Exercise, Nutrition, and Homocysteine

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Homocysteine is an independent cardiovascular disease (CVD) risk factor modifiable by nutrition and possibly exercise. While individuals participating in regular physical activity can modify CVD risk factors, such as total blood cholesterol levels, the impact physical activity has on blood homocysteine concentrations is unclear. This review examines the influence of nutrition and exercise on blood homocysteine levels, the mechanisms of how physical activity may alter homocysteine levels, the role of homocysteine in CVD, evidence to support homocysteine as an independent risk factor for CVD, mechanisms of how homocysteine increases CVD risk, and cut-off values for homocysteinemia. Research examining the impact of physical activity on blood homocysteine levels is equivocal, which is partially due to a lack of control for confounding variables that impact homocysteine. Duration, intensity, and mode of exercise appear to impact blood homocysteine levels differently, and may be dependent on individual fitness levels.

Key Words: physical activity, cardiovascular disease risk factor, diet, vitamin, intensity, nutrient status

The beneficial effects of exercise for the primary and secondary prevention of cardiovascular disease (CVD) are well established (4). Among patients with established CVD, mortality is lower among those who undertake regular exercise with the level of protection dependent on the type, duration, frequency, and intensity of the activity stimulus (38). In addition, increased physical activity in previously sedentary individuals improves known CVD risk factors, such as blood pressure, total blood cholesterol levels, and blood lipid profiles. However, it is unclear if exercise improves or modifies recently identified CVD risk factors such as circulating levels of C-reactive protein and homocysteine.

Homocysteine was first identified as an important biological compound in 1932 (11) and linked with human disease in 1962 (89) when elevated urinary homocysteine levels were found in children with mental retardation. This condition, called homocysteinuria, was later associated with premature occlusive CVD, even in children. These observations led to research investigating the relationship of elevated homocysteine levels and CVD in a wide variety of populations including middle age and elderly men and women with and without traditional risk factors for CVD (6, 16, 62).
Based on the above studies, researchers have identified elevated blood homocysteine as an independent risk factor for CVD further stimulating research examining various factors that impact blood homocysteine levels. We now know a number of factors influence homocysteine levels, such as age, gender, genetics, medications, and lifestyle factors, such as alcohol intake, smoking, nutrition, and physical activity (83).

This review focuses on the impact of two lifestyle factors, nutrition and physical activity, on blood homocysteine concentrations. First, homocysteine metabolism is described. Second, the role that nutrition, especially the B-vitamins, has on homocysteine metabolism is reviewed. Third, the mechanisms of how physical activity may alter homocysteine levels are presented. Fourth, the role of homocysteine in CVD is discussed including evidence to support homocysteine as an independent risk factor, mechanisms related to homocysteine and CVD, and a definition of homocysteinemia. Finally, a review of the epidemiological, cross-sectional, and experimental research examining the impact of physical activity on blood homocysteine levels are presented.

**Homocysteine Metabolism**

Homocysteine is a normal metabolite of the essential amino acid methionine (Figure 1). Structurally, it closely resembles methionine and cysteine; all three amino acids contain sulfur. They are metabolically linked to each other as shown in Figure 1. Methionine is first converted to s-adenosyl-methionine and further

*Figure 1 — Vitamins and enzymes involved in homocysteine metabolism. Creatine is formed from guanidinoacetate and s-adenosyl-methionine. Adapted with permission from reference (45)*

ATP, adenosine triphosphate; Pi, inorganic phosphate; Ppi, inorganic pyrophosphate; TH4, tetrahydrofolate
processed to s-adenosyl-homocysteine before conversion to homocysteine. Once converted to homocysteine, it has two fates: 1) remethylation from homocysteine back into methionine or 2) transsulfuration from homocysteine into cystathionine with further metabolism to cysteine. Four key enzymes are involved in this complex metabolic pathway, methyl-tetrahydrofolate reductase, methionine synthase, cystathionine-β-synthase, and cystathionine-γ-lyase, which rely on vitamins as cofactors to function properly (Figure 1). For more biochemical details of homocysteine metabolism, refer to references 12 and 72.

Role of Nutrition

Homocysteine metabolism is driven by several B-complex cofactors. Folate, vitamin B-6, and vitamin B-12 are used in the remethylation pathway, and vitamin B-6 is used in the transsulfuration pathway (Figure 1). Deficiencies of folate, vitamin B-6, or vitamin B-12 can lead to impaired homocysteine metabolism. In addition, the amount of dietary methionine consumed influences homocysteine metabolism. Thus, these factors will either increase or decrease blood homocysteine, depending on dietary levels.

**Dietary Methionine.** When dietary methionine intake is high, the transsulfuration pathway is promoted over remethylation resulting in homocysteine catabolism to cysteine. Conversely, when dietary methionine is low, the remethylation pathway is favored over transsulfuration to elicit de novo methionine synthesis. A methionine-rich meal (e.g., meal high in animal protein) has been shown to cause an acute increase in homocysteine, which may last up to 24 h in healthy adults (35). Further, methionine-loading tests are often used to study the efficiency of homocysteine metabolism (2). Dietary methionine is found in protein-rich foods such as red meat, poultry, and cheese. Individuals consuming diets high in animal protein have reported increased methionine metabolism and higher homocysteine levels than vegetarians (50). However, some vegetarians and strict vegans may have low dietary intakes of methionine and vitamin B-12 resulting in high blood homocysteine levels (44). Thus, individuals consuming lower meat and animal protein diets with adequate intakes of B-vitamins may be able to keep homocysteine concentrations low (24).

