Insulin in methionine and homocysteine kinetics in healthy humans: plasma vs. intracellular models

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Tessari, Paolo, Edward Kiwanuka, Anna Coracina, Michela Zaramella, Monica Vettore, Anna Valerio, and Giacomo Garibotto. Insulin in methionine and homocysteine kinetics in healthy humans: plasma vs. intracellular models. Am J Physiol Endocrinol Metab 288: E1270–E1276, 2005. Published January 11, 2005.; doi:10.1152/ajpendo.00383.2004.—Methionine is a sulfur-containing amino acid that is reversibly converted into homocysteine. Homocysteine is an independent cardiovascular risk factor frequently associated with the insulin resistance syndrome. The effects of insulin on methionine and homocysteine kinetics in vivo are not known. Six middle-aged male volunteers were infused with L-[methyl-2H3, 1-13C]methionine before (for 3 h) and after (for 3 additional hours) an euglycemic hyperinsulinemic (150 mU/L) clamp. Steady-state methionine and homocysteine kinetics were determined using either plasma (i.e., those of methionine) or intracellular (i.e., those of plasma homocysteine) enrichments. By use of plasma enrichments, insulin decreased methionine rate of appearance (Ra; both methyl- and carbon Ra) by 25% (P < 0.003 vs. basal) and methionine disposal into proteins by 50% (P < 0.0005), whereas it increased homocysteine clearance by ~70% (P < 0.025). With intracellular enrichments, insulin increased all kinetic rates, mainly because homocysteine enrichment decreased by ~40% (P < 0.001). In particular, transmethylation increased sixfold (P < 0.02), transsulfuration fourfold (P = 0.01), remethylation eightfold (P < 0.025), and clearance eightfold (P < 0.004). In summary, 1) physiological hyperinsulinemia stimulated homocysteine metabolic clearance irrespective of the model used; and 2) divergent changes in plasma methionine and homocysteine enrichments were observed after hyperinsulinemia, resulting in different changes in methionine and homocysteine kinetics. In conclusion, insulin increases homocysteine clearance in vivo and may thus prevent homocysteine accumulation in body fluids. Use of plasma homocysteine as a surrogate of intracellular methionine enrichment, after acute perturbations such as insulin infusion, needs to be critically reassessed.

clearance; transsulfuration; transmethylation; euglycemic clamp

METHIONINE, A SULFUR-CONTAINING AMINO ACID that is abundant in meat and other animal proteins, is converted intracellularly to homocysteine through transmethylation with a methyl acceptor (4). Homocysteine, i.e., the demethylated form of methionine, is not normally present in the amino acid sequences of proteins, but it can accumulate in blood as well as in intracellular fluids in a number of conditions, such as arteriosclerosis (21), renal failure (33), Alzheimer’s disease (2), neural tube defects (17), and others. Although not yet included in the cluster of factors associated with the insulin resistance syndromes (30, 36), homocysteine is usually increased in many of these conditions, such as type 2 diabetes, in particular with kidney insufficiency and/or proteinuria (35), obesity (13), and hypertension, particularly associated with diabetes (19). Thus a disturbance in the regulation by insulin of methionine/homocysteine metabolism can be suspected.

Physiological hyperinsulinemia inhibited whole body leucine and phenylalanine appearance from endogenous body proteins in a dose-response fashion (7, 27). Insulin also either decreased or did not affect leucine oxidation (7, 10, 28, 29), whereas it did not stimulate whole body leucine incorporation into proteins when no exogenous amino acids were supplemented (7, 10, 28, 29). No data exist so far on the effects of hyperinsulinemia on methionine and homocysteine kinetics in vivo.

In vitro studies have shown that insulin inhibits methionine/ homocysteine catabolism at the step of transsulfuration, which is the first step of homocysteine irreversible degradation (1, 12). Thus insulin would exhibit a homocysteine-sparing effect in vitro. Therefore, under conditions of insulin resistance, homocysteine concentrations would be decreased. This concept, however, is not consistent with the increased homocysteine concentrations frequently observed in insulin resistance syndromes should insulin resistance be extended from glucose to amino acid (and methionine) metabolism. Furthermore, the effects of insulin on the regulation of other methionine/homocysteine metabolic steps, such as transmethylation and remethylation, are not known. Given these inconsistencies and/or insufficiency of data, the effects of hyperinsulinemia on homocysteine transsulfuration, as well as on other aspects of methionine and homocysteine kinetics in vivo, are worthy of study.

