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Review

Betaine: a key modulator of one-carbon metabolism and homocysteine status

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Abstract

Betaine serves as a methyl donor in a reaction converting homocysteine to methionine, catalysed by the enzyme betaine-homocysteine methyltransferase. It has been used for years to lower the concentration of plasma total homocysteine (tHcy) in patients with homocystinuria, and has recently been shown to reduce fasting and in particular post-methionine load (PML) tHcy in healthy subjects.

Betaine exists in plasma at concentrations of about 30 μmol/L; it varies 10-fold (from 9 to 90 μmol/L) between individuals, but the intra-individual variability is small. Major determinants are choline, dimethylglycine and folate in plasma, folic acid intake and gender.

Recent studies have demonstrated that plasma betaine is a stronger determinant of PML tHcy than are vitamin B_6 and folate. The betaine-PML tHcy relationship is attenuated after supplementation with B-vitamins, and is most pronounced in subjects with low folate. Betaine shows a weaker association with fasting tHcy (than with PML tHcy), and also this association is most pronounced in subjects with low folate. In pregnancy, plasma betaine declines until gestational week 20, and thereafter remains constant. From gestational week 20 onwards, fasting tHcy shows a strong inverse association with plasma betaine, and betaine becomes a stronger predictor than folate of fasting tHcy.

To conclude, betaine status is a component of an individual's biochemical make-up with ramifications to one-carbon metabolism. Betaine status should be investigated in pathologies related to altered metabolism of homocysteine and folate, including cardiovascular disease, cancer and neural tube defects.

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Introduction

Betaine (*N,N,N*-trimethylglycine; Figure 1) was named after its discovery in sugar beets (*Beta vulgaris*) in the 19th century. It is a small N-trimethylated amino acid, existing in zwitterionic form at neutral pH. This substance is often called ''glycine betaine'' to distinguish it from analogous compounds that are widely distributed in microorganisms, plants and animals. Many serve as organic osmolytes, i.e., as substances synthesised or taken up from the environment by cells for protection against osmotic stress, drought, high salinity or high temperature (1). Intracellular accumulation of betaines, non-perturbing to enzyme function, protein structure and membrane integrity, permits water retention in cells, thus protecting a number of vital metabolic pathways from the effects of dehydration (2).

In humans, glycine betaine, hereafter denoted betaine, and carnitine (3-hydroxy-4-N-trimethylammoniumbutyrate) are betaines with additional biological functions and play important roles in cellular metabolism (3). Other dietary betaines (like proline betaine and trigonelline) have no known physiological function in man.

Nutritional and metabolic sources of betaine

Betaine is obtained by humans from foods, either as betaine or choline-containing compounds. Food items with the highest content of betaine are wheat, spinach, shellfish and sugar beets (4, 5). Estimates of betaine intake are from 0.1 to 1 g/day (3, 6) and as high as 2.5 g/day for a diet high in whole wheat and seafood (3). Thus, the intake depends on food composition, but is probably also related to production of the food items, including growing and osmotic conditions (3). Betaine is rapidly absorbed after oral administration in humans, and the bioavailability is assumed to

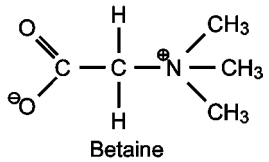

Figure 1 Chemical structure of betaine.

