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1. Review of existing homocysteine clinical trial results

Several clinical trials have been conducted to investigate interventions for lowering homocysteine. A detailed review of nine studies is included in Table 1. The average overall result was to lower homocysteine 23%. Table 1 divides the trials into the categories of a review paper of twenty-five trials, trials in subjects who have just experienced a major health event such as a kidney transplant or heart attack, trials in healthy subjects, and trials that included genetic analysis. In all cases, homocysteine levels decreased and blood plasma levels of folate increased.

The review paper found that a 25% reduction in homocysteine was possible from folic acid supplementation, together with an additional 7% reduction from vitamin B-12 supplementation. The trials following major health events had older participants (average age 58), higher baseline measures of homocysteine (14.0 µmol/L on average), and greater reductions in homocysteine (28% on average). The studies with healthy subjects had younger populations (average age 41), lower...
baseline levels of homocysteine (10.5 on average), and smaller proportional reductions in homocysteine (13% on average).

Two studies examined genetic polymorphisms, #8 Ashfield-Watt and a clinical trial currently in process, #9 Flugelman. Another study, #5 Lamers, realized the potential impact of genomics and stratified experimental cohorts by genotype. Ashfield-Watt reviewed the main MTHFR 677C>T polymorphism (rs1801133) and found that results for the three different genotypes were on a gradient, both in baseline homocysteine levels, and in improvements. For the three genotypes, TT (two copies of the polymorphism), CT, and CC (normal), baseline homocysteine was 12.5, 9.3, and 8.8 respectively, and reduction percentages were 21%, 12%, and 9% respectively.

The interventions investigated were folate-rich food, folate-fortified food, folic acid supplementation, folate supplementation, and 5-MTHF (5-methyltetrahydrofolate) supplementation (the metabolically active form of folate). Supplementation had better results than folate-rich food or folate-fortified food. Folic acid and folate supplements were both effective in reducing homocysteine. Two studies (#2 Akoglu and #5 Lamers) specifically compared folic acid with the active form of folate, 5-MTHF. Both found that the active formulation was more effective in reducing homocysteine levels that the folic acid (Akoglu 37% versus 24%; Lamers 19% versus 12%).

The time period for the studies was generally three to twelve weeks, however the biggest changes occurred in the first few weeks. #3 Akoglu reported several timeframes, at one, two, four, and eight weeks. Respective decreases for the 5-MTHF group and the folic acid group were 16%, 27%, 28%, and 37%, and 6%, 19%, 25%, and 24%, indicating that significant results were starting to be seen after one or two weeks, and that the active formulation had a bigger impact.

Conclusion regarding existing homocysteine clinical trial results

There may be several factors influencing baseline homocysteine levels, particularly age, health status, and genotype. Individuals who are older, have just experienced a major health event, or who have one or more polymorphisms in the main MTHFR gene variant rs1801133 may be more likely to have higher baseline homocysteine levels than those who do not. Further, the reduction proportion from the baseline level may be greater for those with higher initial levels of homocysteine.

Table 1: Summary of existing homocysteine clinical trial results.
2. Metabolism of homocysteine (MTHFR pathway and mechanism analysis)

Homocysteine is a naturally-occurring amino acid in the blood. It is broken down (metabolized) through three pathways, the interconnected, cyclical biochemical pathways of the folate cycle and the methionine cycle, and the transsulfuration pathway (Figure 1). These pathways involve a number of enzymes, in conjunction with B-vitamin cofactors (Table 2), that function to maintain normal homocysteine levels within the body either by conservation or degradation.10

Figure 1: Homocysteine metabolism.
The folate cycle (light grey background), methionine cycle (light blue background), and the transsulfuration pathway (light yellow background) maintain homocysteine levels by conservation and degradation. The conservation and degradation of homocysteine are well-regulated processes that work to keep plasma levels of homocysteine within a defined range. These pathways also function to enable normal DNA synthesis and normal DNA methylation patterns. The interventions used in the pilot study are noted in the figure either in rounded rectangles indicating vitamin alone or in square corner rectangles indicating a B vitamin acting as a co-factor for the associated enzyme. Including folic acid (B-9), the multivitamin intervention included three additional B vitamins, B-2, B-6, and B-12, which act as cofactors for the enzymes depicted in the red rectangles. The L-methylfolate intervention is depicted in the pathway as a rounded green rectangle. The enzyme, BHMT (Betaine-homocysteine S-methyltransferase), depicted by the purple rectangle represents a B vitamin independent means of converting homocysteine to methionine.

