Betaine, Vitamin B12 And Folinic Acid Supplementation Associated With Improved Body Composition And Inflammatory Markers Of An Overweight Elderly Women: A Case Report And Literature Review

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ABSTRACT

Betaine is naturally found in beets and serves as a methyl donor and osmolyte. It has been associated with improvement of body composition, including decreased fat mass and increased muscle mass. We report here the case of a Chinese woman in her 60s diagnosed with metabolic syndrome (obesity, hypertension, hypercholesterolemia, borderline sarcopenia, high level of glucose, insulin and glycated hemoglobin), who was administered a dietary supplement consisting of Betaine (trimethylglycine), Vitamin B12 and folic acid once daily for 3 months. The woman responded well with a reduction of body fat, increase of muscle mass, improvement of insulin level, lowering of total cholesterol level and lipoprotein level, reduction in the level of inflammatory markers (i.e. cross-reactive C protein). This intervention should be considered in overweight/obese subjects, especially those with at risk alleles of the methylenetetrahydrofolate reductase MTHFR gene (which is important in folate metabolism) as in the case study reported here.

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INTRODUCTION:

The desire of attaining or maintaining an optimal body weight is an important goal for better health for the elderly, especially in women, who, in addition to health considerations, often experience societal pressure to have a slim body. However, successful weight reduction can be accompanied by some loss in muscle mass, and in pathological cases lead to sarcopenia (or the degenerative loss of significant skeletal muscle mass quality), which has an estimated prevalence rate of 10% in healthy adults aged \( \geq 60 \) years (Shafiee et al. 2017). Some studies reported an alarming number of sarcopenia of up to 22.9% for Korean women older than 65 years (Park et al. 2016). Sarcopenia can lead to reduced strength and subsequent problems with joint pain and lower capacity for regular exercises, which further exacerbate the problem. Furthermore, muscle weakness in the elderly (dynapenia) can predict future all-cause mortality and is associated with cardiovascular diseases in addition to an increase in falls and disability. In a 5-year study of 869 men and 934 women (aged 70-79), it was found that loss of intermuscular thigh muscle area (as assessed with computed tomography and dual x-ray absorptiometry) was associated with higher mortality. This effect was particularly strong both in normal weight individuals (BMI<25 kg/m^2) and those undergoing weight reduction, while changes in visceral fat area were not found to be associated with mortality outcomes (Santanasto et al. 2017).

A 63-year-old woman has attempted to lose weight and during a period of 6 months lost 3.1 kg in body mass (weight reduction from 64.9 to 61.8 kg) by a combination of moderate regular physical exercises (average of 8,000 to 10,000 daily steps), however she also lost 2.6 Kg in muscle mass in the process (from 43.9 to 41.2 Kg). Thus an attempt was made to prevent further loss of muscle mass by the use of a supplement consisting of Betaine 500mg (also known also trimethylglycine), with folinic acid and Vitamin B12, in the form of methylcobalamin (400mcg and 6mcg respectively). Betaine, an amino acid derivative, was named after its discovery in sugar beet (Beta vulgaris subsp. vulgaris) in the 19th century and has been reported to help with development of improved body composition (Gao et al. 2016; Huang et al. 2016; Chen et al. 2015) and an increase of body strength. The mechanism of improvement in muscle mass was suggested to be due to increased carnitine synthesis in the liver and hormone-sensitive lipase activity in abdominal fat (Zhan et al. 2006). In mice, the increase in metabolites of the L-carnitine biosynthesis pathway (which in turn lead to improvement of fat utilization) can occur even when the animals were fed with a high-fat diet (Pekkinen et al. 2013). A human study has also reported a correlation between carnitine and betaine (Lever et al. 2012). However, other studies have failed to show its ergogenic effectiveness (Lee et al. 2010; Ismaeel 2017).

