


2-28-2012

Tissue-specific alterations of methyl group metabolism with DNA hypermethylation in the Zucker (type 2) diabetic fatty rat.

Kelly T. Williams
Iowa State University

Kevin Schalinske
Iowa State University, kschalin@iastate.edu

Follow this and additional works at: http://lib.dr.iastate.edu/fshn_hs_pubs

 Part of the [Food Chemistry Commons](#), [Human and Clinical Nutrition Commons](#), [Molecular, Genetic, and Biochemical Nutrition Commons](#), and the [Other Food Science Commons](#)

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/fshn_hs_pubs/9. For information on how to cite this item, please visit <http://lib.dr.iastate.edu/howtocite.html>.

This Article is brought to you for free and open access by the Food Science and Human Nutrition at Iowa State University Digital Repository. It has been accepted for inclusion in Food Science and Human Nutrition Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

Tissue-specific alterations of methyl group metabolism with DNA hypermethylation in the Zucker (type 2) diabetic fatty rat.

Abstract

Background Altered methyl group and homocysteine metabolism were tissue-specific, persistent, and preceded hepatic DNA hypomethylation in type 1 diabetic rats. Similar metabolic perturbations have been shown in the Zucker (type 2) diabetic fatty (ZDF) rat in the pre-diabetic and early diabetic stages, but tissue specificity and potential impact on epigenetic marks are unknown, particularly during pathogenesis.

Methods ZDF (*fa/fa*) and lean (+/?) control rats were killed at 12 and 21 weeks of age, representing early and advanced diabetic conditions. Blood and tissues were analysed with respect to methyl group and homocysteine metabolism, including DNA methylation.

Results At 12 weeks, hepatic glycine *N*-methyltransferase (GNMT), methionine synthase, and cystathionine β -synthase (CBS) activity and/or abundance were increased in ZDF rats. At 21 weeks, GNMT activity was increased in liver and kidney; however, only hepatic CBS protein abundance (12 weeks) and betaine-homocysteine *S*-methyltransferase mRNA expression (21 weeks) were significantly elevated (78 and 100%, respectively). Hepatic phosphatidylethanolamine *N*-methyltransferase expression was also elevated in the ZDF rat. Homocysteine concentrations were decreased in plasma and kidney, but not in liver, at 12 and 21 weeks. In contrast to hepatic DNA hypomethylation in the type 1 diabetic rat, genomic DNA was hypermethylated at 12 and 21 weeks in the liver of ZDF rats, concomitant with an increase in DNA methyltransferase 1 expression at 21 weeks.

Conclusions The pathogenesis of type 2 diabetes in the ZDF rat was associated with tissue and disease stage-specific aberrations of methyl group and homocysteine metabolism, with persistent hepatic global DNA hypermethylation.

Disciplines

Food Chemistry | Food Science | Human and Clinical Nutrition | Molecular, Genetic, and Biochemical Nutrition | Other Food Science

Comments

This is the peer reviewed version of the following article is from *Diabetes / Metabolism Research and Reviews*, February 2012, 28(2); 123-131. Which has been published in final form at Doi: [10.1002/dmrr.1281](https://doi.org/10.1002/dmrr.1281). This article may be used for non-commercial purposes in accordance With Wiley Terms and Conditions for self-archiving.

Diabetes/Metabolism Research and Reviews -

Tissue-specific alterations of methyl group metabolism and DNA hypermethylation in the Zucker (type 2) diabetic fatty rat

Journal:	<i>Diabetes/Metabolism Research and Reviews</i>
Manuscript ID:	DMRR-11-RES-066.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Williams, Kelly; Iowa State University, Food Science and Human Nutrition Schalinske, Kevin; Iowa State University, Department of Food Science and Human Nutrition
Keyword primary:	diabetes, ZDF rat, methyl group metabolism, DNA methylation, GNMT
Abstract:	<p>Background Altered methyl group and homocysteine metabolism was tissue-specific, persistent, and preceded hepatic DNA hypomethylation in type 1 diabetic rats. Similar metabolic perturbations have been shown in the Zucker (type 2) diabetic fatty (ZDF) rat in the pre- and early diabetic stages, but tissue specificity and potential impact on epigenetic marks are unknown, particularly during pathogenesis.</p> <p>Methods ZDF (fa/fa) and lean (+/?) control rats were killed at 12 and 21 wk of age, representing early and advanced diabetic conditions. Blood and tissues were analyzed with respect to methyl group and homocysteine metabolism, including DNA methylation.</p> <p>Results At 12 wk, hepatic glycine N-methyltransferase (GNMT), methionine synthase, and cystathionine β-synthase (CBS) activity and/or abundance were increased in ZDF rats. At 21 wk, GNMT activity was increased in liver and kidney; however, only hepatic CBS protein abundance (12 wk) and betaine-homocysteine S-methyltransferase mRNA expression (21 wk) were significantly elevated (78 and 100%, respectively). Hepatic phosphatidylethanolamine N-methyltransferase expression was also elevated in the ZDF rat. Homocysteine concentrations were decreased in plasma and kidney, but not in liver, at 12 and 21 wk. In contrast to hepatic DNA hypomethylation in the type 1 diabetic rat, genomic DNA was hypermethylated at 12 and 21 wk in the liver of ZDF rats, concomitant with an increase in DNMT1 expression at 21 wk.</p> <p>Conclusions The pathogenesis of type 2 diabetes in the ZDF rat was associated with tissue- and disease stage-specific aberrations of methyl group and homocysteine metabolism, with persistent</p>

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



SCHOLARONE™
Manuscripts

FOR REVIEW ONLY

1
2
3 **Tissue-specific alterations of methyl group metabolism and DNA hypermethylation in the**
4
5
6 **Zucker (type 2) diabetic fatty rat**
7

8
9 Kelly T. Williams and Kevin L. Schalinske
10

11
12
13
14
15 Running title: Methyl group metabolism in the ZDF rat
16
17

18
19
20
21
22 Interdepartmental Graduate Program in Nutritional Sciences, and the Department of Food
23
24 Science and Human Nutrition, Iowa State University, Ames IA
25
26

27
28
29
30
31 To whom correspondence should be addressed:
32

33
34 Kevin L. Schalinske
35

36
37 Iowa State University
38

39
40 220 MacKay Hall
41

42
43 Ames IA 50011
44

45
46 Phone: 515-294-9230
47

48
49 Fax: 515-294-6193
50
51
52
53
54
55
56
57
58
59
60

Abstract

Background Altered methyl group and homocysteine metabolism was tissue-specific, persistent, and preceded hepatic DNA hypomethylation in type 1 diabetic rats. Similar metabolic perturbations have been shown in the Zucker (type 2) diabetic fatty (ZDF) rat in the pre- and early diabetic stages, but tissue specificity and potential impact on epigenetic marks are unknown, particularly during pathogenesis.