**B-Vitamin Status.** The B-vitamins play critical roles in both the remethylation and transsulfuration pathways of methionine metabolism (Figure 1). The enzyme, methionine synthase, which is involved with the remethylation reaction converting homocysteine to methionine, requires both folate and vitamin B-12 as coenzymes. The transsulfuration pathway converts homocysteine to cysteine in a series of vitamin B-6 dependent reactions, which requires vitamin B-6 as a coenzyme for the enzymes cystathionine-β-synthase and cystathionine-γ-lyase. Therefore, adequate folate, vitamin B-12, and vitamin B-6 are critical for the enzymes in these pathways to function optimally in order to maintain homocysteine homeostasis. Both the amount of dietary intake and the blood vitamin levels of an individual contribute to their nutritional status. Reduced dietary intake and/or blood levels of these vitamins contribute to an accumulation of blood homocysteine.

Folate insufficiency is the predominant nutritional cause of elevated blood homocysteine in most healthy populations. Research indicates that individuals with diets high in folate or folic acid supplements are able to reduce blood homocysteine
concentrations (9, 10, 87), especially in individuals with low folate status and high homocysteine levels (20). Research also shows that folic acid supplements given to individuals with normal folate status can further lower homocysteine concentrations (46). Vitamin B-12 and, to a lesser extent, vitamin B-6 supplements are also effective at lowering homocysteine, either individually or in combination with folic acid supplements in individuals who have high blood homocysteine levels (77, 80). Therefore, a major contributing factor to high levels of blood homocysteine is a diet low in these three vitamins.

Based on recent data, not all Americans, especially women, consume enough folate, vitamin B-6, or vitamin B-12, even with the 1998 mandate of folic acid fortification to cereal grain products (25). According to NHANES data (2001 to 2002), the percentage of females (19 to 50 y) consuming less than the Estimated Average Requirement (EAR) of folate, vitamin B-6, and vitamin B-12 were 15%, 22%, and 8%, respectively (61). In addition, not all Americans, including active individuals, have optimal blood B-vitamin levels (64). Fogelholm et al. examined vitamin B-6 status in 42 physically active college students (18 to 32 y) before and after 5 wk of vitamin B-complex supplementation. Prior to supplementation, they reported 43% had poor vitamin B-6 status using erythrocyte aspartate aminotransferase activity coefficient as a marker for vitamin B-6 status (29). Herrmann et al. recently examined vitamin B-12 and folate status in 72 recreational endurance athletes (31 to 45 y) (41). They found 10% of the athletes deficient in vitamin B-12 and 15% deficient in folate based on serum levels. Thus, being physically active does not necessarily equate to a healthier nutritional status. Active and inactive individuals may have poor nutritional status, which may influence homocysteine levels independent of the amount, intensity, or type of exercise.

Role of Physical Activity

In addition to adequate nutrition, there is evidence that physical activity may also alter homocysteine production by increasing protein and/or methyl group turnover. These hypothesized mechanisms are discussed below.

**Protein Turnover.** During exercise, protein turnover could alter homocysteine concentrations by either increasing methionine catabolism, thus lowering homocysteine, or by decreasing B-vitamin availability, which would increase homocysteine.

It is well documented that prolonged high-intensity exercise (33, 84) increases protein metabolism and alters blood concentrations of certain amino acids (30). For example, Weiss et al. reported a 33% reduction in blood methionine levels in college students after a 2.5 h moderate intensity run (88). Reduced methionine availability would promote de novo methionine synthesis and, thus, reduce accumulation of homocysteine. In this way, the protein turnover mechanism would lower homocysteine concentrations during high intensity prolonged exercise, as long as folate, vitamins B-6 and B-12 were adequate.

Conversely, prolonged exercise, where glycogen reserves are reduced, places an increased demand on vitamin B-6 dependent reactions. Pyridoxal 5’ phosphate (PLP), the most biologically active form of vitamin B-6, is a coenzyme for transaminases, decarboxylases, and other enzymes used in the metabolic transformations of amino acids and nitrogen containing compounds. PLP is also required for glycogen phosphorylase, the key enzyme in the breakdown of muscle glycogen.
In addition, during exercise, gluconeogenesis involves the breakdown of amino acids, with the carbon skeleton used for energy. If exercise increases the demand for vitamin B-6 or increases its losses, less vitamin B-6 would be available for homocysteine catabolism. In this way, increased protein turnover during prolonged exercise would increase homocysteine concentrations.

