliale | proliferazione delle cellule muscolari lisce progressiva | stenosi arteriosa | alterazioni emostatiche suggestive di uno stato protrombotico |
Possible mechanisms of endothelial dysfunction, atherosclerosis, and thrombosis in hyperhomocysteinemic mice
Hyperhomocysteinemia and its role in the development of atherosclerosis

Atrophy of the tunica media and rupture of the elastic laminae were often observed (arrow in B).

Atherosclerotic lesions
(A) Aortic root of mice fed control
(B) High methionine diet
(C) Lesion sizes in the hyperhomocysteinemic mice
Potential cellular mechanisms by which homocysteine promotes atherosclerosis

• Homocysteine enhances the production of several proinflammatory cytokines
• Expression of monocyte chemoattractant protein 1 (MCP-1) is increased in cultured human vascular endothelial cells, smooth muscle cells and monocytes treated with homocysteine
• Homocysteine has also been shown to increase expression of IL-8 a T-lymphocyte and neutrophil chemoattractant, in cultured endothelial cells.
The immunoregulatory effects of homocysteine and its intermediates on T-lymphocyte function

The effects of Hcy on T cell activation, differentiation and apoptosis. (A) Diagram of molecular mechanisms of Hcy-induced T-cell apoptosis and cell death. (B) Summary of effects of Hcy on T cell function.
A bi-directional link seems to connect Hcy and the immuno-inflammatory activation characterizing Autoimmune Diseases, in which immuno-inflammatory activation may contribute to Hcy increase, and Hcy, in its turn, may act as a pro-inflammatory and immuno-stimulating molecule putatively cooperating at the injury of the disease-specific target organs, at least in rheumatoid arthritis and inflammatory bowel disease.
Omocisteina e artrite reumatoide
RA is a chronic systemic autoimmune inflammatory arthritis associated with extra-articular manifestations.

**Joint Inflammation**
- Joint pain and swelling\(^1\)
- Tenosynovitis/bursitis\(^2\)
- Localised bone resorption\(^3\)
- Cartilage destruction (joint space narrowing)\(^1\)
- Subchondral bone erosions\(^1,3\)
- Misalignment/dislocation, ankylosis
- Limited range of motion\(^4\)

**Fatigue\(^4\)**

**Cardiovascular Disease** (up to four-fold increased risk)\(^5–7\)

**Anaemia** (Up to 60% of patients)\(^8\)

**Malignancy** (up to 2-fold increased risk of lymphoma)\(^9\)

**Myocardial infarction**

**Stroke**

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A significant mortality gap exists between patients with RA and the general US population

- Over the past 4–5 decades:
  - The overall mortality rates of the general population have declined substantially
  - Patients with RA have had a relatively stable mortality rate

Mechanisms linking RA and increased vascular risk

FFA=free fatty acids; HDL=high-density lipoprotein; LDL=low-density lipoprotein; TC=total cholesterol; IR=insulin-resistant; ICAM-1=intercellular adhesion molecule 1; VCAM-1=vascular cell adhesion molecule 1; NO=nitric oxide

Mechanisms accounting for increased CV risk in RA

Inflammation → Coronary Heart Disease

Dyslipidemia
Insulin resistance
Hypercoagulation
Endothelial dysfunction

Vascular disease or risk is undertreated in RA → Drug therapy
NSAIDs, coxibs, steroids

Shared risk factors:
Smoking, obesity, low physical activity
Ischemic heart disease and heart failure: the most common causes of death in RA

- RA may be an independent risk factor for ischaemic heart disease, similar to diabetes mellitus

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio (95% CI)*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic controls</td>
<td>1.00 (REF)</td>
<td></td>
</tr>
<tr>
<td>Type-2 diabetes mellitus</td>
<td>2.01 (0.90-4.51)</td>
<td>0.090</td>
</tr>
<tr>
<td>RA</td>
<td>2.70 (1.24-5.86)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Data from RA patients in the **CARRE study** compared with participants of population-based cohort study on diabetes and cardiovascular disease (the Hoorn study)

*Adjusted for cardiovascular risk factors

Diagram of the pathophysiology of atherosclerosis in rheumatoid arthritis (RA)

Synovial inflammation

- TNF-α
- IL-1β
- IL-6

Autoimmunity
- T-Cell activation
- Activation of monocytes and macrophages

Liver
- CRP and fibrinogen release

Adipose tissue
- FFA release
- Pro-oxidative dyslipidemia

Skeletal muscle
- Impaired glucose uptake
- Insulin resistance

Accelerated atherosclerosis
- Arterial stiffening
- Endothelial dysfunction
- Decreased endothelial progenitor cells

Preclinical atherosclerosis

Saurabh S Dhawan et al, Current Atherosclerosis Reports 2008, 10:128-133
Traditional risk factors for atherosclerosis in patients with autoimmune rheumatic diseases

- Age
- Male gender
- Hypertension
- Diabetes
- Smoking
- The metabolic syndrome
- Hyperlipidemia (total cholesterol, LDL)
- Biomarkers: ESR, CRP, piHDL, OxLDL, Hyperhomocysteinemia, etc
Hyperhomocysteinemia

• L’omocisteina è elevata nei pazienti affetti da AR.

