Original Article

Homocysteine Metabolism in ZDF (Type 2) Diabetic Rats

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Mild hyperhomocysteinemia is a risk factor for many diseases, including cardiovascular disease. We determined the effects of insulin resistance and of type 2 diabetes on homocysteine (Hcy) metabolism using Zucker diabetic fatty rats (ZDF/Gmi *fa/fa* and ZDF/Gmi *fa/?*). Plasma total Hcy was reduced in ZDF *fa/fa* rats by 24% in the prediabetic insulin-resistant stage, while in the frank diabetic stage there was a 59% reduction. Hepatic activities of several enzymes that play a role in the removal of Hcy: cystathionine β-synthase (CBS), cystathionine γ-lyase, and betaine:Hcy methyltransferase (BHMT) were increased as was methionine adenosyltransferase. CBS and BHMT mRNA levels and the hepatic level of S-adenosylmethionine were also increased in the ZDF *fa/fa* rats. Studies with primary hepatocytes showed that Hcy export and the transsulfuration flux in cells from ZDF *fa/fa* rats were particularly sensitive to betaine. Interestingly, liver betaine concentration was found to be significantly lower in the ZDf *fa/fa* rats at both 5 and 11 weeks. These results emphasize the importance of betaine metabolism in determining plasma Hcy levels in type 2 diabetes. *Diabetes* 54: 3245–3251, 2005

Patients with coronary, cerebrovascular, or peripheral arterial disease have mean plasma total homocysteine (tHcy) levels significantly higher than control subjects (1). Over the last decade, these findings led to the identification of hyperhomocysteinemia as an independent risk factor for vascular disease (1). In addition, hyperhomocysteinemia has also been identified as a risk factor for Alzheimer's disease (2) and osteoporotic fractures (3).

Homocysteine (Hcy) is formed after the donation of a methyl group from S-adenosylmethionine (SAM). It can then be metabolized by one of three enzymes. Methionine synthase and betaine:Hcy methyltransferase (BHMT) catalyze the remethylation of Hcy to methionine, and cystathionine β-synthase (CBS) forms part of the transsulfuration pathway where Hcy condenses with serine to form cystathionine. Figure 1 shows the pathway of methionine metabolism.

Diabetes, whether type 1 or type 2, is associated with an increased risk of cardiovascular mortality, with the prevalence of atherosclerosis being two- to sixfold higher in diabetic patients than in people without diabetes (4). Insulin resistance, which immediately precedes the development of type 2 diabetes, is also associated with an increased risk of coronary artery disease (5). Hyperhomocysteinemia has been shown to be a stronger risk factor for cardiovascular disease and for mortality in patients with type 2 diabetes than in subjects without diabetes (6).

Plasma tHcy in diabetic patients is known to be dependent on the presence or absence of nephropathy. Both type 1 and type 2 diabetic patients with nephropathy have elevated levels of tHcy (6,7). However, type 1 diabetic patients with no renal complications have plasma tHcy levels lower than controls (8). This decrease was also shown in an animal model of type 1 diabetes (9).

We, therefore, examined the effect of insulin resistance and type 2 diabetes on plasma tHcy and its metabolism in the liver. We used the leptin receptor–defective Zucker diabetic fatty rat (ZDF), an excellent model for type 2 diabetes (10). Before developing frank diabetes, they go through a phase of insulin resistance, thereby giving us an opportunity to study the effects of both insulin resistance and of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Animals and tissue sampling. All procedures were approved by Memorial University's Institutional Animal Care Committee. Male ZDF rats (ZDF/Gmi *fa/fa*) aged 5 and 11 weeks and male lean rats (ZDF/Gmi *fa/?*) of the same ages were obtained from Charles River Laboratories, Indianapolis, Indiana. They were fed Purina 5008 chow ad libitum and had free access to water. On the day of the experiment, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (65 mg/kg), and blood was collected from the abdominal aorta into heparinized syringes. Before removal of the syringe, a piece of the liver was rapidly removed and freeze-clamped with aluminum tongs precooled in liquid nitrogen. Another portion was removed for immediate enzyme assays. The freeze-clamped liver was stored at −70°C until further use. The blood was centrifuged at 3,700g for 15 min, and the plasma was kept at −20°C until further use.