The key steps of methionine and homocysteine metabolism can be measured in vivo with isotope dilution methods by means of the labeled L-[methyl-2H3, 1-13C]methionine stable isotope (14, 26). A common problem in isotope dilution studies is the choice of the precursor pool on which kinetics are measured (16, 23). Because methionine is demethylated to homocysteine intracellularly, homocysteine enrichment has been proposed as a surrogate of intracellular methionine enrichment (15), in analogy with α-ketoisocaprate (KIC) for leucine (16, 23, 34).

Therefore, the aim of this study was to determine the effects of physiological hyperinsulinemia on methionine rate of appearance (Ra; both methyl- and carbon), transmethylation (TM), remethylation (RM), transsulfuration (TS), disposal into proteins (PS), and clearance in metabolically healthy male
volunteers. Both plasma and “intracellular” enrichments were used, and the kinetic data obtained with these models are compared and discussed.

MATERIALS AND METHODS

Subjects. Six middle-aged male volunteers (age 51 ± 5 yr, body mass index 26.7 ± 0.9 kg/m²) were recruited. No subject had any history of diabetes or impaired glucose tolerance, and their fasting plasma glucose concentrations, Hb A₁C, and general blood chemistry were normal. They had been adjusted for ≤4 wk to a diet containing ~50% calories as carbohydrates, 20% as proteins, and 30% as lipids. Protein intake ranged between 80 and 100 g/day. One patient was under mild hypotensive treatment (with a low-dose angiotensin-converting enzyme inhibitor), which was suspended 1 wk before the study. The aims of the study were explained in detail, and each subject signed his consent to the study. The protocol was approved by the Ethics Committee of the Medical Faculty at the University of Padova, Italy, and it was performed according to the Helsinki Declaration (as revised in 1983).

Experimental design. All subjects were admitted to the Metabolic Unit of the Department of Metabolic Diseases at 7 AM on the study day after the overnight fast. An 18-gauge polyethylene catheter was placed in an antecubital vein of the right arm for insulin, insulin, and glucose infusions. A contralateral wrist vein was cannulated in a retrograde fashion, and the hand was placed in a box heated at 55°C for venous arterialized blood.

The study was divided into two experimental periods, i.e., a basal and a clamp period. After baseline blood and expired air samples were taken for determination of natural isotopic enrichments, a primed (45 min) and a clamp period. After baseline blood and expired air samples were collected every 20 min for an additional 60 min for the instruments, Fullerton, CA) were frequently performed. After 120 min by means of two reflectometers. Independent calibrations against maize starch. Plasma glucose concentration was monitored every 10 min by cation exchange chromatography using a Beckman amino acid analyzer.

Calculations. The values of plasma methionine enrichments at the two steady-state periods, i.e., in the last 60 min of the basal, postabsorptive state, and in the last 60 min of the insulin clamp (i.e., between 120 and 180 min), were averaged. All calculations were performed using these mean values. Plasma methionine kinetics were calculated using the model of Storch et al. (26). Briefly, the model is based on the dilution of the infused doubly labeled methionine by the appearance in plasma of unlabeled methionine as well as by the differences in tracer enrichment attained in plasma of the [methyl-²H₃,1-¹³C](M + 4), and the [¹³C](M + 1) species according to the following formulas. At variance with the suggestion of Storch et al., no correction of the data for estimated intracellular enrichments was performed.

\[ Q_m = I_m \times (E_{m}/E_{i} - 1) \]  
and

\[ Q_e = I_e \times (E_{e}/E_{i} + E_{i}) - 1 \]

where Qm and Qe are the methionine rate of appearance (Rm) in plasma using either the (M + 4) or the (M + 1) masses; \([E_{m}]\) is the rate of tracer infusion (in \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\)); \([E_{i}]\) is tracer enrichment; and \([E_{e}]\) and \([E_{i}]\) are [methyl-²H₃,1-¹³C](M + 4), and [¹³C](M + 1) tracer enrichments in plasma, respectively. The (M + 1) mass is generated by the demethylation of the doubly labeled methyl-²H₃,1-¹³C tracer due to the irreversible loss of the methyl-²H₃ group into the methyl pool(s) intracellularly. Total \([¹³C](M + 1)\) tracer enrichment is the sum of the \([¹³C](M + 1)\) and of the [methyl-²H₃,1-¹³C](M + 4) tracer, since both contain a ³C carbon (26).

