
# Diet- and microbiota-related metabolite, 5-aminovaleric acid betaine (5-AVAB), in health and disease



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Review

# Diet- and microbiota-related metabolite, 5-aminovaleric acid betaine (5-AVAB), in health and disease

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**5-Aminovaleric acid betaine (5-AVAB) is a trimethylated compound associated with the gut microbiota, potentially produced endogenously, and related to the dietary intake of certain foods such as whole grains. 5-AVAB accumulates within the metabolically active tissues and has been typically found in higher concentrations in the heart, muscle, and brown adipose tissue. Furthermore, 5-AVAB has been associated with positive health effects such as fetal brain development, insulin secretion, and reduced cancer risk. However, it also has been linked with some negative health outcomes such as cardiovascular disease and fatty liver disease. At the cellular level, 5-AVAB can influence cellular energy metabolism by reducing β-oxidation of fatty acids. This review will focus on the metabolic role of 5-AVAB with respect to both physiology and pathology. Moreover, the analytics and origin of 5-AVAB and related compounds will be reviewed.**

## Highlights

5-Aminovaleric acid betaine (5-AVAB) is a trimethylated compound that has recently emerged as a metabolically important compound in numerous studies.

Lack of microbiota reduces 5-AVAB and certain microbiota taxa are essential for 5-AVAB production.

Diets influence levels of 5-AVAB, with certain diets promoting bacterial biosynthesis of 5-AVAB and some readily containing 5-AVAB.

Current evidence for the metabolic role of 5-AVAB is rather contradictory, including both beneficial and harmful observations.

A strong relationship of 5-AVAB is shown (e.g., with gut–brain axis, liver and heart function, and adiposity).

## Interest in small metabolites has been increasing

Recent advances in mass spectrometry (MS)-based analytical methods and the increased use of metabolomics approaches have opened novel avenues to permit a much more detailed molecular-level assessment of human metabolism and its linkage with gut microbiota, as well as cellular metabolic routes. Novel compounds, and even compound groups, with putative roles in metabolic processes, are continually being described. One such compound is 5-AVAB, which belongs to the trimethylated compounds commonly called **betaines** (see Glossary). Glycine betaine [1] is the best-known betaine compound, but a wealth of information is currently accumulating on the metabolic roles of several other trimethylated compounds, including 5-AVAB, pipecolic acid betaine [2], proline betaine [3], ergothioneine [4], and trimethylamine *N*-oxide (TMAO) [5].

Several studies have reported positive associations between the gut microbiota and the abundance of 5-AVAB [2,6,7], and there is convincing evidence that this metabolite is at least partially synthesized by gut microbes. However, 5-AVAB has also been reported to be present in certain food sources, including milk and meat [8,9] and the kinds of marine algae used as foodstuffs [10]. During recent years, 5-AVAB has been linked with whole-grain consumption [11], demonstrated to have a role in fetal tissues [12], and claimed to be a vital part of brain development [2]. Additionally, liver health status has been connected to 5-AVAB [7]. Here, we review the origin of 5-AVAB from different sources, before discussing the multiple roles that 5-AVAB has been suggested to have in the body and tissues.

## Origin of 5-AVAB

### Production of 5-AVAB by microbiota

The importance of gut flora in synthesizing 5-AVAB has been reported recently in several publications, and the key role of the gut microbiota has been demonstrated in studies with **germ-free**

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**(GF) mice**, where 5-AVAB levels were significantly reduced in plasma and tissue samples as compared with conventionally housed mice [2,6,7,12,13]. Furthermore, the absence of maternal microbiota decreased 5-AVAB levels in fetal tissues, indicating that decreased synthesis of 5-AVAB is one outcome evoked by the absence of a normal microbiome in the gut [2,12]. Notably, most of the listed publications tend to utilize several nomenclatures for 5-AVAB (Box 1), thus hindering the efficient utilization of the existing information.

Bacterial species in the gut probably have similar properties to **halophilic microorganisms**, which can synthesize a significant amount of glycine betaine from glycine by adding methyl groups, for example, from a methionine source. These halophilic microorganisms, namely, *Actinopolyspora halophila* and *Ectothiorhodospira halochloris*, utilize a three-step catalytic procedure with two methyltransferase enzymes, glycine sarcosine methyltransferase and sarcosine dimethylglycine methyltransferase [14,15]. However, glycine betaine can also act as a methyl donor, similar to the transformation of homocysteine into methionine [1,16]. Pallister *et al*. [17] suggested that 5-AVAB was likely to be a methylated product of **5-aminovaleric acid (5-AVA)**. In addition, we propose that glycine betaine can act as a methyl substrate donor in biosynthesis pathways either in microorganisms or endogenously, where 5-AVAB is formed from 5-AVA (Figure 1A) [6]. However, this pathway still needs to be fully characterized, for instance, with an *in vitro* fermentation model based on human gut microbiota and isotope-labeled glycine betaine or other methyl donors.

Although there have been rather few mechanistic studies demonstrating *in vitro* production of 5-AVAB by certain microbial species, the normal microbiome contains many important bacteria, abundances of which have been correlated with 5-AVAB syntheses, such as bifidobacteria and *Coriobacteriaceae* [6]. Bifidobacteria are known to be beneficial bacteria for human health [18], and *Coriobacteriaceae* can confer protection against liver pathologies [19]. Further evidence supporting the role of microbiota in the production of 5-AVAB from dietary precursors has been obtained from studies demonstrating that even though 5-AVAB is absent in cereal-based foods [20], the addition of cereal bran or whole grains to the diet has been associated with increased circulating and tissue levels of 5-AVAB [6,11]. In the absence of the appropriate gut

## Box 1. Uniform naming is essential for 5-AVAB

5-AVAB has been analyzed and reported during recent years concomitantly in several laboratories, resulting in extensive heterogeneity in its nomenclature. So far, it has been annotated at least as 5-AVAB [45], aminovaleric acid betaine [92], δ-valerobetaine (δVB) [44], δ-aminovaleric acid betaine (δ-AVAB) [49], 5-trimethylammoniopentanoate [35], *N*-trimethyl 5-aminovalerate [80], trimethyl-*N*-aminovalerate [17], 5-trimethylaminovalerate (GBB-5) [93], *N*,*N*,*N*-trimethyl-5-aminovaleric acid (TMAVA) [7], N,N,N-trimethyl-5-aminovalerate (TMAV) [2], δ-*N*-trimethylaminovaleric acid [30], 5-*N*-trimethylaminopentanoate [34], and X-21365 (on the metabolon platform) [64] (see Table 1 in main text).

We propose the uniform name of 5-AVAB and the systematic use of Chemical Abstract Service (CAS) (6778-33-2) and PubChem ID (14274897) in the future to efficiently find all the related articles and authors. This name describes the molecular features comprehensively and the abbreviation gives a short but unique description of the molecule, as the term betaine in the nomenclature is commonly used for compounds with a quaternary ammonium where hydrogens have been substituted with methyl groups such as glycine betaine, proline betaine, and pipecolic acid betaine. Ideally, the nomenclature should not be too complex, while still describing the compound accurately. Furthermore, the IUPAC International Chemical Identifier (InChI) InChI=1S/C8H17NO2/c1-9(2,3)7-5-4-6-8(10)11/h4-7H2,1-3H3 and InChI Key CDLVFVFTRQPQFU-UHFFFAOYSA-N should be used to increase knowledge. The scientific community should avoid using multiple names for a single molecule because it complicates database and publication searches. Additionally, the Greek alphabet should also be avoided in names and abbreviations for two reasons. First, Greek letters can be written with one character or with Latin letters, for example, δVB or delta-valerobetaine, respectively. Second, the encoding used in a lot of software cannot accommodate Greek characters, meaning that this information could be lost during data processing. For the aforementioned reasons, 5-AVAB is the preferred nomenclature for the compound in question.

## Glossary

**Alkylresorcinols (AR C17:0/AR C21:0):** ratio of 17 and 21 carbon chain resorcinolic lipids, respectively, which are phenolic lipids composed of long aliphatic chains and a resorcinol-type phenolic ring.
**5-Aminovaleric acid (5-AVA):** 5-AVAB precursor without a quaternary ammonium.
**Angiotensin II:** a peptide hormone that increases blood pressure.
**Bax/Blc-2:** heterodimer functioning as an apoptotic activator.
**Betaines:** a neutral chemical compound with a positively charged quaternary ammonium and with a negatively charged carboxylate. They are metabolites with numerous roles in the regulation of metabolic homeostasis, such as protection against osmotic stress.
**β-Oxidation:** an energy productive catabolic process that breaks down fatty acids.
**Caspases:** protease enzymes important in programmed cell death.
**Cyclins:** a family of proteins controlling the cell cycle in the cell progression cascade.
**Diabetic neuropathy:** nerve damage that can occur in people with diabetes.
**Early mitral inflow velocity:** the instantaneous pressure difference between the left atrium and the left ventricle; it will increase in the presence of diastolic dysfunction.
**Fibrates:** pharmaceuticals that are used in the treatment of hypercholesterolemia.
**Germ-free (GF) mice:** mice with no microorganisms (i.e., gut microbiota).
**G2/M and SubG1 phases:** growing phases in the cell cycle.
**Halophilic microorganisms:** a group of microorganisms that can grow and often thrive in areas with a high salt concentration.
**Meldonium:** an anti-ischemic medication.
**Microalbuminuria:** the term describing an increase in the level of albumin in urine.
**Mitophagy:** a process of recycling aberrant mitochondria.
**Mitral annular early diastolic velocity:** reflecting the myocardial relaxation in the long-axis direction; it will be rather low in the presence of diastolic dysfunction.
**Nuclear factor-kappa B (NF-κB):** the protein complex that controls cell survival.