Analytical procedures

Plasma metabolites. Plasma tHcy was measured as described (11). Plasma glucose was determined enzymatically (12). Plasma creatinine was measured using a kit (catalog no. 555A) from Sigma Diagnostics (St. Louis, MO). Plasma insulin was measured with a rat insulin enzyme-linked immunosorbent assay kit (catalog no. INSKR020) from Crystal Chem (Chicago, IL) using anti-rat/mouse insulin antibody and a rat insulin standard.

Enzyme assays. Fresh liver samples were diluted 1:5 with 50 mmol/l K phosphate buffer (pH 7.0) and homogenized with a Polytron (Brinkman Instruments, Toronto, Canada) for 20 s at 50% output. The homogenates were centrifuged at 20,000g for 30 min at 4°C, and the supernatant was removed and used immediately to measure the activities of five of the enzymes of methionine metabolism. Methionine adenosyltransferase (MAT) (13,14), CBS (13,15), cystathionine γ-lyase (CGL) (16), methionine synthase (17), and BHMT (18) were assayed using previously described methods. Methylenetet-
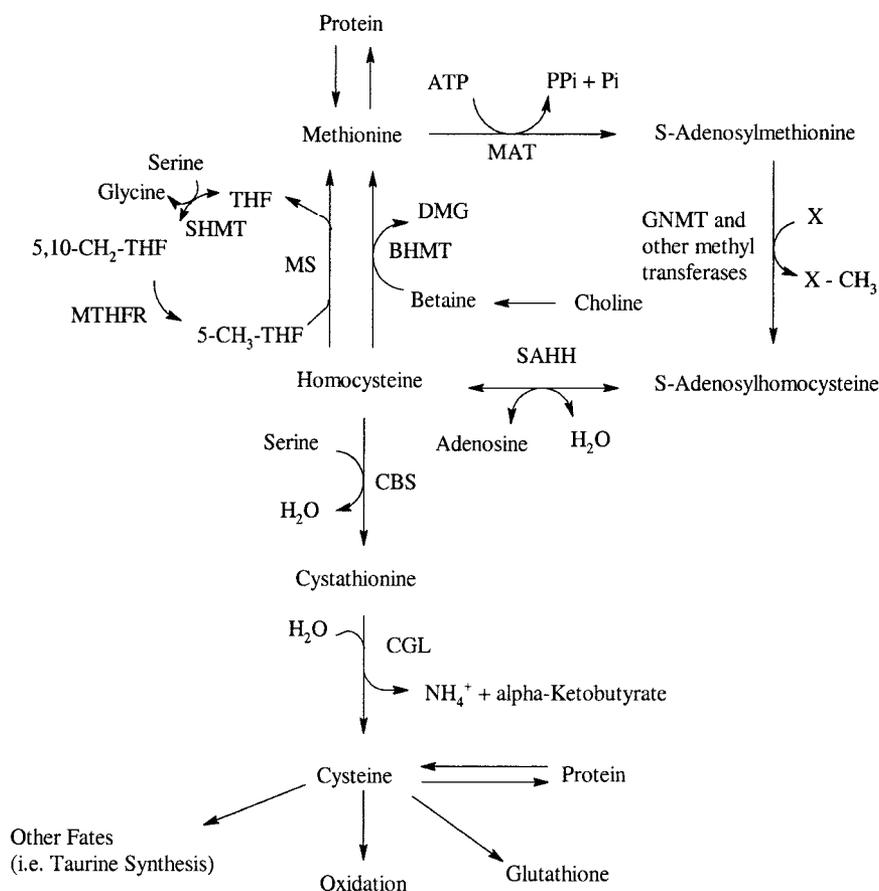
FIG. 1. Methionine metabolic pathway.

rahydrofolate reductase (MTHFR) (19) and glycine *N*-methyltransferase (GNMT) (20) were measured using previously frozen liver tissue. Choline dehydrogenase was measured using liver homogenates (21,22). Protein concentrations were determined by the Biuret method using BSA as a standard. All enzyme assays were determined to be linear with time and with protein.