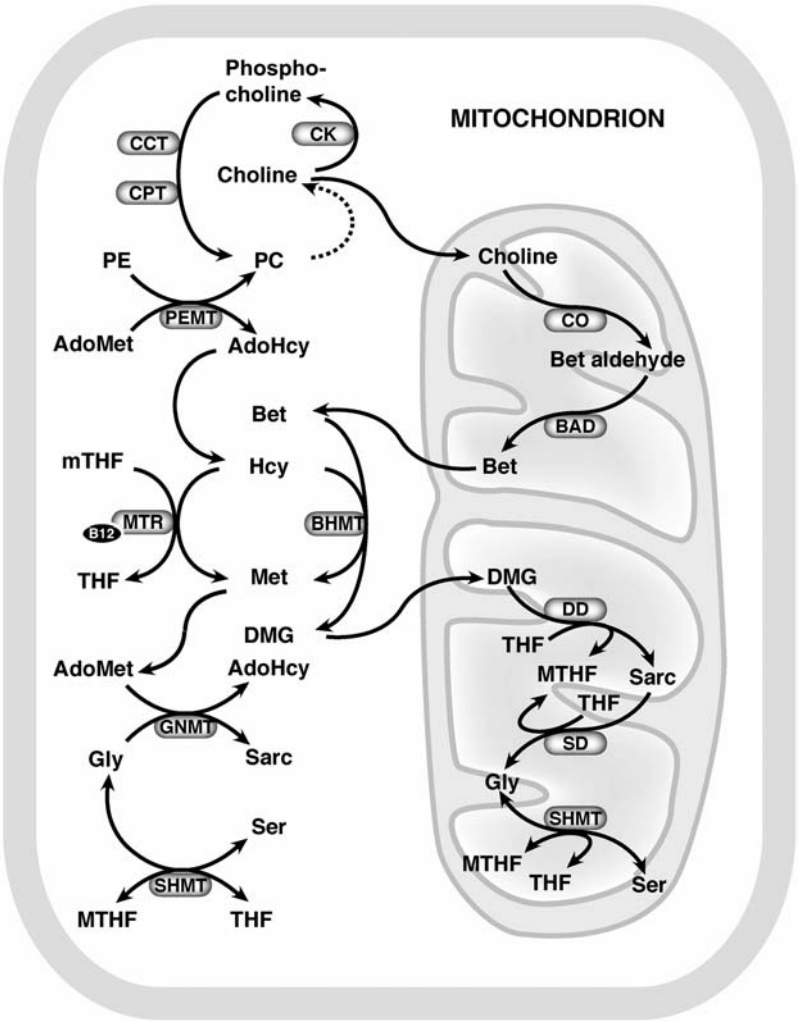

Figure 2 Formation and metabolism of betaine and its role in homocysteine remethylation and one-carbon metabolism. The scheme encompasses reactions known to take place in the mammalian liver. AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; BAD, betaine aldehyde dehydrogenase; Bet, betaine; BHMT, betaine-homocysteine S-methyltransferase; CCT, CTP-phosphocholine cytidylyltransferase; CK, choline kinase; CO, choline oxidase; CPT, choline phosphotransferase; DD, dimethylglycine dehydrogenase; DMG, dimethylglycine; Gly, glycine; Hcy, homocysteine; Met, methionine; mTHF, 5-methyltetrahydrofolate; MTHF, methylenetetrahydrofolate; MTR, methionine synthase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine N-methyltransferase; Sarc, sarcosine (monomethylglycine); SD, sarcosine dehydrogenase; Ser, serine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate.

be close to 100% (7). It is absorbed in the ileum via 'imino' porters along with other dietary betaines such as proline betaine (mainly citrus) and trigonelline (predominantly coffee) (8). Most intracellular betaine is probably obtained by uptake from the extracellular medium rather than synthesis (9).

Alternatively, betaine is formed from choline. Choline is mainly derived from phosphatidylcholine, which is either synthesised de novo (Figure 2) or obtained through the diet. Foods rich in phosphatidylcholine and other choline compounds are beef, chicken, liver and eggs (5).

The conversion of choline to betaine is a two-step enzymic process, which takes place in the liver and kidney. Choline is first oxidised to betaine aldehyde, a reaction catalysed by the mitochondrial choline oxidase (choline dehydrogenase, EC 1.1.99.1), and betaine aldehyde is further oxidised in the mitochondria or cytoplasm to betaine by betaine aldehyde dehydrogenase (EC 1.1.1.8). Other metabolic routes of choline metabolism are formation of acetylcholine and phospholipids, including phosphatidylcholine (10, 11) (Figure 2).

Free choline can either be transported into the mitochondria where it is oxidised to betaine, or can be converted to phosphocholine and thereby directed into phospholipid synthesis (Figure 2). Only minor amounts are used for acetylcholine synthesis. Enzyme kinetic studies (12, 13) and metabolic tracer studies in rat liver suggest that the synthesis of phosphocholine is favoured at low choline concentration, as observed during choline deficiency, whereas conversion into betaine (and further to glycine) becomes predominant at high choline levels. Choline oxidation may therefore serve as a spillover pathway under conditions of high dietary choline (14).

Betaine function and metabolism in mammals

Betaine has three known functions in mammals. It is an organic osmolyte that accumulates in renal medullary cells and some other tissues to balance extracellular hypertonicity (9, 15–17). Secondly, it also acts like a chaperone to stabilise protein structure under denaturing conditions (9). Finally, it serves as a methyl donor in the betaine homocysteine methyltransferase (BHMT) reaction which converts homocysteine to methionine (18).