Betaine supplementation has also been employed agriculturally for the improvement of meat quality. Pigs fed with the highest betaine level had a 23% increase in protein level with 10-26% decrease in fat deposition (Fernández-Figares et al. 2002). Also, commercial broilers were shown to have increased breast muscle yield and decreased abdominal fat content due to betaine’s effect on increase of glutathione peroxidase, catalase and superoxide dismutase, thus leading to a decrease in lipid peroxidation in the muscle of chickens (Alirezaei et al. 2012). The mechanism of the effect of betaine supplementation in broilers has been investigated and DNA methylation studies of the fat pad revealed that expression of lipogenesis genes (lipoprotein lipase and fatty acid synthase genes) were decreased, accompanied by alterations in CpG methylation pattern (Xing et al. 2011).

Lower muscle mass has also been associated with lower levels of vitamin B12 levels (<400pg/mL) (Bulut et al. 2017). The serum vitamin B12 was 15% lower in the sarcopenic group in non-malnourished older European adults (n=66) (Verlaan et al. 2017). Deficiencies of folate (which are associated with hyperhomocysteinemia) has also been associated with sarcopenia, and folate levels were predictive of muscle strength (corrected for BMI) in a group of 56 patients >65 years old with diabetes in Singapore (Wee 2016).

Betaine supplementation was shown to reduce the inflammatory adipokines such as IL-6 and TNF-alpha caused by hypoxia in human fat cells (Olli et al. 2011).
2013). Since obesity is considered as a state of low-grade inflammation and often leads to increased biomarkers of endothelial dysfunction and inflammation hs-CRP (Salgado-Bernabé et al. 2016), we also tested whether hs-CRP levels are affected by supplementation of Betaine in this case.

**MATERIALS AND METHODS**

**Supplement**

The dietary supplement was a combination of Betaine 500mg (Trimethylglycine), Methylcobalamin B12 (6mcg), and Folinic Acid (400 mcg) per capsule (From Kirkman Lab). One capsule was taken once a day before going to bed.

**Biochemical Tests**

The biochemical lab tests were done by a commercial laboratory (MJ Healthcare, Hong Kong), before and after 3 months of daily supplementation. HOMA Calculator v2.2.3 (Diabetes Trials Unit, The Oxford Center for Diabetes, Endocrinology and Metabolism, Oxford, UK) (Levy et al. 1998) was used to calculate the HOMA2-IR score. See https://www.dtu.ox.ac.uk/homacalculator/.

**Weighing Scale**

The weight and other biometric data (including body weight, BMI, fat, water, muscle and bone mass content) and pulse rate and pulse wave velocity was taken with a Withings Body Cardio Electronic Scale (Item model no. 3700546701535) daily (or sometimes twice a day) in the morning or before going to bed in the evening. All measurements were made in a quiet environment with constant room temperature, with the subject avoiding moving around or talking. The free mobile apps “Withings” was used to capture the data by automatic Synchronization via wifi with an Android mobile phone (Android version 6.01; Samsung Galaxy S7 edge). In the first attempt at measuring pulse wave velocity, 5 consecutive weights were taken. The accuracy of the scale is +/- 0.1 lb.

**Genotyping analysis**

The subject’s saliva was collected and the genotype was performed by customer service of 23and me. The raw data was reviewed to examine the genotype in position C667T (rs 1801133) and A2989C (rs 1801131) of MTHFR.

**RESULTS**

**Body Composition**

The overall weight after 3 months of dietary supplementation increased by 1 Kg (from 62-63 Kg, corresponding to a BMI increase of 26.5-26.9). During this period, the body fat composition decreased by 4.5% (from 30.1 to 25.6%) Figure 1. The muscle mass increased by 3.3 Kg (from 41.2 to 44.5 Kg), while the bone mass was slightly increased by 0.2 from 2.2 to 2.4 Kg (Figure 2). Taking 62.5 Kg as the average weight of the subject, according to the Nokia health web site https://dashboard.health.nokia.com, the normal range for fat mass is 25%-36% (for 60-79 year old women), the normal range for bone mass in women (across all age groups)is 2.5% - 4 % (or 1.56 to 2.5 Kg for this subject of 62.5 kg), the normal range of muscle mass is 60% - 72.5% for (elderly of 60-79 year old) women (i.e. 37.5 to 45.31 Kg for a 62.5 Kg woman). Thus the body composition of the study subject is significantly improved considering the decrease of fat%, and increase of muscle mass.
Figure 1:
Upper panel: The Fat mass (in percentages) in the X-axis, days in the Y-axis
Lower panel: The water percentage (in percentages) in the X-axis, days in the Y-axis
The lightly shaded lines indicate the line joining the actual raw data, while the solid lines are the best fit curves.