• L’omocisteina è significativamente aumentata nei pazienti con AR e comorbidità cardiovascolare
  Cisternas M et al J Rheumatol 2002; 29: 1619-22

• L’utilizzo a lungo termine del methotrexate e della sulfasalazina determina un incremento dell’omocisteina
  Haagsma CJ et al Ann Rheum Dis 1999; 58: 79-84

• La supplementazione di folati previene l’innalzamento dei livelli di omocisteina con l’uso di MTX
  Van Ede Rheumatology 2002; 41: 658-65
Abnormal homocysteine metabolism in rheumatoid arthritis

Fasting levels of tHcy were 33% higher in the RA patients than in the control subjects (mean +/- SD 11.7 +/- 1.5 nmoles/ml versus 8.8 +/- 1.1 nmoles/ml; P < 0.01).

Four hours after Met challenge, the increase in plasma tHcy levels (delta tHcy) was higher in the RA patients (20.9 +/- 10.4 nmoles/ml) than in the control subjects (15.5 +/- 1.6 nmoles/ml) (P < 0.02).

In a subgroup analysis, the delta tHcy in patients taking methotrexate (12.9 +/- 2.2 nmoles/ml) did not differ from that in the control group, while the delta tHcy in patients not taking methotrexate (25.3 +/- 1.7 nmoles/ml) was significantly higher (P < 0.0001).

Twenty-five RA patients and 5 controls reported a history of thrombotic events. Eleven and 5 of RA patients were found to have been previously affected by venous or arterial thrombosis, respectively.

Plasma levels of homocysteine in aPL antibody positive patients with thrombosis were found to be significantly higher than in aPL antibody positive RA patients without thrombosis (p <0.001).

When RA patients with thromboses were analyzed, a significant increase of plasma homocysteine levels was found in aPL antibody-positive RA patients versus aPL antibody negative RA patients (p < 0.04) and versus related controls (p< 0.003).
The role of homocysteine as a significant risk factor for white matter lesions in Japanese women with rheumatoid arthritis

Futoshi Anan\textsuperscript{a,*}, Takayuki Masaki\textsuperscript{b}, Hiroshi Tatsukawa\textsuperscript{c}, Shuji Nagano\textsuperscript{c}, Motohiro Oribe\textsuperscript{d}, Nobuoki Eshima\textsuperscript{e}, Tetsunori Saikawa\textsuperscript{f}, Hironobu Yoshimatsu\textsuperscript{b}

Multivariate logistic analysis revealed that WML was independently predicted by the tHcy (odds ratio, 1.35; 95% confidence interval, 1.12-1.63; $P$ b .0001).

Our findings indicate that the presence of WML was associated with the tHcy in Japanese patients with rheumatoid arthritis.
Omocisteina e methotrexate
Methotrexate in rheumatology

- Rheumatoid arthritis
- Psoriatic arthritis
- Systemic vasculitis
- Polymyalgia rheumatica
- Connective tissue disease
- Juvenile chronic arthritis
Mechanism of action of methotrexate

(1) Reduction of cell proliferation,
(2) Increase of apoptosis of T cells,
(3) Increase of endogenous adenosine release,
(4) Alteration of expression of cellular adhesion molecules,
(5) Influence on production of cytokines, humoral responses, and bone formation.

Basic metabolic processes associated with MTX cellular uptake and PG. Progressive glutamic acid moieties are added slowly by the enzyme FPGS and are removed by FPGH. Polyglutamated forms of MTX inhibit several key enzymes in folate metabolism (dihydrofolate reductase and thymidylate synthase) and prevent de-novo purine biosynthesis. The methylation of dUMP is needed for DNA synthesis to generate dTMP. Enzyme inhibition, folate depletion, and direct or indirect effects on cytokine release signaling pathways all create routes via which MTX could suppress RA.
Figure 1  Schematic diagram of mechanism of action of methotrexate. The anti-proliferative actions of methotrexate (MTX) are mediated via the inhibition of folate-dependent pathways. The anti-inflammatory actions are thought to be due to the upregulation of adenosine resultant from the increase in the level of aminomimidazole-carboxamide-ribonucleoside (AICAR). GAR, glycaminamide ribonucleotide.
Simplified metabolic scheme illustrating folate metabolism and its relationship to homocysteine–methionine metabolism.

