The kidney is the major site of S-adenosylhomocysteine disposal in humans

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S-adenosylhomocysteine (SAH), the metabolic precursor of homocysteine in the body, is a potent inhibitor of methylation reactions. Several methylation reactions play a major role in epigenetic regulation of protein expression, atherosclerosis, and cancer development. Here we studied the mechanisms responsible for the maintenance of circulating SAH levels by measurement of the arterio-venous differences across the kidney, splanchnic organs, and the lung in humans. The lungs did not remove or add any circulating SAH, whereas the liver released it into the hepatic veins. The kidney extracted 40% of SAH and the SAH arterio–venous difference across the kidney was directly and significantly related to its arterial levels. Thus, the kidney plays a major role in maintaining SAH levels and may, indirectly, control tissue transmethylation reactions. Our findings of a pivotal role for the human kidney in sulfur amino acid metabolism may also account for the increased plasma levels of SAH in patients with chronic kidney diseases.

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The biological effects of methionine transmethylation, whereby the methyl group of *S*-adenosylmethionine (SAM) is donated to a large variety of acceptor substrates, is of obvious importance for the biosynthesis of a wide range of compounds such as membrane phospholipids, neurotransmitters, proteins, creatine, and hormones.[1] Methylation processes also play a major role in the epigenetic regulation of protein expression and changes in human DNA methylation patterns are an important feature of many diseases, including atherosclerosis and cancer.[2,3] *S*-Adenosylhomocysteine (SAH) is the by-product of methionine transmethylation and the metabolic precursor of homocysteine in all tissues (Figure 1). As SAH is a potent feedback inhibitor of most methyltransferases,[4] including the methionine remethylation pathway, this compound plays an essential role in the control of the overall transmethylation rates.[4,5] Thus, the efficiency of methyltransferase reactions is dependent on the efficient tissue removal of SAH.[4,5]

About 30 years ago, Wilcken and Wilcken[6] observed that increased plasma sulfur amino acids levels were associated with atherosclerosis and cardiovascular damage. Among the sulfur compounds, homocysteine has been considered to be the culprit for this association. However, the recent homocysteine-lowering intervention trials have cast into doubt the issue of causality.[7] One potential explanation for these conflicting results is that hyperhomocysteinemia itself may not be the causative agent in vascular dysfunction, but instead may be a marker for another risk factor.[8,9] Recent studies suggest an indirect mechanism for homocysteine toxicity, secondary to SAH accumulation.[9] An increase in intracellular SAH is associated with DNA hypomethylation in endothelial cells.[10] SAH levels are also associated with endothelial dysfunction in mice with a deficiency of the cystathionine β-synthase gene.[11] Recent experimental[12] and clinical evidence[13–15] also suggests that the accumulation of SAH in body fluids, rather than increased homocysteine levels, is associated with vascular disease and tissue damage. Post-translational modification of proteins, associated with high SAH intracellular accumulation, has been described in patients with chronic kidney diseases.[16,17]

The sites and mechanisms which prevent the body accumulation of SAH in the body are not well understood.[9]

Like plasma homocysteine, plasma SAH levels are inversely related to glomerular filtration rate.[18,19] However, plasma SAH appears to be more sensitive than plasma homocysteine to small decrements in glomerular filtration rate.[19] In patients with chronic kidney diseases a 45-fold increase in SAH as compared with a sixfold increase in SAM and fourfold increase in plasma homocysteine is observed.[13] These changes are not likely dependent on different urine excretion of these compounds, as the fractional excretion of SAM and SAH compared with that of creatinine has been reported to be 93% for SAM and 39% for SAH, which may explain the high ratio of SAM to SAH in normal urine.[20] Stable-isotope studies in non-diabetic[21,22] and diabetic chronic kidney disease patients[23] have shown impaired metabolic clearance of homocysteine by both the trans-sulfuration and the remethylation pathways. It is interesting to note that Stam *et al.*[22] observed that in chronic kidney disease patients the methionine remethylation and trans-methylation rates are inversely related to plasma SAH, suggesting a strong inhibitory action of SAH on methionine-dependent remethylation pathway.

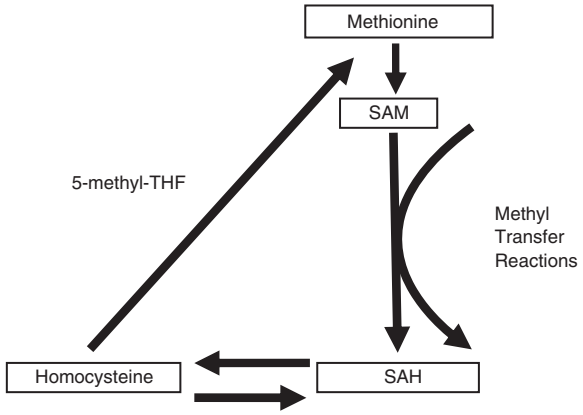

Figure 1 | The metabolic conversion of methionine to homocysteine. SAM = *S*-adenosylmethionine, SAH = *S*-adenosylhomocysteine, 5-methyl-THF = 5-methyl-tetrahydrofolate.