The rate of methionine cation exchange (RM) was calculated as follows:

\[ RM = Q_m - Q_e \]

The rate of the irreversible homocysteine loss through transsulfuration (TS) has been assumed to be equivalent to that of oxidation (15, 26). Therefore, methionine oxidation was calculated by dividing the rate of \(¹³C\)O₂ expiration (in \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\), corrected for an 80% fixation in body bicarbonate pool), over plasma \([¹³C]\)methionine total TTR, i.e., the sum of the (M + 4) and the (M + 1) TTR:

\[ TS = V^{¹³C}_ο₂ \times (I_f^{¹³C}\text{Met E}_{i} - I_I^{¹³C}\text{Met}_e) \]

where \(V^{¹³C}_ο₂\) is the rate of \(¹³C\)O₂ expiration (in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)), in turn calculated by multiplying the atom percent excess of the expired \(¹³C\)O₂, converted into micromoles and corrected for bicarbonate fixation, times total CO₂ production (\(V_{CO₂}\), in \(\text{ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)).
[13C]Met E0 is total [13C]methionine enrichment in plasma, i.e., the sum of the (M + 4) and the (M + 1) TTR. TS was also corrected for the contribution of the infused glucose during the clamp, by subtracting the rate of 13CO2 expiration measured in the four control studies, from the rate of total 13CO2 expiration in the corresponding clamp studies performed with the infusion of the methyl-2H3,1-13C tracer. In the remaining two subjects in whom the repeated control study was not performed, we used the mean correction factor obtained in the four control studies (see below).

The rate of methionine transmethylation (TM) was calculated as follows:

\[ TM = RM + TS \] (5)

The rate of nonoxidative methionine disposal, e.g., the index of whole body protein synthesis (PS), was calculated by subtracting TS from Qc.

\[ PS = Qc − TS \] (6)

Finally, homocysteine clearance was calculated by dividing the sum of homocysteine removal through remethylation and transsulfuration over its concentration, as follows:

\[ \frac{[RM + TS]}{[Hcy]} \] (7)

where [Hcy] is plasma homocysteine concentration (in μmol/l).

All rates described in Eqs. 1–7 were also recalculated using the plasma enrichments corrected for (i.e., multiplied times) the homocysteine/methionine 13C TTR (15). This approach has been proposed to estimate intracellular methionine kinetics (15) in analogy with leucine and valine kinetics (3, 23).

Statistical analysis. All data are expressed as means ± SE. The comparison between the clamp and the basal values was performed with the two-tailed Student’s t-test for paired data. A two-way ANOVA for repeated measurements was employed to compare the plasma vs. intracellular kinetic data. A P value of <0.05 was considered statistically significant.

RESULTS

Control studies. In the four subjects in whom the 180-min euglycemic clamp was repeated without tracer infusion, insulin was increased from 4.8 ± 1.6 to 168.8 ± 16.8 μU/ml. The rate of 13CO2 expiration rose to 33.61 ± 4.8 μmol·kg\(^{-1}\)·h\(^{-1}\) above basal. The insulin-mediated glucose disposal was 7.5 ± 0.9 mg·kg\(^{-1}\)·min\(^{-1}\). The calculated increase above background in the rate of 13CO2 expiration, normalized per gram of glucose infused, was 4.63 ± 0.78 μmol·kg\(^{-1}\)·h\(^{-1}\) per gram of glucose. In these four subjects, the individually determined increase above baseline of the rate of 13CO2 expiration in the control study was subtracted from the 13CO2 expired during the tracer infusion to calculate the “true” rate of 13CO2 expiration from methionine oxidation. In the two remaining subjects in whom the repeated control study was not performed, the aforementioned mean factor was used to recalculate the true rate of 13CO2 expiration during the tracer infusion clamp.

