

**Report on Heart Health and Strawberries
Study
Health Research and Study Center (HRSC), Los
Altos, California
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Background

Homocysteine is an amino acid currently under investigation for its relationship to heart disease due to the finding that individuals with elevated serum homocysteine (homocysteinemia) are at increased risk for cardiovascular disease. Strawberries are a good source of folate (30 µg/6-oz serving) and homocysteine levels are known to decrease with supplemental folate. This study aimed at investigating whether a daily serving of strawberries (6-7medium) would increase plasma folate levels and decrease serum homocysteine levels in healthy human subjects. CRP levels were also analyzed because CRP is a general marker of inflammation, recently become the focus of much research, and appears to be associated with risk of future myocardial infarction, stroke, peripheral vascular disease and cardiovascular death, even in apparently healthy populations. In addition, serum triglycerides (TG) and lipoproteins - total cholesterol (TC), low-density-lipoprotein (LDL) and high-density-lipoprotein (HDL) cholesterol - were measured as secondary objective, given their established relationship to cardiovascular disease. Finally, blood pressure was measured to monitor any potential changes.

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Subjects

Thirty-nine healthy adults, all residents of the San Francisco Bay area, volunteered to be screened for the study. In addition, 8 participants of a previous pilot study who qualified for the screening criterion volunteered to participate. Twenty-six of the screened participants qualified for the study and volunteered to participate, to give a total of 34 participants. During the study, however, 2 participants left for personal reasons (one went travelling and the other had time constraints) and one left because she started taking cholesterol-lowering medication mid-way through the study. Finally, 2 participants could not attend the final blood draw; hence a total of 29 subjects completed the study.

There were 11 males and 18 females, between the ages of 34 and 92 (mean age 66 years). All subjects signed a consent form and a standard subjects' Bill of Rights. In addition, they completed a health questionnaire to ensure that they were healthy and had no allergy to strawberries. There was no compensation for participation in the study. However, each participant received a half-flat of strawberries per week for 8 weeks at no cost and the results of the analyses.

Study Design

Before being accepted into the study, the volunteers were screened for serum homocysteine and a fasting blood sample was taken for homocysteine analysis. The inclusion criterion was a serum homocysteine level ≤ 6.9 $\mu\text{mol/L}$. In addition, participants had to be in general good health and free from cardiovascular diseases, kidney diseases, diabetes or cancer.

The participants who qualified for the study were asked to consume at least one serving of strawberries 6 days a week (7 days if possible) for 8 weeks. A serving of

strawberries comprised about 6-7 medium (about 6-7 oz) California strawberries. They were allowed to eat more if they wished and were given a few extra strawberries to account for losses due perishability. Fresh strawberries were picked up by the participants weekly at HRSC to ensure freshness. Participants were asked not to change their typical diet or their supplement routine. They were asked to fill out a weekly questionnaire on bowel function, evaluating frequency, amount, effort and consistency of stools, as well as laxative or stool softener use. In addition, they completed 3 basic food frequency questionnaire to ensure that there were no major changes in dietary patterns: one reflecting their pre-study diet, one during the first week of the study (week 1), and one during the final week (week 8). The participants' height was taken at the first blood draw visit and their weight was measured at both blood draw visits (week 1 and week 8) in order to calculate the Body Mass Index (BMI) and monitor any weight changes.

Fasting blood samples were taken at the beginning and at the end of the study. The main blood measurements were homocysteine, folate, and CRP. Triglycerides, TC, and HDL were also measured; LDL values were estimated with the Friedewald equation. The subjects were asked to consume their serving of strawberries in the last meal prior to the final blood draw. In addition, triplicate blood pressure measurements were taken by two certified nurses, either on the same day of the blood draw or on the previous day. The 3 measurements were taken 5 minutes apart, in a quiet room. If the participants were having their blood pressure taken the same day of their blood draw, they were allowed to rest about 30 minutes after the blood draw, or had their blood pressure taken before the blood draw. To ensure that baseline and end of study data could be properly compared, plasma samples were packed in dry ice for shipping and

stored at about -70°C at Pacific Biometrics Laboratories (Seattle, WA), where the tests were performed. Samples were pooled and analyzed together at the end of the study.

Data Analysis

The results of the blood measurements are described as mean \pm standard deviation (SD). Paired t -tests were used to compare the changes between the beginning and end values of the parameters analyzed. In the LDL calculation, one subject was excluded because having triglycerides > 400 mg/dL, which does not allow an accurate calculation of LDL. Description of blood pressure measurements reflects 28 of the 29 subjects because one subject was unable to come for the first blood draw and blood pressure exam and had his baseline blood draw one week later.

