Homocysteine metabolism in diabetes

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Abstract

An increase in the plasma level of Hcy (homocysteine), an intermediate in the catabolism of methionine, has been identified as a risk factor for many diseases including CVD (cardiovascular disease). CVD is the major cause of death in patients with diabetes mellitus. Therefore the study of Hcy metabolism in diabetes mellitus has been a major focus of current research. Studies conducted in our laboratory were able to show that in both Type 1 and Type 2 diabetes with no renal complications, the plasma Hcy levels were lower than in controls. In Type 1 diabetes, increased activities of the trans-sulfuration enzymes were the major cause for the reduction in plasma Hcy. In Type 2 diabetes, BHMT (betaine:homocysteine methyltransferase) was also observed to play a major role in the increased catabolism of Hcy in addition to the trans-sulfuration enzymes. We were also able to demonstrate the direct effect of insulin and the counter-regulatory hormones on the regulation of cystathionine β-synthase and BHMT, which accounts for the changes in the activities of these two enzymes seen in diabetes mellitus.

Background

Since the observation, by McCully [1], of severe arteriosclerotic lesions in children with HHcy (hyperhomocysteinaemia) and homocysteinuria, a large number of studies have linked moderate HHcy to atherosclerotic disease. Mean plasma tHcy [total Hcy (homocysteine)] was found to be significantly higher both in male and female patients with coronary artery disease compared with controls with angiographically normal coronary arteries [2]. An increase in plasma Hcy of only 12% greater than the upper limit of normal was shown to be associated with an increase by 3.4-fold in the risk of myocardial infarction [3]. After adjusting for possible confounders, Arnesen et al. [4] found a relative risk for coronary heart disease of 1.32 for an increase in serum Hcy of 4 μmol/l. A meta-analysis of 27 studies relating Hcy to coronary, cerebrovascular and peripheral arterial vascular diseases showed a very strong relationship between these diseases and tHcy [5]. More recently, a meta-analysis that examined 30 prospective and retrospective studies confirmed that increased plasma Hcy is an independent predictor of ischaemic heart disease although of moderate strength [6].

There are certain situations in which the risk posed by HHcy may be appreciably elevated. One of these is diabetes mellitus. There is evidence that HHcy is a stronger risk factor in patients with Type 2 diabetes [7,8] and in patients with existing coronary disease [9]. It is important, therefore, to understand the origin and metabolism of Hcy as well as the ways in which this is modified in diabetes.

Metabolism

The liver has been shown to play a significant role in the regulation of plasma Hcy levels [10] because of its full complement of enzymes involved in methionine and Hcy metabolism. Hcy is an intermediate in the pathway of methionine metabolism (Figure 1) and lies at a central position between its production via transmethylation and its removal through either the remethylation or the trans-sulfuration pathways.

In transmethylation, methionine is converted into the high-energy sulfonium compound SAM (S-adenosyl-methionine) in a reaction catalysed by MAT (methionine adenosyltransferase), with ATP providing the adenosyl moiety. SAM serves as the methyl donor for virtually all known biological methylation reactions whereby it transfers its methyl group to a suitable acceptor. Essentially all of these methyltransferases but one, GNMT (glycine N-methyltransferase), are strongly inhibited by the common product, SAH (S-adenosylhomocysteine). GNMT is unique in that it is only weakly inhibited by SAH, has a relatively high \( K_m \) for SAM and shows a sigmoidal dependence on SAM concentration [11]. GNMT thus functions as a benign, high-capacity, SAM-dependent methyltransferase responsible for the metabolism of excess methionine. SAH, once formed, is hydrolysed to Hcy and adenosine by SAHH (S-adenosylhomocysteine hydrolase).

Remethylation of Hcy to methionine functions to conserve the carbon skeleton of methionine. Two different methyl donors, N⁵-methyltetrahydrofolate and betaine, provide the methyl group necessary to convert Hcy into methionine by two independent enzymes, methionine synthase and BHMT (betaine:homocysteine methyltransferase). If the carbon chain of methionine is not to be conserved, or if cysteine is required, Hcy can be irreversibly converted into cysteine through the trans-sulfuration pathway, which consists of two pyridoxal 5’-phosphate-containing enzymes:

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Key words: betaine, cystathionine β-synthase, diabetes, insulin resistance, liver, trans-sulfuration.

Abbreviations used: BHMT, betaine homocysteine methyltransferase; CBS, cystathionine β-synthase; GL, cystathionine γ-lyase; CV, cardiovascular disease; GNMT, glycine N-methyltransferase; Hcy, homocysteine; HHcy, hyperhomocysteaemia; SAM, Σ-adenosylhomocysteine; S-adenosylmethionine; tHcy, total Hcy.

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CBS (cystathionine β-synthase) and CGL (cystathionine γ-lyase). CBS catalyses the condensation of Hcy with serine to form cystathionine and commits the Hcy moiety to the trans-sulfuration pathway. Cystathionine is hydrolysed by CGL to form cysteine, α-oxobutyrate and ammonium.