van Ede A E et al. Rheumatology 2002;41:658-665
Homocysteine levels (with standard errors) during the 48 weeks of the study.

van Ede A E et al. Rheumatology 2002;41:658-665
The enzymes and polymorphisms of potential importance for MTX toxicity are as follows:

1. methylenetetrahydrofolate reductase (MTHFR),
   a. 5,10-MTHFR C677T and A1298C
2. folylpolyglutamyl synthase (FPGS),
3. thymidylate synthase (TYMS),
4. ATP-binding cassette transporter B1 (ABCB1), C1 (ABCC1), and C2 (ABCC2),
5. g-glutamyl hydrolase (GGH),
6. ATIC,
7. reduced folate carrier,
   a. RFC1 A80G
8. P-glycoprotein,
   a. multidrug resistance gene 1 (MDR1) G2677T>A/C and C3435T
9. methionine synthase,
   a. MS A2756G
10. methionine synthase reductase
    a. MTRR A66G
A common polymorphism exists in the MTHFR [methylenetetrahydrofolate reductase (NAD(P)H)] gene, which encodes the methylenetetrahydrofolate reductase enzyme.

**People in nonsupplemented populations who have a C-to-T substitution at base 677 of the MTHFR gene have homocysteine concentrations that are about 25% higher than those with the CC genotype.**

A metaanalysis of 40 such studies, involving 12,000 CHD cases, found that individuals with the TT genotype had a 25% higher homocysteine than those with the CC genotype and had a 16% (95% CI, 5%–28%) higher risk of CHD, a result apparently providing support for a causal relationship with CHD.

The TT genotype for MTHFR was associated with an increased risk of CHD in Asian and European populations, but not in populations in North America (where flour is fortified with folic acid).
A metaanalysis of 40 such studies, involving 12 000 CHD cases, found that individuals with the TT genotype had a 25% higher homocysteine than those with the CC genotype and had a 16% (95% CI, 5%–28%) higher risk of CHD, a result apparently providing support for a causal relationship with CHD.

The TT genotype for MTHFR was associated with an increased risk of CHD in Asian and European populations, but not in populations in North America (where flour is fortified with folic acid).
Pharmacogenetics

Genetic polymorphisms of enzymes that modify MTX transport and metabolic effects were studied in 213 RA patients. Overall, 26% of the patients discontinued MTX treatment due to poor response or toxicity or both.

- Reduced folate carrier (RFC-1 A80G) and P-glycoprotein (MDR1 C3435T) polymorphisms increased the risk for MTX toxicity (OR, 3.6 and 7.8, respectively)
- 5,10-methylenetetrahydrofolate reductase (MTHFR A1298C) polymorphism was protective (OR, 0.17).

**CONCLUSION:** Results suggest that genetic polymorphisms in the folate metabolic pathway and MTX transporters modify the toxicity but not the efficacy of MTX treatment

Pharmacogenetics

- Patients (n=205) with active RA received MTX at an initial dosage of 7.5 mg/week, which was increased to 15 mg/week and combined with folic acid (1 mg/day) after 4 weeks.

- The following SNPs were analyzed: methylenetetrahydrofolate reductase (MTHFR) 677C>T, MTHFR 1298A>C, dihydrofolate reductase (DHFR) -473G>A, DHFR 35289G>A, and reduced folate carrier 80G>A.

At 6 months,
MTHFR 1298AA was associated with good improvement relative to 1298C (OR 2.3, 95% confidence interval [95% CI] 1.18-4.41), which increased with increased copies of the MTHFR 677CC haplotype.
In contrast, MTHFR 1298C allele carriers developed more ADEs (OR 2.5, 95% CI 1.32-4.72).

**Take home message: MTHFR genotypes may help determine which patients will benefit most from MTX treatment**

To study genetic polymorphisms in the folate pathway, a site of action of methotrexate (MTX) and sulfasalazine (SSZ), as predictors of efficacy of combination disease modifying antirheumatic drug (DMARD) regimens containing MTX and SSZ in 98 early rheumatoid arthritis (RA).

Two favorable allele combinations associated with responder status at 12 months were identified: the MTR 2756A allele in combination with either the SLC19A1 80A allele or the TYMS 3R-del6 haplotype (multivariate analysis, p = 0.0002, p = 0.009 respectively).

Seventy of the 72 patients with these allele combinations responded compared to 12/24 patients without [odds ratio (OR) 35.0, 95% confidence interval (CI) 6.9-176, p < 0.0001]. An association with remission (DAS28 < 2.6) was also observed (OR 3.4, 95% CI 1.1-10.0, p = 0.04).

Take home message: Allele combinations of these genes may predict response to combination DMARD and assist in treatment decisions in patients with early RA.