![Fat content graph](image)

Figure 2
Upper panel: The muscle mass in Kg in the X-axis, days in the Y-axis
Lower panel: The bone mass in Kg in the X-axis, days in the Y-axis
The lightly shaded lines indicate the line joining the actual raw data, while the solid lines are the best fit curves.

![Muscle and bone mass graph](image)

Figure 3: Heart Wave Variability is shown in the Y-axis. Days are shown in the X-axis. Lower values indicate less stiffness of the blood vessels. The top dotted line is the upper limit of the the normal range, while the bottom dotted line is the lower limit of the normal range or the cutoff between normal value and optimal value (taking the person’s age into consideration). Any reading between the dotted line indicates normal values while readings below the bottom indicates optimal values.

![Heart wave variability graph](image)
Pulse wave velocity

There was no significant change in pulse rate, while there is a trend of decrease of pulse wave velocity (Figure 3) ranging between 6 to 7.6, hitting the lowest number of 6 three times during supplementation, while never having reached as low as 6 in the 3 previous months before starting the dietary supplementation. The normal range for 60 to 69 year olds was 9.7 with 7.9 as the 10 percentile and 13.1 as the 90th percentile (See results released by Nokia in June 2016, in the European hypertension society conference held in Paris).

### Biochemical Tests (Table 1)

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After 3 months</th>
<th>unit</th>
<th>Optimal Range</th>
<th>Normal Range</th>
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<tbody>
<tr>
<td><strong>Metabolic Assessment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>6.96</td>
<td>7.58</td>
<td>mmol/L</td>
<td>3.88-4.71</td>
<td>6.96 to 7.58</td>
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<tr>
<td>HbA1c</td>
<td>7.0</td>
<td>7.1</td>
<td>%</td>
<td>4.1-4.5</td>
<td>4.3-5.8</td>
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<tr>
<td>Insulin</td>
<td>12.3</td>
<td>9.6</td>
<td>uU/ml</td>
<td>2.0-5.0</td>
<td>5.0-15.0</td>
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<td>HOMA2-IR</td>
<td>1.71</td>
<td>1.30</td>
<td></td>
<td>&lt;1.4</td>
<td>&lt;1.67</td>
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<tr>
<td>Triglyceride</td>
<td>2.95</td>
<td>4.51</td>
<td>mmol/L</td>
<td>0.79-1.24</td>
<td>0.4-2.26</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>7.1</td>
<td>6.4</td>
<td>mmol/L</td>
<td>4.7-5.1</td>
<td>3.4-5.2</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.14</td>
<td>1.07</td>
<td>mmol/L</td>
<td>1.41-2.04</td>
<td>&gt;0.91</td>
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<tr>
<td>LDL-C</td>
<td>4.06</td>
<td>2.86</td>
<td>mmol/L</td>
<td>&lt;=2.59</td>
<td>&lt;4.14</td>
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<tr>
<td><strong>Inflammation Assessment</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>4.00</td>
<td>2.12</td>
<td>mg/L</td>
<td>F &lt;=1.5</td>
<td>F: 1-3</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Unit</td>
<td>Optimal Range</td>
<td>Normal Range</td>
</tr>
<tr>
<td>----------------</td>
<td>-----</td>
<td>------</td>
<td>--------</td>
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</tr>
<tr>
<td>Fibrinogen</td>
<td>347</td>
<td>323</td>
<td>mg/dl</td>
<td>200-300</td>
<td>200-400</td>
</tr>
<tr>
<td>Ferritin</td>
<td>479</td>
<td>402</td>
<td>ng/ml</td>
<td>90-110</td>
<td>F: 4.63-204</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>430</td>
<td>456</td>
<td>umol/l</td>
<td>F: 175-327</td>
<td>F: 160-390</td>
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<tr>
<td>Homocysteine</td>
<td>8.0</td>
<td>7.0</td>
<td>umol/l</td>
<td>4.0-7.2</td>
<td>5-15</td>
</tr>
</tbody>
</table>