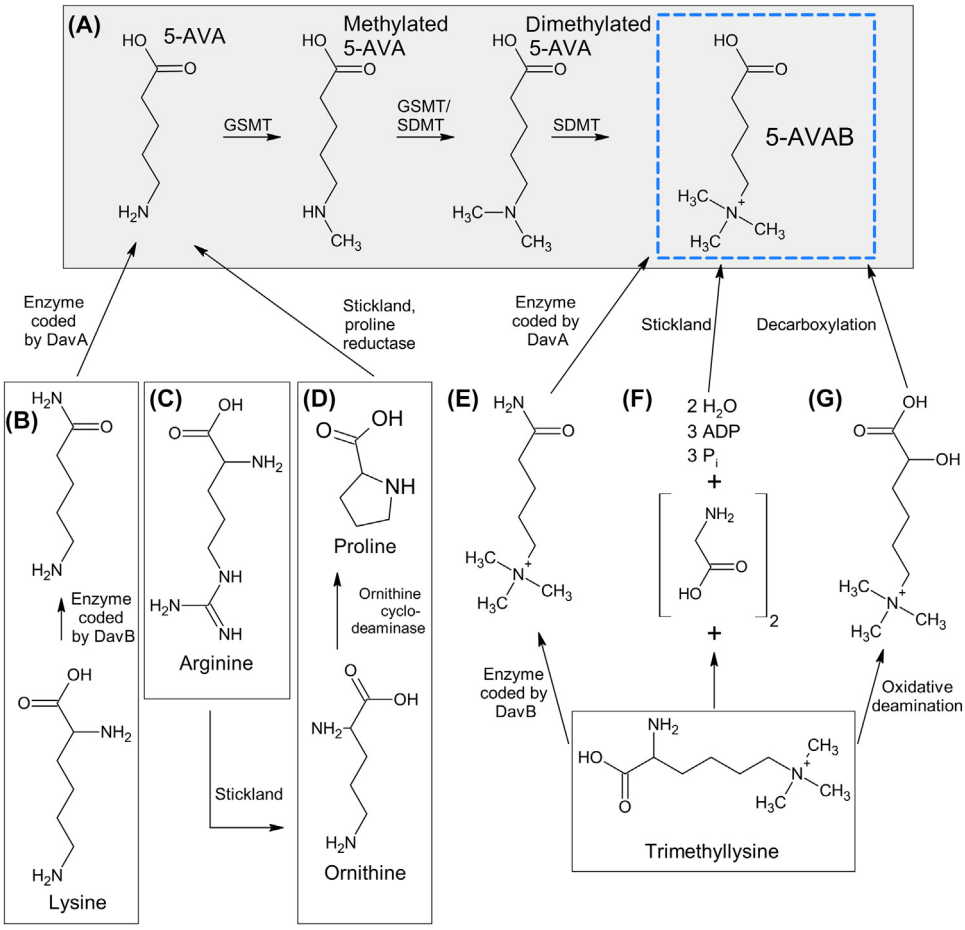


Figure 1. Tentative pathways in 5-AVAB biosynthesis. (A) Predicted microbiota and endogenous pathway forming 5-AVAB from 5-AVA, similar to how glycine betaine is formed from glycine [14]. (B) The formation of 5-aminovaleramide and 5-AVA utilizing the L-lysine DavB/DavA pathway, possibly in the microbiota [22]. (C) Microbial species possibly transforming arginine to 5-AVA via ornithine and proline by the Stickland reaction in the gut flora [24]. (D) Microbial species transforming ornithine and proline to 5-aminovaleramide by ornithine cyclodeaminase (EC 4.3.1.12), proline reductase (EC 1.21.4.1), and Stickland reaction in the gut flora [24,25,27,29]. (E) Pathway for the synthesis of 5-AVAB from trimethyllysine utilizing L-lysine monooxygenase (DavB) and 5-aminovaleramide amidohydrolase (DavA) enzymes in the gut microbiota [7]. (F) Predicted pathway to produce 5-AVAB from trimethyllysine utilizing a Stickland reaction, which may occur in bovine rumen [9]. This could be possible also in microbiota colonizing the human intestine [30]. (G) Endogenous pathway predicted by Hoppel *et al*. [35] to oxidatively deaminate and decarboxylate trimethyllysine to 5-AVAB. Abbreviations: 5-AVA, 5-aminovaleric acid; 5-AVAB, 5-aminovaleric acid betaine; DavA, gene coding 5-aminovaleramide amidohydrolase; DavB, gene coding lysine monooxygenase; GSMT, glycine sarcosine methyltransferase; SDMT, sarcosine dimethylglycine methyltransferase.

flora, then there are no microbes able to produce 5-AVAB, even though there could be starting materials present in the gut. Thus, the observed 5-AVAB traces detected in GF mice [6] have been directly obtained from nutrition or potentially produced via endogenous metabolism.

One of the most likely precursors en route to 5-AVAB biosynthesis is 5-AVA, which can also be synthesized by the gut microbiome. There is good evidence that bacteria belonging to the genus *Pseudomonas* can produce 5-AVA using L-lysine as the starting material [21,22]. This pathway utilizes two important enzymes, namely, L-lysine monooxygenase (EC 1.13.12.2, enzyme

**OCTN2:** $Na^+$-dependent carnitine transporter. Widely expressed in human tissues (e.g., especially abundant in heart and skeletal muscle).
**Phytochemicals:** compounds produced in plants.
**Pre-eclampsia:** a complication during pregnancy characterized by high blood pressure. Untreated pre-eclampsia can lead to serious complications for both mother and baby.
**Reactive oxygen species (ROS):** ROS species that have important roles in cell signaling and homeostasis.
**Sirtuin 1:** an autophagy-related protein having deacetylating properties.
**Sirtuin 3:** protein whose functions are to eliminate reactive oxygen species and prevent apoptosis.
**Sirtuin 6:** stress-responsive protein involved in aging and DNA reparation.
**Sourdough:** dough fermentation utilizing lactic acid bacteria.
**Stickland reaction:** an energy forming fermentation reaction involving coupled amino acid oxidation and reduction reactions.

coded by DavB) and 5-aminovaleramide amidohydrolase (EC 3.5.1.30, enzyme coded by DavA), that oxidize L-lysine to 5-aminovaleramide and hydrolyze 5-aminovaleramide to 5-AVA (Figure 1B), respectively [7,22,23,105].

In addition to the demonstrated DavB/DavA pathway, *Clostridia* bacteria species can utilize a **Stickland reaction** in energy production, and, as a side effect, arginine or proline can be converted into 5-AVA (Figure 1C,D) [24]. Proline is reduced directly to 5-AVA by proline reductase (EC 1.21.4.1) as part of the Stickland reaction [25]. Additionally, arginine and ornithine treatment is demonstrated to elevate 5-AVA concentration in *Clostridia* bacteria [26,27]. 5-AVA biosynthesis from arginine utilizes a Stickland reaction, ornithine cyclodeaminase (EC 4.3.1.12), and proline reductase in *Clostridia* bacteria with proline and ornithine as intermediates (Figure 1C,D) [27–29]. 5-AVA can be further converted to 5-AVAB [2], potentially by glycine sarcosine methyltransferase and sarcosine dimethylglycine methyltransferase (Figure 1A).

Furthermore, 5-AVAB can be biosynthesized directly without the need for a 5-AVA intermediate from trimethyllysine by *Enterococcus faecalis* utilizing the DavB and DavA enzyme pathway (Figure 1E) [7], similarly as demonstrated for lysine (Figure 1B). In addition, trimethyllysine can be directly converted to 5-AVAB by a Stickland reaction (Figure 1F), as demonstrated by Servillo *et al*. [9]. They showed that the bovine ruminal microbiome forms 5-AVAB after just 2 h of incubation and, according to their hypothesis, there is an oxidation–reduction reaction, which can catalyze 5-AVAB production. Moreover, Rebouche *et al*. [30] reported an increased amount of methyl-$^{3}$H-labeled 5-AVAB in rat feces after the animals were fed with methyl-$^{3}$H-labeled trimethyllysine, thus demonstrating 5-AVAB biosynthesis in microbiota *per se*. However, the chemical transformation and microbiome species behind 5-AVAB synthesis need to be characterized more thoroughly to identify possible similarities in the 5-AVAB biosynthetic capacity between other species and the human gut microbiome.

Although the origin of 5-AVAB is not fully clear, the biosynthesis of other betaines, for example, glycine betaine, has been better characterized. For instance, there are at least two well-characterized glycine betaine synthetic pathways in the body that could be extended to 5-AVAB production. The first pathway produces glycine betaine by utilizing two methyltransferase enzymes, and this may also be possible in the biosynthesis of 5-AVAB (Figure 1A). The second glycine betaine biosynthesis pathway that is typically encountered in bacteria also takes place endogenously in the inner membrane of mitochondria. This pathway utilizes choline as its starting material and produces glycine betaine by oxidation [31–33]. Similarly, 5-hydroxypentyltrimethylammonium could be oxidized to produce 5-AVAB, although this pathway has not been examined in any detail in mechanistic studies.

### Endogenous synthesis of 5-AVAB

Although the role of gut microbiota in 5-AVAB biosynthesis has been postulated several times, it needs to be highlighted that most likely there is also some endogenous synthesis of 5-AVAB. For example, this concept is supported by Koistinen *et al*. [6] who demonstrated that a comparison between GF and normally housed mice did not reveal a significant difference in 5-AVAB levels in the heart or muscle tissues. However, more than fivefold differences in 5-AVAB levels were found in intestinal samples between control and gut microbiota-depleted mice.

Interestingly, C$^{14}$-labeled 5-AVAB was detected in urine after C$^{14}$-labeled trimethyllysine was injected intravenously into rats [34], indicating that there are endogenous 5-AVAB synthesis pathways. Furthermore, intraperitonially injected deuterium-labeled trimethyllysine increased 5-AVAB level in blood [105], and after a methyl-$^{3}$H-labeled trimethyllysine injection, methyl-$^{3}$H-labeled 5-

AVAB was found to be tenfold more abundant in urine as compared with feces, whereas tenfold lower amounts of methyl-$^{3}$H-labeled 5-AVAB were found in urine compared with feces after feeding with methyl-$^{3}$H-labeled trimethyllysine [30], further supporting the concept that there is endogenous 5-AVAB synthesis.

The endogenous synthesis pathway for 5-AVAB could resemble the endogenous carnitine biosynthesis pathway, as demonstrated by Hoppel *et al*. [35]. It is hypothesized that in this biosynthesis pathway, trimethyllysine can be oxidatively deaminated and further decarboxylated to 5-AVAB (Figure 1G) [35]. Furthermore, the human body can synthesize 5-AVA [36] and could hypothetically utilize methyl groups from glycine betaine to methylate 5-AVA to 5-AVAB (Figure 1A).

### Dietary sources of 5-AVAB

5-AVAB has been positively associated with various dietary items, either being actually present in the food or providing precursor compounds for its postulated microbial production. In a human trial, consumption of a whole grain-rich diet increased the concentration of 5-AVAB in plasma [11]. When mice were fed a diet rich in whole grains, this led to increased levels of 5-AVAB in intestinal and internal organs and fat samples, with the highest concentrations observed in the heart tissue [11]. If there is a high level of glycine betaine in whole grains, this appears to induce positive changes in the 5-AVAB levels [6]. Furthermore, since there was a correlation between the 5-AVAB concentration and the ratio of two **alkylresorcinols (AR C17:0/AR C21:0)**, which is typically considered as a biomarker of rye intake, it was postulated that an intake of rye-containing foodstuffs could elevate 5-AVAB levels, potentially via gut metabolism [37].