Prevention of side effects

- **Supplementation with folic acid** is an effective measure to reduce hepatic adverse effects associated with MTX treatment.

- There is **no difference between folinic acid and folic acid**, but the latter is less costly.

- However, as clinical experience shows, the addition of especially **larger quantities of folic acid will likely lead to loss of efficacy**.

Folate supplementation and methotrexate treatment in rheumatoid arthritis: a review

Suggested folate use in RA patients taking MTX

<table>
<thead>
<tr>
<th>Indication</th>
<th>Dose</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid All patients taking MTX</td>
<td>5–10 mg once weekly by mouth given morning after MTX dose</td>
<td>Reduction of side-effects (LFT abnormalities, GI intolerance, ± cytopenia) [9, 16]. Reduction of plasma homocysteine [30–32]</td>
</tr>
<tr>
<td>Folinic acid MTX overdose or acute haematological toxicity</td>
<td>15 mg by mouth every 6h, for 2–8 doses (depending on the dose of MTX) [39]</td>
<td>Reversal of haematological toxicity</td>
</tr>
</tbody>
</table>

LFT, liver function tests.
### Key messages

Supplementation of MTX with folic acid improves tolerability. Five milligrams of folic acid should be given on the morning after MTX. Folic acid supplementation has not been shown to reduce the efficacy of MTX in RA. Folic acid offsets the increase in plasma homocysteine associated with MTX treatment and may reduce cardiovascular risk in RA.
# Systematic Review and Meta-analysis of Methotrexate Use and Risk of Cardiovascular Disease

Renata Mich,a RD PhD,a Fumiaki Imamura, PhDa, Moritz Wyler von Ballmoos, MD PhDb, Daniel H. Solomon, MDc, Miguel A. Hernán, MD DrPHa,e Paul M Ridker, MDD, and Dariush Mozaffarian, MD DrPHa,d

<table>
<thead>
<tr>
<th>Study</th>
<th>Underlying disease</th>
<th>Disease Outcome, Incident or recurrent</th>
<th>RR (95% CI)</th>
<th>% Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choi (2002)</td>
<td>RA</td>
<td>CVD fatal, not specified</td>
<td>0.30 (0.13, 0.70)</td>
<td>1.08</td>
</tr>
<tr>
<td>Goodson (2008)</td>
<td>Polyarthritis</td>
<td>CVD fatal, not specified</td>
<td>0.53 (0.25, 1.14)</td>
<td>1.33</td>
</tr>
<tr>
<td>van Halo (2006)</td>
<td>RA</td>
<td>CVD total, incident</td>
<td>0.53 (0.24, 1.19)</td>
<td>1.21</td>
</tr>
<tr>
<td>Trochsen (2007)</td>
<td>RA, Polyarthritis</td>
<td>IHD hospitalization, not specified</td>
<td>0.66 (0.20, 1.80)</td>
<td>0.64</td>
</tr>
<tr>
<td>Prodanovich (2005)</td>
<td>Psoriasis</td>
<td>CVD total, incident</td>
<td>0.73 (0.54, 0.98)</td>
<td>8.93</td>
</tr>
<tr>
<td>Solomon (2006)*</td>
<td>RA</td>
<td>MI or stroke hospitalization, recurrent</td>
<td>0.74 (0.62, 0.88)</td>
<td>23.78</td>
</tr>
<tr>
<td>Nadareishvili (2008)</td>
<td>RA</td>
<td>Ischemic stroke total, incident</td>
<td>0.77 (0.38, 1.54)</td>
<td>1.61</td>
</tr>
<tr>
<td>Suissa (2006)</td>
<td>RA</td>
<td>MI hospitalization, incident</td>
<td>0.81 (0.61, 1.08)</td>
<td>9.36</td>
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<tr>
<td>Prodanovich (2005)</td>
<td>RA</td>
<td>CVD total, incident</td>
<td>0.83 (0.72, 0.96)</td>
<td>36.64</td>
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<tr>
<td>Edwards (2008)</td>
<td>RA</td>
<td>MI total, incident</td>
<td>0.86 (0.56, 1.32)</td>
<td>4.17</td>
</tr>
<tr>
<td>Wolfe (2008)</td>
<td>RA</td>
<td>MI total, incident</td>
<td>1.06 (0.77, 1.30)</td>
<td>11.25</td>
</tr>
</tbody>
</table>

**Overall Pooled Estimate** (I²= 41.8%, p = 0.30)

0.79 (0.73, 0.87) 100.00

Weights are from fixed effects analysis

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Am J Cardiol. 2011 November 1; 108(9): 1362–1370.
Systematic Review and Meta-analysis of Methotrexate Use and Risk of Cardiovascular Disease