Liver metabolites. Choline and its metabolites were extracted from tissue and plasma by the method of Bligh and Dyer (23). Aqueous and organic compounds were separated, analyzed, and quantified directly by high-performance liquid chromatography–mass spectrometry (liquid chromatography–electrospray ionization–isotope dilution mass spectrometry) after the addition of internal standards labeled with stable isotopes that were used to correct for recovery (24). The levels of SAM and S-adenosylhomocysteine (SAH) were measured in freeze-clamped liver tissue (25).

mRNA abundance. Total RNA was isolated from the fresh liver samples as previously described (26). Two micrograms of RNA were reverse transcribed using a one-step reverse transcription kit (Qiagen, Mississauga, Canada) and was amplified by 27 cycles. An upstream primer (5′-GCCTTCAGGACATCCA GTGT-3′) and a downstream primer (5′-TCTTTCCGGGTCTGCTCACGGGC-3′) corresponding to the type 3 rat CBS cDNA (27) were used to amplify a 1,644-bp PCR fragment. For BHMT, an upstream primer (5′-ACCGATTGCCG GCAAGAA-3′) and a downstream primer (5′-CTGTGCGGATTTGAATTTTTG-3′) corresponding to the rat BHMT cDNA (NM-030850) were used to amplify a 1,216-bp PCR fragment. A 768-bp fragment of the rat β-actin was coamplified using amplimer set primers (CLONTECH, Palo Alto, CA). Once amplified, the PCR products were separated on 0.8% agarose. The bands were stained with ethidium bromide and were visualized under UV illumination.

Homocysteine metabolism in isolated hepatocytes. Hepatocytes were isolated from ZDF *fa/fa* and ZDF *fa/?* rats (28). Approximately 5 mg of hepatocytes (dry weight) were preincubated for 20 min at 37°C with Krebs-Henseleit saline with or without 0.3 mmol/l betaine. At the end of the preincubation L-[1-^{14}C] methionine was added to a final concentration of 0.1 mmol/l and the incubation continued for a further 30 min. The total transsulfuration flux was determined as the sum of the ^{14}C present in α-ketobutyrate and in CO_2 as previously described (29). In a separate series of experiments, hepatocytes were incubated with 0.1 mmol/l methionine ± 0.3 mmol/l betaine to measure Hcy export (29).

Statistical analysis. Data are presented as means ± SD. Means were compared using Student's unpaired *t* test, paired *t* test, or one-way ANOVA as appropriate. $P < 0.05$ was taken to indicate a significant difference.

RESULTS

The body weight, hepatosomatic index, plasma insulin, creatinine, and glucose concentrations of these rats have been reported (30). At 5 weeks, the concentrations of plasma glucose (12.1 ± 1.7 vs. 8.4 ± 1.3 mmol/l) and insulin (1.5 ± 0.5 vs. 0.14 ± 0.07 nmol/l) in the ZDF *fa/fa* and ZDF *fa/?* rats, respectively, showed that the ZDF *fa/fa* rats had become insulin resistant. At 11 weeks, the concentrations of plasma glucose (30.3 ± 1.6 vs. 10.9 ± 0.8 mmol/l) and insulin (0.61 ± 0.14 vs. 0.68 ± 0.1 nmol/l) in the ZDF *fa/fa* and the ZDF *fa/?* rats, respectively, showed that the ZDF *fa/fa* rats had become diabetic. Creatinine levels of ZDF *fa/fa* and control rats at 5 weeks were similar (32.4 ± 3.8 vs. 28.9 ± 3.1 μmol/l), but at 11 weeks were lower in the ZDF *fa/fa* rats (18.9 ± 2.7 vs. 29.7 ± 6.1 μmol/l)

Plasma Hcy and hepatic enzymes of Hcy metabolism. At 5 weeks, plasma tHcy was about 25% lower in the ZDF *fa/fa* rats, while at 11 weeks it was reduced by about 60% (Table 1). The plasma tHcy level in the ZDF *fa/fa* rats at 11 weeks was further reduced from its value at 5 weeks, whereas the ZDF *fa/?* rats showed an increase in the plasma tHcy level with age. The reduced plasma tHcy at 5 weeks in the ZDF *fa/fa* rats was accompanied by changes in several enzymes that metabolize methionine (Table 1). Of the enzymes responsible for producing Hcy, MAT and GNMT showed slight but significant increases. CBS, CGL, BHMT, and MTHFR were increased, respectively, by about 79, 35, 80, and 22%. Methionine synthase showed a slight but significant decrease in the ZDF *fa/fa* rats. At 11 weeks, MAT, GNMT, CBS, CGL, and BHMT all showed significant increases in activity as did choline dehydrogenase.