Trimethyllysine is one proposed dietary precursor of 5-AVAB [7,9]. Cereal flours do not seem to contain any detectable amounts of 5-AVAB, in contrast to trimethyllysine, which is present in whole-grain rye flour, as well as in the dough and bread made with this flour as compared with their refined equivalents [20,38]. Nonetheless, to our knowledge, elevated 5-AVAB levels have not been reported after consumption of vegetables, although trimethyllysine is ubiquitous in these foodstuffs [39]. This does not support the hypothesis that the trimethyllysine pathway would be significant in 5-AVAB biosynthesis (i.e., even though trimethyllysine has been demonstrated to be a potential precursor, it does seem that glycine betaine is more important in this respect). It should be noted that whole-grain rye and wheat are rich sources of glycine betaine.

Furthermore, the presence of 5-AVAB has not been detected from any of the bakery products or flours studied to date [20,38,40]. Therefore, the elevation in the amounts of 5-AVAB cannot explain the decrease of trimethyllysine, which occurs in **sourdough** fermented rye products compared with rye flours, nor why the amount of trimethyllysine is lower in whole-grain rye sourdough bread compared with the yeast-fermented counterpart [20]. These findings provide the basis for a hypothesis that there is a catalyzing environment induced by sourdough fermentation that can synthesize new biochemical compounds. Interestingly, a diet rich in fermented food was reported to increase the microbiota diversity [41], thus making it possible to create new biosynthesis pathways within the gut, for example, to catalyze the biosynthesis of 5-AVAB.

Moreover, it is highly plausible that sourdough fermentation replaces the first part of the molecular synthesis pathway, which typically takes place in the gut. Nevertheless, 5-AVAB has not been detected after sourdough fermentation [20], so it must be assumed that it is produced in the gut by bacteria other than those lactic acid bacteria responsible for the sourdough fermentation. The biochemical mechanisms and pathways behind sourdough fermentation are mostly unknown, but clear changes in **phytochemicals** have been demonstrated [42]. For example, γ-butyrobetaine was one of the 1000 or so molecular features that significantly changed after

fermentation [20]. Evidently, the role of sourdough fermentation in 5-AVAB biosynthesis needs to be clarified.

Meat and milk are foods that have been reported to contain 5-AVAB [8,9,43,44]. Moreover, ruminant meat contains more 5-AVAB than non-ruminant meat [9]. Interestingly, the 5-AVAB concentration in Mediterranean buffalo bull meat increased dramatically after both the animals' space allocation was increased and green forage feeding surpassed over 70 mg/kg [8]. Furthermore, a post-dry-aging maturation process of 60 days almost doubled the 5-AVAB concentration in the meat, although water loss might have made some contribution to this change [8]. Typically, high levels of 5-AVAB have been found in metabolically active tissues, including muscle tissue [11], since 5-AVAB is likely accumulating in tissues with large amounts of cell membrane carnitine transporters (**OCTN2**), for which 5-AVAB is a substrate [45].

A similar trend is evident also in milk, as ruminants have 20-fold higher 5-AVAB concentrations in their milk than non-ruminants [9,44]. In addition, the carnitine levels in milk follow the same pattern as the 5-AVAB levels in the milk from sheep, goats, and cows, respectively [9,46], suggesting that 5-AVAB accumulates similar to carnitine. Similar to the meat, by increasing the space allocation and by feeding with green forage, the 5-AVAB concentration in Mediterranean buffalo milk was increased significantly, to above 22 mg/l [43,47]. The 5-AVAB level in human serum has also been reported to correlate with milk consumption [48] and even suggested to be a biomarker of milk consumption but not of overall dairy products [17].

In addition to milk and meat, commercially produced seaweed has been demonstrated to contain 5-AVAB [10,49]. For example, the dried brown seaweed used in both human and animal nutrition contains 117–203 μg/g of 5-AVAB [50]. Most of the studied direct dietary sources have a high 5-AVAB concentration as compared with the human plasma concentration, which has been assayed as 148 ng/ml (standard deviation 53.1 ng/ml) [37] (i.e., the above reported commercial products are good 5-AVAB sources).

## The metabolic role of 5-AVAB

Although many details related to the biological role of 5-AVAB are still unknown, 5-AVAB has been linked with various metabolic phenomena, often discovered in hypothesis-free nontargeted metabolite profiling studies (Table 1). Many of these research efforts are trying to understand the biology and relationship between different compounds with diseases; often the conclusion has highlighted the importance of microbiota, with 5-AVAB as one of the contributing metabolites.

### Fetal brain development

The importance of the relationship between maternal gut health and fetus development has been only recently noted, with interest being focused on the gut–brain axis [51]. 5-AVAB has been claimed to be a key player in fetal development, as it is absent from the fetal brain tissue of GF mice, and this has affected normal brain development [2,12]. Furthermore, it seems that betainized compounds, in general, are positively associated with neurite outgrowth and normal offspring development [2], even though elevated 5-AVAB levels have been associated with the complex pregnancy disorder **pre-eclampsia** [52,53]. It is noteworthy that 5-AVAB has been detected in human postmortem brain samples, pointing to the potential importance of the metabolite also in human cerebral metabolism [54].

The importance of the availability of methyl donors for fetus development has been postulated [55], and current knowledge suggests that glycine betaine can donate its methyl groups for the production of 5-AVAB [6]. Both neurodevelopment and 5-AVAB biosynthesis could be active

Table 1. Nomenclature, related foods, samples, and biological relevance to 5-AVAB

| Publication year | Reported as | Food (related/analyzed) | Human/animal/cell | Tissue | Biological relevance | 5-AVAB beneficial/not | Refs |
|---|---|---|---|---|---|---|---|
| 1965 | 5-Trimethylaminovaleric acid | | Mouse | Urine and carcass | Carnitine is not biosynthesized from 5-AVAB | I[a] | [101] |
| 1973 | 5-*N*-Trimethylaminopentanoate | | Rat | Urine | Endogenous biosynthesis from trimethyllysine | I | [34] |
| 1977 | 5-Trimethylaminovaleric acid | | Cell | Heart cells | Cellular carnitine intake is blocked by 5-AVAB | I | [85] |
| 1980 | 5-Trimethylammoniopentanoate | | Rat | Urine | Endogenous biosynthesis from trimethyllysine | I | [35] |
| 1986 | δ-Aminovaleric acid betaine (δ-AVAB) | | Marine algae | | | I | [49] |
| 1986 | δ-*N*-Trimethylaminovaleric acid | | Rat | Urine and feces | 5-AVAB identified | I | [30] |
| 1992 | δ-Aminovaleric acid betaine (δ-AVAB) | | Marine algae | | | I | [10] |
| 2009 | δ-Aminovaleric acid betaine (AVAB) | | Marine algae | | | I | [50] |
| 2010 | 5-Aminovaleric acid betaine | | Coral | | | I | [102] |
| 2013 | 3-Dehydrocarnitine[b] | | Human | Serum | Increase intermuscular adipose tissue, abdominal adiposity | – | [84] |
| 2014 | 3-Dehydrocarnitine[b] | | Human | Serum | Fibrate drug is positively associated with 5-AVAB | I | [83] |
| 2014 | 3-Dehydrocarnitine[b] | | Human | Stool | Inverse association with colorectal cancer | + | [62] |
| 2014 | 5-Trimethylaminovalerate (GBB-5) | | | | 5-AVAB hydroxylation by BBOX | I | [93] |
| 2015 | δ-Aminovaleric acid betaine (AVAB) | | Marine algae | | | I | [96] |
| 2016 | X-21365 | | Human/mouse | Serum/(plasma/liver/muscle/adipose) | Metformin increases 5-AVAB | I | [64] |
| 2016 | Unidentified, $C_8H_{18}NO_2$ | | Cell | Breast cancer cells | Correlation with hypoxic regions | I | [65] |
| 2016 | Trimethyl-*N*-aminovalerate | Milk (R) | Human | Urine and serum | Milk intake biomarker | I | [17] |
| 2016 | X-21365 | Milk (R) | Human | Plasma/serum | Associated with low-fat milk | I | [48] |
| 2018 | *N*-Trimethyl-5-aminovalerate | | Human | Urine and serum | Biomarker for microalbuminuria, diabetes | – | [80] |
| 2018 | 5-Aminovaleric acid betaine (5-AVAB) | | Human | Umbilical cord plasma | Increased 5-AVAB in pre-eclampsia | I | [52] |
| 2018 | 5-Aminovaleric acid betaine (5-AVAB) | Whole grain (R) | Human/mouse | Plasma, gastrointestinal tract parts, inner organs, and muscle tissues | Elevated 5-AVAB concentration | I | [11] |

*(continued on next page)*

Table 1. (continued)