**Hormone Assessment**

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>Unit</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>FSH</td>
<td>44.17</td>
<td>40.14</td>
<td>mIU/ml</td>
<td>follicular: 3.0 to 10.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Luteal: 0.90-9.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Postmenopausal: 26.72-133.4</td>
</tr>
<tr>
<td>LH</td>
<td>18.84</td>
<td>19.53</td>
<td>mIU/ml</td>
<td>follicular: 2.39 to 6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Luteal: 0.90-9.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Postmenopausal: 10.39-64.57</td>
</tr>
<tr>
<td>Estradiol</td>
<td>10.0</td>
<td>12.0</td>
<td>pg/ml</td>
<td>follicular: 173 to 375</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Luteal: 92-165</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Postmenopausal: 2-60</td>
</tr>
<tr>
<td>TSH</td>
<td>2.00</td>
<td>1.59</td>
<td>ng/dl</td>
<td>1.00-1.50</td>
</tr>
</tbody>
</table>

**Metabolic Assessment**

While the fasting glucose and HbA1c levels did not change significantly (fasting glucose increased from 6.96 to 7.58 mmol/L - optimal range of glucose 3.88-4.71 mmol/L, normal range of glucose 3.60-5.55 mmol/L; HbA1c increased from 7.0 to 7.1 %, optimal range of HbA1c 4.1-4.5% and normal range of HbA1c is 4.3-5.8%) , the insulin level has decreased from 12.3 uU/ml to 9.6 uU/ml, thus the insulin resistance score (HOMA2-IR) has decreased from 1.71 to 1.36. The triglyceride level did not improve and in fact increased from 2.95 to 4.51 mmol/L (optimal range 0.79-1.24 mmol/L and normal range 0.4-2.26 mmol/L). The total cholesterol did improve decreasing from 7.1 to 6.4 mmol/L (optimal range 4.7-5.1, normal range 3.4-5.2 mmol/L). While the HDL-C did not improve (dropping slightly from 1.14 to 1.07 mmol/L (optimal range 1.41-2.04, normal value >0.91), the LDL-C improved from 4.06 to 2.86 mmol/L (optimal value <=2.59, normal value <4.14). Thus overall, considering the decrease in insulin (HOMA2-IR), total cholesterol, and LDL-C, the metabolic assessment has improved significantly.

**Inflammation Assessment**

The hs-CRP level decreased by 1.88 mg/L (from 4.0 to 2.12 mg/L), (optimal range for female <=1.5 and normal range is 1-3 mg/L). The level of fibrinogen decreased from 347 to 323 mg/dL (optimal range 200-300 mg/L., normal range 200-400 mg/L). The level of ferritin decreased from 479 to 402 ng/ml (optimal range 90-110 ng/ml and normal range for women is 4.63-204 ng/ml). The level of uric acid did not improve and was in fact slightly increased from 430 to 456 umol/L (the optimal level for women is 175-327 and the normal range for female is 160-390 umol/L). The level of homocysteine is improved by 1 umol/L (a decrease from 8.0 umol/L to 7.0 umol/L).
(the optimal range is 4.0-7.2 umol/L and the normal range 5-15 umol/L). Thus four out of the five inflammation marker assessments show an improvement.