Methods ZDF (*fa/fa*) and lean (+/?) control rats were killed at 12 and 21 wk of age, representing early and advanced diabetic conditions. Blood and tissues were analyzed with respect to methyl group and homocysteine metabolism, including DNA methylation.

Results At 12 wk, hepatic glycine *N*-methyltransferase (GNMT), methionine synthase, and cystathionine β -synthase (CBS) activity and/or abundance were increased in ZDF rats. At 21 wk, GNMT activity was increased in liver and kidney; however, only hepatic CBS protein abundance (12 wk) and betaine-homocysteine *S*-methyltransferase mRNA expression (21 wk) were significantly elevated (78 and 100%, respectively). Hepatic phosphatidylethanolamine *N*-methyltransferase expression was also elevated in the ZDF rat. Homocysteine concentrations were decreased in plasma and kidney, but not in liver, at 12 and 21 wk. In contrast to hepatic DNA hypomethylation in the type 1 diabetic rat, genomic DNA was hypermethylated at 12 and 21 wk in the liver of ZDF rats, concomitant with an increase in DNMT1 expression at 21 wk.

Conclusions The pathogenesis of type 2 diabetes in the ZDF rat was associated with tissue- and disease stage-specific aberrations of methyl group and homocysteine metabolism, with persistent hepatic global DNA hypermethylation.

Key Words: diabetes; ZDF rat; methyl group metabolism; DNA methylation; GNMT

Introduction

It is estimated that over 12% of the U.S. population is afflicted with diabetes, with type 2 diabetes representing 95% of all cases [1]. Diabetes has recently been identified as a condition that can profoundly alter methyl group and homocysteine metabolism [2,3]. There are three primary pathways involved in the metabolism of methyl groups and homocysteine: transmethylation, remethylation via folate/B₁₂-dependent or –independent mechanisms, and transsulfuration (Figure 1). For transmethylation reactions, methionine is activated to *S*-adenosylmethionine (SAM), which serves as the universal methyl group donor for numerous methyltransferases, resulting in the methylation of substrates such as nucleic acids, lipids, and proteins. All SAM-dependent methyltransferase reactions result in the generation of homocysteine, which can be remethylated back to methionine using methyl groups donated by either 5-methyltetrahydrofolate (5-CH₃-THF) or betaine. For folate-dependent remethylation, the B₁₂-dependent enzyme methionine synthase (MS) utilizes a methyl group from 5-CH₃-THF, whereas betaine-homocysteine *S*-methyltransferase (BHMT) catalyzes the folate-independent remethylation of homocysteine using betaine, a methyl group donor derived from the oxidation of choline. Alternatively, homocysteine can be catabolized through the transsulfuration pathway to cysteine, beginning with its irreversible conversion to cystathionine by cystathionine β-synthase (CBS).

We, and others, have previously shown that an acute diabetic condition alters methyl group and homocysteine metabolism [4-9]. Diabetes induced hepatic protein abundance and activity of glycine *N*-methyltransferase (GNMT) and phosphatidylethanolamine *N*-methyltransferase (PEMT), enzymes that have important roles in regulation of methyl groups and homocysteine. GNMT is proposed to be a regulator of the transmethylation potential via

1
2
3 maintenance of the SAM:S-adenosylhomocysteine (SAH) ratio, whereas PEMT catalyzes the
4
5 sequential trimethylation of phosphatidylethanolamine to phosphatidylcholine and is purported
6
7 to be the greatest consumer of SAM-derived methyl groups [10]. Induction of BHMT and CBS
8
9 are also consistently observed in diabetic or glucocorticoid-treated conditions [4,5,7-9,11,12],
10
11 whereas the effect of diabetes on MS is not consistent. Taken together, these metabolic
12
13 alterations result in hypohomocysteinemia in the early diabetic condition. Importantly, similar
14
15 metabolic aberrations have been observed in both type 1 and type 2 diabetic rat models, as well
16
17 as diabetic patients [4,5,7-9,13,14]
18
19
20
21

22 Progression of diabetes is associated with the development of secondary complications,
23
24 including cardiovascular disease, nephropathy, neuropathy, and retinopathy. As renal
25
26 dysfunction in diabetic patients develops, there is a shift from hypo- to hyperhomocysteinemia
27
28 and suppression of 5,10-methylenetetrahydrofolate reductase (MTHFR) activity that correlates
29
30 with severity of nephropathy [14,15]. Type 2 diabetics with nephropathy also have decreased
31
32 flux through the transsulfuration pathway, which likely contributes to a reduction in metabolic
33
34 homocysteine clearance [16]. Using the streptozotocin (STZ)-treated rat, we have reported
35
36 tissue-specific and sustained alterations of hepatic methyl group/ homocysteine metabolism
37
38 appeared to result in a functional methyl deficient condition, as evidenced by hepatic genomic
39
40 DNA hypomethylation [7]).
41
42
43
44
45

46 The progression of type 2 diabetes from prediabetes to the early diabetic state in the
47
48 Zucker (type 2) diabetic fatty (ZDF) rat with respect to methyl group and homocysteine
49
50 metabolism has been reported [9]; however, the more advanced diabetic condition has not been
51
52 fully characterized. Moreover, the consequences of perturbed methyl group metabolism on DNA
53
54 methylation has not been examined as a function of type 2 diabetes progression. Similar
55
56
57
58
59
60

alterations in methyl group metabolism have been reported when comparing type 1 and type 2 diabetes [14]. Therefore, we hypothesized that ZDF rats with advanced type 2 diabetes would have perturbed methyl group and homocysteine metabolism, including chronic upregulation of GNMT, and develop a methyl-deficient condition similar to type 1 diabetes [7]. Furthermore, due to the functional methyl deficiency, we hypothesized that the animals would have abnormal DNA methylation and expression of epigenetic regulatory proteins, similar to that observed in the STZ-diabetic rat [7] or rats fed methyl-deficient diets [16]. To date, abnormal DNA methylation as a function of type 2 diabetes has not been examined or reported.