| Publication year | Reported as | Food (related/analyzed) | Human/animal/cell | Tissue | Biological relevance | 5-AVAB beneficial/not | Refs |
|---|---|---|---|---|---|---|---|
| 2018 | 5-Aminovaleric acid betaine (5-AVAB) | | Human/mouse | Heart | Decreased β-oxidation | + | [45] |
| 2018 | δ-Valerobetaine (δVB) | Milk (A) | | | | I | [44] |
| 2018 | δ-Valerobetaine (δVB) | Milk/meat (A) | | | | I | [9] |
| 2019 | *N*,*N*,*N*-Trimethyl-5-aminovalerate | | Mouse | Plasma and feces | Gut produces 5-AVAB and angiotensin II downregulates 5-AVAB | I | [13] |
| 2019 | δ-Valerobetaine (δVB) | | Cell | Endothelial cells | Antioxidant and anti-inflammatory | + | [88] |
| 2019 | δ-Valerobetaine (δVB) | | Cell | Endothelial cells | Reduced intracellular lipid peroxidation, reactive oxygen species, and cytokine release | + | [47] |
| 2019 | 5-Aminovaleric acid betaine (5-AVAB) | | Human | Plasma | Concentration average 147 ng/ml | I | [37] |
| 2020 | δ-Valerobetaine (δVB) | | Cell | Adenocarcinoma | Anticancer properties | + | [63] |
| 2020 | δ-Valerobetaine (δVB) | | Cell | Head and neck squamous cell carcinoma | Anticancer properties | + | [60] |
| 2020 | Delta-valerobetaine | | Mouse | Liver | Ranitidine and finasteride medication decreased 5-AVAB levels in liver | I | [71] |
| 2020 | *N*-Trimethyl 5-aminovalerate | | Human | Serum | Biomarker, cardiovascular disease | – | [67] |
| 2020 | 5-Aminovaleric acid betaine (5-AVAB) | | Dog/human | Saliva | | I | [103] |
| 2020 | *N*,*N*,*N*-Trimethyl-5-aminovalerate (TMAV) | | Mouse | Brain | Promotes axonogenesis | + | [2] |
| 2020 | *N*,*N*,*N*-Trimethyl-5-aminovaleric acid (TMAVA) | | Human/mouse | Plasma/(plasma/liver/feces) | Correlation with liver steatosis | – | [7] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2021 | 5-Aminovaleric acid betaine (5-AVAB) | | Human | Umbilical cord plasma | Increased 5-AVAB in pre-eclampsia | I | [53] |
| 2021 | 5-Aminovaleric acid betaine (5-AVAB) | | Human | Brain | 5-AVAB identified | I | [54] |
| 2021 | δ-Valerobetaine (δVB) | Buffalo meat (A) | Italian Mediterranean buffalo bull | Muscle | | I | [8] |
| 2021 | δ-Valerobetaine | Buffalo milk (A) | Italian Mediterranean dairy buffalo | | Buffalo milk has higher antioxidant and antineoplastic properties after green feeding compared with control milk | + | [43] |
| 2021 | δ-Valerobetaine (δVB) | | Cell | Adenocarcinoma | Anticancer properties | + | [61] |
| 2021 | N-Trimethyl-5-aminovalerate (TMAVA) | | Human | Plasma | A diet with several metabolic activators reduced 5-AVAB levels in plasma | I | [77] |
| 2021 | 5-Aminovaleric acid betaine | Dog food (A) | | | 5-AVAB levels correlating with diet associated with dilated cardiomyopathy disease | – | [69] |
| 2021 | δ-Valerobetaine (VB) | | Mouse | Liver and serum | 5-AVAB is an obesogen | – | [72] |
| 2021 | δ-Valerobetaine | | Mouse | Brain and serum | 5-AVAB correlates with cognitive decline | – | [59] |
| 2022 | 5-Aminovaleric acid betaine (5-AVAB) | | Mouse | Brain/placenta/intestine | Gut–brain interaction | I | [12] |
| 2022 | N,N,N-Trimethyl-5-aminovalerate (TMAVA) | | Human | Plasma | Elevated 5-AVAB plasma levels correlate with fatty liver disease | – | [74] |
| 2022 | N,N,N-Trimethyl-5-aminovaleric acid (TMAVA) | | Human/mouse | Plasma | 5-AVAB accelerates cardiac hypertrophy by blocking L-carnitine synthesis and uptake | – | [105] |

[a]I, No beneficial or harmful features described.
[b]Reported misidentification [17,64].

simultaneously, thus making 5-AVAB a possible biomarker providing evidence that there is a sufficient amount of methyl donors in the system. However, 5-AVAB could be biosynthesized utilizing methyl groups originating from glycine betaine and play a critical role in fetal growth, similar to glycine betaine [56,57], and/or protect the neurodevelopment of the fetus as has been shown for a high choline intake during gestation in mice and rat experiments [58]. Interestingly, a very recent article claimed that the extent of the increase in 5-AVAB concentration correlated positively with age-related cognitive decline [59], further strengthening the evidence that this compound is a significant mediator in the gut–brain axis, although its precise role has yet to be established.

### Cancer prevention and inhibition

There is a growing body of evidence supporting the possibilities of using betaines, including 5-AVAB, in cancer treatment and prevention [43,60–63]. For instance, it has been claimed that 5-AVAB may protect an individual from colorectal cancer. When three human colon cancer cell lines (LoVo, SW480, and SW620) were treated with 5-AVAB *in vitro*, their growth was arrested in the **G2/M and SubG1 phases** while non-malignant control cells proliferated normally [61,63]. 5-AVAB treatment activated **cyclins**, **caspases**, **reactive oxygen species (ROS)**, and **Bax/Blc-2** (Figure 2) apoptotic pathways in the LoVo colon cancer cell line [63]. Furthermore, 5-AVAB treatment upregulated the synthesis of **Sirtuin 6** protein [63]. The exposure to 5-AVAB also downregulated **Sirtuin 3** and activated **mitophagy** (Figure 2) in SW480 and SW620 colon cancer cells [61]. Healthy individuals typically have higher levels of 5-AVAB in feces than colorectal cancer patients and colorectal cancer was nearly three times less likely if the fecal sample contained 5-AVAB (misidentified at first as 3-dehydrocarnitine in Goedert *et al*., [62] but later correctly identified to be 5-AVAB [17,64]). It should be noted that there is extensive variation in the fecal samples, meaning that studies with larger sample sizes will be needed to confirm if there is an inverse association between the 5-AVAB level and colorectal cancer.

In addition to colorectal cancer cells, human oral carcinoma Cal 27 cells became apoptotic when treated with 5-AVAB together with another betaine, γ-butyrobetaine [43,60]. Furthermore, 5-AVAB was detected in hypoxic regions in MDA-MB-231 breast cancer cell samples that were grown in mice mammary fat pad [65]. The biological mechanism behind the effect of 5-AVAB to inhibit the viability of cancer cells could be attributable to the reduction of **β-oxidation** of fatty acids (Figure 2) although the role of hypoxia remains unknown. It is known that reduced β-oxidation of fatty acids can slow down cancer development as some cancer cells are dependent on β-oxidation for their energy production [66].

### Heart muscle regulation

Heart muscle seems to be one of the tissues in which 5-AVAB accumulates [11]. 5-AVAB levels in human plasma have recently been positively associated with impaired left ventricular filling pressure, indicating that 5-AVAB may be an early biomarker of left ventricle diastolic dysfunction [67]. In the same trial, 5-AVAB was associated with the ratio of **early mitral inflow velocity** and **mitral annular early diastolic velocity**, a novel indicator for heart failure with preserved ejection fraction [67,68]. In addition, investigators examined a cardiac condition called dilated cardiomyopathy, a disease affecting human and dog hearts, and they reported that dog diets associated with this condition contain a higher level of 5-AVAB compared with the diets that are not associated with it [69]. Somewhat at odds with these results, it has been demonstrated that 5-AVAB reduces β-oxidation in cardiomyocytes by decreasing the carnitine level via inhibition of the cell membrane carnitine transporter (Figure 2) [45]. This could be beneficial after ischemia, as some drugs, such as mildronate, that decrease β-oxidation of fatty acids, are used to improve cardiac function after ischemia [70]. Taken together, it seems that 5-AVAB can exert both positive and negative effects on the heart.

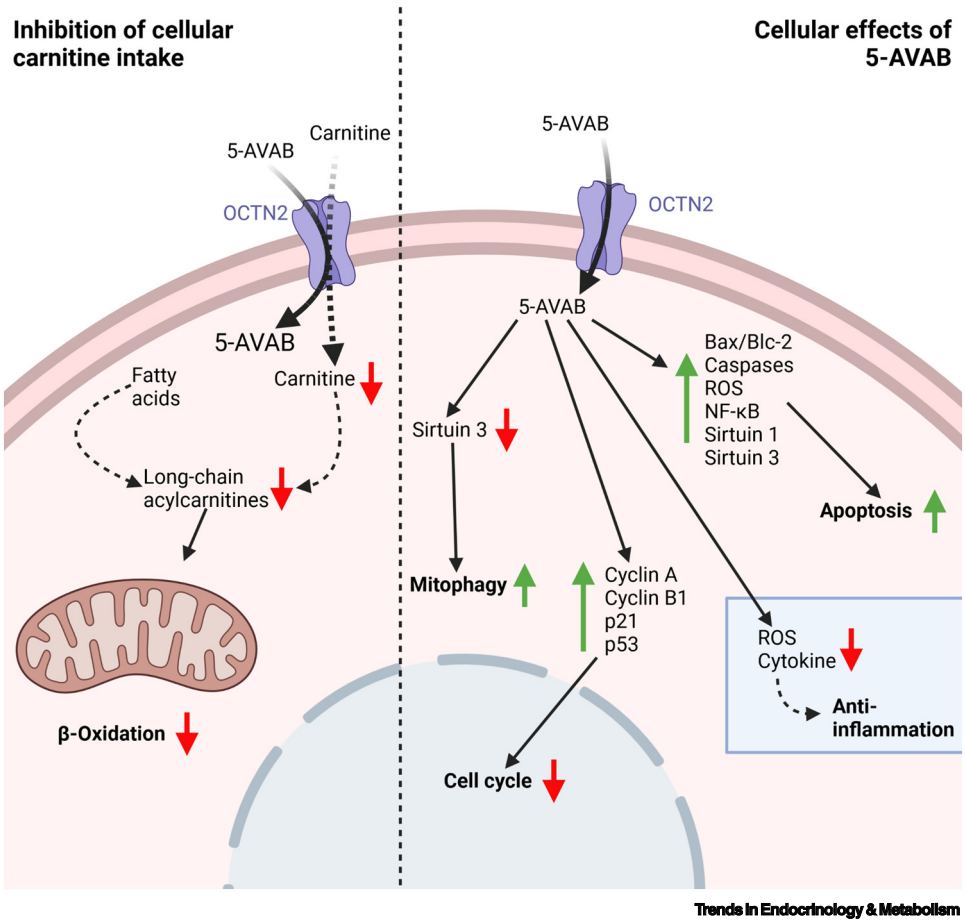


Figure 2. Molecular mechanisms induced by 5-aminovaleric acid betaine (5-AVAB). 5-AVAB inhibits β-oxidation of fatty acids. This effect has been shown in cardiomyocytes and liver cells [7,45], but likely also occurs in other tissues. 5-AVAB is a substrate for the cell membrane carnitine transporter (OCTN2) and can compete with carnitine on cellular uptake. This leads to reduced cellular carnitine levels and reduced β-oxidation of fatty acids. Furthermore, 5-AVAB ameliorates cytotoxicity in endothelial cells by decreasing reactive oxygen species (ROS) and cytokine release [88]. In cancer cells, 5-AVAB treatment has promoted apoptosis and reduced the cell cycle, suggesting anticancer properties [61,63]. This figure was created using BioRender (https://biorender.com/). Abbreviation: NF- κB, nuclear factor-kappa B.