**Hormone Assessment**

The level of thyroid stimulating hormone (TSH) improved from 2.00 to 1.59 uIU/ml (optimal range 0.90 - 2.09 uIU/ml and normal range is 0.47-5.0 uIU/ml). The level of estradiol improved slightly from 10.0 to 12.0 pg/ml (the optimal range for postmenopausal women is 26.0 - 60.0 pg/ml, and the normal range is <26 pg/ml). The follicle stimulating hormone (FSH) level in blood has improved from 44.17 to 40.14 (optimal level for postmenopausal women is 26.72-133.4 mIU/ml and the optimal level for premenopausal women ranges from 2.39 to 6.6 mIU/ml during the follicular phase and 0.90-9.33 mIU/ml during the luteal phase). The luteinizing hormone (LH) did not change significantly (from 18.84 to 19.53 mIU/ml) (the optimal range for postmenopausal women is 10.39-64.57mIU/ml, and the optimal range for premenopausal women range from 2.39-6.60 mIU/ml during the follicular phase and 0.90 to 9.33 mIU/ml). The levels of testosterone, SHBG, albumin, free testosterone, bio-testosterone did not change significantly over the course of the study.

**Genotype**

The subject has compound heterozygotic mutations in both the A677C and C2987T mutations of the MTHFR. Therefore she would be considered as a compound heterozygote with reduced MTHFR enzyme activity compared to that of normal genotype (Kang et al. 1991).

**DISCUSSION**

**Body composition and Metabolic Assessment**

Overall the body composition of the subject was improved as evidenced by the lower fat mass and higher muscle mass. This is consistent with a cross-sectional study showing that lower body fat was associated with higher plasma betaine in a Norwegian population (Konstantinova et al. 2008; Slow et al. 2011). The improvement in cholesterol levels in plasma is also consistent with a report that higher betaine plasma level was associated with lowering of non-high-density lipoprotein cholesterol in an acute coronary syndrome cohort (Lever et al. 2011) and also associated with lesser severity of nonalcoholic fatty liver (NAFLD) in a Southern Chinese cohort (Chen et al. 2016). This further confirms an earlier animal study that betaine can improve adipose tissue function in mice fed a high-fat diet (Wang et al. 2010). The improvement in insulin resistance score (HOMA2-IR decreased from 1.71 to 1.36) is encouraging and surpassed the cut off of 1.4 for dysglycemia group (Ghasemi et al. 2015; Lee et al. 2016). The lower HOMA2-IR is consistent with another report that the increase in muscle mass is correlated with improvement of insulin resistance (Srikanthan and Karlamangla 2011). Increase in muscle mass is also beneficial in that it is inversely associated with coronary artery calcification in middle-aged asymptomatic adults (Ko et al. 2016).

**Heart rate and pulse wave velocity**

Since the body composition has improved, it is not surprising to observe a trend of improvement of pulse wave velocity (Pwv). Some variability of the value over time can be seen during the study period since the Pwv value can be influenced by consumption of coffee, stress level, physical activity level, and diet. As the subject does not consume alcohol and also does not smoke, we rule out that the change in variability of Pwv was due to either of those two factors.

**Inflammation assessment**

The decrease in high-sensitivity C-reactive protein (hs-CRP) should be one very positive aspect of the intervention as elevated hsCRP is associated with type 2 diabetes (Phosat et al. 2017). Elevated hs-CRP is also correlated with haemorrhagic stroke (HS) in a Chinese Han population cohort (Xue et al. 2017). A case-control study shown that haemorrhagic stroke cases have higher expression of hsCRP (median of 5.4 mg/L) than controls and certain variants of CRP are associated with hsCRP elevation. Therefore it might be more important for those susceptible individuals with familial history of haemorrhagic stroke to
monitor their hsCRP and take steps to lower them in case of exposure to risk factors that could elevate hsCRP, e.g. obesity as one such risk factor.

**Hormonal Assessment**

The decrease in FSH is likely to be a favorable sign as the beneficial effect of the diabetes drug metformin has been shown to act via inhibition of FSH action (Rice et al. 2013). The increase in estradiol is also encouraging since this hormone has been implicated as having a protective role against diabetes (Louet et al. 2004). Furthermore the estradiol receptor plays an important role in beta cells as a regulator of insulin synthesis in vivo (Wong et al. 2010). TSH was reported to have a strong positive association with HOMA-IR in non-obese diabetic subjects (Jayanthi et al. 2017), leading us to posit that the observed decrease in TSH is a good sign. There was no significant change in the other hormones such as Thyroxine T4 which make it unlikely that the elevated HbA1c is due to deficiencies of Zinc and Magnesium (as there is no thyroid comorbidity), which have been reported to be significantly correlated with Hb1Ac in obese T2 diabetics (Jayanthi et al. 2017).