Results

Dietary Intake and Weight

The food frequency questionnaires were reviewed to compare the consumption of three categories of food intake - fruits, vegetables, and dairy products - prior to the study and at week 8. These categories were selected as being those that may influence folate intake and blood pressure measurements. This review revealed that the majority of the participants (52%) increased their intake of fruits of 4-5 servings/week; in addition, intake of vegetables increased by 3-4 servings/week in 31% of the participants; and consumption of dairy products also increased by 3-4 servings/week in 40% of the study subjects.

The mean weight of the subjects did not change for the duration of the study (77 ± 17 kg both at week 1 and week 8; BMI = 27 ± 4 kg/m²).

Folate, Homocysteine and CRP

After 8 weeks of daily strawberry consumption, there was a statistically significant increase of 22% in plasma folate

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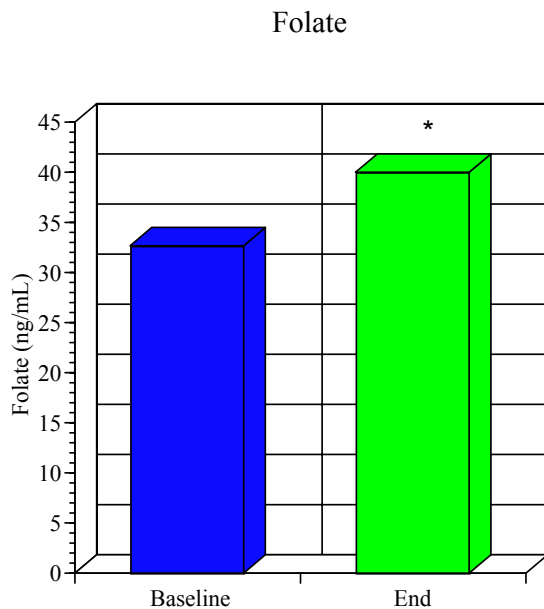
levels ($P < 0.001$) (Table 1 and Figure 1). However, no significant changes were observed in serum homocysteine and CRP (Table 1). Figures 2, 5, and 8 illustrate the changes in folate (fig. 2), homocysteine (fig. 5) and CRP (fig. 8) for each subject. Figures 3, 6, and 9 show the same changes ordered by baseline values. Finally, figures 4, 7, and 10 show the changes in each subject ordered by baseline values. No specific trend can be observed (see Additional Figures).

Table 1. Homocysteine, folate, and C-reactive proteins (CRP) at baseline and end of study (means \pm SD).

<u>Parameter</u>	<u>Baseline</u>	<u>End</u>	<u>Change</u>
Homocysteine ($\mu\text{mol/L}$)	9.9 \pm 3.2	9.5 \pm 2.2	-0.4 \pm 2.8
Folate (ng/mL)	32.7 \pm 10.6	40.0 \pm 11.8	7.3 \pm 6.3 *
CRP (mg/L)	2.1 \pm 1.7	2.1 \pm 2.0	- 0.1 \pm 1.0

*statistically significant ($P < 0.001$).

Figure 1. Mean folate levels at baseline and end of the



study.

* significantly different from baseline ($P < 0.001$).

Blood Pressure

At the end of the study there was a statistically significant reduction (4%, $P < 0.001$) in systolic blood pressure, whereas diastolic blood pressure did not change over the course of the study (Table 2).

Table 2. Blood pressure at baseline and end of study (means \pm SD).

<u>Blood Pressure</u>	<u>Baseline</u>	<u>End</u>	<u>Change</u>
Systolic (mm/Hg)	121 \pm 9	116 \pm 7	-5 \pm 7 *
Diastolic (mm/Hg)	71 \pm 4	71 \pm 5	0.3 \pm 6.2

*statistically significant ($P < 0.001$).

Serum Lipids and Lipoproteins

Finally, no significant changes were observed in serum triglycerides, TC, LDL, VLDL or HDL at the end of the study (Table 3).

Table 3. Serum lipids and lipoproteins at baseline and end of study (means \pm SD).

<u>Serum lipids/lipoproteins</u>	<u>Baseline</u>	<u>End</u>	<u>Change</u>
Triglycerides	156 \pm 108	151 \pm 90	-5 \pm 56
Total cholesterol	208 \pm 41	209 \pm 35	1.0 \pm 22
LDL cholesterol	128 \pm 40	129 \pm 35	1.0 \pm 18
VLDL cholesterol	28.8 \pm 17.8	28.4 \pm 15.5	0.4 \pm 11
HDL cholesterol	52 \pm 15	52 \pm 14	0 \pm 6

Bowel Function

The review of the bowel function questionnaires reveals no pattern of bowel function improvement. Most people (62%) did not experience any change in bowel function. Nine participants (31%) experienced an increase in stool frequency; 6 (21%) reported softer stools and 7 (24%) increased stool amount; lastly, only 2 participants (7%) experienced lessened effort in stool elimination. Among the 29 participants, only 2 were making habitual use of laxatives/stool softeners and continued to use them with the same or increased frequency during the study.