Materials and Methods

Chemicals and reagents

Reagents were obtained as follows: 3T3 fully methylated DNA, *Bss*HII, *Hpa*II, and *Msp*I restriction enzymes, New England Biolabs, Inc. (Ipswich, MA); 5-³H]-dCTP, MP Biomedicals (Solon, OH); enhanced chemiluminescence Western blotting detection reagents and 5-¹⁴C]-methyl-THF, Amersham/GE Healthcare (Piscataway, NJ); CBS antibody (H-300, sc-67154), goat anti-rabbit and goat anti-chicken horseradish peroxidase secondary antibodies, Santa Cruz Biotechnology, Inc (Santa Cruz, CA); and *S*-adenosyl-L-³H]-methionine, Perkin Elmer Life Sciences (Waltham, MA). All other reagents were of analytical grade.

Animals

All animal protocols were approved by and conducted in accordance with the guidelines set forth by the Iowa State University Institutional Animal Care and Use Committee. For the first study, male rats were obtained at 11 wk of age, allowed to acclimate for 1 wk, and killed at 12 wk of age. For the second study, male rats were obtained at 10 wk of age and killed at 21 wk of age.

1
2
3 Each study utilized six lean (ZDF/Gmi +/?) and six ZDF (ZDF/Gmi *fa/fa*) rats (Charles River
4 Laboratories, Wilmington, MA) kept in individual cages with a 12-h light-dark cycle and given
5
6 *ad libitum* access to water and Purina 5008 diet. Following a 12-h fast, rats were anesthetized by
7
8 intraperitoneal injection with ketamine: xylazine (90: 10 mg/kg body weight, respectively),
9
10 whole blood samples were collected by cardiac puncture, and blood glucose levels were
11
12 immediately assessed using a glucometer. Plasma and serum samples were obtained by
13
14 centrifugation and stored at -20°C. Serum insulin levels were analyzed by Rat/Mouse Insulin
15
16 ELISA Kit (Linco, Inc., St Charles, MO). Tissues were rapidly removed and a portion of the
17
18 liver was immediately homogenized for preparation of cytosolic extracts as previously described
19
20 [7]. The remainder of the liver and all other tissues were frozen in liquid nitrogen and stored at -
21
22 70°C. The total soluble protein concentration of extracts was determined using the Bradford
23
24 assay with Coomassie Protein Plus Reagent (Thermo Scientific).
25
26
27
28
29
30

31 **Enzyme assays**

32
33 The enzymatic activity of GNMT, PEMT, and MS were determined using radioisotopic assays as
34
35 previously described [5-7,17,18]. Briefly, GNMT and MS activity in the liver, kidney, and heart
36
37 were determined by incubation of cytosolic extracts with reaction mixes containing *S*-adenosyl-
38
39 L-[³H-*methyl*]-methionine or 5-[¹⁴C]-methyl-THF, respectively. After terminating the reaction,
40
41 the unreacted radiolabeled substrate was removed using activated charcoal or anion exchange
42
43 resin, respectively. Using microsomal extracts, hepatic PEMT activity was assessed by
44
45 determining the incorporation of *S*-adenosyl- L-[³H-*methyl*]-methionine into the lipid fractions.
46
47
48
49
50
51
52 Liquid scintillation counting was used to determine radiolabel incorporation for all assays.

53 **Immunoblotting**

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Relative protein abundance was determined using methods previously described [7] with minor modifications. Proteins were separated using SDS-PAGE and subsequently transferred to nitrocellulose. For assessment of GNMT protein abundance, blots were incubated with an affinity-purified chicken antibody (Aves Labs, Inc., Tigard, OR) directed against the peptide sequence KER WNR RKE PAF DK (GNMT residues #97-110) diluted 1:40,000 in TTBS/ 10% BSA. CBS primary antibody was prepared 1:400 in TTBS/ 10% BSA, and goat anti-chicken or anti-rabbit horseradish peroxidase secondary antibodies were diluted 1:5,000 in TTBS. Following incubation with chemiluminescence reagents and subsequent exposure to film, relative protein abundance was quantified by densitometric analysis with QuantityOne software (Bio-Rad Laboratories, Hercules, CA).

DNA methylation

Using the method of Pogribny et al [19] and as described previously [7], digestion of DNA followed by cytosine extension was used for assessment of DNA methylation status in liver, kidney, and heart tissues. Global and CpG island DNA methylation were determined by digesting 1.0 μ g DNA using the methylation-sensitive restriction enzymes *HpaII* and *BssHII*, respectively. For the cytosine extension assay, a reaction mixture of the DNA digest, 10X PCR Buffer II (without $MgCl_2$), 25 mM $MgCl_2$, 0.5 U Amplitaq DNA Polymerase (Applied Biosystems), and [3H]-dCTP was incubated at 55°C for 1 h. Following incubation, samples were applied to Whatman DE-81 ion exchange filter paper and washed in 0.5 mM sodium phosphate buffer (pH 7.0) three times, dried, and 3H incorporation was assessed using liquid scintillation counting. Samples were run in duplicate and reactions using either *MspI*-digested DNA or mouse 3T3 fully-methylated DNA with the appropriate restriction enzyme were used as controls.