**Methyl Group Turnover.** High-intensity exercise elicits an increase in methyl group turnover, which could increase homocysteine production. As shown in Figure 1, methionine is first converted to s-adenosyl-methionine, which is the most important methyl group donor in humans. A sufficient supply of methyl groups is important in several biochemical pathways, of which many are exercise related, such as the synthesis of DNA, RNA, carnitine, choline, acetycholine, phosphatidylcholine, epinephrine, adrenaline, methylhistadine, and creatine (60, 72, 82). Creatine synthesis in the liver accounts for nearly 75% of daily homocysteine formation (72), where s-adenosyl-methionine donates its methyl group to guanidinoacetate to form creatine and s-adenosyl-homocysteine (Figure 1). High-intensity exercise relies on creatine phosphate for muscle contractions, where creatine reacts with the adenosyl-triphosphate (ATP) produced by glycolysis and oxidative phosphorylation, to form ADP and creatine phosphate. During exercise, when muscle ATP is being consumed, the high-energy phosphate group of creatine phosphate is transferred to ADP to restore ATP. Creatine is then recycled or converted to creatinine, which is excreted in the urine. Thus, high-intensity long-duration physical activity, which increases the demand for creatine, increases homocysteine production compared with less-intense short-duration physical activity. Further, when creatine is taken orally, the endogenous production of creatine decreases, which also decreases the endogenous production of homocysteine. A recent study examined the effects of a 4 wk oral creatine supplementation (amount of creatine taken each day was equal to twice their creatinine excretion; 2.1 to 5.1 g/d) in healthy adults ranging in age from 21 to 58 y (48). The experimental group had a significant reduction in homocysteine production \((n = 8; -0.9 \mu\text{mol/L})\) compared to the control group \((n = 8; +0.2 \mu\text{mol/L})\) \((P < 0.05)\), supporting that when endogenous creatine is made, blood homocysteine levels may rise. Thus, an increase in methyl group turnover increases homocysteine production.

**Homocysteine and CVD**

For many individuals, CVD cannot be fully explained by the traditional risk factors such as hypertension and high blood cholesterol levels. For example, 35% of coronary heart disease cases occur in individuals with total cholesterol levels < 200 mg/dL (54). Other risk factors, such as blood homocysteine concentrations, appear to have strong relationships with CVD. This section reviews evidence supporting homocysteine as an independent risk factor for CVD, discusses the proposed mechanisms whereby homocysteine contributes to CVD, and examines the levels of blood homocysteine concentrations associated with increased CVD risk.

**Homocysteine as a CVD Risk Factor**

Extensive research supporting high blood homocysteine levels as an independent CVD risk factor is summarized in current meta-analyses, review articles, and
clinical trials. The first meta-analysis was completed by Boushey et al., who included 27 studies involving more than 4000 patients with occlusive vascular disease (cardiovascular, peripheral, and cerebrovascular) and an equal number of controls (8). Results showed that homocysteine was an independent risk factor for atherosclerotic disease in the coronary, cerebral, and peripheral vessels, and that a 5 µmol/L increment increase in total plasma homocysteine levels was associated with a 60% increased risk for coronary heart disease in men and an 80% increased risk for women (8). This study was followed by two more meta-analyses that also supported the relationship between high homocysteine levels and CVD (15, 86).

The Homocysteine Studies Collaboration (15), a compilation of prospective and retrospective studies using a total of 5073 heart disease events and 1113 stroke events, found that a 25% lower than usual blood homocysteine concentration (~ 3 µmol/L lower) was associated with an 11% lower heart disease risk (15). In addition, Wald et al. completed a meta-analysis that used 72 studies in which the prevalence of a mutation in the methyl-tetrahydrofolate reductase gene, which increases homocysteine, was determined in cases and controls (n = 16,849) as well as 20 prospective studies with 3820 participants (86). They concluded from these studies that by lowering blood homocysteine concentrations by 3 µmol/L from current levels, the risk of ischaemic heart disease was reduced by 16% and deep vein thrombosis reduced by 25% (86). Finally, Refsum and Ueland completed an extensive review examining the relationship between CVD mortality and blood homocysteine concentrations (66). They reviewed 80 studies, including more than 10,000 patients of cross-sectional, case control, nested case control, and cohort populations and found that blood homocysteine concentration was a prevalent and strong risk factor for atherosclerotic vascular disease in the coronary, cerebral, and peripheral vessels, and for arterial and venous thromboembolism. They concluded high blood homocysteine levels confers a graded increased risk with no threshold, is independent of and may enhance the effect of the conventional risk factors, and seems to be a particularly strong predictor of cardiovascular mortality.

Clinical trials have also examined the association between myocardial infarct (MI) and increased homocysteine levels. Stampfer et al. completed a clinical trial using 14,916 male physicians, age 40 to 84 y, with no prior MI (75). They measured plasma homocysteine concentrations at baseline and after 5 y. Blood samples from 271 men who subsequently developed MI were analyzed for homocysteine levels together with paired controls, matched by age and smoking. They concluded that moderately high levels of plasma homocysteine were associated with subsequent risk of MI independent of other coronary risk factors (75). This finding was supported by a more recent clinical trial by Zylberstein et al., where 1368 women were followed for 24 y (93). These researchers found that blood homocysteine levels in excess of 14.2 µmol/L in middle-age women was an independent risk factor for future MI, particularly fatal events (93). Collectively, these studies strongly suggest that elevated blood homocysteine levels increase risk of CVD, independent of other CVD risk factors.