The increase in methyl group donors with the betaine (trimethylglycine), Vitamin B12 and folinic acid supplementation could lead to an increase in DNA methylation levels of genes that reduce the inflammatory response or body fat loss. Such a hypothesis could be further tested by studying the methylation of genes that have been shown to be associated with loss of body mass e.g. FTO gene methylation (Zhou et al. 2017), or those loci that were shown to change in weight loss intervention (Aronica et al. 2017; Volkov et al. 2016).

One possible mechanism of reduction of insulin levels is that perhaps betaine helps in combining with arsenate to form the less toxic arsenobetaine. Chronic arsenic exposure has been shown to be related to type 2 diabetes (Islam et al. 2012). Urine arsenobetaine levels have been found to be associated with beta cell function in non-diabetic Koreans as reflected by their (HOMA) scores (Baek et al. 2017). A pilot study has been carried out by Megan N Hall of Columbia University to find out if betaine supplementation (1,000mg daily for 8 weeks) could be effective for decreasing the arsenate concentration in urine of adults of Bangladesh; however, the results of the study (even though it showed some trend of effectiveness in reduction of arsenate) cannot be analyzed statistically at the moment due to the small sample size of only 15 subjects in each arm of the study and the wide fluctuation of the level of arsenate in the urine of the subjects (ClinicalTrials.gov Identifier: NCT01749982). The proposal is based on the hypothesis that individuals exposed to higher concentrations of arsenic, especially those with a reduced capacity to completely methylate inorganic arsenate (InAs), which is highly toxic to dimethylarsinic acid (DMA), may be more susceptible to arsenic toxicity and could benefit from the effect of increased methyl-donors that results from the betaine supplementation. The inclusion of folinic acid may also lead to further increase of betaine as shown by an intervention study of arsenic-exposed Bangladeshi adults (Hall et al. 2016).

The subject has two mutations in methylenetetrahydrofolate reductase MTHFR gene and this means that the subject will probably have decreased specific MTHFR activity and might have elevated homocysteine as a consequence of this., Compound heterozygotes for C677T/ A1298C may be at risk for hyperhomocysteinemia and low folate levels, which can contribute to many disorders, such as neural tube defects (1) and abortions (2). However the baseline level of homocysteine of the subject at 8umol/L is not considered to be abnormally high. The improvement observed in homocysteine level with betaine supplementation is consistent with animal studies, especially in MTHFR deficient mice (Schwahn et al. 2007; Kelly et al. 2005; Schwahn et al. 2004) and human studies (Mazza et al. 2016). The Nursing Unacquainted Related Stress Etiologies Study found that nurses with a risk allele in the MTHFR C677T gene are more likely to become insulin resistant and subjects taking supplements of folic acid and vitamin B12 have lower HOMA levels (Kheradmand et al. 2017). Similar studies have been performed in a northern Han Chinese population, showing that C677T polymorphism may contribute to insulin resistance by increasing hs-CRP and decreasing vitamin B12. (Chen et al. 2010). The
mechanism of how betaine can lower homocysteine levels have also been studied in a rat model system with a choline and methionine deficient diet for 3 weeks. It was demonstrated that addition of betaine (in drinking water at 1%) could induce betaine-homocysteine methyltransferase (BHMT) and methionine adenosyltransferase (MAT) activities (Ahn et al. 2016).