Renata Micha, RD PhD\textsuperscript{a}, Fumiaki Imamura, PhD\textsuperscript{a}, Moritz Wyler von Ballmoos, MD PhD\textsuperscript{b}, Daniel H. Solomon, MD\textsuperscript{c}, Miguel A. Hernán, MD DrPH\textsuperscript{a,e}, Paul M Ridker, MD\textsuperscript{d}, and Dariush Mozaffarian, MD DrPH\textsuperscript{a,d}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{chart.png}
\caption{Methotrexate use and CVD among studies that adjusted for underlying disease severity}
\end{figure}

Am J Cardiol. 2011 November 1; 108(9): 1362–1370.
Omocisteina e LES
Premature coronary heart disease (CHD) has emerged as a major cause of morbidity and mortality in patients with systemic lupus erythematous (SLE).

Overall SLE patients have a 5–6-fold increased risk of CHD and this excess risk is especially pronounced in younger women where the excess risk may be `50-fold.

Rheumatology 2005;44:1492–1502
Comparison of the prevalence of subclinical atherosclerosis in patients with SLE compared with healthy controls using two different methods of assessment in the Manchester cohort. Patients with SLE had a significantly higher prevalence of both endothelial dysfunction (A) and carotid plaque development (B).

Rheumatology 2005;44:1492–1502
Metabolic risk factors found to be significantly different in a cohort–control study of 235 patients with SLE compared with a primary care population

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>SLE (%)</th>
<th>Controls (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>33</td>
<td>13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5</td>
<td>1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Post-menopause</td>
<td>38</td>
<td>19</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Elevated creatinine</td>
<td>9</td>
<td>0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sedentary lifestyle</td>
<td>15</td>
<td>9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.8 (0.06)</td>
<td>0.78 (0.06)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/l)</td>
<td>0.45 (0.34)</td>
<td>0.38 (0.27)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.4 (0.88)</td>
<td>1.2 (0.67)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Homocysteine &gt;15 mmol/l</td>
<td>11</td>
<td>0.4</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

All results expressed as % with the abnormality except waist:hip ratio, VLDL cholesterol and triglycerides where mean (s.d.) values are shown.
Omocisteina e LES

- uno studio basato su oltre 60 pazienti affetti da LES ha riscontrato una correlazione tra calcificazioni coronariche, valutate con la tecnica della TC electron-bean, ed incremento dei valori di omocisteina e trigliceridi; i valori di LDL e HDL, invece, non presentavano associazione di rilevanza statistica.

Omocisteina e LES

L’aumento dei livelli di omocisteina nei pazienti affetti da LES rappresenta un fattore di rischio per coronaropatia, ictus e trombosi arteriosa

ADMA and homocysteine are biomarkers for and may be mediators of premature arterial stiffening in patients with SLE.

Because arterial stiffness has independent prognostic value for cardiovascular morbidity and mortality, its predictors may identify patients who are at increased risk of cardiovascular disease.
Omocisteina, f. di Raynaud e sclerodermia
Levy et al hanno segnalato che pazienti con fenomeno di Raynaud, sia primitivo sia secondario a sclerodermia, presentavano **concentrazioni plasmatiche di omocisteina superiori a quelli rilevabili nei sani.**

Una successiva esperienza italiana ha confermato il riscontro di livelli moderatamente elevati di omocisteina nei pazienti con fenomeno di Raynaud secondario a sclerodermia, ma non nei soggetti con fenomeno di Raynaud primario.

In questo lavoro sono stati valutati anche i livelli ematici di folato e vitamina B12 come pure i polimorfismi del gene MTHFR, giungendo alla conclusione che **l’iperomocisteinemia nei pazienti sclerodermici sia legata più ad un deficit nutrizionale che a fattori genetici.**

### Clinical studies investigating Hcy plasma levels in patients with RP

<table>
<thead>
<tr>
<th>Author, year, year</th>
<th>Secondary RP</th>
<th>Primary RP</th>
<th>Controls</th>
<th>Plasma Hcy levels</th>
<th>Other findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheng, 2002 [12]</td>
<td>11 SLE</td>
<td>--</td>
<td>23a</td>
<td>Higher in SLE patients with RP than control patients without RP and healthy controls.</td>
<td>No difference in plasma folate and vitamin B12 levels among the three groups of patients.</td>
</tr>
</tbody>
</table>

*Hcy plasma levels positively correlated with the severity of the microvascular involvement in the nailfold bed.*
A schematic representation of the putative mechanisms by which Hcy may affect the arteriolar tone thereby favouring the RP onset. Hcy is able to produce endothelial dysfunction (with reduced NO availability), platelet activation (with increased thromboxane production), and smooth muscle cell remodelling and contraction. All these effects, even in the absence of significant influence on sympathetic tone and PGI2 and ET-1 production, may favour asoconstriction. Hcy: homocysteine; NO: nitric oxide; PGI2: prostacyclin; ET-1: endothelin-1; α2: alpha-2 adrenergic receptors.
A scheme of the relationship among clinical findings (evidence of RP), Hcy plasma levels, and specific therapy. Hcy: homocysteine; RP: Raynaud's phenomenon.
• A dysregulation in the mechanisms of vascular motility resulting in an imbalance between vasodilatation and vasoconstriction represents the key issue in the pathogenesis of Raynaud's phenomenon.