## Regulation of liver function

A high-fat diet can typically cause fat accumulation in the liver. A recently conducted mice model analysis demonstrated that a high-fat diet was associated with increased 5-AVAB concentrations in the liver [71]. Moreover, Zhao *et al*. [7] utilized a mouse model and showed that the presence of 5-AVAB in drinking water could further amplify the fat accumulation in the liver when combined with a high-fat diet. Additionally, a reanalysis of a metabolomics study that focused on youth non-alcoholic fatty liver disease revealed an association between the serum 5-AVAB level and hepatic steatosis [72,73]. Similarly, the 5-AVAB plasma level has been associated with metabolic dysfunction-associated fatty liver disease and, rather interestingly, a di-methylated form of 5-AVAB was claimed to be an important predictor for metabolic dysfunction-associated fatty liver [74].

The 5-AVAB fat accumulation amplification effect contrasts with the property of glycine betaine to protect the liver against non-alcoholic fatty liver disease [75] and alcoholic fatty liver disease [76]. The postulated role of 5-AVAB in fat accumulation in the liver could be explained by the reduction

of β-oxidation, leading to increased levels of triglycerides and free fatty acids [7]. Interestingly, ranitidine and finasteride, drugs typically used for heartburn and hair loss treatment, respectively, seem to decrease the effect of a high-fat diet on 5-AVAB levels in mouse liver samples, possibly via a modification of gut microbiota composition or function [71]. Furthermore, a placebo-controlled intervention involving a diet supplemented with L-carnitine tartrate, nicotinamide riboside, serine, and acetyl-L-cysteine ameliorated fatty liver disease and simultaneously significantly decreased the concentration of 5-AVAB [77]. Again, the level of 5-AVAB and the presence of fatty liver disease displayed a positive correlation; however, the causality remains unknown. Overall, it seems that high-fat diets can increase the biosynthesis of 5-AVAB, possibly via a modulation of the gut microbiota, and consequently, the elevated 5-AVAB levels could lead to increased fat accumulation in the liver.

### Diabetes prevention

Metformin, a drug used to treat type 2 diabetes, has been described to increase the concentration of 5-AVAB in human serum, in both short- and long-term trials [64]. Typically, diets rich in whole grains have been associated with a reduced risk of type 2 diabetes [78,79]; the betainized compounds present in these diets could contribute to their beneficial effects [37]. For example, it has been reported that there are correlations between increased 5-AVAB levels and improved insulin secretion [11]. Additionally, 5-AVAB was demonstrated to improve glucose tolerance and insulin tolerance in mice [7]. For this reason, the higher 5-AVAB concentration in plasma might exert some protection against the diabetogenic Western diet.

Type 1 diabetes is not as common as type 2 diabetes but is a major global health issue. **Microalbuminuria** is an early marker of **diabetic neuropathy**. Even though the condition is severe, it is not often detected sufficiently early, highlighting the need for better biomarkers. A recent study introduced the possibility that 5-AVAB measured from the blood could act as an earlier and thus better biomarker for diabetic neuropathy than microalbuminuria [80]. The kidneys contain high amounts of carnitine transporters, and these are likely responsible for the reabsorption of 5-AVAB from urine back to blood [45,81]. The early stages of diabetic nephropathy could disrupt this process, which could explain why 5-AVAB could be an early biomarker, a hypothesis in need of testing.

### Systemic effects

When 5-AVAB was combined with a high-fat diet, this reduced weight gain in mice as compared with a high-fat diet alone, suggesting that 5-AVAB might protect from diet-induced obesity [7]. Surprisingly, the lean mass of mice remained relatively constant with or without 5-AVAB treatment, but the fat mass was reduced significantly after 5-AVAB treatment. Additionally, it was demonstrated that lipolysis was increased after 5-AVAB treatment in mice [7]. At odds with the protective role of 5-AVAB to combat obesity, there is a result revealing an obesogenic role in mice [72]. However, the weight gain did not differ from that of conventional mice fed without 5-AVAB. Moreover, the plasma metabolomics dataset revealed a significant correlation between increased central adiposity (i.e., a white adipose tissue mass) and 5-AVAB [72,82]. These contradictory results highlight the fact that also in this sense, the metabolic role of 5-AVAB is not yet understood completely.

Importantly, administration of **angiotensin II** in mice was reported to decrease the level of 5-AVAB via altered microbiota [13]. This suggests a possible role for 5-AVAB also in blood pressure regulation.

A series of studies have reported various health implications for 5-AVAB [83,84] (during the time of publication, it had been misidentified as 3-dehydrocarnitine, but this was later corrected to

5-AVAB [17,64]). For instance, the level of 5-AVAB in blood samples was positively associated with the increased amount of intramuscular adipose tissue and there was a trend between the 5-AVAB concentration and abdominal adiposity in elderly people [84]. Moreover, it was demonstrated that **fibrate** therapy was associated with increased 5-AVAB levels in elderly people [83]. Since it is known that 5-AVAB reduces β-oxidation, in this way at least in theory, it could counteract the effects of the fibrates, by increasing the levels of free fatty acids in the blood [7].

Overall, since the current literature is replete with conflicting results, a heterogenic nomenclature (Box 1), and uncertainty about the identification of the compound, it is essential to apply appropriate and accurate analytical methods [i.e., MS combined with liquid chromatographic separation in hydrophilic interaction columns (HILIC)], if one wishes to reliably assay 5-AVAB and gain a better understanding of its role in the body (Box 2).

### Cellular-level effects

Recently, it has been reported that 5-AVAB can reduce β-oxidation of fatty acids in the liver [7] and in cardiomyocytes [45,85]. It has been proposed that 5-AVAB acts in some ways similar to **meldonium**, an anti-ischemia drug [45]. Pharmacological studies have suggested that meldonium reduces the cellular L-carnitine levels by blocking the OCTN2 transporters. The reduced uptake of L-carnitine directs cardiomyocyte energy metabolism towards the consumption of glucose rather than fatty acids [70]. Moreover, both meldonium and 5-AVAB have been analyzed against the OCTN2 in an *in silico* model and their interactions with this protein are similar to L-carnitine [45,86]. Furthermore, recent results demonstrate that 5-AVAB acts similarly as meldonium and inhibits the γ-butyrobetaine dioxygenase [105], the enzyme which synthesizes L-carnitine from γ-butyrobetaine, although this was not reported earlier when structural analogs of meldonium were analyzed [87].

Moreover, 5-AVAB has been demonstrated to have an anti-inflammatory effect against the endothelial damage evoked by high glucose concentrations. 5-AVAB reduced high glucose-activated inflammation signaling via **Sirtuin 1**, Sirtuin 6, and **nuclear factor-kappa B (NF-κB)**. When

#### Box 2. Analytical methods for 5-AVAB

Trimethylated compounds are highly hydrophilic metabolites that can be hard to separate in reversed-phase liquid chromatography. Rapid advances have been made in HPLC methods during recent decades, and HILIC liquid chromatography has been introduced to provide efficient separation for polar components [94,95]. HILIC can separate polar compounds efficiently, thus making it an extremely efficient analytical method for water-soluble betainized compounds.

HILIC is especially useful when separating trimethylated compounds with the same molecular mass, as is demonstrated in Figure I for three compounds, 5-AVAB, 3M-4-TMAB, and 4-AVAB. Synthesized standards for these three compounds (Figure IA) and their presence in tissue samples (Figure IB), as well as tissue samples spiked with synthesized standards (Figure IC), highlight the suitability of HILIC-LC-MS in the analysis of trimethylated compounds possessing the same molecular formulas ($C_8H_{17}NO_2$, *m/z* 160.13375). MS alone is not reliable enough to identify these compounds since the mass-to-charge ratios for 5-AVAB, 4-AVAB, and 3M-4-TMAB are the same and they share many chemical properties, thus giving very similar MS/MS spectra (Figure ID). Instead, an HPLC-MS method coupled with an HILIC [94] column is an efficient way to separate these compounds from each other [37] (Figure IA and figure legend). For instance, this method was used successfully to identify four different betainized compounds in marine algae [96]. The same method was used by [97], demonstrating the superior performance of HILIC compared with reversed-phase chromatography when analyzing extremely polar compounds, such as betaines, from mouse samples. Furthermore, these compounds are not masked by the tissue samples as the spiked compounds are found from the pig brain and muscle samples (Figure IC).

For example, of the three compounds, only 5-AVAB was observed in human and pig brain samples, in line with the previous report where 5-AVAB was detected in human postmortem brain and cerebrospinal fluid samples [54]. However, 3M-4-TMAB and 4-AVAB have been reported to be present in mouse brain tissue when assessed with different analytical methods [98,99].

Moreover, 5-AVAB has been misidentified as 3-dehydrocarnitine [17,64], which implies that these newly found betaines can be difficult to identify. Furthermore, also other trimethylated compounds, like glycine betaine and TMAO, have received inconsistent identifications [100]. Methods such as NMR can face challenges since the concentrations are very low in normal physiological conditions. Reliable identification of compounds with identical mass-to-charge ratios and similar chemical structures typically requires chromatographic separation, and 5-AVAB, 3M-4-TMAB, and 4-AVAB are no exceptions in this respect. The polar character of these compounds hinders separation with reverse-phase chromatography and thus justifies the use of HILIC chromatography combined with MS.