Mechanism: How Does Elevated Blood Homocysteine Increase CVD Risk?

Homocysteine appears to have a wide variety of adverse effects on vascular physiology that contributes to increased cardiovascular risk. The most commonly
suggested mechanisms explaining the link between CVD and homocysteine are endothelial dysfunction and platelet aggregation/thrombosis. These mechanisms are briefly reviewed here.

**Endothelial Dysfunction.** Homocysteine may promote atherosclerosis by inducing endothelial dysfunction, which is characterized as a loss in vasodilation control in blood vessel cells. In cell culture studies, homocysteine inhibits endothelium-dependent anticoagulant reactions (39, 52, 68), induces the expression of procoagulants (31, 68), decreases interactions between endothelial cells and plasminogen activators (36, 37), and impairs the bioavailability of endothelium-derived nitric oxide (74). These findings are supported by animal studies in mini-pigs and rats, which have showed specific structural abnormalities in the large arteries with elevated blood homocysteine levels (13, 59, 69). Studies in cynomolgus monkeys also showed that high blood homocysteine levels impaired responses to endothelium-dependent vasodilators, such as nitric oxide (53). Nitric oxide allows the smooth muscle of blood vessels to relax or dilate, which creates greater blood flow when the demand for oxygen is high, such as during exercise. Therefore, an impaired response to nitric oxide would inhibit blood vessel dilation.

Human studies also support a relationship between high blood homocysteine levels and endothelial dysfunction via impaired nitric oxide release (70, 78). Degradation of nitric oxide via abnormal interaction with the free thiol moiety of homocysteine may decrease the bioavailability of nitric oxide. Thiols are proposed to react with nitric oxide to form s-nitrosothiols, which have both potent vasodilation and antiplatelet effects. Woo et al. found endothelial-dependent dilation was significantly lower in subjects with high homocysteine levels when compared to those with low homocysteine levels ($P < 0.001$) (91). In addition, Dinckal et al. demonstrated the relationship between homocysteine and endothelial-dependent dilation by placing subjects on a 4-wk homocysteine lowering diet, which included B-vitamin supplements (26). They found significant reductions in blood homocysteine concentrations and significant improvements in endothelial-dependent dilation in comparison to the placebo group (26).

**Platelet Aggregation and Thrombosis.** Platelet aggregation is a clustering of platelets and/or blood cell fragments, which can lead to the formation of blood clots. Thrombosis is the formation or presence of one or more blood clots that may partially or completely obstruct the flow of blood through the circulatory system. The most convincing research that supports homocysteine’s role in platelet aggregation and thrombosis is via oxidative stress mechanisms. The oxidative damage caused by hydrogen peroxide during the oxidation of homocysteine may increase platelet activity. Specifically, the SH group of homocysteine is oxidized to a disulfide bond (-S-S-) in a reaction that is coupled to the formation of reactive oxygen species, such as hydrogen peroxide. In turn, these reactive species cause endothelial dysfunction, decrease nitric oxide production, and therefore accelerate atherosclerosis (34).

However, direct generation of reactive oxygen species by homocysteine is unlikely to have physiological relevance, but it is probable that oxidative stress is a secondary effect. See the review by Edirisinghe for this mechanism (28). Animal studies done in mice support a relationship between high blood levels of homocysteine and oxidative stress by activating signal transduction pathways leading to inflammation and apoptosis (90). However, in humans it has been much more difficult to find this relationship. Selhub argues that human studies provided inconclusive results.
when looking for thrombogenic abnormalities in patients with high blood levels of homocysteine (72). He suggested the abnormalities observed in human studies are due to inconsistencies in genetic background, dietary habits, and pathology differences among sample populations.

In summary, cell culture, animal and human research all support a relationship between high homocysteine levels and endothelial dysfunction. It appears high blood homocysteine levels are partially responsible for endothelial dysfunction and high concentrations may attenuate platelet aggregation and thrombosis. Currently, not one unifying hypothesis exists that explains the mechanistic effects of high levels of circulating homocysteine on CVD.

What is Elevated Blood Homocysteine?

What level of blood homocysteine is associated with a higher risk of CVD? Some researchers argue that the lowest level of blood homocysteine possible is ideal (85); however, not everyone agrees on a single value or range of homocysteine that represents the lowest risk of CVD. The term hyperhomocysteinemia is used to describe an individual with elevated blood homocysteine concentrations, yet there is no standard upper level cut-off value. Hyperhomocysteinemia values vary among published reports, with studies typically using the 95th percentile values as a high cut-off point in their reported control samples.