Discussion

Daily consumption of a daily serving of strawberries for 8 weeks resulted in a statistically significant but modest (22%) increase in plasma folate levels in healthy men and women. It also brought a significant but small reduction in systolic blood pressure (4%).

However, serum levels of homocysteine and CRP remained unchanged. Unlike in our previous pilot study, a screening for serum homocysteine levels was executed and resulted in a population with an average baseline level of ~ 10 $\mu\text{mol/L}$. Although no recommended optimal level for serum homocysteine has been established, several studies indicate that the risk for adverse outcomes seems to rise at levels above 10 $\mu\text{mol/L}$. Nonetheless, serum homocysteine did not change significantly with daily consumption of a serving of 6-7 oz of strawberries for 8 weeks. It is possible that the folate present in the amount of strawberries provided in the study (30 $\mu\text{g}/6\text{-oz}$) might not have been sufficient to bring a significant change in serum homocysteine. Significant decreases in serum homocysteine have been observed with supplementation providing 1 mg of folate /day. In addition, these changes have been observed in individuals with confirmed elevated homocysteine.

Additional Figures

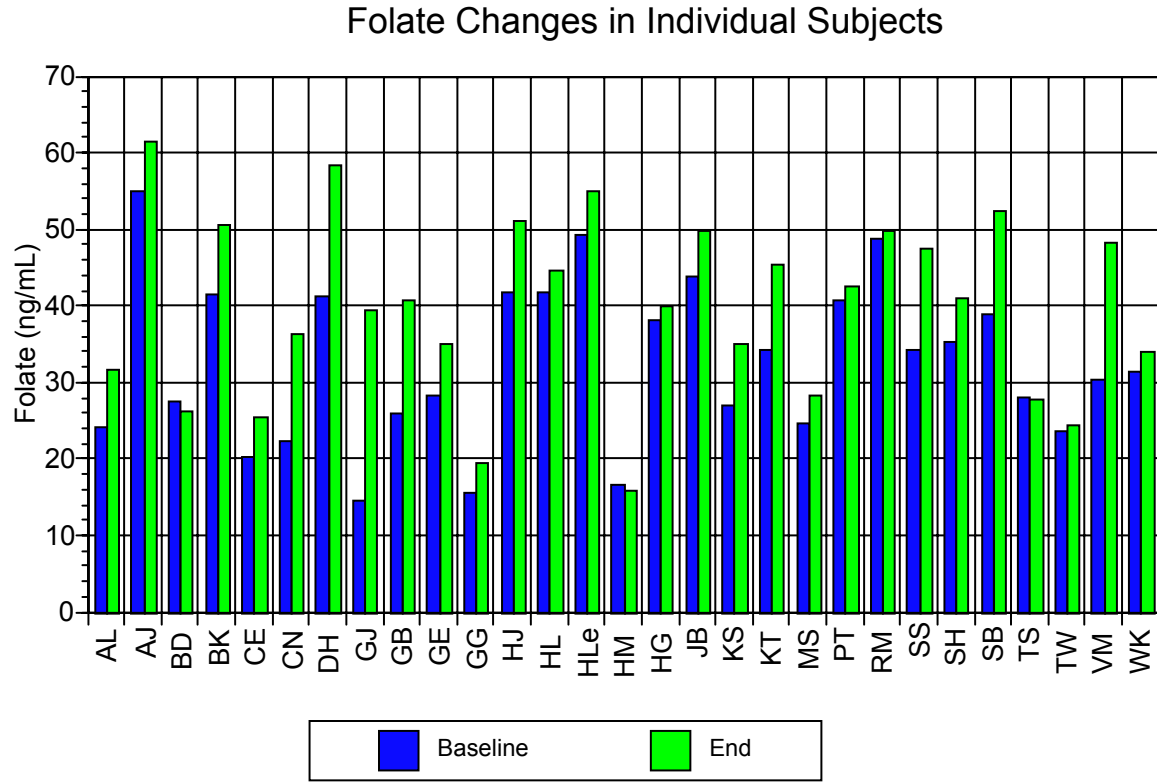


Figure 2. Folate levels (ng/mL) at baseline and end of study for each subject.