The folate cycle and the methionine cycle are responsible for homocysteine conservation. Within the folate cycle, MTHFR, with B-2 as a cofactor, converts folate to its active form, L-methylfolate. The methionine cycle interacts with the folate cycle by using L-methylfolate as a methyl donor to conserve homocysteine by recycling it into methionine. The conversion of homocysteine into methionine is accomplished by the transfer of a methyl group from L-methylfolate to homocysteine by methionine synthase and B-12 as a cofactor. Additionally, homocysteine can be converted to methionine by an alternate biochemical reaction present primarily in the liver and kidneys whereby betaine acts as a methyl donor for betaine homocysteine methyltransferase allowing for the conversion of homocysteine to methionine. The transsulfuration pathway is responsible for homocysteine degradation. Homocysteine degradation is mediated by the enzyme cystathione β-synthase, with B-6 as a cofactor, and involves the conversion of homocysteine to cysteine.11

Table 2: The three enzymes involved in homocysteine metabolism.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cofactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene-tetrahydrofolate reductase (MTHFR)</td>
<td>Folate (5,10-methylenetetrahydrofolate)</td>
</tr>
<tr>
<td>Methionine synthase</td>
<td>Folate (5-methyltetrahydrofolate)</td>
</tr>
<tr>
<td></td>
<td>Vitamin B-12</td>
</tr>
<tr>
<td>Cystathione synthase</td>
<td>Vitamin B-6</td>
</tr>
</tbody>
</table>

There are three enzymes that, in conjunction with B vitamins as cofactors, directly control the level of plasma homocysteine. MTHFR is required to convert folate (5,10-methylenetetrahydrofolate) into its active form, 5-methyltetrahydrofolate (i.e. L-methylfolate). The active form of 5-methyltetrahydrofolate is used as a substrate by the enzyme methionine synthase with B-12 as a cofactor to convert homocysteine into methionine.

3. Variability in homocysteine test results
A key unknown in the MTHFR study is the inherent variability in homocysteine measurements. Within-person, between-person, and methodological variability could all contribute to a false positive or negative result, and/or lead to large sample sizes needed to demonstrate statistical significance in the full cohort study. A reliability coefficient (R) for a given measurement can be generated via serial measurements of the same individual or population. Reliability coefficients range from 0 to 1, and measurements with a high ‘R’ allow for one measurement to more accurately reflect the actual status of an individual. Three studies (n=96, 20, 20) measuring the within-person and between-person variabilities of homocysteine measurements have demonstrated high Reliability Coefficients (R=0.88, 0.84, 0.94), low standard deviations for within-person measurements (0.93, 0.84, 0.35 µmol/L), and reasonable standard deviations for between-person measurements (2.5, 3.8, 7.9 µmol/L).

In the pilot study, all U.S. homocysteine measurements (for six out of seven participants) were conducted by LabCorp, Inc. using DPC Immulite 2000 analyzers (Siemens Medical). The variability of homocysteine measurements on this device has been previously shown to be less than 10% across a broad range of measurement values, and correlation to HPLC measures has been demonstrated (r=0.98). These results reinforce homocysteine as an appropriate target for intervention in preventive medicine research, imply reasonable confidence in the observed individual trends in the pilot study, and indicate the potential to demonstrate a statistically significant result with the full cohort study.

4. Baseline homocysteine levels

To understand more about the expected homocysteine levels in different populations, and what factors may cause variance, a review of the baseline levels of homocysteine was conducted from various homocysteine clinical trials. Figure 2 plots data from the clinical trials reviewed in Table 1 (Clinical trials), the multiple studies discussed in the review paper (Trialists’ 20051), and the pilot study results (Pilot study). The pilot study group seems to be within the general range of other groups. Overall, this analysis suggests that baseline levels of homocysteine may increase with age and major health events.