1
2
3 The degree of genomic methylation fell within the expected range of 70-90% [20] compared to
4 the *MspI* control.
5
6

7 8 **Real-time RT-PCR** 9

10 A 0.1 g sample of frozen liver was preserved in 1.0 mL RNALater-ICE (Ambion, Inc, Austin,
11 TX) and subsequently used for RNA isolation using Trizol Reagent (Invitrogen, Carlsbad, CA)
12 with TURBO DNA-free (Ambion, Inc.) DNase treatment. The reverse-transcription assay was
13 performed using iScript Select cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) and
14 the resulting cDNA was diluted 50-fold for use in the PCR reaction with iQ SYBR Green
15 Supermix (Bio-Rad Laboratories). The specific primers used for RT-PCR (Table 1) were
16 designed using PrimerQuest (Integrated DNA Technologies, Inc., Coralville, IA). Samples were
17 run in duplicate, data were normalized to 18S control, and results were analyzed using the
18 comparative C_t method.
19
20
21
22
23
24
25
26
27
28
29
30

31 **Statistical analysis** 32

33 For each time point, the mean values of each treatment group (lean vs. ZDF) were subjected to a
34 Student's *t* test (SigmaStat, SPSS, Chicago, IL). A Mann-Whitney rank sum test was used when
35 normality or equal variance test failed. Differences were considered significant when $P \leq 0.05$
36 using a one-tailed test.
37
38
39
40
41
42
43
44
45

46 **Results**

47 **Confirmation of a diabetic condition in ZDF rats** 48

49 All ZDF rats were hyperglycemic (blood glucose: 26.2 ± 1.9 and 30.2 ± 0.8 mM) compared to
50 control values (blood glucose: 12.1 ± 0.9 and 17.8 ± 1.1 mM) at 12 and 21 wk, respectively.
51
52
53 Likewise, serum insulin levels were elevated at 12 and 21 wk (0.63 ± 0.10 and 0.81 ± 0.13
54
55
56
57
58
59
60

1
2
3 ng/mL) compared to values (0.32 ± 0.03 and 0.44 ± 0.02 ng/mL), indicating a hyperinsulinemic
4
5
6 state.

8 **Perturbation of methyl group metabolism regulatory proteins in the ZDF rat**

9
10 Diabetes produced tissue- and time-specific changes in the activity (Table 2) and expression
11
12 (Table 3) of a number of key enzymes involved in methyl group metabolism. GNMT and PEMT
13
14 are key proteins in the regulation of methyl group and homocysteine balance. The activity and
15
16 protein abundance of GNMT were elevated in the liver of ZDF rats compared to lean controls at
17
18 both 12 and 21 wk of age (Table 2). In support of this finding, GNMT mRNA abundance was
19
20 both 12 and 21 wk of age (Table 2). In support of this finding, GNMT mRNA abundance was
21
22 also increased in the liver at 21 wk (Table 3). Renal GNMT activity was increased at 21 wk
23
24 (Table 2); however, a **corresponding** increase in renal GNMT protein abundance was not
25
26 observed, suggesting possible post-translational regulation of GNMT activity in the diabetic rat
27
28 kidney. Contrary to our previous results using the type 1 diabetic rat, hepatic PEMT activity was
29
30 unchanged in the ZDF rat at 12 wk and enzyme activity was decreased at 21 wk (lean vs. ZDF:
31
32 88 ± 10 vs. 62 ± 8 pmol/min·mg protein, $P = 0.035$). However, similar to the increase in PEMT
33
34 activity noted in the type 1 diabetic rat (6), there was a trend ($P < 0.10$) towards increased
35
36 hepatic PEMT mRNA abundance at 12 wk and a statistically significant 73% increase in PEMT
37
38 mRNA abundance in ZDF rat liver at 21 wk (Table 3).

39
40
41
42
43 Removal of homocysteine via folate/B₁₂-dependent and -independent remethylation (MS
44
45 and BHMT, respectively), or catabolism by the transsulfuration pathway (CBS), was
46
47 significantly altered in the ZDF rat **that was highly dependent on age and was tissue-specific**. In
48
49 agreement with the type 1 diabetic rat (4), BHMT mRNA abundance was increased in the ZDF
50
51 rat liver at 21 wk (Table 3). In the early diabetic condition (12 wk), hepatic MS activity was
52
53 significantly increased (Table 2), supported by a similar trend for MS mRNA abundance (Table
54
55
56
57
58
59
60

1
2
3 3); however, no significant differences were observed in the liver at 21 wk, or in renal MS
4
5 activity. CBS did not show any changes in mRNA abundance (Table 3), but there was a tissue-
6
7 specific effect on protein abundance (Table 2). Similar to observations from the STZ-diabetic rat
8
9 (7), CBS protein abundance was greater in the liver, whereas it was decreased in the kidney
10
11 when ZDF rats were compared to lean controls at 12 wk. At 21 wk, hepatic CBS abundance was
12
13 reduced in the ZDF rat, and no significant difference was observed in renal CBS protein
14
15 abundance between ZDF and lean rats. For heart tissue, MS activity was increased and CBS
16
17 abundance was decreased 12% in ZDF rats relative to controls (Table 2) at 21 wk. In both the
18
19 early and advanced diabetic conditions, cardiac GNMT protein abundance was similar between
20
21 diabetic rats and controls; however, no appreciable cardiac GNMT activity was detected.
22
23
24
25
26

27 **Perturbation of homocysteine balance in the ZDF rat**

28
29 Plasma total homocysteine concentrations were decreased 67 and 54% in ZDF rats at 12 and 21
30
31 wk, respectively, compared to the lean controls (Table 4). There were no changes in the hepatic
32
33 concentration of homocysteine at either time point. However, homocysteine concentrations in
34
35 the kidney reflected the changes in plasma homocysteine, exhibiting a significant decrease of 63
36
37 and 49% in the ZDF kidney at 12 and 21 wk, respectively.
38
39
40

41 **DNA hypermethylation and upregulation of DNMT1 in the type 2 diabetic rat liver**

42
43 In contrast to the development of hepatic global DNA hypomethylation in the advanced STZ-
44
45 diabetic rat (7), global DNA hypermethylation was characteristic of ZDF rat liver at both 12 and
46
47 21 wk (Figure 2). This effect was specific to genomic DNA in the liver; global DNA changes
48
49 were not observed in the kidney (Figure 2) and heart, nor in CpG island methylation status as
50
51 measured by the overall incorporation of [³H]-dCTP. DNMT1 mRNA abundance was increased
52
53 95% in the ZDF rat liver at 21 wk (Table 3). No significant changes were observed in the
54
55
56
57
58
59
60

1
2
3 mRNA abundance of other key nuclear proteins involved in DNA methylation, including DNMT
4
5 3a and 3b, as well as MBD 1,2,3,4 and MeCP2 (data not shown).
6
7
8
9