Folate Changes in Individual Subjects Ordered by Increasing Baseline Levels

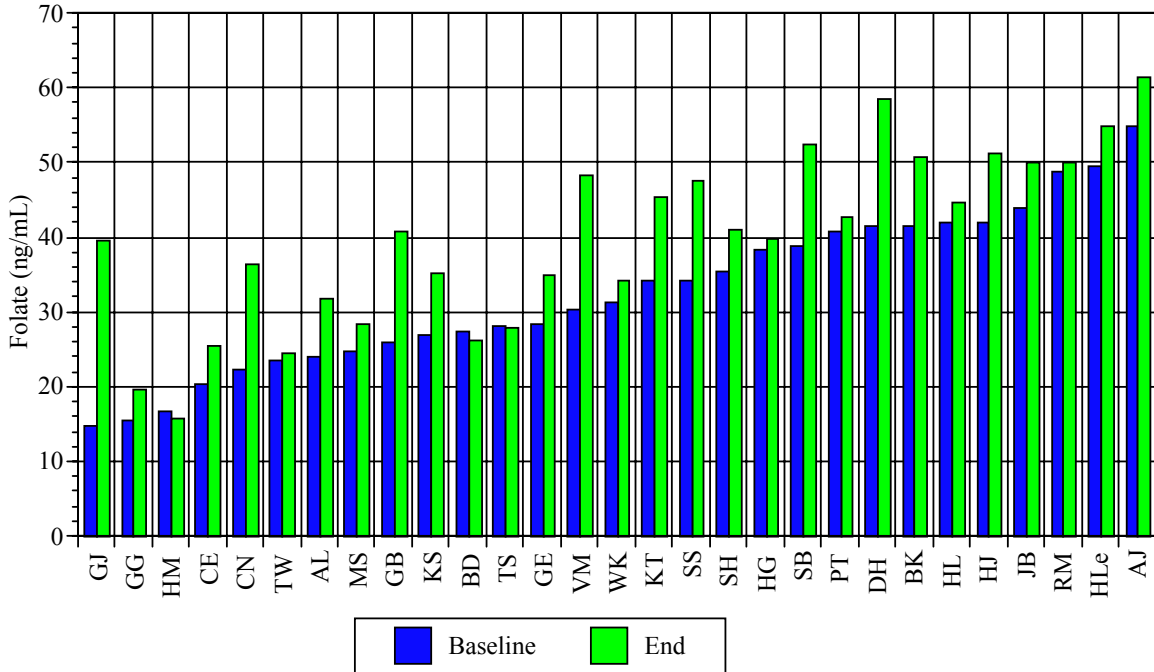


Figure 3. Folate levels (ng/mL) at baseline and end of study for each subject according to baseline levels.

Changes in folate according to baseline values

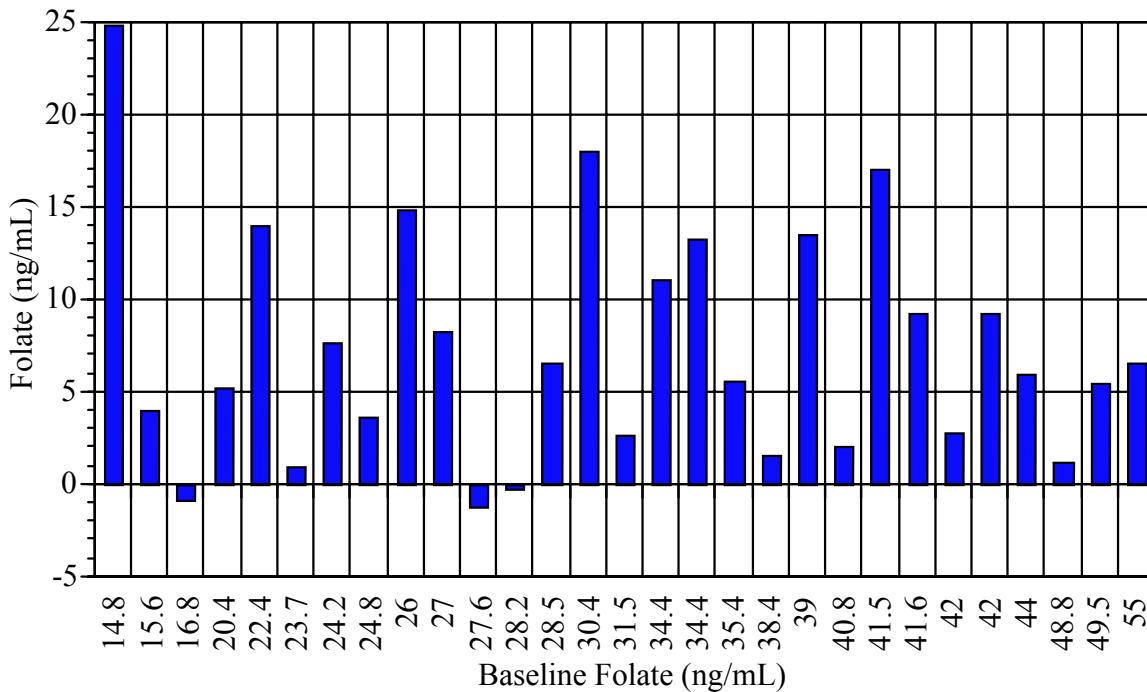


Figure 4. Changes (post-pre) in folate levels (ng/mL) for each subject according to baseline levels