In addition to the differences in enzyme activity between

TABLE 1
Plasma total homocysteine and hepatic activities of enzymes of homocysteine metabolism

	5 weeks		11 weeks	
	ZDF fa/?	ZDF fa/fa	ZDF fa/?	ZDF fa/fa
Plasma total homocysteine (μmol/l)	4.8 ± 0.5[a]	3.6 ± 0.2[b]	7.5 ± 0.4[a]	3.1 ± 0.3[b]
MAT	0.49 ± 0.02[a]	0.6 ± 0.04[b]	1.3 ± 0.09[a]	1.7 ± 0.09[b]
CBS	3.9 ± 0.4[a]	7.0 ± 1.0[b]	6.3 ± 0.9[a]	11.2 ± 1.9[b]
GNMT	0.52 ± 0.06[a]	0.73 ± 0.04[b]	0.67 ± 0.08[a]	1.0 ± 0.09[b]
CGL	16.5 ± 2.3[a]	22.3 ± 2.8[b]	16.5 ± 1.4[a]	38.4 ± 4.2[b]
Methionine synthase	0.033 ± 0.002[a]	0.029 ± 0.002[b]	0.032 ± 0.003	0.035 ± 0.003
MTHFR	0.09 ± 0.01[a]	0.11 ± 0.01[b]	0.09 ± 0.02	0.1 ± 0.02
BHMT	0.4 ± 0.04[a]	0.72 ± 0.06[b]	0.34 ± 0.06[a]	0.76 ± 0.08[b]
Choline dehydrogenase	2.7 ± 0.5	3.2 ± 0.7	2.4 ± 0.4[a]	3.1 ± 0.6[b]

Data are means ± SD for six rats. In each group, enzyme activities are expressed as micromoles of product synthesized per minute per 100 g body weight. Differences in superscript letters signify significant differences within each age-group ($P < 0.05$).

the control and the experimental animals at each age-group, MAT, CBS, and GNMT all showed significant increases in their activity with age in the ZDF fa/? rats.

mRNA levels. At 5 weeks, the increased activity of CBS in the ZDF fa/fa rats was accompanied by a comparable increase in CBS mRNA level (Fig. 2A). The increased BHMT activity observed in the ZDF fa/fa rats at 5 and 11

weeks was also accompanied by comparable increases in mRNA levels (Fig. 2B).

SAM, SAH, and SAM-to-SAH ratio. The hepatic levels of SAM and SAH and the SAM-to-SAH ratio are shown in Table 2. At both 5 and 11 weeks, SAM was significantly increased in the ZDF fa/fa rats. The SAM-to-SAH ratio was significantly higher in the ZDF fa/fa rats at 5 weeks. Both
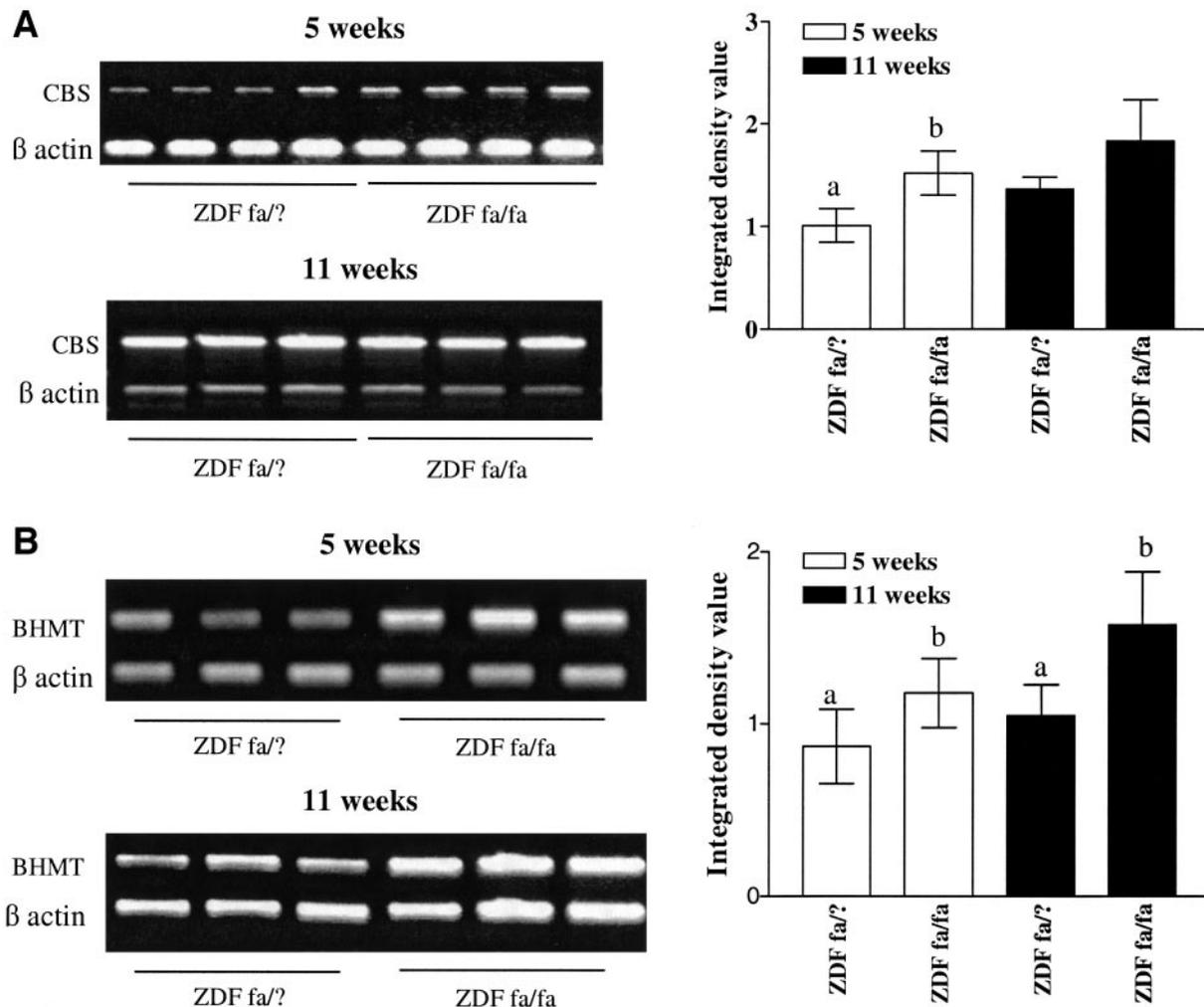