BHMT is a cytosolic zinc metalloenzyme, which is expressed at a high level in human liver and kidney. The enzyme catalyses a methyl transfer reaction from betaine to homocysteine, thereby converting them to dimethylglycine (DMG) and methionine, respectively. Homocysteine remethylation is also catalysed by the ubiquitous methionine synthase, which uses 5-methyltetrahydrofolate as methyl donor. Thus, homocysteine resides at the point of convergence of betaine and folate metabolism (18). Furthermore, the BHMT reaction represents the first step along a pathway where three methyl groups of choline are made available to the one-carbon pool. The other two methyl groups from betaine enter the folate pool through the formation of 5,10-methylenetetrahydrofolate in the mitochondria, as detailed in Figure 2.

Studies on nutritional effects on the BHMT activity indicate an important role of this enzyme in one-carbon metabolism. The BHMT activity, in the livers of both rats and chicken, increases drastically during methionine restriction, but only in the presence of sufficient dietary choline or betaine. Thus, BHMT is up-regulated and will conserve the homocysteine backbone only when betaine is available (14).

Hepatic BHMT also increases moderately during methionine excess. This remarkable biphasic response has led to the suggestion that BHMT may have multiple roles, i.e., methionine conservation during methionine restriction, but also removal of excess choline, betaine or homocysteine. Methionine excess decreases the activity of the folate-dependent methionine synthase, which agrees with the role of this enzyme in methionine conservation (18). Notably, animal experiments suggest that the overall homocysteine remethylation to methionine during methionine balance is shared about equally between BHMT and methionine synthase (19).

Nutritional experiments in rats, studies of betaine and choline effects in methylenetetrahydrofolate reductase (*MTHFR*) knock-out mice (20, 21), and observations in humans, all point to a strong interrelationship between the BHMT and methionine synthase pathways (22). During choline deprivation leading to low betaine content, more 5-methyltetrahydrofolate is used for homocysteine remethylation, thereby increasing folate requirements. Conversely, during folate deficiency, methyl groups from choline and betaine are used, thereby increasing choline requirements. Thus, 5-methyltetrahydrofolate and choline/betaine have been regarded as fungible sources of methyl groups (22).

BHMT is a component of the methionine cycle. A model for the regulation of this cycle has been composed, based on data on enzymes involved, their activities, kinetic characteristics, and levels of substrates and products. Both BHMT and methionine synthase have a low K_m for homocysteine, and methionine synthase activity is highest at low methionine levels. Elevated S-adenosylmethionine (AdoMet), resulting from methionine excess, inhibits BHMT and formation of 5-methyltetrafolate catalysed by MTHFR (18). Thus, homocysteine remethylation is favoured at low levels of homocysteine and methionine. Cystathionine β-synthase and methionine adenosyltransferase-III are high K_m enzymes which are stimulated by AdoMet, and the expression is enhanced at high methionine levels. Thus, during methionine excess, homocysteine is directed towards the transsulfuration pathway and methionine is converted into AdoMet (19, 23, 24).

Plasma and urinary betaine and its determinants

The median concentration of betaine in plasma/serum is about 30 μmol/L (25), but varies substantially between individuals, i.e., the concentrations may range from 9 to 90 μmol/L, and 10–90 percentiles typically are 20–40 μmol/L. Men have about 15% higher levels than women (26–29). The plasma concentration decreases during pregnancy to the same extent as total homocysteine (tHcy) and related metabolites, and reaches a plateau at about gestational week 20 (30). This reduction may partly be attributed to plasma volume expansion, but could, like the effect of gender, result from hormonal effects on betaine metabolism (31). Such hormonal control could be explained by the presence of consensus sites for steroid hormones, including oestrogen and androgen binding sites, in the human *BHMT* gene (14).

Notably, despite the large inter-individual variability, there is a small intra-individual variation in plasma betaine concentration over a 3-year period. Thus, plasma betaine seems to be under strict metabolic control, giving individual set points for betaine concentration in serum/plasma (32). However, plasma betaine increases moderately (<30%) 2–3 h after a light meal (28, 33), shows a transient increase (about 15%) 4 h after methionine loading (29), and increases in a dose-dependent manner following supplementation with folic acid at doses ranging from 50 to 800 μg/day (34). The effect of diet is explained by increased intake of betaine or its precursor, choline; high doses of methionine may either enhance betaine synthesis or decrease betaine utilisation (through AdoMet inhibition of BHMT), whereas long-term folate supplementation probably has a betaine-sparing effect.

The effect of folic acid supplementation on plasma betaine is in agreement with the observation that serum folate shows a positive association with plasma betaine in some (34, 35), but not all (29), studies.

Such a relation becomes evident in a large population with a wide range of folate concentrations. Choline is an even stronger predictor of plasma betaine and shows a positive linear association across the whole range of plasma choline concentrations (35). There is also a positive association between plasma betaine and plasma DMG, which can be explained by DMG production from betaine (35). At DMG concentrations above the 80th percentile, there is no further increases in betaine (25).