Other dietary interventions have been designed to increase muscle mass, e.g. the Nordic diet taken by over a thousand participants belonging to the Helsinki Birth Cohort (born 1934-44) was found to increase muscle strength and muscle mass 10 years later for women but not for men (Perälä et al. 2017). A combination of special nutritional (NT) - physiotherapeutic (PT) intervention was used to prevent sarcopenia in a group of 34 elderly patients (average age 66.47) in Hungary, where it was found the physical exercises itself (PT) did not significantly affect muscle strength, whereas the combined effect of NT and PT was effective in the treatment of sarcopenia in the elderly (Molnár et al. 2016).

LIMITATIONS
The following limitations need to be taken into consideration in this report:

1. The subject’s body composition and pulse wave velocity was measured with an electronic scale based on electric impedance instead of the gold standard or more sophisticated methods such as whole body magnetic resonance imaging (MRI) or dual x-ray absorptiometric determinations (Lukaski and Siders 2003). Dehydration is known to cause an overestimation of body fat (Lukaski et al. 1986). In this study, attempts were made to reduce errors by not employing the electronic scale as a single measure device but rather used it to monitor change in body composition over a period of time in a consistent manner of measurement with one single scale for one individual.

2. The other inflammatory markers such as TNF-α, sTNF-R p55, and sTNF-R p75 which have been associated with cardiovascular events were not measured (Schnabel et al. 2013). Pro-inflammatory markers such as IL-1, IL-6, IL-8, IL-18, tumor necrosis factor alpha have also been associated with metabolic syndrome (Fatima et al. 2017; Ellulu et al. 2017) and it would be interesting to do further investigations about whether Betaine supplementation also decrease other pro-inflammatory markers.

3. Other genetic factors besides the MTHFR polymorphism of the subject have not been considered and there are many reports about the importance of other genes related to the one-carbon metabolic pathway that could affect the outcome of the supplementation with betaine, vitamin B12 and folinic acid (Matone et al. 2016). Polymorphism in the MTRR 66GG gene was known to further increase the risk for deep vein thrombosis for a cohort of South Indians in the presence of the MTHFR 677CT/1298AC genotype (which is the MTHFR genotype of this study’s subject) (Naushad et al. 2008). Genes related to homocysteine metabolism (e.g. deficiency in cystationine beta-synthase (CBS) could also cause hyperhomocysteinemia (Blom 2000). Other genetic factors may also interact with diet to affect the risk of obesity and metabolic syndrome (Greenhill 2015; Ussar et al. 2015; Ganz et al. 2016).

4. The intestinal microbiome composition may also exert an important risk factor in metabolic syndrome (Chassaing et al. 2017; Cavalcante-Silva et al. 2015; Festi et al. 2014; Chassaing et al. 2015). There are reports that betaine may play an important role in establishment and survival of surface-attached microbial communities in certain marine environments (Kapfhammer et al. 2005). Betaine has also been reported to improve lactic acid fermentation by B. coagulans and raised the productivity by 22% (Xu and Xu 2014). Moreover B. Coagulans has been shown to be a potential agent in the management of diarrhea predominant irritable bowel syndrome (IBS) patients (Urgesi et al. 2014; Majeed et al. 2016). This study did not include any determination of the changes of microbial composition during the period of supplementation. However it would not be surprising if betaine supplementation has a positive effect on gut health since betaine
supplementation was found to reverse weight loss in parasite infected chicks (Fetterer et al. 2003).

5. Although the subject did not change her exercise habits during the intervention, an ideal study would include a more detailed diary of the exercise or physical activities undertaken. Exercise has been shown to decrease homocysteine levels independent of MTHFR risk alleles and plasma vitamin B12 status (Dankner et al. 2007). Besides improvement in lipid profiles, resistance exercises are known to affect body composition and inflammatory markers of post-menopausal women (Nunes et al. 2016).

CONCLUSION
An intervention with betaine, vitamin B12 and folinic acid was shown to offer some benefits in improvement of body composition, lipid profile, and inflammatory biomarkers in a 63 year old woman who is likely to be deficient in methyl donors due to her genetic heterozygosity in the risk MTHFR alleles. Thus our findings are consistent with the suggestion that betaine supplementation could provide beneficial myocardio protection as well as helping in the anti-stress response.

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