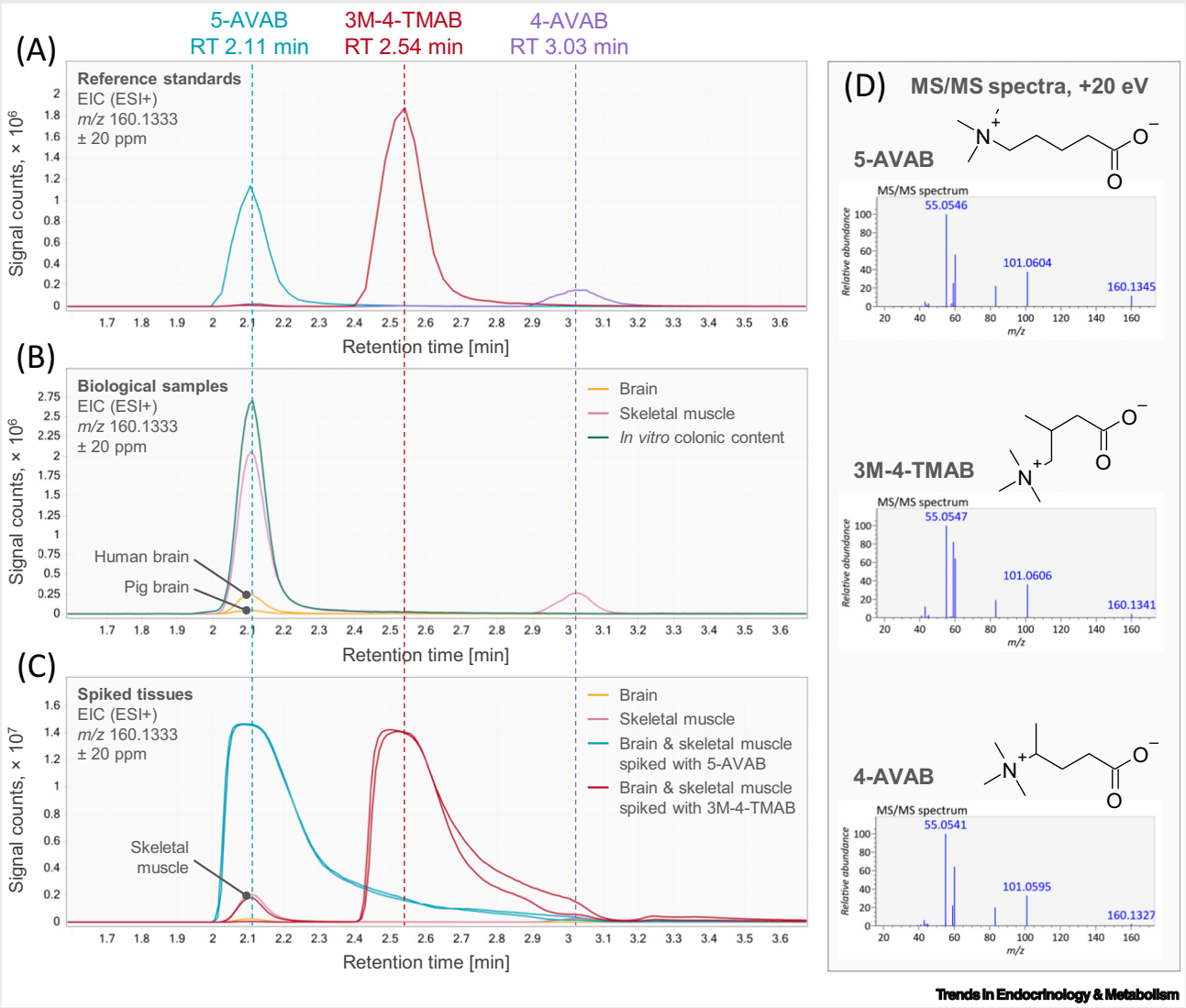


Figure I. Hydrophilic interaction column (HILIC) chromatography enables the identification of isomeric betainized compounds. (A) Identification of in-house synthesized standard compounds. Retention times are 2.11 min (5-AVAB), 2.54 min (3M-4-TMAB), and 3.03 min (4-AVAB). (B) Brain samples from pig and human had lower abundances for 5-AVAB compared with muscle and feces samples. For skeletal muscle, a low peak of 4-AVAB can be identified, but the rest of the samples showed no signals for 3M-4-TMAB and 4-AVAB. (C) Plain tissue samples plotted with 5-AVAB and 3M-4-TMAB spiked samples. (D) Molecular formulas and MS/MS spectrums of 5-AVAB, 3M-4-TMAB, and 4-AVAB. 5-AVAB CAS Num. 6778-33-2, CID Num. 14274897, MS/MS spectrum [CID voltage: m/z (intensity%)] 20 V: 55.054 (100), 60.081 (74), 101.060 (41), 83.049 (27), 59.049 (25), 160.133 (13; [M + H]+), 58.065 (4). 3M-4-TMAB CAS Num. –, CID Num. 20606311, MS/MS spectrum 20 V: 55.054 (100), 59.048 (92), 60.080 (85), 101.059 (45), 83.049 (30), 43.017 (16), 160.134 (10; [M + H]+), 45.056 (4). 4-AVAB CAS Num. 67066-58-4, CID Num. 90655245, MS/MS spectrum 20 V: 60.080 (100), 55.054 (68), 59.050 (22), 101.060 (11), 83.050 (5), 45.056 (5). Compounds observed utilizing HILIC chromatography in an ultra-performance liquid chromatography coupled to quadrupole time of flight mass spectrometry (UPLC-QTOF-MS) system, as described previously [104]. Abbreviations: 4-AVAB, 4-aminovaleric acid betaine; 5-AVAB, 5-aminovaleric acid betaine; EIC, extracted ion chromatogram; ESI, electrospray ionization; 3M-4-TMAB, 3-methyl-4-(trimethylammonio)butanoate; MS/MS, fragmented precursor ion mass spectrum; m/z, mass-to-charge ratio; ppm, parts per million; RT, retention time.

endothelial cells were exposed to high glucose concentrations, Sirtuin 1 and Sirtuin 6 were down-regulated and NF-κB was upregulated, and 5-AVAB treatment was able to counteract these changes [88]. Last, 5-AVAB may have both antioxidant and anti-inflammatory properties; when

## Key figure

Overall view of the origin, effects, and analysis of 5-aminovaleric acid betaine (5-AVAB)

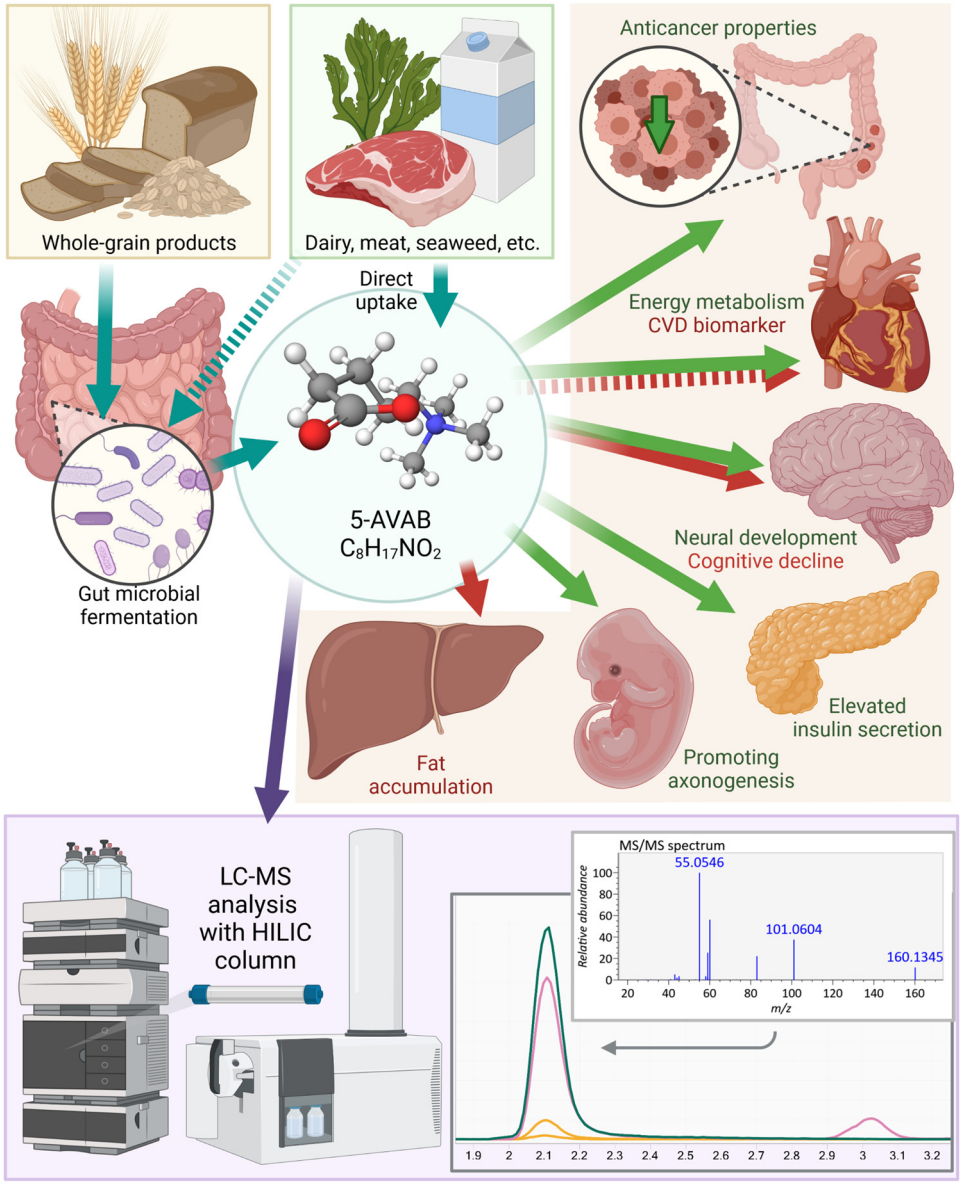


Figure 3. 5-AVAB is derived directly or indirectly from nutrition. It has anticancer properties and beneficial health effects (e.g., in the heart, brain, pancreas, and fetal development). 5-AVAB has been demonstrated to be a cardiovascular disease (CVD) marker and to assist in fat accumulation in the liver. It can be efficiently analyzed with liquid chromatography–mass spectrometry (LC-MS) with a hydrophilic interaction column (HILIC). This figure was created using BioRender (https://biorender.com/).

colon cancer cells were treated with 5-AVAB, there was an increase in the amounts of mitochondrial ROS as well as Sirtuin 1 upregulation [60,88]. Because sirtuins, enzymes that are also associated with lifespan, display a dual role in tumorigenesis by acting both as a cancer suppressor and promoter [89–91], these results can be challenging to interpret. Nonetheless, the overall cellular level effects caused by 5-AVAB point more towards a positive health outcome (Figure 3, Key figure).

## Concluding remarks

Recently, the scientific community has taken more interest in 5-AVAB, as the number of publications reporting the microbial origin and various metabolic roles of the compound is steadily increasing. Most importantly, the role of gut microbial interaction with multiple tissues via 5-AVAB has been demonstrated several times; however, numerous questions remain open (see Outstanding questions). Based on the current experimental and epidemiological evidence, 5-AVAB has been linked to some negative health outcomes, including fatty liver disease and pre-eclampsia, but it has also been associated with a growing number of overall beneficial health effects and suggested to have many potential preventative roles, namely, anticancer, anti-inflammatory, and antioxidant properties. In addition, 5-AVAB seems to exert a protective role related to heart diseases and diabetes and be essential for fetal brain development, but the causality is double-edged with both negative and positive health outcomes being described. Current evidence suggests that the key molecular mechanism of action of 5-AVAB is its ability to influence lipid and energy metabolism via the inhibition of β-oxidation of fatty acids, but further research will be needed to obtain a fuller picture of its effects.

