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Joshua C. Neuman  
*Iowa State University*

Kelsey A. Albright  
*Iowa State University*

Kevin Schalinske  
*Iowa State University, kschalin@iastate.edu*

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# Exercise prevents hyperhomocysteinemia in a dietary folate-restricted mouse model.

## Abstract

Hyperhomocysteinemia is a condition that results from altered methyl group metabolism and is associated with numerous pathological conditions. A number of nutritional and hormonal factors have been shown to influence circulating homocysteine concentrations; however, the impact of exercise on homocysteine and methyl group balance is not well understood. Our hypothesis was that exercise represents an effective means to prevent hyperhomocysteinemia in a folate-independent manner. The purpose of this study was to determine the influence of exercise on homocysteine metabolism in a dietary folate-restricted mouse model characterized by moderate hyperhomocysteinemia. Female outbred mice (12 weeks old) were assigned to either a sedentary or free-access wheel exercise group. Following a 4-week acclimation period, half of the mice in each group were provided a folate-restricted diet for 7-weeks prior to euthanasia and tissue collection. As expected, folate-restricted sedentary mice exhibited a 2-fold increase in plasma total homocysteine concentrations; however, exercise completely prevented the increase in circulating homocysteine concentrations. Moreover, exercise reduced plasma homocysteine concentrations 36% within the group fed only the control diet. The prevention of hyperhomocysteinemia by exercise appears, at least in part, to be the result of increased folate-independent homocysteine remethylation owing to a 2-fold increase in renal betaine homocysteine S-methyltransferase. To our knowledge, this is the first report demonstrating the prevention of hyperhomocysteinemia by exercise in a dietary folate-restriction model. Future research will be directed at determining if exercise can have a positive impact on other nutritional, hormonal, and genetic models of hyperhomocysteinemia relevant to humans.

## Keywords

folate, hyperhomocysteinemia, exercise, betaine-homocysteine S-methyltransferase, mouse

## Comments

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Exercise prevents hyperhomocysteinemia in a dietary folate-restricted mouse model

Joshua C. Neuman<sup>a,b</sup>, Kelsey A. Albright<sup>a</sup>, and Kevin L. Schalinske<sup>a,b</sup>

<sup>a</sup>Department of Food Science and Human Nutrition and <sup>b</sup>Interdepartmental Graduate Program in  
Nutritional Sciences, Iowa State University, Ames, Iowa 50011.

Address for correspondence:  
Kevin L. Schalinske  
Department of Food Science and Human Nutrition  
220 MacKay Hall  
Iowa State University  
Ames, IA 50011  
Tel: 515-294-9230 Fax: 515-294-6193  
E-mail address: [kschalin@iastate.edu](mailto:kschalin@iastate.edu)

- 19 **Abbreviations:**
- 20 BMT; betaine-homocysteine S-methyltransferase
- 21 CBS; cystathionine  $\beta$ -synthase
- 22 GNMT; glycine N-methyltransferase
- 23 MS; methionine synthase
- 24 MTHFR; 5,10-methylene-tetrahydrofolate reductase
- 25 PEMT; phosphatidylethanolamine N-methyltransferase
- 26 SAH; S-adenosylhomocysteine
- 27 SAM; S-adenosylmethionine
- 28 THF; tetrahydrofolate

29 **Abstract**

30 Hyperhomocysteinemia is a condition that results from altered methyl group metabolism and is  
31 associated with numerous pathological conditions. A number of nutritional and hormonal factors  
32 have been shown to influence circulating homocysteine concentrations; however, the impact of  
33 exercise on homocysteine and methyl group balance is not well understood. Our hypothesis was  
34 that exercise represents an effective means to prevent hyperhomocysteinemia in a folate-  
35 independent manner. The purpose of this study was to determine the influence of exercise on  
36 homocysteine metabolism in a dietary folate-restricted mouse model characterized by moderate  
37 hyperhomocysteinemia. Female outbred mice (12 wk old) were assigned to either a sedentary or  
38 free-access wheel exercise group. Following a 4-wk acclimation period, half of the mice in each  
39 group were provided a folate-restricted diet for 7-wk prior to euthanasia and tissue collection. As  
40 expected, folate-restricted sedentary mice exhibited a 2-fold increase in plasma total  
41 homocysteine concentrations; however, exercise completely prevented the increase in circulating  
42 homocysteine concentrations. Moreover, exercise reduced plasma homocysteine concentrations  
43 36% within the group fed only the control diet. The prevention of hyperhomocysteinemia by  
44 exercise appears, at least in part, to be the result of increased folate-independent homocysteine  
45 remethylation owing to a 2-fold increase in renal betaine homocysteine *S*-methyltransferase. To  
46 our knowledge, this is the first report demonstrating the prevention of hyperhomocysteinemia by  
47 exercise in a dietary folate-restriction model. Future research will be directed at determining if  
48 exercise can have a positive impact on other nutritional, hormonal, and genetic models of  
49 hyperhomocysteinemia relevant to humans.