In this study, to explore the sites and mechanisms underlying the regulation of circulating SAH levels, we measured plasma SAH across the kidney, splanchnic bed and lung in humans. Our results show for the first time that the human kidney plays a unique role in the removal of SAH from circulation indicating that the kidney may have an important role in the control of body transmethylation reactions.

RESULTS

Individual arterial and venous levels of SAH, as well as their arterio-venous difference across the kidney and splanchnic organs are reported in Table 1. Renal vein SAH concentrations were remarkably lower (by ~40%; $P<0.001$) than the corresponding arterial values measured in the same subjects, demonstrating that plasma SAH decreases substantially after a single pass across the kidney. Fractional extraction of SAH was twofold greater than that of creatinine ($P<0.01$). The SAH arterio-venous difference across the kidney was directly related to SAH arterial levels ($r=0.72$; $P<0.02$) (Figure 2).

SAH levels in the hepatic veins were ~20% greater than those in the artery ($P<0.02$), indicating that splanchnic organs are a major source for SAH into the circulation. No significant A–V gradient of SAH was observed across the lung.

Mean homocysteine levels in the renal and in the liver veins were not statistically different from those in the artery (Femoral artery 10.4 ± 1, Renal vein 10.3 ± 1, Liver vein $10.1\pm1\,\mu mol\,l^{-1}$; $P=$NS). Similarly, no significant arterio-venous homocysteine gradient was observed across the lung (Femoral artery 10.4 ± 1, Lung artery $10.1\pm1\,\mu mol\,l^{-1}$).

DISCUSSION

We observed for the first time that the kidney is a major site for the disposal of plasma SAH in humans. The SAH arterio-venous difference across the kidney represents ~40% of the SAH arterial plasma concentration, positioning the human kidney as a major tissue in the metabolic disposal of plasma SAH. It is interesting to note that the arterio-venous

Table 1 | SAH plasma levels and fractional extractions across the kidney, splanchnic organs, and lung

Subject	Arterial SAH (nmol/l)	Renal vein SAH (nmol/l)	SAH kidney A–V (nmol/l)	SAH kidney FE (%)	Creatinine kidney FE (%)	Liver vein SAH (nmol/l)	SAH splanchnic AV (nmol/l)	SAH splanchnic FE (%)	Lung artery SAH (nmol/l)	SAH lung V–A (nmol/l)
1	26.0	25.0	1.0	+3.8	+22	40.0	−14	−53.8	21.0	−5
2	24.9	19.9	5.0	+20	+21.2	34.0	−9.1	−36.0	24.9	0
3	42.0	20.0	22.0	+52.4	+19	44.0	−2	−4.8	38.0	−4
4	22.6	14.6	7.9	+34.8	+18	25.9	−3.3	−15.0	42.0	19
5	29.9	10.1	19.8	+66.7	+28	33.1	−3.2	−10.5	17.0	−13
6	28.9	14.0	14.9	+51.6	+17	34.6	−5.7	−19.7	34.9	5.2
7	17.8	8.0	9.8	+55.1	+18	19.6	−1.8	−10.1	17.9	0.2
8	17.6	10.4	7.2	+40.9	+23	25.0	−7.4	−42.8	20.4	2.8
9	18.1	11.9	6.2	+34.2	+18	20.0	−1.9	−10.5	21.2	3.1
10	12.2	6.65	5.6	+45.7	+23	13.0	−0.8	−6.1	12.6	0.4
Mean ± s.e.m.	24.1 ± 2.7	14.1 ± 1.9	10.0 ± 2.2**	40.5 ± 5.8***	21 ± 1	28.9 ± 3.1	−4.9 ± 1.12*	−21 ± 5.0	24.9 ± 3.1	−0.8 ± 2.6

A–V, arterio-venous; FE, fractional extraction (negative extraction means production); SAH, *S*-adenosylhomocysteine, V–A, venous–arterial.
*$P<0.02$ and **$P<0.001$ or less Artery vs Vein; ***$P<0.01$ vs creatinine FE across the kidney. 'Splanchnic' indicates the metabolic activity of the liver + intestine.