In healthy subjects, plasma betaine is not related to urinary excretion of betaine, which is less than 5% of the creatinine excretion (26). The urinary excretion of betaine is strongly related to plasma tHcy (33). It increases transiently in parallel with that of other osmolytes in response to a water load, but remains low during water deprivation (36). The plasma betaine level seems to be strictly controlled under these conditions, and is not influenced by acute diuresis or antidiuresis (32).

There are few studies on plasma betaine and its relation to disease. Plasma betaine is normal or slightly reduced in subjects with renal dysfunction (25, 27), which is in agreement with no or a weak, inverse association between betaine and creatinine (35). The plasma betaine reduction in renal patients does not correlate with an increase in urinary betaine excretion (27), and contrasts to the marked increase in plasma DMG in renal patients (37) and the strong positive association of serum creatinine with plasma DMG and choline (29).

In diabetic patients, plasma betaine is normal, but betaine excretion is increased up to fivefold (27). The betaine excretion is not strongly related to impaired renal function (27), but is positively related to markers of proximal tubular dysfunction (retinol binding protein) and indicators of poor glycaemic control, including elevated plasma glucose (38). However, an animal experiment demonstrated no change in betaine excretion during acute increase in plasma glucose following glucose infusion (39). It is therefore unlikely that increased urinary betaine excretion in diabetics results from an acute effect of high glucose on renal osmoregulation. Increased excretion may reflect renal damage from long-term hyperglycaemia or a renal complication of diabetes unrelated to blood glucose per se.

Effect of betaine or choline supplementation on plasma tHcy

High doses (6 g/day and over) of betaine, alone or in combination with B-vitamins, have been used for years to treat patients with homocystinuria. This includes patients with cystathionine β-synthase deficiency who do not respond to vitamin B_6 (40), and rare forms caused by MTHFR deficiency or by defects in cobalamin metabolism (41). Such treatment reduces plasma tHcy and partly corrects other biochemical abnormalities, but also improves the clinical condition. No side effects have been observed (40, 41).

Betaine has recently been investigated as a tHcy-lowering agent in subjects without homocystinuria (42). In healthy subjects, betaine at doses between 1.5 and 6 g/day reduces the increase in tHcy after methionine loading in a dose-dependent manner, and at 6 g, the reduction is about 40% (43, 44). Folic acid is not effective. Studies on rats injected with betaine suggest that the reduction of post-methionine load (PML) tHcy is an immediate effect occurring within minutes (45).

Fasting tHcy is reduced in a dose-dependent manner following oral betaine given at doses of 1–6 g/day. The reduction obtained with the highest dose is up to 20%, but is related to the pretreatment tHcy level (44, 46, 47). Thus, the reduction is of a similar magnitude to that observed with folic acid, and only a marginal additional effect was observed by adding folic acid. Preliminary observation suggests that the betaine effect is fast and becomes manifest within hours (44, 47).

Betaine supplementation reduces PML tHcy but not fasting tHcy in renal patients who are folate- and vitamin B_6-replete (48).

A recent study demonstrated a reduction of fasting tHcy by oral intake of choline (given as phosphatidylcholine), which was of similar magnitude to that obtained with an equivalent dose of betaine. A single dose of choline also reduced PML tHcy, but seemed to be less effective than betaine, possibly because oxidation to betaine is required before one-carbon units become available for homocysteine remethylation (49). Furthermore, dietary choline deficiency has the opposite effect. Such a dietary intervention increases PML tHcy by 35% in humans and 100% in mice (50).

In conclusion, oral betaine or choline, at doses similar to the amounts found in some diets, reduce fasting tHcy and, in particular, PML tHcy.

Betaine and serum lipids

Betaine supplementation, at doses of 4–6 g/day for 12 weeks, increases total serum cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triacylglycerol concentrations by up to 10% in patients with renal failure (48), and increases serum total cholesterol, LDL cholesterol and triacylglycerol by 12–14% in obese subjects who are on a weight-loss diet (46). Similar effects from betaine supplementation on serum lipids have been observed in healthy subjects (42). Other studies have shown that choline-deficient humans (51) and patients receiving parenteral nutrition without choline (52) have low total cholesterol, and choline supplementation in rats increases serum cholesterol (53). These observations suggest that dietary choline increases serum lipids in humans, but conclusive studies in humans have not been conducted (42).