50 **Key Words:** folate; hyperhomocysteinemia; exercise; betaine-homocysteine *S*-  
51 methyltransferase; mouse

## 52 **1. Introduction**

53

54 The maintenance of the folate-dependent one-carbon pool and methyl group metabolism is  
55 essential for optimization of health. Perturbations of these interrelated metabolic pathways have  
56 been implicated in a number of diseases, including cancer development, cardiovascular disease,  
57 neural tube defects, and cognitive disorders [1-4]. Homocysteine is an important intermediate in  
58 methyl group metabolism and is partially dependent on folate/ B<sub>12</sub> for its metabolism.  
59 Hyperhomocysteinemia, a condition that can result from a lack of methyl group donors,  
60 cofactors, and/ or relevant genetic anomalies, has been shown to be an independent risk factor in  
61 the development of cardiovascular disease [5].

62

63 Homocysteine is a product of transmethylation reactions involving *S*-adenosylmethionine  
64 (SAM), the activated form of methionine, in which a methyl group is donated to a number of  
65 acceptors, including proteins, lipids, and nucleic acids (Fig. 1) [6]. Homocysteine can be  
66 remethylated back to methionine by folate-dependent or -independent mechanisms, or undergo  
67 irreversible catabolism by transsulfuration. Folate-dependent remethylation utilizes folic acid in  
68 its most reduced form to transfer a methyl group to homocysteine and generate methionine via  
69 the vitamin B<sub>12</sub>-dependent enzyme methionine synthase (MS). Conversely, folate-independent  
70 remethylation of homocysteine utilizes the enzyme betaine-homocysteine *S*-methyltransferase  
71 (BHMT) and a methyl group from betaine, a compound derived from the oxidation of choline.  
72 Transsulfuration of homocysteine by the vitamin B<sub>6</sub>-dependent enzymes cystathionine β-  
73 synthase (CBS) and cystathionine γ-lyase leads to irreversible catabolism and the eventual  
74 formation of cysteine. Thus, homocysteine balance and the prevention of

75 hyperhomocysteinemia are dependent on a number of substrates, cofactors, and the proper  
76 expression and function of key enzymes.

77

78         As the regulation of homocysteine balance is vital to maintain optimal health, the  
79 establishment of homocysteine management-based therapies is necessary to prevent or treat  
80 diseases related to hyperhomocysteinemia. Recent studies examining the role of exercise as a  
81 potential means to reduce circulating homocysteine concentrations have been inconclusive,  
82 owing in large part to the variations in study design and exercise regimes [7-15]. Moreover,  
83 discrepancies within these human studies, including B-vitamin and subject training status, as  
84 well as variations in mode, intensity, and duration of test exercises, limit the strength of their  
85 conclusions [16, 17]. Mechanistically, reductions in homocysteine concentrations by exercise  
86 may be related to increased protein turnover owing to increased plasma methionine  
87 concentrations during exercise, followed by reduced concentrations below basal levels after  
88 exercise [18-21]. This fluctuation in methionine availability for methyl group metabolism may  
89 be due, in part, to the increased need of methionine for muscle anabolism, potentially resulting in  
90 diminished homocysteine production [17, 21]. However, exercise also increases the demand of  
91 vitamin B<sub>6</sub> to support increased muscle catabolism, thereby potentially limiting its availability  
92 for transsulfuration and subsequently resulting in homocysteine accumulation [22].