FIG. 2. RT-PCR analysis of gepatic cystathionine β-synthase mRNA (A) and BHMT mRNA (B). The relative amounts wif each mRNA, measured by spot densitometry, are graphically represented. Both CBS and BHMT mRNA were normalized to β-actin mRNA. Values are means ± SD for six rats. Differing superscripts at each age-group indicate significant difference from each other. P < 0.05.

TABLE 2
SAM, SAH, and hepatic SAM-to-SAH ratio

	5 weeks		11 weeks	
	ZDF *fa/?*	ZDF *fa/fa*	ZDF *fa/?*	ZDF *fa/fa*
SAM	88 ± 8.4^a	142.1 ± 11.3^b	172.9 ± 20.0^a	199.9 ± 15.5^b
SAH	12.3 ± 1.2	11.6 ± 1.3	11.9 ± 2.3	11.7 ± 2.2
SAM-to-SAH	7.2 ± 0.7^a	12.3 ± 2.0^b	15.1 ± 4.7	17.5 ± 3.5

Data are means $\pm$ SD for six rats in each group, and values for SAM and SAH are expressed as nmol/g liver. At each age-group, differences in superscript letters indicate significant difference from the ZDF *fa/?* rats ($P < 0.05$).

SAM and the SAM-to-SAH ratio showed increases with age in ZDF *fa/?* rats, consistent with the age-related increase in MAT activity.

Transsulfuration flux. In a preliminary study, we investigated the effect of betaine on the transsulfuration flux, as measured by the conversion of L-[1-^{14}C] methionine to $^{14}CO_2$ and ^{14}C-α-ketobutyrate by isolated hepatocytes from Sprague Dawley rats. All concentrations of betaine used (0.1, 0.3, or 1 mmol/l) significantly reduced the catabolism of methionine, compared with incubations of hepatocytes with methionine alone. We then conducted studies with hepatocytes from ZDF rats using 0.1 mmol/l L-[1-^{14}C] methionine and 0.3 mmol/l betaine. Incubation with 0.3 mmol/l betaine significantly decreased the transsulfuration flux in hepatocytes of ZDF *fa/fa* rats at both ages, but the reduction seen in the hepatocytes of ZDF *fa/?* rats was significant only at 11 weeks of age (Table 3).

Hcy export. Hepatocytes from ZDF *fa/fa* and ZDF *fa/?* rats, at both ages, showed a significant reduction in Hcy export when incubated with 0.3 mmol/l betaine (Table 4). However, at both ages the decrease observed in the ZDF *fa/fa* rats was about twofold greater than that of the ZDF *fa/?* rats.