Isotope enrichments, total V\(\dot{C}O_2\), and expired 13CO2. Near-steady-state conditions were attained in both the basal and the clamp periods (Fig. 1). In the fasting state, the ratio between homocysteine and methionine 13C enrichment was 0.652 ± 0.128, in good agreement with a published report (15). During the clamp, both the [M + 4] and the [M + 1] methionine TTR increased significantly vs. basal by 28–67%, respectively (P < 0.001), whereas that of homocysteine decreased by ~45% (P < 0.001) (Table 1). Consequently, the ratio between homocysteine and methionine 13C enrichment decreased to 0.189 ± 0.027 (P < 0.0001). During the clamp, the rate of total V\(\dot{C}O_2\) increased by ~16% (P < 0.0002). An increase in the rate of expiration of 13CO2 was also observed, which was, however, not significant when this was corrected for the glucose infused (Table 1).

Methionine and homocysteine kinetics. By use of plasma enrichments, fasting methionine Ra, TS, TM, and RM were in good agreement with published reports in postabsorptive healthy volunteers (Table 2) (15, 26). After the hyperinsulinemic clamp, methionine Ra was decreased by ~25% (P < 0.003 vs. basal) and PS by ~50% (P < 0.0005), whereas clearance was increased by ~70% (P < 0.025). No significant changes on TS, RM, and TM were observed.

With intracellular enrichments (i.e., by correcting for the homocysteine enrichment in plasma), fasting methionine Ra, TS, TM, and RM were again in good agreement with the data of MacCoss et al. (15) in postabsorptive healthy volunteers. All rates were significantly greater than those determined with plasma methionine enrichments (P < 0.01 or less by ANOVA). During the hyperinsulinemic clamp, however, in contrast with the plasma data, the methionine Ra (both methyl- and carbon), was increased by ~170% (P < 0.05) and by ~40% (P = not significant) vs. basal, respectively. TS was increased more than twofold (P < 0.012), TM about sixfold (P < 0.02), RM about eightfold (P = 0.025), and clearance about eightfold (P < 0.003). Methionine disposal into PS was not significantly changed. The data of the single hypertensive subject did not deviate from the group’s means (data not shown).
Plasma insulin, insulin-mediated glucose disposal, vitamin status, and methylenetetrahydrofolate reductase isoforms. During the euglycemic hyperinsulinemic clamp, insulin was raised from 5.0 ± 1.1 to 147.8 ± 14.5 μU/ml. Plasma glucose was maintained at the fasting values of 85 ± 2 mg/dl by means of the variable 20% dextrose infusion. The insulin-mediated glucose disposal was 7.6 ± 1.2 mg·kg⁻¹·min⁻¹. Plasma folate concentration was 4.9 ± 1 ± 3 μg/l (normal range: 1.8–14) and that of the vitamin B₁₂ was 339 ± 85 ng/l (normal range: 180–900). Two subjects were heterozygotic for the [677C → T] mutation of the methylenetetrahydrofolate reductase (MTHFR) alleles, and the other four were homocysteine for the common phenotype (i.e., no mutation). Plasma amino acid concentrations are reported in Table 3. Most amino acids decreased with hyperinsulinemia, but homocysteine concentrations did not change.

Correlations. Significant positive correlations were found between the insulin-mediated glucose disposal rate and the relative increase vs. basal of methionine transmethylation (r = 0.8, P < 0.03) and of homocysteine clearance (r = 0.81, P < 0.025), using the plasma calculations.

DISCUSSION

Methionine is a sulfur-containing essential amino acid that is the precursor of homocysteine, a powerful and independent risk factor for cardiovascular disease and for other conditions (2, 8, 13, 17, 19). In insulin-resistant states, homocysteine concentration is usually increased (8). Therefore, knowledge of the physiological effects of insulin on methionine and homocysteine kinetics in vivo is necessary to test the effects of the hormone in insulin-resistant states with the purpose to investigate the potential mechanism(s) linking hyperhomocysteinemia to insulin resistance.

In this study, we used the doubly labeled [1-3H,Methyl]methionine stable isotope tracer, which allowed us to measure a number of methionine and homocysteine metabolic steps (26). Because methionine is transmethylated to homocysteine intracellularly and then released into plasma (4), plasma homocysteine enrichment has been proposed as a surrogate of intracellular methionine enrichment to calculate precisely methionine and homocysteine kinetics in vivo (15) in analogy to the leucine-KIC (23) as well as to the valine-isovaleric acid (3) systems. Therefore, in this study, the kinetic data were presented using both plasma (i.e., based on plasma methionine enrichments) and intracellular (based on plasma homocysteine enrichments) calculations.