93

94         Our hypothesis was that exercise represents an effective means to prevent  
95 hyperhomocysteinemia in a folate-independent manner. This was based on our previous  
96 research demonstrating that a gluconeogenic state and related hormonal alterations, similar to  
97 what is exhibited as a function of exercise, results in reduced homocysteine concentrations via

98 enhanced folate-independent remethylation of homocysteine, as well as increased catabolism  
99 [23-26]. The aim of the present study was to assess the influence of voluntary exercise on  
100 homocysteine balance using a folate-restricted mouse model of hyperhomocysteinemia. This  
101 moderate hyperhomocysteinemia model was utilized to represent populations that experience  
102 poor folate absorption or intake, as well as individuals with relevant polymorphisms associated  
103 with modestly high circulating homocysteine concentrations, such as the 5,10-  
104 methylenetetrahydrofolate reductase (MTHFR) C677T gene [27].

105

106



107 **2. Methods and materials**

108

109 *2.1. Chemicals and reagents.*

110

111 Reagents were obtained as follows: [<sup>14</sup>CH<sub>3</sub>]-betaine, Moravek; DL-homocysteine thiolactone,  
112 Sigma-Aldrich Chemical; 5-[<sup>14</sup>CH<sub>3</sub>]-tetrahydrofolate, Amersham Pharmacia; S-adenosyl-L-  
113 [*methyl*-<sup>3</sup>H] methionine, New England Nuclear. All other reagents were of analytical grade.

114

115 *2.2. Animals and diets.*

116

117 All animal procedures and protocols were approved by and conducted in accordance with  
118 guidelines established by Iowa State University Laboratory Animal Resources. Female  
119 intercrossing outbred mice (9-10 wk of age) were obtained from Harlan (Indianapolis, IN) and  
120 initially housed in groups of 2 or 3 in a 12-h light: dark cycle and provided an AIN-93G semi-  
121 purified diet (Table 1) and water ad libitum [28]. No antibiotics were added to the diets or  
122 drinking water, resulting in a moderate degree of folate deficiency as we have previously  
123 reported [24]. After an acclimation period of 3 d, mice were randomly assigned to one of 2  
124 groups: sedentary or free-access wheel exercised. Wheel exercised-mice were housed  
125 individually to obtain accurate distance calculation. For the duration of the study, wheel  
126 exercised-mice had free-access to their wheels 24 h/ d for 5 d/ wk. After 4 wk, half of the mice  
127 in each group were switched to a folate-restricted diet resulting in 4 groups: sedentary with  
128 control diet; sedentary with folate-restricted diet; wheel-exercised with control diet; and wheel-  
129 exercised with folate-restricted diet. After 11 wk, mice were fasted for 12 h and given an

130 intraperitoneal injection of freshly prepared ketamine: xylazine (90: 10 mg/kg body wt).  
131 Euthanasia consisted of exsanguination and removal of major organs for their subsequent  
132 processing as described previously [23-26]. Heparinized whole blood was collected via cardiac  
133 puncture, centrifuged at 4,000 g for 6 min, and plasma was stored at -20°C for subsequent  
134 homocysteine analysis. Liver tissue was rapidly removed and 0.5 g portions were homogenized  
135 in 2 ml of an ice-cold buffer containing 10 mM sodium phosphate (pH 7.0), 1 mM EDTA, 1 mM  
136 sodium azide, 0.25 M sucrose, and 0.1 mM phenylmethylsulfonyl fluoride. Homogenates were  
137 centrifuged at 20,000 g for 30 min at 4°C and the resulting supernatant was stored at -80°C for  
138 enzyme activity analysis. One kidney was removed, homogenized in 4 vol of the same buffer,  
139 and extracts were stored similar to liver samples. Total soluble protein concentrations were  
140 determined utilizing the Pierce Bicinchoninic Acid method (Thermo Scientific) with bovine  
141 serum albumin as the standard.