10 **Discussion**

11
12 Here, we demonstrate that during the progression of type 2 diabetes in the ZDF rat there are
13
14 tissue-specific alterations of methyl group and homocysteine metabolism consistent with type 1
15
16 diabetes [4-7], accompanied by concomitant changes in epigenetic marks, namely the
17
18 methylation of DNA. However, unlike the effects of the chronic type 1 diabetic condition in
19
20 which we observed hepatic genomic DNA hypomethylation [7], genomic DNA was
21
22 hypermethylated in the ZDF rat liver, even in the early (12 wk) stages of the disease. In early
23
24 diabetes, both ZDF and type 1-diabetic rat models have been shown to exhibit similar
25
26 perturbations in hepatic methyl group and homocysteine metabolism [4-9], consistent with our
27
28 observations with ZDF rats at 12 wk of age. The net effect of these metabolic perturbations may
29
30 result in the loss of methyl groups via induction of GNMT and an increase in the flux through the
31
32 transsulfuration pathway, although this was not directly measured. This might be anticipated
33
34 based on the abundance of evidence supporting GNMT as a regulator of the SAM:SAH ratio
35
36 [22], and the known induction of CBS expression and activity by glucocorticoids and SAM,
37
38 respectively [23,24]. The upregulation of CBS may be a compensatory mechanism for self-
39
40 protection of the liver and kidney against the oxidative stress associated with a diabetic
41
42 condition. CBS activity has been shown to be enhanced under conditions of oxidative stress [25]
43
44 and the antioxidant glutathione, a by-product of the transsulfuration pathway, has been reported
45
46 to be depleted in diabetic tissues [26].
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

It appears that the pathogenesis of type 2 diabetes from 12 to 21 wk results in further alterations in methyl group metabolism. The induction of GNMT at 21 wk was not as robust as that observed for 12 wk with only a 19 and 8% increase in activity and protein abundance, respectively. However, there was a significant increase in BHMT mRNA abundance at 21 wk that was not observed in the early (12 wk) diabetic condition. For diabetes, increases in BHMT mRNA abundance have previously been associated with increased BHMT activity [12], suggesting enhanced homocysteine remethylation. Interestingly, PEMT was upregulated at the mRNA level, whereas PEMT activity was decreased at 21 wk. Taken together, a decrease in production of homocysteine by PEMT and GNMT, combined with an increase in BHMT activity, might be expected to contribute to the hypohomocysteinemia that persisted in the advanced diabetic state despite no change in CBS protein abundance. **Others have shown that diabetes also increases homocysteine catabolism via transsulfuration [9,12].**

The response of the kidney to a diabetic condition appears to be less stringently regulated compared to the liver. Whereas hepatic enzymes relevant to methyl group and homocysteine metabolism appear to be regulated at a transcriptional and/or translational level during diabetes progression, there was a lesser impact on these enzymes in the kidney. Thus, a diabetic condition appears to have a direct impact on hepatic methyl group metabolism via alterations in gene expression, whereas renal methyl group metabolism **may** reflect the diminished supply of methyl groups from the liver. Renal GNMT activity was only increased in the more advanced diabetic state (21 wk) and there were not concomitant increases in protein abundance. We have previously established that GNMT regulation is tissue-specific in response to retinoids [26], as well as diabetes [7]. Aside from the increase in GNMT activity, there were no observed alternations of other methyl group-related enzymes, nor was DNA methylation status affected in

1
2
3 the kidney. House et al [27] also found a lack of effect of either glucagon treatment or STZ-
4
5 diabetes on renal methyl group metabolism, whereas Jacobs et al [8] reported that renal MS and
6
7 MTHFR activities were suppressed by a type 1 diabetic condition.
8
9

10 Homocysteine balance within a tissue, and subsequently the circulation, reflects the
11
12 collective metabolic routes linked to its production, remethylation, and catabolism. The liver is
13
14 unique in that it contains the full complement of pathways involved in methyl group metabolism.
15
16 The kidney also exhibits all of the pathways [28]; however, BHMT is expressed at low levels in
17
18 the rat kidney compared to human tissue [29] and MS activity was found not to be quantitatively
19
20 significant [28,30], resulting in transsulfuration being the primary route for removal of
21
22 homocysteine in the kidney. Homocysteine levels in the ZDF liver were not different from
23
24 controls at 12 or 21 wk, despite decreased homocysteine concentrations in both the plasma and
25
26 kidney. It is unlikely that the low levels of homocysteine in the kidney are due to increased
27
28 urinary excretion, because this was shown not to be a significant route for homocysteine removal
29
30 in healthy or STZ-diabetic kidneys [8,30]. However, this may be different for the ZDF rat,
31
32 where glomerular filtration rate was elevated at 3 months of age [31]. Thus, the reduction in
33
34 renal and plasma homocysteine concentrations may be the result of increased excretion in the
35
36 ZDF rat model. The kidney has been shown to contribute significantly to the removal of
37
38 homocysteine from the circulation [32] and data suggests it contains adequate CBS to
39
40 compensate for acute or chronic increases in plasma homocysteine [28]. Taken together, it
41
42 appears that the persistent hypohomocysteinemia observed in type 2 diabetes rat models might
43
44 be due to an irreversible loss of homocysteine via the renal transsulfuration pathway and/ or
45
46 excretion. In contrast, homocysteine may be remethylated to methionine in the kidney owing to
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 a markedly elevated expression of BHMT in humans compared to the rat, thereby conserving the
4
5 homocysteine moiety and contributing to the eventual development of hyperhomocysteinemia.
6
7