While many microbial and direct nutritional sources, such as *E. faecalis* and meat, respectively, have been confirmed, the possibility of endogenous biosynthesis needs further research. One way to gain a better understanding of 5-AVAB synthesis in the gut and endogenous pathways would be to test the effects of several precursors with and without isotopic labeling, followed by analysis with state-of-the-art methods, such as liquid chromatography tandem MS (LC-MS/MS) with hydrophilic interaction chromatography. The confusing nomenclature must be eliminated because this complicates the sharing of novel and existing information around the scientific community. It would be important to identify different tissues where 5-AVAB can exert effects as well as interactions of this betainized compound with other physiological and pathological compounds and thus reveal still unknown mechanisms of action for 5-AVAB. Overall, 5-AVAB has proven its significance as an important gut-related metabolic player and it will be interesting to continue evaluating its cellular effects and molecular mechanisms in the brain, heart, and human health in general.

## Outstanding questions

Current knowledge about the metabolic role of 5-AVAB derives mostly from *in vitro* and *in vivo* studies. Does 5-AVAB also have a similar effect on metabolic processes occurring in humans?

What are the overall physiological and health outcomes of 5-AVAB? What are the key mechanisms behind these effects?

From which precursors can gut microbiota synthetize 5-AVAB? Can 5-AVAB be endogenously biosynthesized? What is the role of direct absorption of 5-AVAB from diet?

What is the pharmacokinetics (absorption, distribution, metabolism, and elimination) of 5-AVAB in humans?

The results related to different diseases are very limited and in part contradictory. Could 5-AVAB be used as a biomarker for some diseases? Could 5-AVAB prevent other diseases?

Does 5-AVAB have a double-edged role, that is, being important for normal fetal development but at the same time harmful in other instances (e.g., when there is a high fat intake from diet)?

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### Declaration of interests

The authors declare that the review was written in the absence of any commercial or financial relationships.

### References

1. Craig, S.A. (2004) Betaine in human nutrition. *Am. J. Clin. Nutr.* 80, 539–549
2. Vuong, H.E. *et al.* (2020) The maternal microbiome modulates fetal neurodevelopment in mice. *Nature* 586, 281–286
3. Cheng, F. *et al.* (2020) A review of pharmacological and pharmacokinetic properties of stachydrine. *Pharmacol. Res.* 155, 104755
4. Borodina, I. *et al.* (2020) The biology of ergothioneine, an antioxidant nutraceutical. *Nutr. Res. Rev.* 33, 190–217

5. Janeiro, M.H. *et al.* (2018) Implication of trimethylamine N-oxide (TMAO) in disease: potential biomarker or new therapeutic target. *Nutrients* 10, 1398
6. Koistinen, V.M. *et al.* (2019) Contribution of gut microbiota to metabolism of dietary glycine betaine in mice and in vitro colonic fermentation. *Microbiome* 7 103–102
7. Zhao, M. *et al.* (2020) TMAVA, a metabolite of intestinal microbes, is increased in plasma from patients with liver steatosis, inhibits γ-butyrobetaine hydroxylase, and exacerbates fatty liver in mice. *Gastroenterology* 158, 2266–2281
8. Salzano, A. *et al.* (2021) Effect of breeding techniques and prolonged post dry aging maturation process on biomolecule levels in raw buffalo meat. *Vet. Sci.* 8, 66
9. Servillo, L. *et al.* (2018) Ruminant meat and milk contain δ-valerobetaine, another precursor of trimethylamine N-oxide (TMAO) like γ-butyrobetaine. *Food Chem.* 260, 193–199
10. Blunden, G. *et al.* (1992) Betaines and tertiary sulphonium compounds from 62 species of marine algae. *Biochem. Syst. Ecol.* 20, 373–388
11. Kärkkäinen, O. *et al.* (2018) Diets rich in whole grains increase betainized compounds associated with glucose metabolism. *Am. J. Clin. Nutr.* 108, 971–979
12. Pessa-Morikawa, T. *et al.* (2022) Maternal microbiota-derived metabolic profile in fetal murine intestine, brain and placenta. *BMC Microbiol.* 22, 46
13. Cheema, M.U. and Pluznick, J.L. (2019) Gut microbiota plays a central role to modulate the plasma and fecal metabolomes in response to angiotensin II. *Hypertension* 74, 184–193
14. Nyyssölä, A. *et al.* (2000) Extreme halophiles synthesize betaine from glycine by methylation. *J. Biol. Chem.* 275, 22196–22201
15. Nyyssölä, A. *et al.* (2001) Characterization of glycine sarcosine N-methyltransferase and sarcosine dimethylglycine N-methyltransferase. *Appl. Environ. Microbiol.* 67, 2044–2050
16. Ueland, P.M. *et al.* (2005) Betaine: a key modulator of one-carbon metabolism and homocysteine status. *Clin. Chem. Lab. Med.* 43, 1069–1075
17. Pallister, T. *et al.* (2016) Metabolites of milk intake: a metabolomic approach in UK twins with findings replicated in two European cohorts. *Eur. J. Nutr.* 56, 2379–2391
18. Mitsuoka, T. (1990) Bifidobacteria and their role in human health. *J. Ind. Microbiol.* 6, 263–267
19. Clavel, T. *et al.* (2014) Intestinal microbiota in metabolic diseases. *Gut Microbes* 5, 544–551
20. Koistinen, V.M. *et al.* (2018) Metabolic profiling of sourdough fermented wheat and rye bread. *Sci. Rep.* 8 5684–5611
21. Fothergill, J.C. and Guest, J.R. (1977) Catabolism of L-lysine by *Pseudomonas aeruginosa*. *J. Gen. Microbiol.* 99, 139–155
22. Revelles, O. *et al.* (2005) Multiple and interconnected pathways for L-lysine catabolism in *Pseudomonas putida* KT2440. *J. Bacteriol.* 187, 7500–7510
23. Liu, P. *et al.* (2014) Enzymatic production of 5-aminovalerate from L-lysine using L-lysine monooxygenase and 5-aminovaleramide amidohydrolase. *Sci. Rep.* 4, 5657
24. Barker, H.A. (1981) Amino acid degradation by anaerobic bacteria. *Annu. Rev. Biochem.* 50, 23–40
25. Bouillaut, L. *et al.* (2013) Proline-dependent regulation of *Clostridium difficile* Stickland metabolism. *J. Bacteriol.* 195, 844–854
26. Wildenauer, F.X. and Winter, J. (1986) Fermentation of isoleucine and arginine by pure and syntrophic cultures of *Clostridium sporogenes*. *FEMS Microbiol. Lett.* 38, 373–379
27. Costilow, R.N. and Laycock, L. (1968) Proline as an intermediate in the reductive deamination of ornithine to δ-aminovaleric acid. *J. Bacteriol.* 96, 1011–1020
28. Costilow, R.N. and Laycock, L. (1971) Ornithine cyclase (deaminating). *J. Biol. Chem.* 246, 6655–6660
29. Muth, W.L. and Costilow, R.N. (1974) Ornithine cyclase (deaminating). *J. Biol. Chem.* 249, 7463–7467
30. Rebouche, C.J. *et al.* (1986) -N-Trimethyllysine availability regulates the rate of carnitine biosynthesis in the growing rat. *J. Nutr.* 116, 751–759
31. Boch, J. *et al.* (1996) Synthesis of the osmoprotectant glycine betaine in *Bacillus subtilis*: characterization of the gbsAB genes. *J. Bacteriol.* 178, 5121–5129
32. Pocard, J.-A. *et al.* (1997) Molecular characterization of the bet genes encoding glycine betaine synthesis in *Sinorhizobium meliloti* 102F34. *Microbiology (Soc. Gen. Microbiol.)* 143, 1369–1379
33. Salvi, F. and Gadda, G. (2013) Human choline dehydrogenase: medical promises and biochemical challenges. *Arch. Biochem. Biophys.* 537, 243–252
34. Cox, R.A. and Hoppel, C.L. (1973) Biosynthesis of carnitine and 4-N-trimethylaminobutyrate from 6-N-trimethyl-lysine. *136* 1083–1090
35. Hoppel, C.L. *et al.* (1980) $N^6$-Trimethyl-lysine metabolism. 3-Hydroxy-$N^6$-trimethyl-lysine and carnitine biosynthesis. *Biochem. J.* 188, 509–519
36. Callery, P.S. and Geelhaar, L.A. (1984) Biosynthesis of 5-aminopentanoic acid and 2-piperidone from cadaverine and 1-piperideine in mouse. *J. Neurochem.* 43, 1631–1634
37. Tuomainen, M. *et al.* (2019) Quantitative assessment of betainized compounds and associations with dietary and metabolic biomarkers in the randomized study of the healthy Nordic diet (SYSDIET). *Am. J. Clin. Nutr.* 110, 1108–1118
38. Servillo, L. *et al.* (2018) The betaine profile of cereal flours unveils new and uncommon betaines. *Food Chem.* 239, 234–241
39. Servillo, L. *et al.* (2014) Where does $N^{\varepsilon}$-trimethyllysine for the carnitine biosynthesis in mammals come from? *PLoS One* 9, e84589
40. Koistinen, V.M. and Hanhineva, K. (2017) Mass spectrometry-based analysis of whole-grain phytochemicals. *Crit. Rev. Food Sci. Nutr.* 57, 1688–1709
41. Wastyk, H.C. *et al.* (2021) Gut-microbiota-targeted diets modulate human immune status. *Cell* 184, 4137–4153
42. Katina, K. *et al.* (2005) Potential of sourdough for healthier cereal products. *Trends Food Sci. Technol.* 16, 104–112
43. Salzano, A. *et al.* (2021) Green feed increases antioxidant and antineoplastic activity of buffalo milk: a globally significant livestock. *Food Chem.* 344, 128669
44. Servillo, L. *et al.* (2018) Carnitine precursors and short-chain acylcarnitines in water buffalo milk. *J. Agric. Food Chem.* 66, 8142–8149
45. Kärkkäinen, O. *et al.* (2018) Whole grain intake associated molecule 5-aminovaleric acid betaine decreases β-oxidation of fatty acids in mouse cardiomyocytes. *Sci. Rep.* 8, 13036–13037
46. Woollard, D.C. *et al.* (1999) Carnitine in milk: a survey of content, distribution and temporal variation. *Food Chem.* 66, 121–127
47. Salzano, A. *et al.* (2019) Short communication: space allocation in intensive Mediterranean buffalo production influences the profile of functional biomolecules in milk and dairy products. *J. Dairy Sci.* 102, 7717–7722
48. Pallister, T. *et al.* (2016) Characterizing blood metabolomics profiles associated with self-reported food intakes in female twins. *PLoS One* 11, e0158568
49. Blunden, G. *et al.* (1986) The characterisation and quantitative estimation of betaines in commercial seaweed extracts. *Bot. Mar.* 29, 155
50. MacKinnon, S.L. *et al.* (2009) Improved methods of analysis for betaines in *Ascophyllum nodosum* and its commercial seaweed extracts. *J. Appl. Phycol.* 22, 489–494
51. Vuong, H.E. *et al.* (2017) The microbiome and host behavior. *Annu. Rev. Neurosci.* 40, 21–49
52. Jääskeläinen, T. *et al.* (2018) A non-targeted LC-MS profiling reveals elevated levels of carnitine precursors and trimethylated compounds in the cord plasma of pre-eclamptic infants. *Sci. Rep.* 8 14616–12
53. Jääskeläinen, T. *et al.* (2021) A non-targeted LC-MS metabolic profiling of pregnancy: longitudinal evidence from healthy and pre-eclamptic pregnancies. *Metabolomics* 17, 20
54. Kärkkäinen, O. *et al.* (2021) Changes in the metabolic profile of human male postmortem frontal cortex and cerebrospinal fluid samples associated with heavy alcohol use. *Addict. Biol.* 26, e13035
55. Visentin, C.E. *et al.* (2015) Maternal choline status, but not fetal genotype, influences cord plasma choline metabolite concentrations. *J. Nutr.* 145, 1491–1497