142

## 143 *2.2. Determination of homocysteine concentrations.*

144

145 Plasma homocysteine concentrations were determined as described by Ubbink et al. [29] with  
146 modifications [25]. For intracellular homocysteine determination, hepatic and renal tissue were  
147 homogenized in 2 volumes of 0.4 M perchloric acid, centrifuged at 9,000 g for 10 min at 25°C,  
148 and the resulting supernatant neutralized with 8 M potassium hydroxide and treated in the same  
149 manner as the plasma samples [30]. Both intracellular and plasma samples were incubated at  
150 4°C for 30 min in a solution containing 1 mM N-acetylcysteine as an internal standard and 10%  
151 tributylphosphine in dimethylformamide. Addition of 10% trichloroacetic acid with 1 mM  
152 EDTA was used to stop the reaction and centrifuged at 1,000 g for 5 min at 4°C. For

153 derivatization, the supernatant was collected and added to a solution containing 0.125 M borate  
154 buffer (pH 9.5), 0.1% 4-fluoro-7-sulfobenzofurazan, and 1.55 M sodium hydroxide.

155 Homocysteine detection and quantification was performed by HPLC in combination with  
156 fluorescence detection using a  $\mu$ Bondapak C<sub>18</sub> Radial-Pak column (Waters Associates) and a  
157 mobile phase containing 4% acetonitrile in 0.1 M potassium phosphate buffer (pH 2.1).

158

### 159 *2.3. Enzyme activity determinations.*

160

161 Measurement of BHMT activity was based on the method described by Garrow [31] and  
162 performed in triplicate. Protein aliquots of 40 and 100  $\mu$ g for hepatic and renal tissue,  
163 respectively, were added to a reaction mixture containing the following: 50 mM [<sup>14</sup>CH<sub>3</sub>]-betaine,  
164 100 mM DL-homocysteine thiolactone, 500 mM Tris (pH 7.5), 50 g/L bovine serum albumin,  
165 10% 2-mercaptoethanol solution, and deionized water. Following incubation at 37°C for 1 h, the  
166 reaction was terminated with 2.5 ml of deionized water and samples were immediately applied to  
167 Dowex 1 $\times$ 4 (OH form) resin columns. Eluted fractions were collected in scintillation vials and  
168 radioactivity measured by liquid scintillation counting.

169

170 MS activity measurements were performed as described [32] with 600  $\mu$ g protein added to 100  $\mu$ l  
171 of a reaction mixture containing freshly prepared 100 mM DL-homocysteine thiolactone, 1.3 mM  
172 cyanocobalamin, 500 mM sodium phosphate buffer (pH 7.5), 10 mM S-adenosylmethionine,  
173 82.4 mM 2-mercaptoethanol, 1 mM dithiothreitol, 15 mM 5-[<sup>14</sup>CH<sub>3</sub>]-tetrahydrofolate, and  
174 deionized water. Following incubation at 37°C for 1 h, the reaction was terminated with 800  $\mu$ l

175 of ice-cold deionized water, applied to AG 1-X8 resin (Cl form) column, and the effluent (3 ml  
176 total) was collected in vials for liquid scintillation counting.

177

178 *2.4. Statistical analyses.*

179

180 Means for individual treatment groups were analyzed by two-way ANOVA using SigmaStat  
181 software (SPSS, Chicago, IL). A Student's *t*-test was used to compare sedentary and exercise  
182 means within a diet group. When means were statistically different ( $P \leq 0.05$ ), Fisher's least  
183 significant difference procedure was used for comparison [33].

184

### 185 **3. Results**

186

187 *3.1. Exercise decreased weight gain in both control-fed and folate-restricted mice.*

188

189 Initial body weight measurements of mice across all groups were not statistically different.  
190 However, control diet exercised mice and folate-restricted diet exercised mice exhibited 24 and  
191 18% decrease, respectively, in final body weight (Table 2). Folate-restriction was without effect  
192 on weight gain in either the sedentary or exercised group. Thus, this experimental design can be  
193 considered a moderate degree of folate deficiency, similar to our previous report [23]. The total  
194 distance (km) of exercise was not significantly different between control diet and folate-  
195 restricted diet groups.

196

197 *3.2. Exercise prevented hyperhomocysteinemia in the folate-restricted dietary treatment group.*

198

199 As expected, a folate-restricted diet increased plasma homocysteine concentrations >2-fold in the  
200 sedentary group (Fig. 2). However, the addition of wheel exercise in the folate-restricted diet  
201 completely prevented the increase in homocysteine concentrations compared to the folate-  
202 restricted diet sedentary group. Moreover, exercise alone decreased plasma homocysteine  
203 concentrations 36% in the control diet group.