Stamper et al. was the first to publish reference ranges based on the 95th percentile cut-off point. They reported a 95th percentile value of 15.8 µmol/L for US white men (75). In South Africa, Ubbink et al. used men’s responses to vitamin supplementation to develop a mathematical prediction model to calculate the plasma homocysteine concentration that could be expected for individuals treated with a vitamin supplement (81). They predicted that plasma homocysteine concentrations would approach a normal frequency distribution with a 95% reference range of 4.9 to 11.7 µmol/L for adult white men, provided that the vitamin status of the study population is improved (81). Data extracted from the Third National Health and Nutrition Examination Survey (NHANES 1991-1994) helped to identify reference ranges for serum total homocysteine concentration in US men and women (73). For individuals age 20 to 39 y, the 95th percentile for blood homocysteine concentrations were ≥ 11.4 µmol/L for men and ≥ 10.4 µmol/L for women. Finally, the Nutrition Committee of the American Heart Association suggested a blood homocysteine level < 10 µmol/L is a reasonable therapeutic goal for individuals at increased risk. They determined it was better to have a goal level of blood homocysteine rather than using the definition of “normal” based on population statistical values of the mean ± 2 standard deviations (56).

Regardless of the upper cut-off values for blood homocysteine, risk for CVD rises with increasing homocysteine concentrations. In general, blood homocysteine concentrations increase with age and are higher in men than in women. A review by Boushey et al. found a 60 to 80% greater risk for CVD for each 5 µmol/L increment of plasma homocysteine concentration > 10 µmol/L and suggested this increase in risk is similar to a 0.5 mmol/L (20 mg/dL) increase in total blood cholesterol (8). Furthermore, as mentioned earlier, some reports suggest that reducing blood homocysteine concentrations by 3 µmol/L reduces chronic disease risk by as much as 25% (15, 86). However, recent clinical trials using blood homocysteine lowering therapies (B-vitamin supplementation vs. placebo) in individuals with previous
heart attacks or strokes have not been successful at lowering risk of recurrent events with supplementation (7, 79). These data suggest maintaining the lowest possible blood homocysteine concentration throughout the lifespan may help keep chronic disease risk low.

**Physical Activity and Homocysteine**

Increased physical activity in previously sedentary individuals modifies known risk factors for CVD, including improvements in circulating levels of total serum cholesterol, blood pressure, and cardiorespiratory fitness (VO$_{2\text{max}}$) (5). Although the effects of exercise on these traditional risk factors are well documented, few studies have addressed whether physical activity can modify blood homocysteine. This section reviews the current literature that examines the relationship between physical activity and blood homocysteine concentrations in epidemiology, cross-sectional, and intervention/experimental studies.

**Epidemiology Research**

Currently, there has only been one large population-based study that examines the relationship between lifestyle activeness and blood homocysteine concentrations. Nygard et al. examined the relationship between physical activity and homocysteine in the Hordland Homocysteine Study (Norway) (63). They found self-reported leisure time physical activity was negatively associated with blood homocysteine concentrations ($P < 0.001$) in both men and women. Participants reported their leisure time physical activity for the year prior to the study in one of four categories (sedentary/none, moderate activity, active exercise, heavy training). Those individuals with the highest leisure time physical activity had the lowest levels of blood homocysteine. The difference in blood homocysteine levels between the sedentary and highly active groups was 0.76 µmol/L in men and 0.94 µmol/L in women; however, very few individuals were in the highly active groups (147 men and 51 women; total $N = 12,263$). Unfortunately, B-vitamin status was not reported for these individuals. However, when the researchers statistically adjusted for higher fruit and vegetable consumption and supplement use, they found significantly lower blood homocysteine levels ($P < 0.01$) (63). This study stimulated interest in the relationship between physical activity, diet, and homocysteine levels and prompted many of the research studies discussed in the next section.

**Cross-Sectional Research**

To date, nine cross-sectional studies have examined the association between physical activity and homocysteine by using self-reported questionnaires, personal standardized interviews, and/or fitness levels determined by measured VO$_{2\text{max}}$ to describe individual physical activity levels (Table 1). Two of these studies (55, 71) found no significant relationship between the level of blood homocysteine concentrations and physical activity levels. One study by Saw et al. found higher amounts of physical activity was associated with lower homocysteine levels in Chinese men and women before statistically controlling for folate status, but after multivariate adjustment there was no difference between exercisers and non-exercisers (71). Unfortunately, individuals in this study were considered exercisers if they self-reported
Table 1  Cross Sectional Studies Examining Relationships Between Levels of Physical Activity and Blood Homocysteine Concentrations