• Measurement of homocysteine plasma level in patients with Raynaud's phenomenon is recommended, also in consideration of the fact that hyperhomocysteinemia can be effectively treated with vitamins.
Correlation between homocysteine plasma levels and nailfold videocapillaroscopic patterns in systemic sclerosis

Paola Caramaschi • Alessandro Volpe • Sabrina Canestrini • Lisa M. Bambara • Giovanni Faccini • Antonio Carletto • Domenico Biasi

Fig. 1: Homocysteine levels in patients affected by SSc and in healthy controls ($p<0.001$).

Fig. 3: Homocysteine levels in different grades of skin involvement ($P=0.04$).
Hcy plasma level is related to microvascular involvement in patients affected by SSc; the concentration increases with the progression of the nailfold videocapilaroscopic pattern.

Hyperhomocysteinemia may represent an aggravating factor among the complex mechanisms involved in scleroderma damage contributing to the injury of endothelium.
Homocysteine plasma concentration is related to severity of lung impairment in scleroderma

Figure 1. A. Mean plasma homocysteine concentrations (95% CI) in control group and in the 3 scleroderma subgroups with different pulmonary involvement. Group A: Patients without lung involvement. Group B: Patients with mild or moderate lung involvement. Group C: Patients with severe or endstage lung involvement. ANOVA: F = 15.673; p < 0.001. *Significantly different by Tukey post-hoc comparison (p < 0.001). #Significantly different by Tukey post-hoc comparison (p = 0.048). B. Plasma homocysteine concentrations in individual subjects.
Omocisteina e osteoporosi
Recently, high circulating homocysteine (HCY) concentrations have been linked to an increased risk of fragility fractures and osteoporosis.

- However, the mechanisms behind these observations are largely unknown. Some first mechanistic studies indicate that HCY stimulates osteoclasts and induces a dysbalance between osteoclasts and osteoblasts in favour of the osteoclasts.

- Extracellular mechanisms seem to be involved. Saito et al. demonstrated a decreased concentration of enzymatic cross-links in the bones of hyperhomocysteinemic female fracture patients indicating a disturbed collagen cross-linking.
Hyperhomocysteinemia induces a tissue specific accumulation of homocysteine in bone by collagen binding and adversely affects bone
Markus Herrmann, Andrea Tami et al. Bone 44 (2009) 467–475

HCY tissue concentration. Mean (95% CI) HCY tissue concentration in bone (filled circles) and myocardium (open circles). Due to analytical differences results are given as percentage of controls. **p<0.001 vs. CO, ‡‡p<0.001 vs. bone tissue. CO — controls, METH — methionine-group, HOMO — homocysteine group.
Hyperhomocysteinemia induces a tissue specific accumulation of homocysteine in bone by collagen binding and adversely affects bone

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SAH and SAM. Mean (95% CI) SAH (A), SAM (B) and SAH / SAM–ratio (C) in bone tissue (filled circles) and plasma (open circles). **p<0.001 vs. CO, ‡‡p<0.001 vs. bone tissue.

SAH — S adenosylhomocysteine, SAM — S-adenosylmethionine, CO — controls, METH — methionine-group, HOMO — homocysteine group
Hyperhomocysteinemia induces a tissue specific accumulation of homocysteine in bone by collagen binding and adversely affects bone
Markus Herrmann, Andrea Tami et al. Bone 44 (2009) 467–475

μCT. Representative μCT scans showing the reduction of bone volume in hyperhomocysteinemic animals. The bottom row illustrates the increased porosity of the trabecular network in METH and HOMO animals.
Homocysteine Levels and the Risk of Osteoporotic Fracture