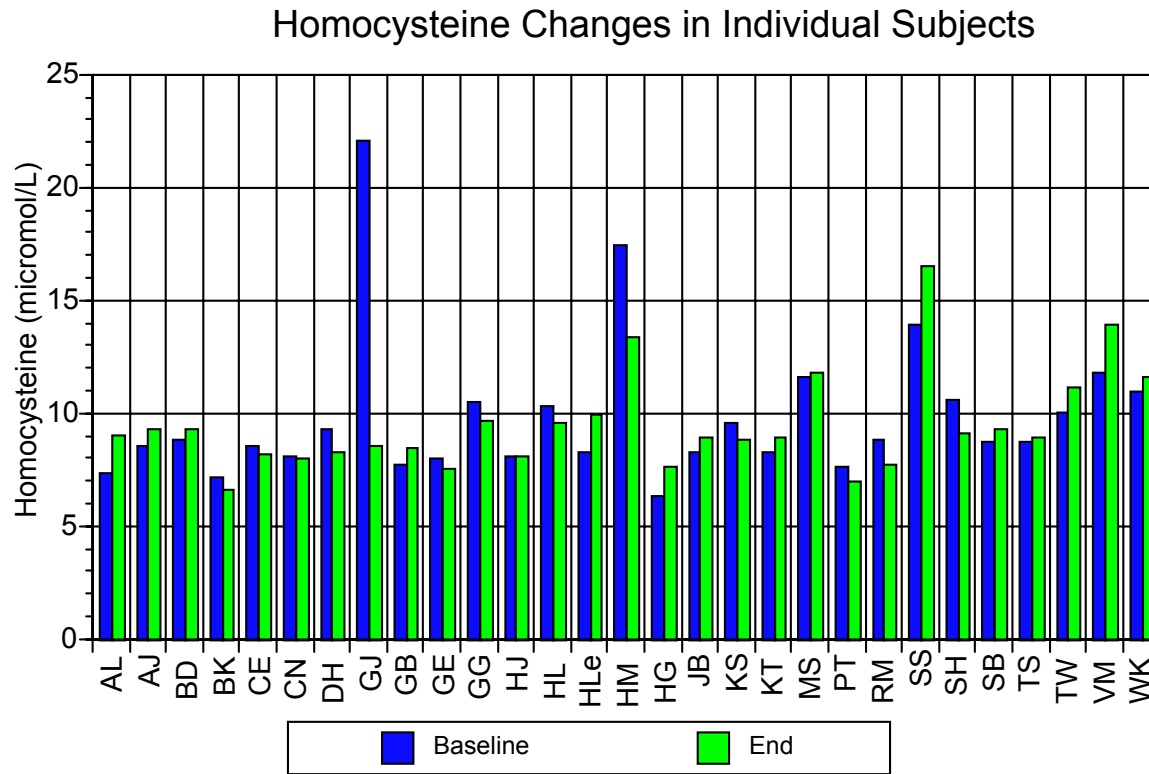


Figure 5. Homocysteine levels ($\mu\text{mol/L}$) at baseline and end of study for each subject.

Homocysteine Changes in Individual Subjects
Ordered by Decreasing Baseline Levels