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample number/ gender</th>
<th>Measure of physical activeness</th>
<th>[Hcy]$^3$ &amp; physical activity level</th>
<th>Vitamin status Blood/intake$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(55)</td>
<td>204 F 380 M</td>
<td>interview &amp; questionnaire: activity levels</td>
<td>No relationship</td>
<td>Blood (folate, B-6, B-12)</td>
</tr>
<tr>
<td>(67)</td>
<td>20 M</td>
<td>$VO_{2\text{max}}$ &amp; questionnaire: h/wk</td>
<td>↑</td>
<td>Not reported</td>
</tr>
<tr>
<td>(19)</td>
<td>1319 F 1297 M</td>
<td>questionnaire: h/wk</td>
<td>↑ females only</td>
<td>Intake (folate)</td>
</tr>
<tr>
<td>(71)</td>
<td>270 F 216 M</td>
<td>interview &amp; questionnaire: h/wk</td>
<td>No relationship</td>
<td>Blood &amp; intake (folate, B-6, B-12)</td>
</tr>
<tr>
<td>(14)</td>
<td>1154 F 1128 M</td>
<td>questionnaire: activity levels</td>
<td>↓ endurance exercisers only*</td>
<td>Intake</td>
</tr>
<tr>
<td>(18)</td>
<td>192 F 231 M</td>
<td>interview: h/wk</td>
<td>↓</td>
<td>Not reported</td>
</tr>
<tr>
<td>(49)</td>
<td>90 F 87 M</td>
<td>questionnaire: activity levels</td>
<td>↓</td>
<td>Intake (folate, B-6, B-12)</td>
</tr>
<tr>
<td>(40)</td>
<td>191 F 196 M</td>
<td>interview: activity levels</td>
<td>↓</td>
<td>Not reported</td>
</tr>
<tr>
<td>(51)</td>
<td>730 F 714 M</td>
<td>$VO_{2\text{max}}$: fitness levels</td>
<td>↓</td>
<td>Blood (folate &amp; B-12)</td>
</tr>
</tbody>
</table>

$^1$M, male; F, female; $^2$Physical activity was measured with questionnaires, interviews, and/or estimated $VO_{2\text{max}}$ values. Activity levels were determined by activity h/wk, category activity levels, such as low, moderate, and heavy, or $VO_{2\text{max}}$ values; $^3$[Hcy] Blood homocysteine concentrations; $^4$All dietary intake data was reported using Food Frequency Questionnaires, which measured average intake per week over the previous year for consumption of particular foods or food groups. Approximate frequency of consumption was then quantified in terms of the number of times a month a food was consumed.

↓ Lower homocysteine concentrations in the group most physically active
↑ Higher homocysteine concentrations in the group most physically active
* Compared with resistance exercise or a sedentary lifestyle

participating in at least 0.5 h of physical activity a week, such as brisk walking, bowling, tai chi, chi kung, jogging, tennis, or swimming laps. This duration and/or frequency of activity may be too low to determine whether blood homocysteine levels are different between exercisers and non-exercisers. Two strengths of this study were that B-vitamin status was evaluated using both dietary intake and blood measurements and hours of television watching was reported to further understand the activeness of their participants (71).

Five cross-sectional studies showed lower blood homocysteine levels in individuals reporting higher levels of physical activity (14, 18, 40, 49, 51), but none of
these studies statistically adjusted for B-vitamin status based on blood measures and dietary intake.

Finally, two cross-sectional studies showed a positive relationship between homocysteine and physical activity levels. Rinder et al. reported higher homocysteine levels in exercisers when compared to non-exercisers. They compared ten competitive master male athletes (68.5 ± 1.4 y) to inactive controls (64.5 ± 2.3 y) and found blood homocysteine concentrations in the athletes to be significantly higher (10.7 ± 1.3 µmol/L) when compared to controls (9.2 ± 1.4 µmol/L) \((P = 0.02)\) (67). De Bree et al. also found a similar relationship between physical activity and homocysteine, but only in women (19). They examined a random sample of Dutch men and women (20 to 65 y) and found no association between physical activity and homocysteine levels in men. However, for women, they found a positive relationship between physical activity and plasma homocysteine levels while adjusting for age, dietary folate intake, vitamin supplement use, and other lifestyle factors. Thus, as hours of physical activity increased, so did blood homocysteine levels in females (19).

In summary, these nine cross-sectional studies reported equivocal results with no clear consensus as to whether physical activity negatively or positively impacts blood homocysteine levels. There are a number of confounders that may have contributed to the mixed observations in these studies: 1) nutritional status parameters were not assessed or accounted for; 2) age ranged widely within each study; 3) individuals self-reported their physical activity levels, which are often overestimated; and 4) level of physical activity and/or fitness level was defined differently among the studies making comparisons between studies difficult.

**Exercise Interventions**

Many experimental studies have examined the impact of physical activity on homocysteine levels (Tables 2 through 4) with varying degrees of exercise intensity and length of exercise interventions. Therefore, these studies are divided into three categories: 1) **acute exercise**, defined as one episode of physical activity lasting between 10 to 210 min; 2) **chronic exercise**, defined as a physical activity program lasting 10 d or more in previously active individuals; and 3) in previously sedentary individuals.

**Acute Exercise.** Seven studies have examined the effect of acute exercise on blood homocysteine levels in active individuals. As summarized in Table 2, three studies found no effect (21, 22, 92), three studies found exercise to increase homocysteine levels (42, 47, 88), while one study found acute exercise to decrease blood homocysteine levels (32).

The three studies reporting an increase in homocysteine levels with acute exercise had 60 min or longer duration of physical activity, but none of these studies statistically adjusted for B-vitamin status based on both blood measures and dietary intake (42, 47, 88). The three studies finding no effects of acute exercise on blood homocysteine concentrations were all ≤ 60 min in duration and used moderate-intensity stationary cycling, possibly not rigorous enough to elicit changes (21, 22, 92).