By use of plasma calculations, physiological hyperinsulinemia decreased methionine Rₘ from endogenous proteolysis, expressed both as the methyl and as the carbon Rₘ, by ~25% at the present insulin concentration of ~150 μU/ml. This finding is in agreement with the effects of insulin on the Rₘ of several essential amino acids and is consistent with an inhibition of endogenous protein breakdown (7, 10, 11, 27–29). Insulin also decreased methionine disposal into proteins, also in agreement with data obtained with other amino acids tracers, confirming the failure of the hormone to stimulate whole body protein synthesis without amino acid supplementation (7, 10, 11, 27–29). In addition, insulin increased homocysteine metabolic clearance (2). Thus hyperinsulinemia on the one hand inhibited methionine appearance into the plasma space but on the other hand stimulated homocysteine removal from plasma, both effects possibly restraining homocysteine accumulation into plasma. The finding of a positive correlation between the insulin sensitivity index and the increment of both
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transmethylation and homocysteine clearance is in agreement with this concept.

This study was performed in middle-aged male volunteers. Although the age of our subjects was greater than that of usual “young healthy volunteers,” the extent of the inhibition of endogenous methionine Ra was well within the range of previous studies in younger subjects with the leucine isotope method (7, 10, 27). Nevertheless, it will be interesting in future studies to assess the effects of age on methionine and homocysteine kinetics. Also, the response of male vs. female subjects should be investigated.

The use of intracellular calculations led to markedly greater kinetic rates, as was expected because of the ~45% lower plasma homocysteine than methionine enrichment (Table 1). In the postabsorptive state, the absolute rates of methionine/ homocysteine kinetics were in fair agreement with what reported by MacCoss et al. (15). During hyperinsulinemia, however, there were sharply different responses from those observed with the plasma calculations. Hyperinsulinemia increased methionine Ra by ~40–170%, although this was significant only for the methyl Ra (Table 2). It also markedly increased transsulfuration, transmethylation, remethylation, and homocysteine clearance, whereas it did not significantly suppress methionine incorporation into proteins. Thus insulin apparently increased both methionine release into plasma from intracellular sources and its disposal through transmethylation and homocysteine irreversible loss (through transsulfuration/oxidation). Therefore, there was a net exit (by 10.57 µmol·kg⁻¹·h⁻¹, i.e., corresponding to the increase of TS), of homocysteine molecules from the intracellular space(s).

A key point to correctly interpret these data is the achievement of a full equilibration between plasma (as reflected by methionine) and intracellular (as reflected by homocysteine) enrichments. In our study, the steady state in both methionine and homocysteine plasma enrichments was apparently attained in the final 60 min of each 180-min experimental period (i.e., those defined as basal and clamp periods; Fig. 1). It has been previously reported that, after the intravenous infusion of [1-¹³C]methionine, achievement of the steady state in plasma methionine enrichment required ~1 h, whereas that of homocysteine required up to 8 h (15). Notably, in the referenced study (see Fig. 3 of Ref. 15), plasma homocysteine enrichment after ~3 h was only ~25% lower than that after 8 h. As a matter of fact, the shorter experimental time of the basal and of the clamp periods of our study may have resulted in both an underestimation of homocysteine enrichment in the basal state and an overestimation following the clamp, since a clear-cut decrease was observed with insulin (Fig. 1 and Table 1). Nevertheless, in our study, the basal ratio between plasma homocysteine and methionine enrichment (0.65) was in good agreement with that of MacCoss et al. (15) (0.58). Although it may be possible that subtle additional changes in plasma homocysteine enrichments occurred beyond the 3 h of each experimental period, in the direction of a greater enrichment in the basal state and of a lower enrichment after the insulin infusion, our data should be considered, if anything, as conservative.