Choline and related metabolites. Table 5 shows the liver concentrations of choline and related metabolites. The liver betaine concentration was lower in the ZDF *fa/fa* rats at both 5 and 11 weeks, while there was no difference in the choline concentration. One of the most remarkable findings was the high level of betaine found in the liver of the young animals and the reduction that occurs with age. Hepatic glycerophosphorylcholine was significantly higher in the ZDF *fa/fa* rats at 5 weeks, though at 11 weeks it was significantly lower. Phosphorylcholine was significantly higher in the ZDF *fa/fa* rats at both ages. Both glycerophosphorylcholine and phosphorylcholine levels increased with age in the ZDF *fa/?* rats. Phosphatidylcholine was significantly decreased in ZDF *fa/fa* rats at 11 weeks, while sphingomyelin was significantly reduced at both 5 and 11 weeks.

Table 6 shows choline and related metabolite concentrations in the plasma. The only significant difference in plasma metabolites between the different rats was an increased phosphatidylcholine level in the ZDF *fa/fa* rats at 11 weeks. However, the plasma betaine concentration decreased with age in both groups of rats while the concentration of phosphatidylcholine increased in the ZDF *fa/fa* rats from 5 to 11 weeks.

DISCUSSION

This study describes the altered metabolism of methionine and Hcy that occurs in insulin resistance and in early type 2 diabetes. It is clear that the hormonal changes that are prevalent in these two disease states act at several sites in the pathway of methionine metabolism. Hyperphagia in the diabetic rats may also contribute to these metabolic changes. Indeed, we observed a twofold increase in food intake per 100 g body weight at the diabetic stage. However, there was no difference at 5 weeks. The increased hepatic CBS and BHMT activity at 5 weeks cannot, therefore, be attributed to increased protein intake. In addition, we have shown a direct effect of insulin in repressing CBS expression in both human and rat cultured hepatocytes (42). Our finding of lowered plasma tHcy in the 5-week ZDF *fa/fa* rats shows that insulin resistance alone can decrease plasma tHcy levels.

The most striking finding in the present study concerns the role of betaine and BHMT in determining plasma tHcy. Not only was BHMT activity and mRNA levels elevated but the hepatic betaine concentration was remarkably reduced. Furthermore, this increased BHMT was clearly effective in decreasing hepatic Hcy output. This is evident from the data showing that addition of betaine to hepatocytes from ZDF *fa/fa* rats virtually eliminated Hcy export from cells compared with a lesser effect in hepatocytes from ZDF *fa/?* rats (Table 4). Increased remethylation of Hcy by BHMT also reduces the Hcy available for flux through the transsulfuration pathway, which decreased in these cells (Table 3). Although the importance of the BHMT-catalyzed remethylation reaction in determining Hcy levels has not received as much attention as the folate-dependent remethylation pathway, it should be recalled that Schwahn et al. (31) found a highly significant negative correlation between plasma betaine and plasma tHcy concentrations in humans. Supplementation with betaine was shown to lower plasma tHcy in humans (32) and in *MTHFR* +/+, +/−, and −/− mice (31). Because of the elevated BHMT activity, betaine may be particularly efficacious in reducing plasma tHcy in the later stages of type 2 diabetes when renal complications bring about hyperhomocysteinemia.

Our data suggest that there is enhanced utilization of betaine in the ZDF *fa/fa* rat. The observed decrease in

TABLE 3
Transsulfuration flux in isolated hepatocytes

	Transsulfuration flux (nmol $\cdot$ mg dry wt^{-1} $\cdot$ 30 min$^-$)			
	5 weeks		11 weeks	
	ZDF *fa/?*	ZDF *fa/fa*	ZDF *fa/?*	ZDF *fa/fa*
L-[1-^{14}C] methionine	1.00 ± 0.25	1.08 ± 0.05	1.18 ± 0.21	1.08 ± 0.15
L-[1-^{14}C] methionine + betaine	0.91 ± 0.32	$0.87 \pm 0.12*$	$0.96 \pm 0.15*$	$0.86 \pm 0.13*$

Data are means $\pm$ SD; $n = 3$ for 5 weeks and $n = 5$ for 11 weeks. Hepatocytes were incubated with 0.1 mmol/l L-[1-^{14}C] methionine $\pm$ 0.3 mmol/l betaine to determine the transsulfuration flux. *Significant difference from the corresponding incubations without betaine ($P < 0.05$).