A likely mechanism for the cholesterol elevation by betaine or choline supplementation is enhanced synthesis of phosphatidylcholine which enhances assembly of very-low-density lipoprotein (VLDL), a transport

vehicle for lipids from the liver into plasma (54). A possible clinical implication is that cholesterol elevation may offset some benefits of the tHcy-lowering effect of betaine or choline (42).

A recent study demonstrated a significant inverse correlation between plasma betaine and plasma lipids, including triglycerides, total cholesterol, HDL cholesterol and apolipoprotein A1 in 502 cardiovascular patients (55). This observation is apparently in conflict with the increased levels of serum lipids observed after betaine supplementation. The relation of lipids in serum/plasma to betaine and choline concentrations in subjects on a normal diet vs. subjects supplemented with high doses of these lipotropic agents should be further investigated.

Betaine as determinant of plasma tHcy

It is important to investigate the relation between endogenous betaine and tHcy in plasma/serum for two reasons. tHcy is a risk factor for various diseases, including cardiovascular disease, impaired cognitive function and birth defects. Secondly, plasma tHcy is a measure of the function of several B-vitamins, which is explained by the converging actions of these vitamins on homocysteine metabolism. Likewise, plasma tHcy may reflect the role of betaine in overall homocysteine remethylation in humans (56).

The increase in tHcy following methionine loading is inversely associated with plasma betaine in cardiovascular patients. This effect is attenuated after the patients have been supplemented with B-vitamins (folate, vitamin B_6 and cobalamin) for 3 months (29). In a large study on 500 healthy subjects (35), plasma betaine was the strongest predictor of the PML increases in tHcy, with an (adjusted) mean change in tHcy of 7.2 μmol/L when comparing the extreme betaine quartiles. This is in agreement with the pronounced reduction in PML tHcy by betaine supplementation (42). Similar estimates of mean PML tHcy changes according to folate, cobalamin and vitamin B_6 were 3.2, 3.2 and 3.7 μmol/L, respectively. The inverse association between the PML increase in tHcy and plasma betaine was strongest at low folate, and with an interaction between folate and betaine of borderline significance (35).

Early studies on the association between plasma betaine and fasting tHcy showed variable results. Plasma betaine showed a moderate inverse association with fasting tHcy in 120 cardiovascular (20) and in 99 renal patients (37), whereas no such relation was observed by simple correlation analysis in 60 healthy blood donors (25), in 500 healthy subjects (35) or in 158 patients attending a lipid clinic (33). In three studies, an inverse relation was observed after appropriate multiple adjustments for factors showing a positive relation to both betaine and tHcy (33, 35, 57). More important, plasma betaine appears to be a strong predictor of fasting tHcy in subjects with low serum folate, and a strong interaction between folate and betaine has been observed (35). The latter observation has been pursued in a large study on 10,700 healthy subjects, which demonstrated that in subjects with low folate (<9.7 nmol/L) and the *MTHFR* 677TT genotype, betaine is a very strong predictor of plasma tHcy, corresponding to a mean tHcy change of 5.4 μmol/L across the extreme betaine quartiles (58). Thus, these data strongly suggest that betaine takes over as a methyl donor and sustains methionine synthesis under conditions of impaired folate status.

Pregnancy imposes a considerable stress on folate stores, and inadequate folate status is common in pregnancy (59). An increased maternal plasma/serum folate during pregnancy and higher blood folate in the newborn than in the mother probably reflect conditions favouring a maternal-to-foetal folate transfer (59). Likewise, increasing maternal plasma choline during pregnancy, and a plasma choline in the newborn, which is three times higher than in the mother, indicate efficient choline transfer. Under these conditions of folate and choline provision to the foetus, maternal plasma betaine is reduced but becomes a strong determinant of maternal plasma tHcy. More precisely, in early pregnancy, plasma folate, but not plasma betaine, is a strong determinant of plasma tHcy. During the course of pregnancy, the inverse association between maternal betaine and tHcy is strengthened and betaine becomes the strongest determinant at gestational week 36 (30) and at delivery (57). Thus, betaine-dependent homocysteine remethylation seems to be enhanced under conditions of altered folate status imposed by pregnancy.

Conclusion

Betaine is an important nutrient, which is obtained from foods or is synthesised endogenously from choline. It serves as an osmolyte and a methyl donor, and thereby is linked to folate and homocysteine metabolism. The betaine concentration in human serum/plasma shows large inter-individual variations, but the intra-individual variability over time is small, suggesting that betaine is under strict metabolic control. Recent data from studies in humans demonstrate that betaine becomes an important source of methyl groups under conditions of impaired folate status, an idea substantiated by animal experiments. The role of betaine in human health and disease should be further investigated.

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