8 The observation of global DNA hypermethylation in the livers of ZDF rats was
9
10 particularly intriguing and completely divergent from our previous observations in the type 1
11
12 diabetes [7]. Although DNA hypomethylation has been shown to be associated with a number of
13
14 pathological conditions, hypermethylation of DNA can also have deleterious effects. DNA
15
16 methylation at cytosine residues within CpG dinucleotides is an important modulator of
17
18 chromatin structure, repressor of transposable elements, and regulator of gene expression
19
20 [33,34]. The CpG sequence represents only 1-4% of the genome and this is proposed to be due
21
22 to selection against the sequence due to its high potential for deleterious effects [35]. An
23
24 increase in the levels of 5-methylcytosine elevates the chance of point mutations throughout the
25
26 genome by spontaneous deamination [36], whereby an unmethylated cytosine base is deaminated
27
28 to uracil and deamination of methylated cytosine produces thymine. If undetected by DNA
29
30 repair enzymes prior to replication, the transition mutation will be maintained and carried on to
31
32 daughter cells, thereby contributing to genomic instability. Moreover, induction of genomic
33
34 DNA hypermethylation owing to overexpression of DNMT1 was associated with chromosomal
35
36 overcondensation, as well as chromosomal rearrangement and misalignment of sister chromatids
37
38 [37], which could be expected to alter chromosomal segregation during cell division. Genomic
39
40 hypermethylation has been associated with increased methylation at histone 3 lysine 9 [37], a
41
42 finding that was also observed in lymphocytes from type 1 diabetic patients and associated with
43
44 the promoter regions of a number of genes involved in inflammation, which may contribute to
45
46 the development of secondary complications [38]. Likewise, one of the few reports of genomic
47
48 DNA hypermethylation in clinical studies found that leukocytic DNA hypermethylation was
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 associated with increased inflammation and increased mortality in patients with chronic kidney
4 disease [39].
5
6

7
8 The pathophysiology of ZDF rats is characterized by obesity, hyperglycemia,
9 hyperinsulinemia, hyperlipidemia, and hypertension [40]). ZDF rats develop hyperinsulinemia
10 and insulin resistance before 7 wk of age, with an incremental drop in elevated insulin levels
11 around 8 wk of age, and at 12 wk of age blood glucose levels rise, resulting in animals becoming
12 overtly diabetic [41]. Although insulin levels continue to decrease due to pancreatic β -cell
13 exhaustion, ZDF rats may remain hyperinsulinemic relative to lean controls well into the
14 advanced diabetic stage [21], as was observed in this study. In contrast, chemically-induced type
15 1 diabetes also results in hyperglycemia, but is characterized by a lack of insulin production.
16
17 Treatment of rats or hepatic cell lines with glucocorticoids, such as dexamethasone or
18 triamcinolone, has also been shown to induce expression of methionine adenosyltransferase
19 (MAT), GNMT, BHMT, and CBS at the transcriptional level and insulin administration has been
20 shown to prevent these alterations in both rats and/or cell lines [4,11,12,42,43]. Interestingly,
21 insulin stimulates transmethylation, and transsulfuration flux in healthy individuals [44]. Insulin
22 treatment of untreated HepG2 hepatocarcinoma cells was also capable of inducing MAT activity
23 [45]; thus, insulin likely plays a role in the metabolic perturbations observed in the early type 2
24 diabetic state. Treatment of HepG2 cells with glucose also resulted in stimulation of MAT
25 activity and induced genomic hypermethylation [45]. Several recent cell culture studies that
26 were designed to mimic hyperglycemia have reported histone modifications which persist even
27 after restoration of normal glucose concentrations [38,46-48]. Based on genome-wide profiling
28 of specific histone modifications, these studies implicate hyperglycemia in the epigenetic
29 regulation of pathways involved in signal transduction, oxidative stress, immune function, and
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 inflammation. The bulk of evidence indicates that aberrations of methyl group metabolism and
4
5 epigenetic regulation are likely due to the combination of hyperglycemia, a lack of insulin or
6
7 insulin resistance, and elevated counter-regulatory hormones. In support of this, we have recently
8
9 demonstrated that insulin administration prevents many of the alterations in methyl group and
10
11 homocysteine metabolism, including the hypomethylation of DNA, in a type 1 diabetes rat
12
13 model [49]. The differential response in our observed changes in methyl group metabolism as a
14
15 function of type 1 vs. type 2 diabetes may reside in differences in metabolic signals that are
16
17 characteristic of each diabetic state. In both human patients and animal models, C peptide is
18
19 increased in type 2 diabetes, but markedly decreased in type 1 diabetes [49]. C peptide has been
20
21 shown to exhibit a number of insulinomimetic properties, be involved in cell signaling, and has
22
23 been implicated in the development of vascular inflammation and atherosclerosis in type 2
24
25 diabetes [49]. However, the actions of C peptide in type 2 diabetics are largely uncharacterized
26
27 and may provide an opportunity for investigation into the pathological differences between type
28
29 1 and type 2 diabetes, particularly with respect to methyl group metabolism.
30
31
32
33
34
35

36 In summary, we have shown that the metabolism of methyl groups and homocysteine
37
38 were altered in a tissue-specific manner during the pathogenesis of type 2 diabetes in the ZDF
39
40 rat. Although there are many commonalities in the regulation of methyl group and homocysteine
41
42 metabolism in type 1 and type 2 diabetes, the impact on epigenetic regulation (i.e., DNA
43
44 methylation) was divergent between the two diabetic conditions. Abnormalities of enzymatic
45
46 regulation and key metabolite concentrations have been observed as early as 5 wk of age [9] and
47
48 we have shown hepatic DNA hypermethylation was evident at 12 wk of age in ZDF rats. Data
49
50 are lacking prior to these time points; thus, it is unclear when abnormalities in methyl group
51
52 metabolism and epigenetic regulation are initiated in the ZDF rat. However, it is evident that
53
54
55
56
57
58
59
60

1
2
3 perturbations of methyl group metabolism and aberrant DNA methylation represent early events
4
5 in the pathogenesis of type 2 diabetes with potentially persistent effects owing to the stable
6
7 nature of epigenetic marks. The role of DNA methylation in diabetes and its potential relation to
8
9 diabetic complications, such as nephropathy [50], is clearly an important and emerging field.
10
11

12 13 14 15 **Acknowledgements**

16
17 This work was supported by a grant from the American Heart Association (0755760Z). Dr. K.T.
18
19 Williams is currently a postdoctoral fellow at HudsonAlpha Institute for Biotechnology,
20
21 Huntsville, AL.
22
23