TABLE 4
Homocysteine export from isolated hepatocytes

| | Homocysteine export (nmol · mg dry wt^{-1} · 30 min^{-}) | | | |
| | 5 weeks | | 11 weeks | |
	ZDF *fa/?*	ZDF *fa/fa*	ZDF *fa/?*	ZDF *fa/fa*
Methionine	1.17 ± 0.23	1.21 ± 0.25	1.64 ± 0.61	2.03 ± 0.92
Methionine + betaine	0.86 ± 0.12*	0.52 ± 0.08*	0.89 ± 0.45*	0.32 ± 0.26*

Data are means ± SD; $n = 3$ for 5 weeks and $n = 5$ for 11 weeks. Hcy export was measured in hepatocytes isolated from ZDF rats incubated with 0.1 mmol/l methionine ± 0.3 mmol/l betaine. *Significant difference from corresponding incubations without betaine ($P < 0.05$).

hepatic phosphatidylcholine concentration (Table 5) is consistent with enhanced utilization of choline to form betaine in the livers of these rats. Sphingomyelin, which is derived from phosphatidylcholine, is also reduced in *fa/fa* liver. The increase in phosphorylcholine concentrations may reflect increased activity of the pathway for phosphatidylcholine biosynthesis that occurs when tissue phosphatidylcholine concentrations fall. The significant increase in plasma phosphatidylcholine concentrations in ZDF *fa/fa* rats at 11 weeks (Table 6) probably reflects increased secretion of lipoproteins from liver.

Another remarkable feature of betaine metabolism is the extraordinarily high hepatic betaine concentration found in young rats. In the control animals (ZDF *fa/?*), the hepatic betaine level was 17.6 μmol/g at 5 weeks; this decreased to 5.3 μmol/g at 11 weeks. Assuming that hepatic betaine is evenly distributed in intracellular water and that rat liver contains 0.45 ml of cell water per gram (35), we can calculate a betaine concentration in the 5-week-old control animals of 39 mmol/l. This should be compared with total osmolarity of about 305–310 mOs mmol/l in mammalian tissues (except for the renal medulla).

Davies et al. (36) have reported a very large urinary excretion of betaine in normal neonates and in young rats after weaning. High betaine concentrations are found in mammalian kidneys, particularly in the renal medulla. Renal betaine has been characterized as a compatible osmolyte (together with other trimethylamines and polyols) where it can offset the high extracellular osmolarity that arises as a result of the urinary concentrating mechanisms. In addition, these osmolytes may protect these cells from adverse effects of urea, which is present at high concentrations (37).

Wettstein et al. (38) have showed that betaine plays a role in cell volume homeostasis in perfused livers of adult rats. Whether this is also the function of the high betaine concentrations in neonatal liver is a focus of our current work. We must also comment on the ratio of hepatocellular to plasma betaine. Plasma betaine was fairly constant, in the 90–170 μmol/l range, in all of the rats (Table 6). The liver-to-plasma concentration ratios for betaine were about 230, 130, 100, and 60, respectively, for ZDF *fa/?* and ZDF *fa/fa* rats at 5 and 11 weeks of age. How such gradients are produced and maintained are important issues for future work.

Diabetes is characterized not only by the lack of insulin or the resistance to the action of insulin, but also by increases in the counter-regulatory hormones glucocorticoid and glucagon (39,40). Hepatic MAT activity has been shown to be increased by glucocorticoid administration (41). Finkelstein et al. (33) examined the effects of administration of a number of hormones on BHMT activity. The principal findings were that hydrocortisone increased BHMT activity, whereas thyroxin decreased it. This group also demonstrated increased activity in alloxan diabetic rats. This latter finding has been confirmed by Nieman et al. (34) in streptozotocin-induced diabetic rats. We are currently exploring the possibility that insulin may regulate BHMT expression. Insulin treatment of streptozotocin-induced diabetic rats was shown to restore the increased activity of CBS to normal levels (9) by acting at the level of CBS gene transcription to repress CBS promoter activity (42). Cyclic AMP, the intracellular messenger of glucagon, and glucocorticoids increase CBS expression in rat hepatoma cells (43). The increased activity of CBS, brought about both by allosteric activation by the increased hepatic SAM levels (44) and by increased expression, does not imply an increased transsulfuration flux. This flux is determined by the rate at which methionine enters into hepatic metabolism, which will be largely determined by dietary methionine consumption. It seems that the major effect of increased CBS activity is to decrease the steady-state Hcy concentration at which transsulfuration occurs (45). In this way it contributes to the decreased plasma tHcy concentration. Similarly, the increased activity of BHMT does not necessarily imply an increased remethylation flux in vivo but, rather, it decreases the steady-state Hcy concentration at which remethylation via BHMT occurs.