Joyce B.J. van Meurs, Ph.D., Rosalie A.M. Dhonukshe-Rutten, M.Sc., Saskia M.F. Pluijm, Ph.D., Marjolein van der Klift, M.D., Ph.D., Robert de Jonge, Ph.D., Jan Lindemans, Ph.D., Lisette C.P.G.M. de Groot, Ph.D., Albert Hofman, M.D., Ph.D., Jacqueline C.M. Witteman, Ph.D., Johannes P.T.M. van Leeuwen, Ph.D., Monique M.B. Breteler, M.D., Ph.D., Paul Lips, M.D., Ph.D., Huibert A.P. Pols, M.D., Ph.D., and André G. Uitterlinden, Ph.D.
### Table 1. Baseline Characteristics of Study Subjects.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Rotterdam Study</th>
<th></th>
<th>LASA (N=1291)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cohort 1 (N=562)</td>
<td>Cohort 2 (N=553)</td>
<td></td>
</tr>
<tr>
<td>Women — no. (%)</td>
<td>351 (62)</td>
<td>278 (50)</td>
<td>663 (51)</td>
</tr>
<tr>
<td>Age — yr</td>
<td>70.3±8.8</td>
<td>73.6±7.9</td>
<td>75.6±6.6</td>
</tr>
<tr>
<td>Body-mass index †</td>
<td>26.5±3.9</td>
<td>26.3±3.6</td>
<td>26.8±4.2</td>
</tr>
<tr>
<td>Current smoker — %</td>
<td>24</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>Fall in previous year — %</td>
<td>21</td>
<td>NA</td>
<td>32</td>
</tr>
<tr>
<td>Homocysteine level — μmol/liter ‡</td>
<td>15.9±5.7</td>
<td>11.9±4.3</td>
<td>14.7±6.0</td>
</tr>
<tr>
<td>Serum creatinine level — μmol/liter ‡</td>
<td>82.6±20.4</td>
<td>89.1±20.2</td>
<td>93.6±22.0</td>
</tr>
<tr>
<td>Bone mineral density — g/cm² ¶</td>
<td>Lumbar spine</td>
<td>1.09±0.20</td>
<td>1.11±0.21</td>
</tr>
<tr>
<td></td>
<td>Femoral neck</td>
<td>0.85±0.13</td>
<td>0.83±0.15</td>
</tr>
<tr>
<td>Follow-up — yr</td>
<td>8.1±3.7</td>
<td>5.7±1.9</td>
<td>2.7±0.7</td>
</tr>
<tr>
<td>Loss to follow-up — %</td>
<td>2.3</td>
<td>3.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Incidence of fracture — no./1000 person-yr</td>
<td>18.2</td>
<td>15.8</td>
<td>16.4</td>
</tr>
</tbody>
</table>

* Plus–minus values are means ± SD. LASA denotes the Longitudinal Aging Study Amsterdam, and NA not available.
During 11,253 person-years of follow-up, 191 subjects (135 women and 56 men) sustained an osteoporotic fracture; a majority were hip and wrist fractures.
Cumulative Incidence of Fracture among Study Subjects with Homocysteine Levels in the Highest Age and Sex-Specific Quartile as Compared with All Other Subjects.

RR denotes relative risk, and CI confidence interval.
Cumulative Incidence of Fracture among Study Subjects with Homocysteine Levels in the Highest Age and Sex-Specific Quartile as Compared with All Other Subjects.

RR denotes relative risk, and CI confidence interval.
Cumulative Incidence of Fracture among Study Subjects with Homocysteine Levels in the Highest Age and Sex-Specific Quartile as Compared with All Other Subjects.

RR denotes relative risk, and CI confidence interval.
Homocysteine levels were not associated with bone mineral density at either the femoral neck or the lumbar spine.
Homocysteine Levels and the Risk of Osteoporotic Fracture


<table>
<thead>
<tr>
<th>Factor</th>
<th>Relative Risk (95% CI)</th>
<th>Population Attributable Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;75 yr</td>
<td>2.3 (1.7–3.1)</td>
<td>31 (25–48)</td>
</tr>
<tr>
<td>BMD, lowest quartile</td>
<td>1.6 (1.1–2.3)</td>
<td>13 (2–25)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.6 (1.1–2.3)</td>
<td>10 (4–23)</td>
</tr>
<tr>
<td>Fall in previous year†</td>
<td>1.9 (1.2–2.7)</td>
<td>20 (10–35)</td>
</tr>
<tr>
<td>Dementia and cognitive impairment†</td>
<td>2.5 (1.5–4.1)</td>
<td>15 (7–30)</td>
</tr>
<tr>
<td>Homocysteine level, highest quartile</td>
<td>1.9 (1.4–2.6)</td>
<td>19 (10–29)</td>
</tr>
</tbody>
</table>

A serum homocysteine level in the highest quartile doubled the risk of fracture.

A homocysteine level in the highest age-specific quartile conferred a 19 percent attributable risk in our population.
Homocysteine as a Predictive Factor for Hip Fracture in Older Persons


Figure 1. Multivariable-Adjusted Hazard Ratios for the Risk of Hip Fracture, According to the Quartile of Total Homocysteine Concentration.