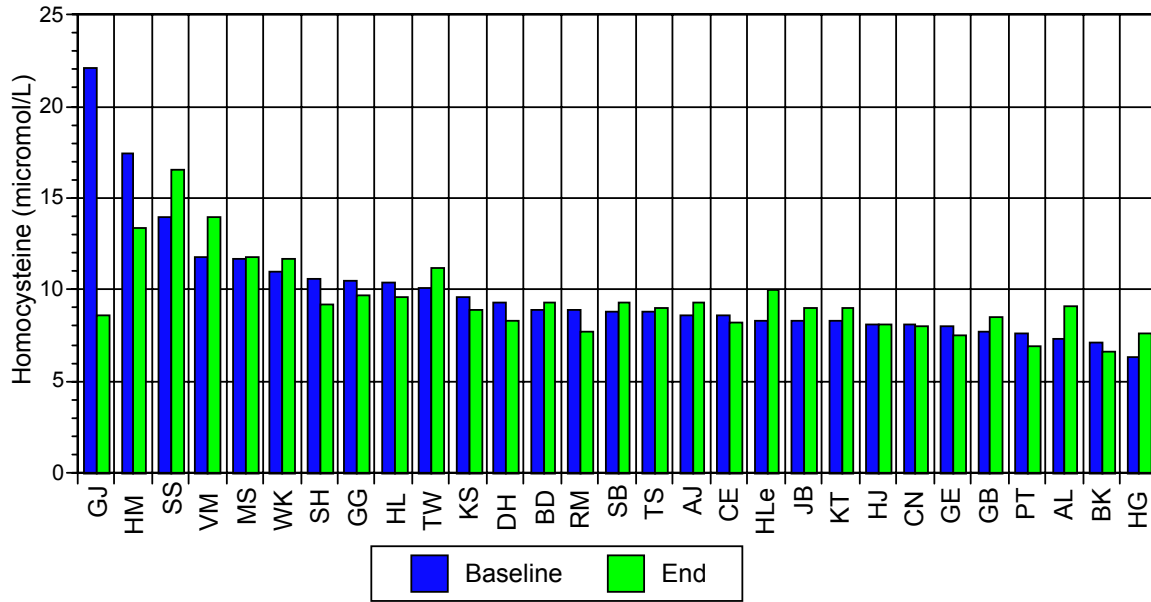


Figure 6. Homocysteine levels ($\mu\text{mol/L}$) at baseline and end of study for each subject according to baseline levels.

Changes in homocysteine according to baseline values

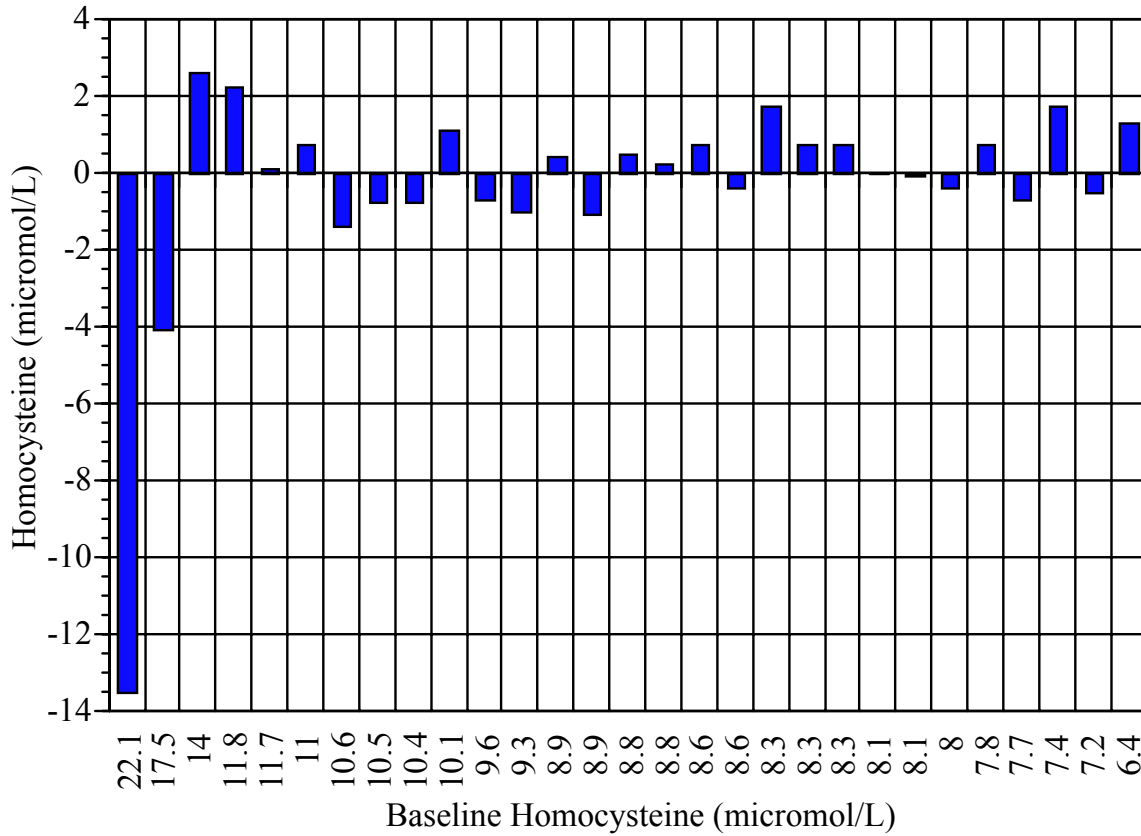


Figure 7. Changes (post-pre) in homocysteine levels ($\mu\text{mol/L}$) for each subject according to baseline levels.