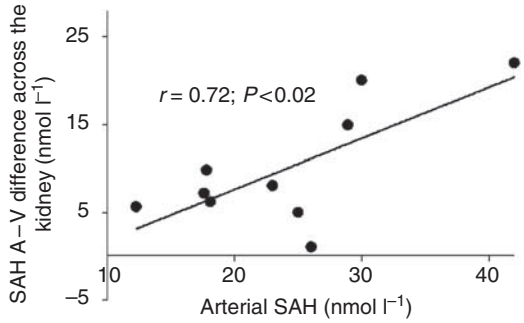

Figure 2 | Relationship between the arterio–venous (A–V) difference of *S*-adenosylhomocysteine (SAH) across the kidney and arterial SAH levels.

difference of SAH is directly related to the arterial levels of the same metabolite, implying that the regulatory role of the kidney on plasma SAH occurs over a wide range of SAH plasma levels. SAH has a molecular mass of 384 D,[1] which is within the filtration range of normal glomeruli. Although SAH may be filtered and lost with the urine, its urinary clearance is remarkably lower than creatinine clearance.[20] In our study, the fractional extraction of SAH across the kidney was ~2-fold greater than that of creatinine. This finding strongly indicates that a large part of SAH is removed by kidney metabolic clearance.

As a new finding, we observed that SAH is released by the splanchnic organs. SAH levels in the hepatic veins were markedly greater (~21%) than those in the artery, indicating that splanchnic organs are a major source for SAH entering into the circulation. In contrast, no significant SAH gradient was observed across the lung.

The efficiency of methyltransferase reactions is dependent on the efficient removal of SAH. This is effectively accomplished by SAH hydrolase, an enzyme that appears to act in close proximity to the methyltransferases. SAH hydrolase is highly expressed in the rat kidney and is homogeneously localized in proximal and distal tubule cells as well as in glomeruli.[24,25] This distribution is likely involved in maintaining efficient transmethylation reactions and thereby low intracellular kidney SAH levels. Considering an average kidney plasma flow of 600 ml/min[26] the removal of SAH by the kidney would according to the results of our study amount to ~8.6 μmol/day. This figure clearly matches the estimated release of SAH by splanchnic organs (about ~6 μmol/day, considering a plasma flow of about 800 ml/min).[26] According to these findings, extracellular SAH pools (~350 nmol, considering a plasma SAH concentration 25 nmol/l and extracellular fluid 14 l in a 70 kg man)[27] are composed of only a small fraction (about 4%) of the estimated kidney daily removal, suggesting that the extracellular SAH pool is renewed by tissue SAH generation and SAH kidney removal several times a day.

In conclusion, this study is the first to show that the human kidney is the major site for the metabolic disposition of plasma SAH in the body. These data display a new role for the kidney in the metabolic regulation of sulfur amino acids and, indirectly, of tissue methyl transfer reactions in humans.

MATERIALS AND METHODS

Patients and procedures

Ten patients (seven men, three women, mean age 67, range 56–74 years, BMI 24 ± 1 kg/m^2) who were scheduled for elective cardiac catheterization for hemodynamic evaluation or the assessment of coronary heart disease were eligible for enrollment in this protocol. The patients were enrolled in the study on a consecutive basis if they met the following exclusion criteria: NYHA Class III congestive heart failure, a recent myocardial infarction, pregnancy, or unstable renal function. Their estimated[28] glomerular filtration rate was 83 ± 5 ml/min per 1.73 m^2. No subject had evidence of liver disease or diabetes mellitus. The study was approved by the Ethical Committee of the Department of Internal Medicine of the University of Genoa. Subjects were informed about the purposes, procedures, and possible risks of the study, before their informed consent was obtained. The procedures were in accordance with the Helsinki declaration. Three sets of blood samples were obtained at ~20-min intervals from the femoral artery as well as from the renal veins, the hepatic veins, and the pulmonary artery, in the postabsorptive state. Plasma SAH and homocysteine levels were determined in triplicate by HPLC.[15] This method measures both SAH and SAM in body fluids. However, although the intra-assay coefficient of variation for SAH is remarkably low (4.7%), coefficient of variation for SAM analysis is greater (~10%), which makes unlikely the possibility to detect small SAM arterio-venous differences.[15] Therefore, data regarding SAM are not reported. All samples from one individual were always run in the same batch. Blood acid-base, glucose, lactate, and creatinine were measured with an ABL800 Flex apparatus (Radiometer, Copenhagen, Denmark).

Calculations and statistical methods

The arterio-venous difference of SAH and homocysteine across the splanchnic organs, lung, and kidney was calculated as: [A]−[V], where [A] and [V] are the metabolite concentrations in arterial and venous plasma. Fractional extractions were calculated as 100 × ([A]−[V]/[A]). Statistical analysis was performed using analysis of variance for repeated measures to compare arterial data with venous data (Statview Statistical Package, Abacus, Berkeley, CA, USA). Linear regression and correlation were employed to evaluate the relation between two variables. A *P*-value of <0.05 was considered statistically significant. All data are expressed as means ± s.e.

DISCLOSURE

Bengt Lindholm is employed by Baxter Healthcare.

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