**Diabetes mellitus**

Approx. 75% of Type 2 diabetic patients in the United States die of cardiovascular complications [14]. They are likely to experience CVD (cardiovascular disease) at a younger age and are more likely to develop congestive heart failure [12]. The prevalence of atherosclerosis is 2–6-fold greater in diabetic patients than in non-diabetic patients [13].

**Hcy levels in insulin resistance and diabetes**

Despite the 4–6-fold increase in CVD risk found in diabetic patients compared with their non-diabetic counterparts, a clear relationship between Hcy levels and diabetes has not been established. Plasma Hcy in diabetes varies depending on the presence or absence of nephropathy with levels being normal or even lower when there is no nephropathy and higher when there is nephropathy. Many studies have shown an association between HHcy and decreased renal function in patients with Type 2 diabetes [14–16]. Type 1 diabetic patients with nephropathy were also found to have HHcy [17], while those with no renal complications were found to have plasma Hcy levels lower than in healthy people [18,19].

A consensus on Hcy levels in insulin resistance has been elusive. Different results may be due to the differences in the methods employed to measure insulin resistance and/or the degree of insulin resistance of the subjects at the time of experimentation. Reports of positive [20–22], negative [23,24] as well as no [25] relationships have been observed between insulin resistance and plasma Hcy levels.

**HHcy and pathogenesis of atherosclerosis**

A unifying hypothesis for the mechanism of Hcy-mediated vascular injury has not yet been established. One frequently described mechanism involves oxidative damage, as Hcy can undergo autoxidation in the plasma or intracellularly to form various reactive oxygen species [26]. Hcy has also been shown to decrease the activity [27] as well as the expression [28] of the antioxidant enzyme glutathione peroxidase. Creation of a prothrombotic environment by the action of Hcy on various factors involved in coagulation has also been proposed [29–31].

The reaction of Hcy with NO (nitric oxide) acts to prevent oxidative damage caused by Hcy but at the same time reduces the bioavailability of NO by trapping it intracellularly as a nitrosothiol [32]. Hcy is also a potent mitogen for vascular smooth-muscle cells [33,34]. Aggregates formed by the combination of Hcy thiolactone, a cyclical product of Hcy, with LDL (low-density lipoprotein) were shown to
Plasma Hcy in Type 1 diabetes, insulin resistance and Type 2 diabetes

Values are expressed as means ± S.D. for six rats. Differing letters indicate significant difference from each other under the three different conditions studied.

Figure 2 | Plasma Hcy in Type 1 diabetes, insulin resistance and Type 2 diabetes

Hcy metabolism in Type 1 diabetes

Much work has been carried out in our laboratory to further the understanding of hormonal regulation of plasma Hcy. Jacobs et al. [38] conducted studies in streptozotocin-induced diabetic rats to examine the role played by insulin in the regulation of plasma Hcy in this Type 1 model. They found the plasma Hcy level to be approx. 40% lower in the diabetic rats than in the control rats (Figure 2). The insulin-treated diabetic rats, however, maintained a plasma Hcy level comparable with the control rats, indicating the possible role of insulin in regulating plasma Hcy. The reduction in plasma Hcy in the untreated diabetic rats was accompanied by increased hepatic activity of both the trans-sulfuration enzymes, CBS and CGL. Insulin treatment of the diabetic rats was successful in preventing the increases in the activities of these two enzymes.

In addition to reduced levels of insulin, Type 1 diabetes is characterized by elevations in the levels of counter-regulatory hormones such as glucagon and glucocorticoids [39]. Jacobs et al. [40] treated rats with high doses of glucagon for 2 days and observed a 30% reduction in plasma tHcy together with increased hepatic activities of GNMT and the two trans-sulfuration enzymes, CBS and CGL. Hepatocytes, isolated from these glucagon-treated rats, were found to export a significantly lower level of Hcy compared with hepatocytes from saline-treated rats. The 5-fold increase seen in the flux through CBS in hepatocytes isolated from glucagon-treated rats, when incubated with L-[1-14C]methionine, further confirms the stimulatory effect of glucagon on the hepatic transsulfuration pathway.

The increase in the activity of CBS seen in the glucagon-treated rats was accompanied by an increase in its mRNA level. This led us to measure the level of CBS mRNA in the livers of streptozotocin-diabetic and insulin-treated diabetic rats [41]. We found CBS mRNA to be markedly elevated in the diabetic rats, which was reduced by treatment with insulin. A similar effect was seen on CBS in H4IIE cells, a rat hepatoma cell culture model, where glucocorticoids increased the levels of both CBS protein and mRNA; insulin inhibited this stimulatory effect. Nuclear run-on assays performed on isolated nuclei from H4IIE cells that had been incubated with triamcinolone in the presence and absence of insulin indicated that both hormones exert their effect at the level of transcription. In HepG2 cells, a human cell culture model, insulin treatment was also able to decrease the activity of CBS together with a reduction in the level of its protein. CBS-1b promoter was shown to be sensitive to insulin, confirming that the effect of insulin was at the level of transcription [41].