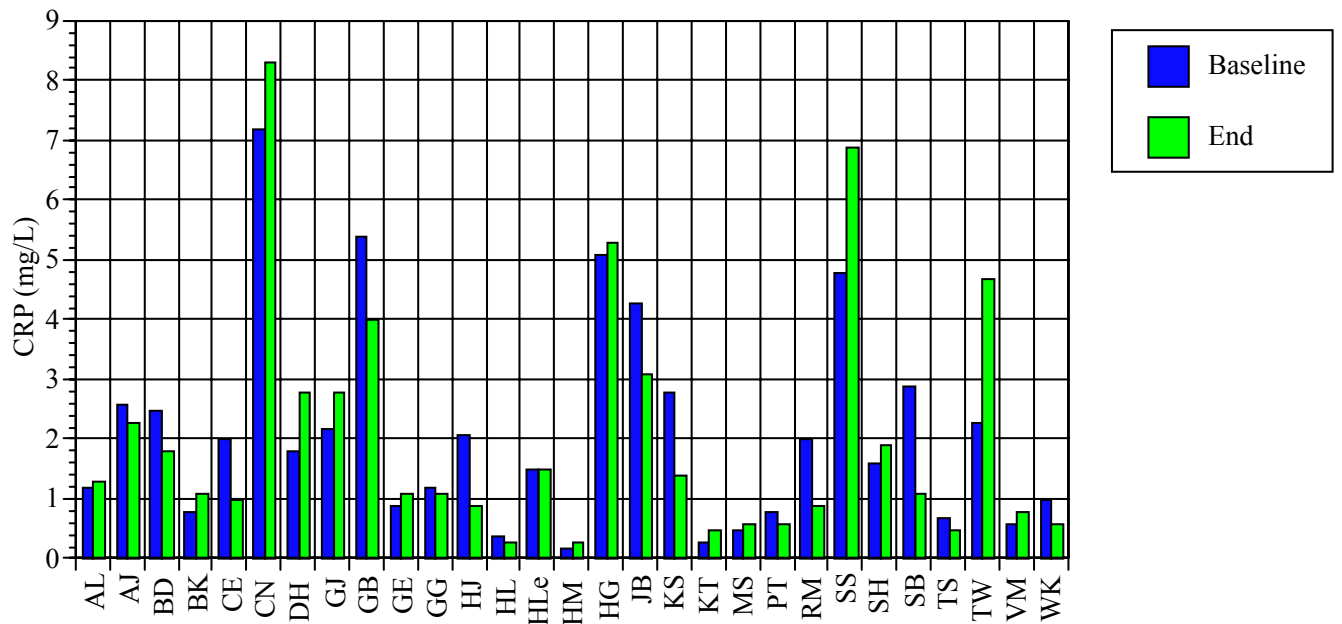
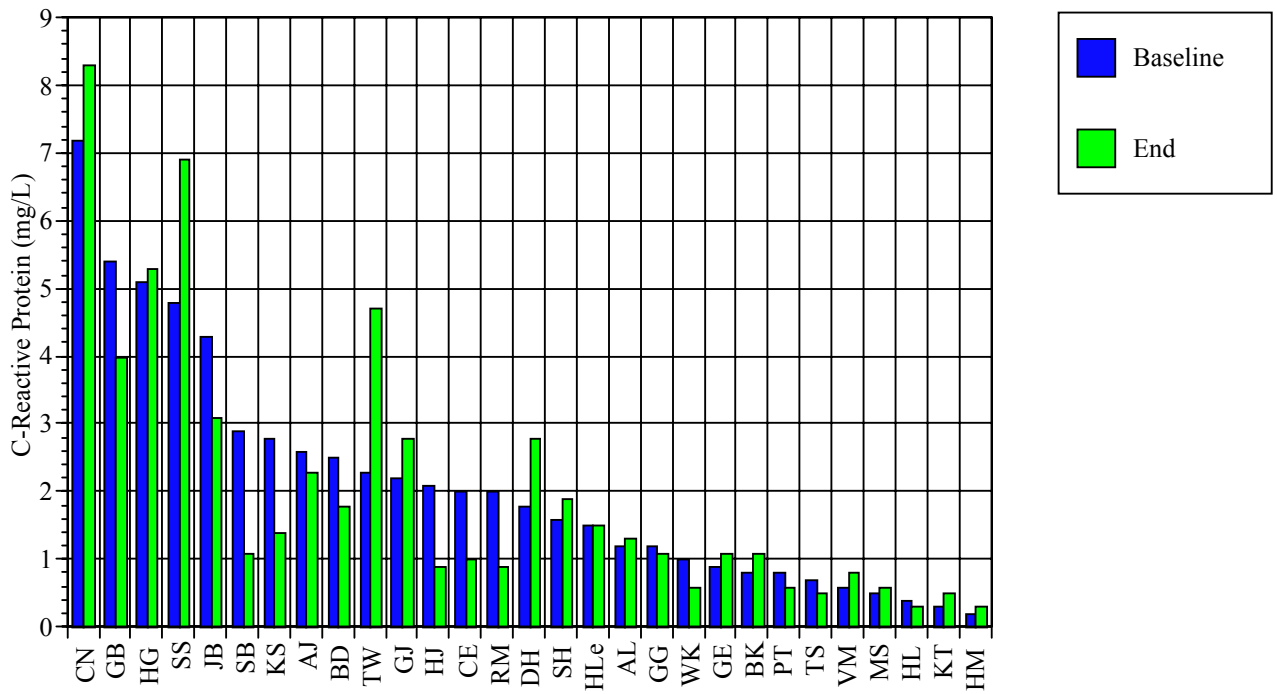