Two factors that may influence the effect of physical activity on blood homocysteine levels are exercise mode and intensity. For example, Herrmann et al. compared blood homocysteine levels of marathon runners, 100 km runners,
Table 2  Blood Homocysteine Response to Acute Exercise in Active Individuals

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample number/gender</th>
<th>Minutes (mode) intensity</th>
<th>[Hcy] response to exercise</th>
<th>Vitamin status Blood/intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>(92)</td>
<td>20 M</td>
<td>30 (cycling) 70% max heart rate</td>
<td>≠</td>
<td>Not reported</td>
</tr>
<tr>
<td>(21)</td>
<td>15 F</td>
<td>~ 10 (cycling) VO&lt;sub&gt;2max&lt;/sub&gt; test</td>
<td>≠</td>
<td>Not reported</td>
</tr>
<tr>
<td>(88)</td>
<td>13 M</td>
<td>150 (running) ~ 70% max heart rate</td>
<td>↑</td>
<td>Not reported</td>
</tr>
<tr>
<td>(22)</td>
<td>7 M</td>
<td>60 (cycling) sustained 60% VO&lt;sub&gt;2max&lt;/sub&gt;</td>
<td>≠</td>
<td>Not reported</td>
</tr>
<tr>
<td>(47)</td>
<td>39 M</td>
<td>~ 67 (triathlon) competitive race</td>
<td>↑</td>
<td>Blood (folate &amp; B-12)</td>
</tr>
<tr>
<td>(42)</td>
<td>100 B</td>
<td>220-264 (running) 328-390 (mtn. biking) 630-715 (100 k running) competitive race</td>
<td>↑ ≠ │ Blood* (folate &amp; B-12)</td>
<td></td>
</tr>
<tr>
<td>(32)</td>
<td>12 M</td>
<td>~ 12 (cycling) VO&lt;sub&gt;2max&lt;/sub&gt; test</td>
<td>↓</td>
<td>Intake (folate, B-6, B-12)</td>
</tr>
</tbody>
</table>

* M, male; F, female; [Hcy] Blood homocysteine concentrations

↓ lower homocysteine concentrations at the end of exercise compared to baseline

↑ higher homocysteine concentrations at the end of exercise compared to baseline

≠ no change in homocysteine concentrations

* blood vitamins only measured/statistically controlled for in individuals with >12 µmol [Hcy] (n = 23)

and mountain bikers before and after their respective races (42). Only marathon runners had significantly higher homocysteine levels after racing when compared to baseline levels (P < 0.05). When groups were compared, homocysteine values were significantly different among the groups at 15 min post-race (median values: marathon runners (16.1 µmol/L), 100 km runners (9.5 µmol/L), and mountain bikers (8.8 µmol/L); P < 0.05). The authors suggest the accumulation of homocysteine is higher in marathon runners because it is a sustained high-intensity, long-duration event, whereas, 100 km running is lower in intensity and may have brief occasions of rest similar to mountain biking downhill (42). To date, no intervention studies have examined the impact of acute exercise on blood homocysteine concentrations in sedentary individuals.

Chronic Exercise. Ten studies have examined the effect of chronic exercise, programs lasting from 2 to 25 wk, on blood homocysteine levels in active (Table 3) and sedentary (Table 4) individuals.

Active Individuals. As shown in Table 3, a total of five studies report no consistent blood homocysteine response to exercise training programs in previously active
individuals (3, 21, 43, 47, 76). One study showed a 10% increase in blood homocysteine levels in 14 active young (22 y) men training (70 to 85% max heart rate) for 4 wk with cycle ergometers ($P < 0.05$) (3). Conversely, Konig et al. found 30 d of triathlon training lowered homocysteine levels in well-trained male triathletes ($n = 39$). However, the significantly lower blood homocysteine concentrations were only found in the triathletes ($n = 9$) reporting the highest amount of training volume (> 14.9 h/wk) when compared to the group with the lowest training volume (< 9.1 h/wk) ($P < 0.05$) (47).

Herrmann et al. examined the impact of swimming on blood homocysteine concentrations and found a 10% increase in blood homocysteine levels induced by both high-intensity training (20 km/wk) and volume-oriented training (30 km/wk), yet these increases were not statistically significant, $P = 0.070$ and $P = 0.054$, respectively (43). De Cree et al. also reported no significant change in blood homocysteine levels with 10 d of high intensity cycle-ergometer training in females (21).
Table 4  Chronic Exercise and Blood Homocysteine Response in Sedentary Individuals

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Number/gender</th>
<th>Training program (duration, intensity, frequency, intervention length)</th>
<th>[Hcy]$^2$ response to training</th>
<th>Vitamin status Blood/intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>11 M</td>
<td>Variety of exercises 60 min, moderate: 3 × wk for 12 wks</td>
<td>↓</td>
<td>Blood (folate)</td>
</tr>
<tr>
<td>(17)</td>
<td>6 M</td>
<td>Walking 30 min, low: 5 × wk for 6 wks</td>
<td>≠</td>
<td>Blood (folate &amp; B-12)</td>
</tr>
<tr>
<td>(23)</td>
<td>30 M/F</td>
<td>Variety of exercises 45 min, low: 2 × wk for 17 wks</td>
<td>≠</td>
<td>Blood (folate, B-6, B-12)</td>
</tr>
<tr>
<td>(65)</td>
<td>12 F</td>
<td>Walking 24 min, low: 3 × wk for 25 wks</td>
<td>↓</td>
<td>Blood (folate &amp; B-12)</td>
</tr>
<tr>
<td>(27)</td>
<td>324 M/F</td>
<td>Walking 30 min, mod or high: 3-4 or 5-7 × wk for 25 wks</td>
<td>≠ ML</td>
<td>Intake (folate, B-6, B-12)</td>
</tr>
</tbody>
</table>