These studies with Type 1 diabetic rats, glucagon-treated rats and various cell culture models provide ample evidence for the regulation of the trans-sulfuration pathway by insulin and its counter-regulatory hormones.

Hcy metabolism in Type 2 diabetes

Work in our laboratory also sought to identify the changes that occur in plasma Hcy in insulin resistance and Type 2 diabetes and to identify the metabolic steps that lead to these changes [42]. For this, we used leptin-receptor-defective ZDF (Zucker diabetic fatty) rats, which are known to go through an initial insulin-resistant phase before going on to develop frank Type 2 diabetes.

The hormonal changes that occur in insulin resistance and Type 2 diabetes appear to act at several points of the methionine metabolic pathway leading to alterations in methionine and Hcy metabolism. Plasma total Hcy was found to be reduced in insulin resistance as well as in Type 2 diabetes, compared with control rats (Figure 2). This reduction appeared to be brought about by increases in the trans-sulfuration as well as the remethylation routes of Hcy removal, through increases in the activities of CBS, CGL and BHMT. The increase in the trans-sulfuration enzymes was similar to that demonstrated in glucagon-treated as well as streptozotocin-diabetic rats [38,40] and agreed with the direct effect of insulin and glucagon on CBS expression [41]. Hepatic BHMT activity and mRNA levels were increased in both the insulin-resistant and diabetic stages with a concomitant reduction in hepatic betaine concentration (Figure 3). These results suggest that increased BHMT activity decreases the Hcy levels and depletes hepatic betaine stores. In support of these findings, Schwahn et al. [43] have previously shown a negative correlation between plasma betaine and plasma tHcy in humans. In addition, supplementation with betaine has been shown to reduce plasma Hcy [44]. The fact that...
BHMT was more active in insulin-resistant and diabetic rats was further evident in the marked reduction, by added betaine, of Hcy export from isolated hepatocytes incubated with methionine (Figure 3). These studies emphasize the significant role played by hormones in regulating plasma Hcy levels and provide a metabolic explanation for the reduced plasma Hcy levels seen in Type 1 and Type 2 diabetes before the onset of renal complications.

Renal metabolism of Hcy
The reduced plasma Hcy levels in diabetes and insulin resistance have been observed in normal or hyperfiltering kidneys [19,38,42]. However, with decreasing renal function, the concentration of plasma Hcy becomes elevated. This has been observed in both Type 1 and Type 2 diabetes mellitus [15,17]. Patients with end-stage renal disease with no diabetes also exhibit elevated plasma Hcy [45]. These observations agree with the finding, in rats, by us that the kidney is a major organ involved in Hcy metabolism [46]. We showed an uptake of plasma Hcy by the rat kidney, in vivo. Since this Hcy did not appear in the urine, this finding implies an appreciable renal Hcy metabolism. We were also able to show that the trans-sulfuration pathway was responsible for most of the renal catabolism of Hcy [47]. The important role played by the kidneys in maintaining plasma Hcy homocostasis was shown by the ability of rat kidneys to handle acute increases in plasma Hcy [48]. It should be pointed out, however, that results available in humans do not support the idea of the kidney being a major remover of plasma Hcy [49]. The fact that human plasma contains a high proportion of protein-bound (and, therefore, unfilterable) Hcy may make it more difficult to demonstrate a renal a–v (arteriovenous) difference.

Conclusion
It is clear that plasma Hcy plays a major role in the aetiology of several chronic diseases, one of the most important being atherosclerotic CVD. CVD is the leading cause of death in diabetic patients. Is there any additional role played by plasma Hcy in the development of CVD in diabetes? The many studies that have been published dealing with plasma Hcy levels in both Type 1 and Type 2 diabetes as well as insulin resistance have yielded a multiplicity of results. This may very well reflect the many mechanisms involved in the regulatory process of plasma Hcy. We have clearly shown that the metabolic regulation of plasma Hcy is altered in diabetes and that two major enzymes involved in the removal of plasma Hcy, CBS and BHMT, are hormonally regulated. Although not quite fully explained as yet, there also appears to be a relationship between kidney function and plasma Hcy. The kidney has been shown to be a major organ in the removal of Hcy in rats but the same is not as evident in humans. However, most studies have been able to show a positive correlation between plasma Hcy and plasma creatinine levels, suggesting the importance of the kidney in the regulation of plasma Hcy. Renal function in Type 2 diabetes appears to change with the progress of the disease: hyperfiltration in the early stages and progressive deterioration with the progression of diabetes. Diabetes therefore provides an interesting situation with changes in kidney functions being superimposed on the already existing changes in the metabolic milieu.

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References

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