That a full equilibration between plasma and intracellular methionine and homocysteine pools may have failed to occur is, however, suggested by the key observation that methionine enrichment increased whereas that of homocysteine decreased during hyperinsulinemia. The complexity of circulating homocysteine pools may have accounted for this apparent discrepancy. Free (i.e., reduced) homocysteine concentration accounts for less than 2% and oxidized homocysteine [as either mixed (homocysteine-cysteine) or homologous (homocysteine-homocysteine) disulfides] for ~20% of total homocysteine, whereas the largest amount is bound to proteins either with thiol or with covalent bounds (4, 21). Erythrocytes may also represent relevant sources of circulating homocysteine (31). Thus a slow equilibration between any of the (reversible) homocysteine pools may have occurred. Another possibility is that plasma homocysteine enrichment does not reflect true intracellular methionine enrichment in all conditions. Further studies are required to elucidate this important point.

The insulin-stimulated increase of homocysteine irreversible disposal, i.e., of transsulfuration (with the intracellular model), as well as the stimulation of homocysteine clearance (with both models), indicates a protective mechanism by insulin against an accumulation of homocysteine in body fluids. Thus, under conditions of normal insulin availability and/or action, the removal of homocysteine from the plasma space is likely increased by insulin, which may be useful particularly after ingestion of a mixed, protein-rich meal. Notably, the calculation of homocysteine clearance in this study is not biased by any change in plasma homocysteine concentrations, which remained constant. In this respect, the finding of unchanged homocysteine concentrations despite the insulin-induced decrease of many other amino acids is surprising. This might be explained by the complexity of the homocysteine pools, as discussed above, and by a possible slow equilibration among them following an acute stimulus such as acute hyperinsulinemia.

The key enzyme of homocysteine irreversible catabolism is cystathionine β-synthase (CBS), which is the rate-limiting step of the transsulfuration reaction (2). In rats, this enzyme is located in liver, kidney, small intestine, pancreas, adipose tissue, and brain (4). CBS activity is stimulated by oxidizing conditions, by phosphorylation/dephosphorylation, by methionine supplementation, and by adenosylmethionine (which is formed in the transmethylation reaction), whereas it is inhibited by adenosylhomocysteine, the direct precursor of homocysteine (4). Our
finding of the insulin stimulation of both transmethylation and transsulfuration with the intracellular model supports a role of the hormone in favoring homocysteine disposal through the two abovementioned biochemical steps. Interestingly, oxidizing conditions would result in increased homocysteine oxidation and glutathione synthesis, thus increasing the availability of a key antioxidant substrate in homeostatic regulation.

In vitro data on the effects of insulin on CBS activity appear, however, to be in contrast with the in vivo findings. Insulin has been reported to regulate CBS activity (25). Experimental, insulin-deficient diabetes in rats increased CBS activity in liver, and this increase was reversed by insulin treatment (12, 20). However, it cannot be excluded that some metabolic abnormality secondary to insulin deficiency directly affected CBS activity. In contrast, in diet-induced insulin resistance models, an inverse relationship between insulin levels and CBS activity was found (5). Thus insulin deficiency vs. insulin resistance may play a different role in the regulation of homocysteine catabolism and, possibly, of its concentrations. In healthy humans, no relation between plasma total homocysteine concentrations and insulin sensitivity was found (9). Euglycemic hyperinsulinemia reduced plasma homocysteine concentrations in normal but not in type 2 diabetic subjects without, however, any dose-response effect (6). Conversely, the improvement of insulin sensitivity and metabolic control did not influence plasma homocysteine concentrations in type 2 diabetes (18). As a matter of fact, the issue of the regulation by insulin and/or insulin resistance conditions on homocysteine concentrations is still unresolved. Therefore, kinetic studies like the present one may add new information on this important issue, particularly in insulin-resistant states characterized also by hyperhomocysteinemia, such as diabetes mellitus with nephropathy, proteinuric syndromes, renal insufficiency, etc.

The vitamin metabolic status of our subjects was normal, as shown by the vitamin B12 and folate concentrations (see RESULTS). Thus no restriction by these cofactors of homocysteine remethylation likely occurred.

In conclusion, this study shows that physiological hyperinsulinemia increased methionine homocysteine clearance (with both the plasma and the intracellular model), as well as transmethylation and transsulfuration (with the intracellular model) in humans. Insulin thus exhibited a possible protective effect against the accumulation in body fluids of homocysteine, a potent cardiovascular risk factor. These insulin effects need to be tested in clinical conditions of hyperhomocysteinemia and/or insulin resistance. The validity of either model (i.e., plasma vs. intracellular) in the evaluation of methionine/homocysteine kinetics should be further investigated.

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