24 25 26 27 28 **Conflict of Interest**

29
30
31
32 None.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

1. Campbell RK. Type 2 diabetes: where we are today: an overview of disease burden, current treatments, and treatment strategies. *J Am Pharm Assoc* 2009; **49**:S3-9.
2. Williams KT, Schalinske KL. New insights into the regulation of methyl group and homocysteine metabolism. *J Nutr* 2007; **137**:311-314.
3. Wijekoon EP, Brosnan ME, Brosnan JT. Homocysteine metabolism in diabetes. *Biochem Soc Trans* 2007; **35**:1175-1179.
4. Nieman KM, Rowling MJ, Garrow TA, *et al.* Modulation of methyl group metabolism by streptozotocin-induced diabetes and all-trans-retinoic acid. *J Biol Chem* 2004; **279**:45708-45712.
5. Nieman KM, Hartz CS, Szegedi SS, *et al.* Folate status modulates the induction of hepatic glycine *N*-methyltransferase and homocysteine metabolism in rats. *Am J Physiol Endocrinol Metab* 2006; **291**:E1235-1242.
6. Hartz CS, Nieman KM, Jacobs RL, *et al.* Hepatic phosphatidylethanolamine *N*-methyltransferase expression is increased in diabetic rats. *J Nutr* 2006; **136**:3005-3009.
7. Williams KT, Garrow TA, Schalinske KL. Type I diabetes leads to tissue-specific DNA hypomethylation in male rats. *J Nutr* 2008; **138**:2064-2069.
8. Jacobs RL, House JD, Brosnan ME, *et al.* Effects of streptozotocin-induced diabetes and of insulin treatment on homocysteine metabolism in the rat. *Diabetes* 1998; **47**:1967-1970.
9. Wijekoon EP, Hall B, Ratnam S, *et al.* Homocysteine metabolism in ZDF (type 2) diabetic rats. *Diabetes* 2005; **54**:3245-3251.

10. Stead LM, Brosnan JT, Brosnan ME, *et al.* Is it time to reevaluate methyl balance in humans? *Am J Clin Nutr* 2006; **83**:5-10.
11. Ratnam S, Maclean KN, Jacobs RL, *et al.* Hormonal regulation of cystathionine beta-synthase expression in liver. *J Biol Chem* 2002; **277**:42912-4918.
12. Ratnam S, Wijekoon EP, Hall B, *et al.* Effects of diabetes and insulin on betaine-homocysteine S-methyltransferase expression in rat liver. *Am J Physiol Endocrinol Metab* 2006; **290**:E933-939.
13. Abu-Lebdeh HS, Barazzoni R, Meek SE, *et al.* Effects of insulin deprivation and treatment on homocysteine metabolism in people with type 1 diabetes. *J Clin Endocrinol Metab* 2006; **91**:3344-3348.
14. Poirier LA, Brown AT, Fink LM, *et al.* Blood S-adenosylmethionine concentrations and lymphocyte methylenetetrahydrofolate reductase activity in diabetes mellitus and diabetic nephropathy. *Metabolism* 2001; **50**:1014-1018.
15. Herrmann W, Schorr H, Obeid R, *et al.* Disturbed homocysteine and methionine cycle intermediates S-adenosylhomocysteine and S-adenosylmethionine are related to degree of renal insufficiency in type 2 diabetes. *Clin Chem* 2005; **51**:891-897.
16. Ghoshal K, Li X, Datta J, *et al.* A folate- and methyl-deficient diet alters the expression of DNA methyltransferases and methyl CpG binding proteins involved in epigenetic gene silencing in livers of F344 rats. *J Nutr* 2006; **136**:1522-1527.
17. Cook RJ, Wagner C. Glycine N-methyltransferase is a folate binding protein of rat liver cytosol. *Proc Natl Acad Sci U S A* 1984; **81**:3631-3634.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
18. Duce AM, Ortiz P, Cabrero C, *et al.* S-adenosyl-L-methionine synthetase and phospholipid methyltransferase are inhibited in human cirrhosis. *Hepatology* 1988; **8**:65-68.
 19. Pogribny I, Yi P, James SJ. A sensitive new method for rapid detection of abnormal methylation patterns in global DNA and within CpG islands. *Biochem Biophys Res Comm* 1999; **262**:624-628.
 20. Ehrlich M, Gama-Sosa MA, Huang LH, *et al.* Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells. *Nucleic Acids Res* 1982; **10**:2709-2721.
 21. Schmidt RE, Dorsey DA, Beaudet LN, *et al.* Analysis of the Zucker Diabetic Fatty (ZDF) type 2 diabetic rat model suggests a neurotrophic role for insulin/IGF-I in diabetic autonomic neuropathy. *Am J Pathol* 2003; **163**:21-128.
 22. Luka Z, Mudd SH, Wagner C. Glycine N-methyltransferase and regulation of S-adenosylmethionine levels. *J Biol Chem* 2009; **284**:22507-22511.
 23. Finkelstein JD, Kyle WE, Martin JJ. Abnormal methionine adenosyltransferase in hypermethioninemia. *Biochem Biophys Res Commun* 1975; **66**:1491-1497.
 24. Goss SJ: Characterization of cystathionine synthase as a selectable, liver specific trait in rat hepatomas. *J Cell Sci* 1986; **82**:309 –320.
 25. Zou CG, Banerjee R. Homocysteine and redox signaling. *Antioxid Redox Signal* 2005; **7**:547-559.
 26. McMullen MH, Rowling MJ, Ozias MK, *et al.* Activation and induction of glycine N-methyltransferase by retinoids are tissue- and gender-specific. *Arch Biochem Biophys* 2002; **401**:73-80.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
27. House JD, Brosnan ME, Brosnan JT. Renal uptake and excretion of homocysteine in rats with acute hyperhomocysteinemia. *Kidney Int* 1998; **54**:1601-1607.
 28. Finkelstein JD. Pathways and regulation of homocysteine metabolism in mammals. *Semin Thromb Memost* 2000; **26**:219-225.
 29. Delgado-Reyes CV, Wallig MA, Garrow TA. Immunohistochemical detection of betaine-homocysteine S-methyltransferase in human, pig, and rat liver and kidney. *Arch Biochem Biophys* 2001; **393**:184-186.
 30. House JD, Brosnan ME, Brosnan JT. Characterization of homocysteine metabolism in the rat kidney. *Biochem J* 1997; **328**:287-292.
 31. Hoshi S, Yoshida F, Ingaki T, *et al.* Podocyte injury promotes progressive nephropathy in Zucker diabetic fatty rats, *Lab Invest* 2002; **82**, 25-35.
 32. Garibotto G, Valli A, Anderstam B, *et al.* The kidney is the major site of S-adenosylhomocysteine disposal in humans. *Kidney Int* 2009; **76**:293-296.
 33. Dunn BK. Hypomethylation: one side of a larger picture. *Ann NY Acad Sci* 2003; **983**:28-42.
 34. Lande-Diner L, Zhang J, Ben-Porath I, *et al.* Role of DNA methylation in stable gene repression. *J Biol Chem* 2007; **282**:12194-12200.
 35. Lindahl T. Instability and decay of the primary structure of DNA. *Nature* 1993; **362**:709-715.
 36. Duncan BK, Miller JH. Mutagenic deamination of cytosine residues in DNA. *Nature* 1980; **287**:560-561.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
37. Weissman F, Muyrers-Chen I, Musch T, *et al.* DNA hypermethylation in *Drosophila melanogaster* causes irregular chromosome condensation and dysregulation of epigenetic histone modifications. *Mol Cell Biol* 2003; **23**:2577-2586.
38. Miao F, Smith DD, Zhang L, *et al.* Lymphocytes from patients with type 1 diabetes display a distinct profile of chromatin histone H3 lysine 9 dimethylation: an epigenetic study in diabetes. *Diabetes* 2008; **57** :3189-3198.
39. Stenvinkel P, Karimi M, Johansson S, *et al.* Impact of inflammation on epigenetic DNA methylation – a novel risk factor for cardiovascular disease? *J Intern Med* 2007; **261**:488-499.
40. Chen D, Wang MW. Development and application of rodent models for type 2 diabetes. *Diabetes Obes Metab* 2005; **7**:307-317.
41. Etgen GJ, Oldham BA. Profiling of Zucker diabetic fatty rats in their progression to the overt diabetic state. *Metabolism* 2000; **49**:684-688.
42. Gil B, Pajares MA, Mato JM, *et al.* Glucocorticoid regulation of hepatic S-adenosylmethionine synthetase gene expression. *Endocrinology* 1997; **138**:1251-8.
43. Rowling MJ, Schalinske KL. Retinoic acid and glucocorticoid treatment induce hepatic glycine N-methyltransferase and lower plasma homocysteine concentrations in rats and rat hepatoma cells. *J Nutr* 2003; **133**:3392-3398.
44. Tessari P, Kiwanuka E, Coracina A, *et al.* Insulin in methionine and homocysteine kinetics in healthy humans: plasma vs. intracellular models. *Am J Physiol Endocrinol Metab* 2005; **288**:E1270-1276.
45. Chiang EP, Wang YC, Chen WW, *et al.* Effects of insulin and glucose on cellular metabolic fluxes in homocysteine transsulfuration, remethylation, S-adenosylmethionine