56. Joselit, Y. *et al.* (2018) Maternal betaine supplementation affects fetal growth and lipid metabolism of high-fat fed mice in a temporal-specific manner. *Nutr. Diabetes* 8 41–11
57. Velzing-Aarts, F.V. *et al.* (2005) Plasma choline and betaine and their relation to plasma homocysteine in normal pregnancy. *Am. J. Clin. Nutr.* 81, 1383–1389
58. Blusztajn, J.K. *et al.* (2017) Neuroprotective actions of dietary choline. *Nutrients* 9, 815
59. Mossad, O. *et al.* (2021) Microbiota-dependent increase in δ-valerobetaine alters neuronal function and is responsible for age-related cognitive decline. *Nat. Aging* 1, 1127–1136
60. D'Onofrio, N. *et al.* (2020) Synergistic effect of dietary betaines on SIRT1-mediated apoptosis in human oral squamous cell carcinoma Cal 27. *Cancers* 12, 2468
61. D'Onofrio, N. *et al.* (2021) Colorectal cancer apoptosis induced by dietary δ-valerobetaine involves PINK1/Parkin dependent-mitophagy and SIRT3. *Int. J. Mol. Sci.* 22, 8117
62. Goedert, J.J. *et al.* (2014) Fecal metabolomics: assay performance and association with colorectal cancer. *Carcinogenesis (New York)* 35, 2089–2096
63. D'Onofrio, N. *et al.* (2020) ROS-mediated apoptotic cell death of human colon cancer LoVo cells by milk δ-valerobetaine. *Sci. Rep.* 10, 8978
64. Adam, J. *et al.* (2016) Metformin effect on nontargeted metabolite profiles in patients with type 2 diabetes and in multiple murine tissues. *Diabetes (New York, N.Y.)* 65, 3776–3785
65. Mascini, N.E. *et al.* (2016) Mass spectrometry imaging of the hypoxia marker pimonidazole in a breast tumor model. *Anal. Chem. (Washington)* 88, 3107–3114
66. Carracedo, A. *et al.* (2013) Cancer metabolism: fatty acid oxidation in the limelight. *Nat. Rev. Cancer* 13, 227–232
67. Razavi, A.C. *et al.* (2020) Novel findings from a metabolomics study of left ventricular diastolic function: the Bogalusa Heart Study. *J. Am. Heart Assoc.* 9, e015118
68. Park, J.-H. and Marwick, T.H. (2011) Use and limitations of E/e' to assess left ventricular filling pressure by echocardiography. *J. Cardiovasc. Ultrasound* 19, 169–173
69. Smith, C.E. *et al.* (2021) Investigation of diets associated with dilated cardiomyopathy in dogs using foodomics analysis. *Sci. Rep.* 11, 15881
70. Dambrova, M. *et al.* (2016) Pharmacological effects of meldonium: biochemical mechanisms and biomarkers of cardiometabolic activity. *Pharmacol. Res.* 113, 771–780
71. Liu, J. *et al.* (2020) Ranitidine and finasteride inhibit the synthesis and release of trimethylamine N-oxide and mitigates its cardiovascular and renal damage through modulating gut microbiota. *Int. J. Biol. Sci.* 16, 790–802
72. Liu, K.H. *et al.* (2021) Microbial metabolite delta-valerobetaine is a diet-dependent obesogen. *Nat. Metab.* 3, 1694–1705
73. Cioffi, C. and Vos, M.B. (2018) Su1506 - comparison of plasma metabolomics profiles of pediatric NASH vs. NAFLD. *Gastroenterology* 154, S-1161
74. Zeybel, M. *et al.* (2022) Multiomics analysis reveals the impact of microbiota on host metabolism in hepatic steatosis. *Adv. Sci.* 9, 2104373
75. Kathirvel, E. *et al.* (2010) Betaine improves nonalcoholic fatty liver and associated hepatic insulin resistance: a potential mechanism for hepatoprotection by betaine. *Am. J. Physiol. Gastrointest. Liver Physiol.* 299, 1068–1077
76. Jung, Y.S. *et al.* (2013) Alleviation of alcoholic liver injury by betaine involves an enhancement of antioxidant defense via regulation of sulfur amino acid metabolism. *Food Chem. Toxicol.* 62, 292–298
77. Zeybel, M. *et al.* (2021) Combined metabolic activators therapy ameliorates liver fat in nonalcoholic fatty liver disease patients. *Mol. Syst. Biol.* 17, e10459
78. de Munter, J. *et al.* (2007) Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review. *PLoS Med.* 4, e261
79. Ye, E.Q. *et al.* (2012) Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. *J. Nutr.* 142, 1304–1313
80. Haukka, J.K. *et al.* (2018) Metabolomic profile predicts development of microalbuminuria in individuals with type 1 diabetes. *Sci. Rep.* 8 13853–10
81. Rebouche, C.J. and Seim, H. (1998) Carnitine metabolism and its regulation in microorganisms and mammals. *Annu. Rev. Nutr.* 18, 39–61
82. Bellissimo, M.P. *et al.* (2019) Plasma high-resolution metabolomics differentiates adults with normal weight obesity from lean individuals. *Obesity (Silver Spring)* 27, 1729–1737
83. Altmaier, E. *et al.* (2014) Metabolomics approach reveals effects of antihypertensives and lipid-lowering drugs on the human metabolism. *Eur. J. Epidemiol.* 29, 325–336
84. Lustgarten, M.S. *et al.* (2013) Serum glycine is associated with regional body fat and insulin resistance in functionally-limited older adults. *PLoS One* 8, e84034
85. Molstad, P. *et al.* (1977) Specificity and characteristics of the carnitine transport in human heart cells (CCL 27) in culture. *Biochim. Biophys. Acta* 471, 296–304
86. Ohashi, R. *et al.* (2002) Studies on functional sites of organic cation/carnitine transporter OCTN2 (SLC22A5) using a Ser467Cys mutant protein. *J. Pharmacol. Exp. Ther.* 302, 1286–1294
87. Tars, K. *et al.* (2014) Targeting carnitine biosynthesis: discovery of new inhibitors against γ-butyrobetaine hydroxylase. *J. Med. Chem.* 57, 2213–2236
88. D'Onofrio, N. *et al.* (2019) Antioxidant and anti-inflammatory activities of buffalo milk δ-valerobetaine. *J. Agric. Food Chem.* 67, 1702–1710
89. Carafa, V. *et al.* (2019) Dual tumor suppressor and tumor promoter action of sirtuins in determining malignant phenotype. *Front. Pharmacol.* 10, 38
90. Lin, Z. and Fang, D. (2013) The roles of SIRT1 in cancer. *Genes Cancer* 4, 97–104
91. Vitiello, M. *et al.* (2016) Multiple pathways of SIRT6 at the crossroads in the control of longevity, cancer, and cardiovascular diseases. *Ageing Res. Rev.* 35, 301–311
92. Wishart, D.S. *et al.* (2018) HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Res.* 46, D608–D617
93. Rydzik, A.M. *et al.* (2014) Comparison of the substrate selectivity and biochemical properties of human and bacterial γ-butyrobetaine hydroxylase. *Org. Biomol. Chem.* 12, 6354–6358
94. Bruce, S.J. *et al.* (2010) Quantitative measurement of betaine and free choline in plasma, cereals and cereal products by isotope dilution LC-MS/MS. *J. Agric. Food Chem.* 58, 2055–2061
95. Alpert, A.J. (1990) Hydrophilic-interaction chromatography for the separation of peptides, nucleic acids and other polar compounds. *J. Chromatogr. A* 499, 177–196
96. MacKinnon, S.L. and Craft, C. (2015) Analysis of betaines from marine algae using LC-MS-MS. *Methods Mol. Biol.* 1308, 267–275
97. Pekkinen, J. *et al.* (2015) Amino acid-derived betaines dominate as urinary markers for rye bran intake in mice fed high-fat diet—a nontargeted metabolomics study. *Mol. Nutr. Food Res.* 59, 1550–1562
98. Hulme, H. *et al.* (2020) Microbiome-derived carnitine mimics as previously unknown mediators of gut-brain axis communication. *Sci. Adv.* 6, eaax6328
99. Hulme, H. *et al.* (2022) Mapping the influence of the gut microbiota on small molecules across the microbiome gut brain axis. *J. Am. Soc. Mass Spectrom.* 33, 649–659
100. Lever, M. and Slow, S. (2010) The clinical significance of betaine, an osmolyte with a key role in methyl group metabolism. *Clin. Biochem.* 43, 732–744
101. Lindstedt, G. and Lindstedt, S. (1965) Studies on the biosynthesis of carnitine. *J. Biol. Chem.* 240, 316–321
102. Li, C. *et al.* (2010) Determination of betaine metabolites and dimethylsulfoniopropionate in coral tissues using liquid chromatography–time-of-flight mass spectrometry and stable isotope-labeled internal standards. *J. Chromatogr. B Anal. Tech. Biomed. Life Sci.* 878, 1809–1816
103. Turunen, S. *et al.* (2020) Metabolome of canine and human saliva: a non-targeted metabolomics study. *Metabolomics* 16, 90
104. Klavus, A. *et al.* (2020) "notame": workflow for non-targeted LC-MS metabolic profiling. *Metabolites* 10, 135
105. Zhao, M. *et al.* (2022) Gut microbiota production of trimethyl-5-aminovaleric acid reduces fatty acid oxidation and accelerates cardiac hypertrophy. *Nat. Commun.* 13, 1757