![Figure 2: Examples of baseline homocysteine levels by age.](image)

While lab tests typically indicate a reference range with an upside boundary of 15 µmol/L for homocysteine, there is considerable support for lower levels being more appropriate. The principal paper cited on the subject recommends an upper cut-off of 11.4 µmol/L for men and 10.4 µmol/L for women at any age. If that is correct, widespread homocysteine-lowering programs could be useful as the average level of homocysteine in typical Western populations was 12 µmol/L in 1998.

5. Blood test data: participant plasma folate

In addition to homocysteine and vitamin B-12, four participants tested plasma folate (B-9) and the results are displayed in Table 3. For all of these individuals, plasma folate levels were at or above the high point of the test reference range (19.9 mg/mL) after the second intervention (L-methylfolate), and persisted in that range throughout the duration of the pilot study. This suggests that L-methylfolate is not impacting the pathway as initially hypothesized to reduce homocysteine levels, but is having the expected immediate impact of generating higher plasma folate levels.

![Table 3: Participant plasma folate (folic acid) levels (mg/mL) at study intervals.](image)
6. Personal statements from study participants

To probe the motivations that individuals may have for joining citizen science research studies, five study participants prepared short personal statements.

From Citizen 1 (AV): “One of the motivating factors in my participation in this study was to assess how the different interventions would affect my homocysteine levels. The results of this study obviously show that not everyone will respond in a similar manner to a particular intervention. Although previous studies have shown that L-methylfolate supplementation can lower homocysteine levels, it was clearly not the case for me. On the other hand, a simple multivitamin was able to lower my homocysteine level, suggesting that this intervention is a better choice for me. My results also suggest that, aside from MTHFR, there could be SNPs in other enzymes controlling homocysteine levels that may be affecting homocysteine regulation.”

From Citizen 2 (RM): “I've been interested in optimizing my nutritional intake, including supplements, and curious about how my personal genetics might contribute actionable information. When I started to poke around, and found the MTHFR/B-9 connection, I was surprised to see there was no definitive answer to how much of a difference it would make to take a given vitamin for a person with a given genotype. Taking an empirical approach, and seeing what works, seemed like a good idea. I was surprised. Being the ‘odd man out’ in the results, with the most paradoxical effects (could there be a feedback loop inhibiting MTHFR metabolism in the presence of inactive B-9?), I'd like to recruit other people with homozygous minor variants in MTHFR and see what their results are. I'm also looking at changing my vitamin regimen.”

From Citizen 3 (MS): “Ever since I received my 23andMe data, I've been interested in doing more with the information. As it has become more of a norm for my friends and colleagues to have their data too, we decided to see if we could make our genomic data more useful with links to phenotypic measurements and interventions to self-manage conditions for improvement. What surprised me the most was how quickly and effectively the regular drug store vitamin reduced my homocysteine level (a 29% percent reduction from 12.9 to 9.1). This stands in opposition to conventional wisdom I grew up with that a well-balanced diet obviates the need for vitamins. I was also glad to see that as a vegetarian, I was able to raise my B-12 levels. For me, this study was more rigorous and analysis than I have brought to supplement-taking previously and enough proof for me that a basic multivitamin may help to reduce my homocysteine level. Since the end of the pilot study, I have been taking the Centrum multivitamin on a daily basis. If blood tests were cheaper, I would conduct similar experiments on the other supplements I take.”

From Citizen 4 (KH): “From the moment I received my 23andMe results, I wanted to know what more I could do. Not only how to learn more about my genome, but to effectively use that information to make positive changes. What surprised me was how much of an improvement the folate made in my results. I'm a believer in the benefits of supplements, but haven’t always been good about actually taking them. Since the study, I take my vitamins with much greater frequency, and have noticed an increased energy level. I would love to see what other combination of supplements would be beneficial to me based on my particular genetic makeup.”

From Citizen 5 (CH): “I was initially drawn to this study by the desire to prove the concept that a group of individual citizens could ask a scientifically valid question, conduct a study with scientific rigor and generate meaningful data and results. While this goal was achieved, I underestimated the personal impact the study would have on me. Through this study I learned that I have very low baseline vitamin B-12 levels and high baseline homocysteine levels, and that these levels were normalized by the addition of a simple multivitamin. This result has led me to add a multi-vitamin to my daily regimen and has increased my interest in learning more about my body through future study.”

References


e-Patients Blog

- No Rhyme or Reason--Nancy Finn  July 1, 2015
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