$^2$Hcy, Blood homocysteine concentrations
↓ lower homocysteine concentrations at the end of program compared to baseline
≠ no change in homocysteine concentrations
↑ higher homocysteine concentrations at the end of program compared to baseline
ML, moderate intensity, low frequency; MH, moderate intensity, high frequency; HH, high intensity, high frequency; HL, high intensity, low frequency

Finally, only one study has examined blood homocysteine response to resistance training (76). There were no differences in blood homocysteine levels found in a small group of women ($n=5$) participating in a weight-training program for 8 wk (76). Details about the weight lifting program were not reported. The intensity or frequency of the training program might have impacted the results.

In summary, these studies do not show a consistent effect of chronic exercise on blood homocysteine levels in active individuals. However, only three of the five studies reported blood B-vitamin levels and none reported B-vitamin intake, thus, adding to the variable results. In addition, it may be that significant effects were not found in three of these studies because individuals were described as active prior to the beginning of the study intervention and therefore, did not perform more exercise than their normal routines, which did not elicit a change in blood homocysteine levels over the course of the intervention.

**Sedentary Individuals.** As shown in Table 4, when sedentary individuals participated in exercise training, blood homocysteine levels varied in response. For example, Cooper et al. and de Jong et al. found no significant changes in blood homocysteine levels in men who participated in a 6-wk walking program or in
elderly adults who completed a variety of low-impact exercises for 17 wk, respectively (17, 23). However, in both of these studies the exercise intensity was very low, and may have not been high enough to elicit a change in blood homocysteine concentrations.

As demonstrated by Duncan et al., intensity of the exercise may be a factor that modifies blood homocysteine (27). In this study, sedentary individuals were placed into one of four different exercise programs, high intensity-high frequency, high intensity-low frequency, moderate intensity-high frequency, or moderate intensity-low frequency. Homocysteine levels significantly increased in the high intensity-high frequency and the high intensity-low frequency groups compared to baseline values after 6 months of training ($P < 0.003$), but not in the moderate intensity groups (27). Conversely, others have found that low to moderate intensity exercise decreases homocysteine in individuals with health issues. Ali et al. reported that a 12-wk moderate-intensity aerobic-type exercise program decreased blood homocysteine levels in male phase II cardiac rehabilitation patients (1). Randeva et al. also reported a decrease in blood homocysteine levels in overweight women with polycystic ovary syndrome participating in a walking exercise program for 25 wk (65).

In summary, all of the exercise intervention programs used diverse exercise programs varying in program length (2 to 25 wk), exercise intensity and mode (brisk walking, cycling, swimming, running, weight training, etc.), and frequency (3 to 7 d/wk), which may be the reason for the varied results. In addition, health status varies among individuals, from those considered healthy to those with compromised health, which influences various metabolic pathways.

Conclusions

As illustrated in Figure 2, there is an interrelationship between nutrition, exercise, and homocysteine. Proper intake of vitamins B-6, B-12, and folate can help to maintain low homocysteine levels and support the increased demands on metabolism during high-intensity and/or prolonged exercise. For example, exercise may increase the need for vitamin B-6 since it is involved in many biochemical reactions necessary to fuel working muscles and repair damaged tissue (57, 58).

Based on research conducted over the last 10 y, no consistent relationship exists between physical activity and blood homocysteine concentrations. The impact of physical activity on blood homocysteine concentrations appears to vary based on fitness levels, nutritional status, genetic, or other factors that were either not measured or accounted for in the studies reviewed. The primary variables, excluding dietary factors, which help explain the inconsistencies in blood homocysteine levels, may be the mode, intensity, and duration of exercise. If these variables impact blood homocysteine concentrations, it may be relative to individual fitness levels.

Further studies are needed to help determine the relationship between exercise and blood homocysteine. These studies need to clearly describe the participant’s fitness level prior to the exercise program and detail the intensity, duration, and frequency of the exercises in the program. Furthermore, researchers need to carefully account for the many factors that influence homocysteine metabolism, such as dietary intake and blood vitamin levels of folate, vitamin B-6, and vitamin B-12, in order to reduce variability between individuals and studies.
Figure 2 — Diagram of relationships between blood homocysteine levels, nutrition, and exercise based on current research literature.

↓ decreases blood homocysteine concentration
↑ increases blood homocysteine concentration
≠ no change in blood homocysteine concentration

* impact of intensity and duration of exercise on blood homocysteine may be relative to individual fitness level

** impact of mode on blood homocysteine may depend on size of muscle mass involved and/or type of muscle contraction/loading

References