204

205 *3.3. Folate-restriction and exercise modulated hepatic homocysteine remethylation enzymes and*  
206 *intracellular homocysteine concentrations.*

207

208 A folate restricted diet increased the activity of BHMT, but was without effect on MS activity in  
209 the liver (Table 3). In contrast, exercise reduced MS activity in both diet groups, but was  
210 without effect on hepatic BHMT activity. Hepatic intracellular homocysteine concentrations  
211 were not statistically different when all four mean values were compared; however, the exercised  
212 groups taken together exhibited diminished homocysteine concentrations compared to the  
213 sedentary groups ( $P = 0.02$ ).

214

215 *3.4. Renal BHMT activity was increased by exercise.*

216

217 Although the amount of BHMT activity in renal tissue is significantly lower than the liver, it was  
218 markedly influenced by exercise (Fig. 3). Exercise increased renal BHMT activity in the control  
219 and folate-restricted groups, 101 and 60%, respectively. In contrast to the liver, renal BHMT  
220 was not altered by a folate-restricted diet alone.

#### 221 4. Discussion

222

223 The benefits of exercise for human health have been well documented, particularly with respect  
224 to improving cardiovascular function [34]. Because hyperhomocysteinemia has been shown to  
225 be an independent risk factor for cardiovascular disease [5], identifying and understanding  
226 intervention strategies to promote homocysteine balance is an important goal for disease  
227 management. To our knowledge, this is the first report clearly demonstrating that exercise can  
228 completely prevent an increase in circulating homocysteine concentrations in a dietary folate-  
229 restricted mouse model of hyperhomocysteinemia, thereby supporting our original hypothesis.

230

231 Although hyperhomocysteinemia has been shown to be an independent risk factor for  
232 cardiovascular disease, it is unclear what influence elevated homocysteine concentrations have  
233 on vasculature and disease progression [35]. There is little doubt that hyperhomocysteinemia  
234 plays a role in the development of cardiovascular disease. This is not only supported by human  
235 population studies identifying it as an independent risk factor, but strong evidence resides in  
236 animal models with diet- and/ or genetic-based elevations in homocysteine concentrations [36,  
237 37]. However, clinical trials targeting homocysteine management by the utilization of B-vitamin  
238 supplementation as a means to lower circulating homocysteine concentrations have not been as  
239 effective as anticipated [38-41]. Numerous reviews have debated the various explanations for  
240 these findings and the associative vs. causal role of homocysteine in vascular disease [42-44].  
241 Nonetheless, it is clear that well-define indices of vascular disease result from animal studies  
242 utilizing genetic- and dietary-induced elevations in the concentration of plasma homocysteine .

243 The specific mechanism by which exercise prevents hyperhomocysteinemia owing to a folate-  
244 restricted diet is not completely clear. Homocysteine balance depends on its production from  
245 SAM-dependent transmethylation reactions, remethylation by folate-dependent and folate-  
246 independent pathways, and irreversible catabolism through the transsulfuration pathway. Here,  
247 we evaluated many of these possibilities by determining the expression and function of key  
248 regulatory enzymes involved in homocysteine production, remethylation, and catabolism. This  
249 analysis did not provide any additional mechanistic insight with respect to the positive effect of  
250 exercise on preventing hyperhomocysteinemia. The increase (53%) in mean hepatic BHMT  
251 activity by exercise in the control diet group did not reach statistical significance ( $P = 0.13$ ),  
252 whereas MS activity was reduced in both groups by exercise. Interestingly, a folate-restricted  
253 diet alone resulted in significant 111% elevations in hepatic BHMT activity. Our previous  
254 folate-restriction studies using a rat model did not exhibit elevations in hepatic BHMT activity to  
255 the extent demonstrated with this mouse model [23]. Others have reported that dietary-mediated  
256 alterations in hepatic BHMT activity resulted in decreased homocysteine concentrations [45].  
257 Moreover, it has been reported that folate-deficiency results in increased concentrations of  
258 dimethylglycine and decreased circulating concentrations of betaine, indicating a potential  
259 elevation in hepatic BHMT activity [46].