Figure 8. C-reactive proteins (CRP) levels (mg/L) at

C-Reactive Protein Changes in Individual Subjects
Ordered by Decreasing Baseline Levels



baseline and end of study for each

Figure 9. C-reactive protein (CRP) levels (mg/L) at baseline and end of study for each subject according to baseline levels.

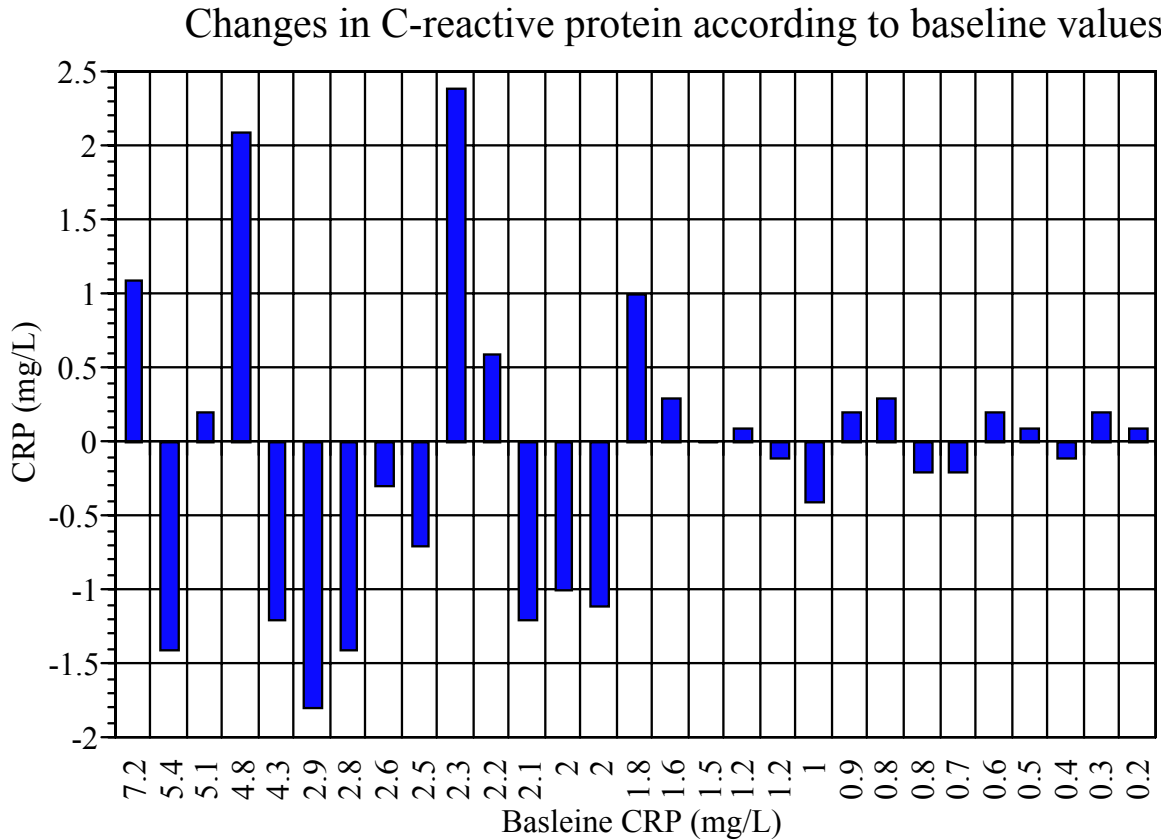


Figure 10. Changes (post-pre) in C-reactive protein (CRP) levels (mg/L) each subject according to baseline levels.