It is also possible that alterations in renal function may

TABLE 5
Choline and related metabolite concentrations in the liver of ZDF *fa/?* and ZDF *fa/fa* rats aged 5 and 11 weeks

| | 5 weeks | | 11 weeks | |
Metabolite	ZDF *fa/?*	ZDF *fa/fa*	ZDF *fa/?*	ZDf *fa/fa*
Betaine	17.6 ± 3.6[a]	9.9 ± 1.4[b]	5.3 ± 0.7[a]	2.5 ± 0.7[b]
Choline	0.06 ± 0.03	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
Glycerophosphorylcholine	0.6 ± 0.04[a]	1.0 ± 0.2[b]	1.5 ± 0.1[a]	0.96 ± 0.1[b]
Phosphorylcholine	1.2 ± 0.27[a]	2.1 ± 0.3[b]	1.9 ± 0.3[a]	2.9 ± 0.9[b]
Phosphatidylcholine	28.1 ± 2.8	29.2 ± 1.4	25.3 ± 5.2[a]	19.3 ± 2.0[b]
Sphingomyelin	3.3 ± 0.3[a]	2.9 ± 0.2[b]	3.4 ± 0.4[a]	2.8 ± 0.3[b]

Data are means ± SD (μmol/g) for six rats. Differences in superscript letters signify a significant difference from the ZDF *fa/?* rats of the same age ($P < 0.05$).

TABLE 6
Choline and related metabolite concentrations in the plasma of ZDF *fa/?* rats and ZDF *fa/fa* rats at 5 and 11 weeks of age

Metabolite	5 weeks		11 weeks	
	ZDF *fa/?*	ZDF *fa/fa*	ZDF *fa/?*	ZDf *fa/fa*
Betaine	0.17 ± 0.04	0.17 ± 0.02	0.12 ± 0.03	0.09 ± 0.03
Choline	0.08 ± 0.02	0.08 ± 0.03	0.07 ± 0.01	0.08 ± 0.02
Phosphatidylcholine	0.86 ± 0.27	0.95 ± 0.23	0.80 ± 0.29[a]	1.69 ± 0.48[b]

Data are means ± SD (μmol/ml). Differences in superscript letters signify a statistically significant difference from the ZDF *fa/?* rats of the same age ($P < 0.05$).

contribute, somewhat, to the decreased plasma tHcy, at least when the rats are diabetic. The kidney is a major site for the removal and subsequent metabolism of Hcy (46). Hyperfiltration is a characteristic sign of renal dysfunction in early diabetes (47). Such hyperfiltration has been reported to occur in ZDF *fa/fa* rats from about 7 weeks of age and to continue until they are about 3 months old (48). Such an occurrence probably accounts for the decreased plasma creatinine seen at 11 weeks. The delivery of an increased quantity of Hcy to the kidney via filtration could result in increased renal catabolism of Hcy and contribute to its reduced plasma concentration. However, this cannot contribute to the decreased Hcy at the insulin-resistant stage where creatinine levels were unchanged.

It is now clear that plasma tHcy levels decrease in both type 1 and type 2 diabetes when there is no renal damage. The present work showed that this is true even at the prediabetic, insulin-resistant stage. However, literature reports on the phenomenon show a variety of responses of Hcy to insulin resistance (5,49,50). This variability is likely to result from different degrees of insulin resistance as well as other factors such as impaired renal function. Our results, which at 5 weeks were obtained in an animal model in which there was no evidence of impaired renal function as well as a consistent degree of insulin resistance, agree remarkably well with Rosolova's study of the relationship between Hcy and insulin resistance in healthy human subjects (5).

In summary, this study shows a decreased plasma tHcy level in both insulin-resistant and type 2 diabetic rats. We show increased activities of BHMT and CBS in these states, as well as increased mRNA levels for these enzymes. We also show increased hepatic SAM levels, which will activate CBS. We report experiments in hepatocytes that directly demonstrate the role of betaine metabolism in enhanced Hcy removal. These results emphasize the importance of BHMT in regulating Hcy metabolism.

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