260

261 A significant amount of homocysteine metabolism occurs in the kidney [47] and this tissue has  
262 been shown to be a major factor under other conditions, such as diabetes, that are characterized  
263 by aberrant homocysteine balance [30]. Although the expression of BHMT in the rodent kidney  
264 is quite low[48], we found that exercise resulted in a significant increase in renal BHMT activity  
265 in the control diet group, as well as the folate-restricted group. It is not clear whether these

266 alterations in renal BHMT activity are biologically sufficient to explain the prevention of  
267 hyperhomocysteinemia by exercise.

268  
269 Prolonged exercise is characterized by numerous changes in circulating hormones that ultimately  
270 promote gluconeogenesis and utilization of free fatty acids. This shift is also reflected in other  
271 gluconeogenic states, such as diabetes. We and others have demonstrated that a diabetic state or  
272 administration of synthetic glucocorticoid compounds has a major impact on methyl group and  
273 homocysteine metabolism [23-26, 49-52]. A consistent finding from these reports is a reduction  
274 in circulating homocysteine concentrations owing to an increase in folate-independent  
275 remethylation (i.e., BHMT) and catabolism of homocysteine through the transsulfuration  
276 pathway. This finding was the basis for our hypothesis and supports our results that exercise can  
277 prevent hyperhomocysteinemia that is the result of dietary folate restriction.

278  
279 It also remains a possibility that the maintenance of homocysteine balance by exercise in folate-  
280 restricted mice may not be the result of direct changes in homocysteine metabolism, but rather  
281 alterations in methionine and/ or cysteine requirements as a function of protein metabolism and  
282 energy needs. Increased muscle anabolism following exercise may increase the methionine  
283 requirement for protein synthesis, thereby limiting its availability for SAM-dependent  
284 transmethylation reactions and subsequently decreasing homocysteine production. Alterations in  
285 intracellular methionine concentrations owing to exercise have been reported in both animal and  
286 human studies [19-22]. Transsulfuration of homocysteine provides cysteine and  $\alpha$ -ketobutyrate,  
287 both of which can be utilized in energy production and may have increased importance in  
288 supplying the cell with energy during exercise [53]. Previous research found plasma cysteine



289 concentrations were decreased in exercised rats, indicating a potential increase in the utilization  
290 of cysteine for both protein synthesis and/or as a source of energy [54].

291

292 In summary, we have demonstrated that exercise represents an effective strategy to maintain  
293 homocysteine balance in a diet-mediated model of hyperhomocysteinemia. A limitation of this  
294 study and goal for future research is to determine the exercise dose (i.e., time, intensity) required  
295 to effectively prevent hyperhomocysteinemia, as well as potential adverse vascular outcomes.

296 We have found in preliminary studies that mice subjected to a treadmill regime consisting of a  
297 specified intensity for a defined time period was nearly as effective as ad libitum wheel exercise,  
298 even though the total distance exercise was markedly less. Future research also needs to be  
299 directed at determining the precise signal and mechanism for the impact of exercise on  
300 prevention of hyperhomocysteinemia. Although additional research is required to define the  
301 precise relation between exercise and homocysteine balance, the impact of our observations has  
302 significant health implications for many individuals. We anticipate that our findings will  
303 stimulate future animal and human studies directed at evaluating the impact of exercise on other  
304 dietary, hormonal, and genetic models of hyperhomocysteinemia.

305

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Table 1 – Ingredient composition of the basal and folate-restricted diets fed to mice

Components <sup>a</sup>	g/kg diet
Casein, vitamin-free	100.0
Cornstarch	402.0
Glucose, monohydrate	393.0
Corn oil	50.0
Vitamin mix <sup>b</sup>	10.0
Mineral mix <sup>c</sup>	40.0
L-methionine	3.0
Choline bitartrate	2.0

<sup>a</sup> All diet ingredients were purchased from Harlan Teklad (Madison, WI), except L-methionine and choline bitartrate (Sigma Aldrich).

<sup>b</sup> AIN-93-VX formulation (Harlan Teklad). For folate-restricted mice, a customized vitamin mix devoid of folate was used (Harlan Teklad).