The y axis is on a log scale. The reference group is quartile 1. The I bars denote 95 percent confidence intervals.
Flow chart of trial selection process for meta-analysis and systematic review.
### RR and 95% CI from the included studies of plasma Hcy level and all fractures

<table>
<thead>
<tr>
<th>Study</th>
<th>RR (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Meur (2004)</td>
<td>2.00 (1.40, 2.70)</td>
<td>13.39</td>
</tr>
<tr>
<td>McLean (Men) (2004)</td>
<td>3.84 (1.38, 10.70)</td>
<td>3.39</td>
</tr>
<tr>
<td>McLean (Women) (2004)</td>
<td>1.92 (1.18, 3.10)</td>
<td>9.61</td>
</tr>
<tr>
<td>Ravaglia (2005)</td>
<td>1.28 (0.55, 2.96)</td>
<td>4.65</td>
</tr>
<tr>
<td>Dhonukshe-Rutten (Men) (2005)</td>
<td>2.60 (1.10, 6.50)</td>
<td>4.27</td>
</tr>
<tr>
<td>Dhonukshe-Rutten (Women) (2005)</td>
<td>1.70 (0.80, 3.50)</td>
<td>5.65</td>
</tr>
<tr>
<td>Gjesdal (Women) (2007)</td>
<td>2.16 (1.20, 3.89)</td>
<td>7.67</td>
</tr>
<tr>
<td>Gjesdal (Men) (2007)</td>
<td>1.52 (0.64, 3.58)</td>
<td>4.48</td>
</tr>
<tr>
<td>Gerdhem (2007)</td>
<td>1.18 (0.89, 1.36)</td>
<td>16.69</td>
</tr>
<tr>
<td>McLean (2008)</td>
<td>1.66 (1.08, 2.56)</td>
<td>10.75</td>
</tr>
<tr>
<td>Zhu (2009)</td>
<td>0.94 (0.65, 1.37)</td>
<td>12.20</td>
</tr>
<tr>
<td>Enneman (2012)</td>
<td>1.50 (0.81, 2.77)</td>
<td>7.25</td>
</tr>
<tr>
<td><strong>Overall</strong> (I-squared = 48.5%, p = 0.030)</td>
<td><strong>1.59 (1.30, 1.96)</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>
RR and 95% CI from the included studies of plasma Hcy level and hip fracture.
Homocysteine level and risk of fracture: A meta-analysis and systematic review

RR and 95% CI from the included studies of plasma Hcy level and all fractures by gender.
### Summary of RCTs included in the review.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Participants</th>
<th>Women n (%)</th>
<th>Age</th>
<th>Dose and duration</th>
<th>Outcomes</th>
<th>Result summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sato et al. (2005)</td>
<td>RCT</td>
<td>314</td>
<td>169 (53.8)</td>
<td>71.6 (5.1)</td>
<td>A daily with 5 mg of folate and 1500 µg of mecobalamin for 2 years.</td>
<td>Plasma Hcy level fracture</td>
<td>The number of hip fractures per 10000 patient-years was 10 and 43 for the treatment and placebo groups. Adjusted RR in the treatment vs placebo groups was 0.24 (95% CI, 0.11–0.53). Plasma Hcy decreased in the treatment group (after 1 year, −36.1 ± 1.7; after 2 years, −38.1 ± 1.7) but increased in the placebo group (after 1 year, 18.2 ± 1.1; after 2 years, 31.2 ± 1.4). 175 fractures in the treatment group and 175 fractures in the placebo group. Adjusted RR in the treatment vs placebo groups was 1.06 (95% CI, 0.81–1.40). Plasma Hcy decreased 0.29 mg/L in the treatment group and increased 0.11/L in the placebo group.</td>
</tr>
<tr>
<td>Anna et al. (2007)</td>
<td>RCT</td>
<td>2764</td>
<td>796 (28.9)</td>
<td>68.8 (7.1)</td>
<td>A daily with 2.5 mg folic acid, 50 mg pyridoxine hydrochloride and 1 mg cyanocobalamin for one year.</td>
<td>Plasma Hcy level fracture</td>
<td></td>
</tr>
<tr>
<td>van Wijngaarden, JP et al. (2007)</td>
<td>RCT</td>
<td>2919</td>
<td>NP</td>
<td>NP</td>
<td>A daily tablet with 500 µg vitamin B12 and 400 µg folic acid for 2 years.</td>
<td>Plasma Hcy level fracture</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** not provide, NP.
Considerazioni conclusive

- Innumerevoli interazioni metaboliche dell’omocisteina
- Valutazione del suo significato in alcune malattie reumatiche
- Link bidirezionale tra rischio cardiovascolare e infiammazione cronica
- Possibile utilizzo di terapia che ne riduca la concentrazione plasmatica e pertanto i rischi connessi.