<sup>c</sup> AIN- 93G-MX formulation (Harlan Teklad).

Table 2 – Body weights and distance exercised from control and folate-restricted rats with and without exercise

	Control		Folate-restricted		2-Way ANOVA P-values		
	- Ex	+ Ex	- Ex	+ Ex	Diet	Ex	Diet × Ex
Initial Weight (g)	28.7±0.7	27.6±0.7	28.3±0.3	27.2±0.6	NS	NS	NS
Final Weight (g)	35.7±2.2 <sup>a</sup>	27.0±1.1 <sup>b</sup>	35.3±1.1 <sup>a</sup>	29. ±1.0 <sup>b</sup>	0.558	<0.001	0.384
Total Distance (km)	NA	930±101	NA	756±102			

Data are means ± S.E.,  $n = 5-6$ . Means within a column without a common superscript letter differ,  $P \leq 0.05$ . Ex, exercise; NA, not applicable; NS, not significant.

Table 3 – Hepatic activity of betaine-homocysteine S-methyltransferase (BHMT) and methionine synthase (MS), and intracellular homocysteine (Hcy) concentrations from control and folate-restricted rats with and without exercise

	Control		Folate-restricted		2-Way ANOVA P-values		
	- Ex	+ Ex	- Ex	+ Ex	Diet	Ex	Diet × Ex
BHMT (pmol/min·mg)	87±15 <sup>a</sup>	133±21 <sup>a,b</sup>	184±22 <sup>b</sup>	159±25 <sup>b</sup>	0.013	0.640	0.125
MS (pmol/min·mg)	108±11 <sup>a</sup>	67±12 <sup>b,c</sup>	89±8 <sup>a,b</sup>	51±8 <sup>c</sup>	0.058	<0.001	0.884
Hcy (nmol/g)	6.5±0.6	4.9±0.4	5.7±0.4	5.0±0.5	0.438	0.019	0.317

Data are means ± S.E.,  $n = 5-6$ . Means within a column without a common superscript letter differ,  $P \leq 0.05$ . Ex, exercise.

## Figure Legends

Fig. 1 – Methyl group and homocysteine metabolism. Enzymes are shown in black boxes, whereas vitamin substrates and/or cofactors are shown in gray boxes. Abbreviations are: betaine-homocysteine *S*-methyltransferase [BHMT]; cystathionine  $\beta$ -synthase [CBS]; dimethylglycine [DMG]; methionine synthase [MS]; methyltransferases [MTs]; 5,10-methylene-THF reductase [MTHFR]; *S*-adenosylhomocysteine [SAH]; SAH hydrolase [SAHH]; *S*-adenosylmethionine [SAM]; tetrahydrofolate [THF]; and methyl acceptor [X]. In addition to THF, this series of interrelated pathways are dependent on a number of other B-vitamins, including riboflavin [B<sub>2</sub>], vitamin B<sub>6</sub>, and vitamin B<sub>12</sub>.

Fig. 2 – Plasma homocysteine concentrations of control and folate-restricted diet sedentary and wheel-exercised mice. Half of the mice in each diet group were allowed access to an exercise wheel for 4 wk, after which they were then fed either a control diet or a diet without folate in the vitamin mix. After an additional 7 wk, plasma samples were obtained for the measurement of total homocysteine concentrations. Values are means  $\pm$  SE; n = 5-6. Bars without a common letter differ,  $P \leq 0.05$ . Bars denoted with an asterisk [\*] are different from control diet sedentary group,  $P \leq 0.05$ . Two-way ANOVA: diet,  $P = 0.002$ ; exercise,  $P < 0.001$ ; interaction,  $P = 0.010$ .

Fig. 3 - Renal betaine-homocysteine *S*-methyltransferase [BHMT] activity of control and folate-restricted diet sedentary and wheel-exercised mice. Kidney samples from the control and folate-restricted mice with or without exercise were homogenized for enzyme activity determination. Values are means  $\pm$  SE; n = 5-6. Bars without a common letter differ,  $P \leq 0.05$ . BHMT activity

is defined as pmol/ [min • mg protein]. Two-way ANOVA: diet,  $P = 0.246$ ; exercise,  $P = 0.007$ ; interaction,  $P = 0.822$ .