- 1
2
3 synthesis, and global deoxyribonucleic acid methylation. *J Clin Endocrinol Metab* 2009;
4
5 **94**:1017-1025.
6
7
- 8 46. Brasacchio D, Okabe J, Tikellis C, Balcerczyk A, George P, Baker EK, Calkin AC,
9
10 Brownlee M, Cooper ME, El-Osta A. Hyperglycemia induces a dynamic cooperativity of
11
12 histone methylase and demethylase enzymes associated with gene-activating epigenetic
13
14 marks that co-exist on the lysine tail. *Diabetes* 2009; **58**:1229-1236.
15
16
- 17 47. Miao F, Wu X, Zhang L, Yuan YC, Riggs AD, Natarajan R. Genome-wide analysis of
18
19 histone lysine methylation variations caused by diabetic conditions in human monocytes.
20
21 *J Biol Chem* 2007; **282**:13854-13863.
22
23
- 24 48. Villeneuve LM, Reddy MA, Lanting LL, Wang M, Meng L, Natarajan R. Epigenetic
25
26 histone H3 lysine 9 methylation in metabolic memory and inflammatory phenotype of
27
28 vascular smooth muscle cells in diabetes. *Proc Natl Acad Sci U S A* 2008; **105**:9047-
29
30 9052.
31
32
- 33 49. Nieman KM, Schalinske KL. Insulin administration abrogates perturbation of methyl
34
35 group and homocysteine metabolism in streptozotocin-treated type 1 diabetic rats. *Am J*
36
37 *Physiol Endocrinol Metab* July 5, 2011, published ahead of print, doi:
38
39 [10.1152/ajpendo.00317.2011](https://doi.org/10.1152/ajpendo.00317.2011).
40
41
42
- 43 50. Hills CE, Brunskill NJ. Cellular and physiological effects of C-peptide. *Clin Sci (Lond)*
44
45 2009; **116**:565-574.
46
47
- 48 51. Bell CG, Teschendorff AE, Rakyan VK, Maxwell AP, Beck S, Savage DA. Genome-
49
50 wide DNA methylation analysis for diabetic nephropathy in type 1 diabetes mellitus.
51
52 *BMC Med Genomics* 2010; **3**:33.
53
54
55
56
57
58
59
60

Figure Legends

Figure 1. Methyl group and homocysteine metabolism. Enzymes are shown in black boxes, whereas vitamin substrates and/or cofactors are shown in gray boxes. Abbreviations are: betaine-homocysteine *S*-methyltransferase (BHMT); cystathionine β -synthase (CBS); dimethylglycine (DMG); methionine synthase (MS); methyltransferases (MTs); 5,10-methylene-THF reductase (MTHFR); *S*-adenosylhomocysteine (SAH); *S*-adenosylmethionine (SAM); tetrahydrofolate (THF); and methyl acceptor (X). Key SAM-dependent methyltransferases include: glycine *N*-methyltransferase (GNMT); phosphatidylethanolamine *N*-methyltransferase (PEMT); and DNA methyltransferase 1 (DNMT1). These three methyltransferases respectively catalyze the conversion of glycine to sarcosine, phosphatidylethanolamine to phosphatidylcholine, and cytosine-DNA to 5-methyl-cytosine-DNA . In addition to THF, this series of interrelated pathways are dependent on a number of other B-vitamins, including riboflavin (B₂), vitamin B₆, and vitamin B₁₂.

Figure 2. Hepatic and renal methylation status of genomic DNA in lean (open bars) and ZDF (black bars) rats at (A) 12 and (B) 21 wk of age. Using the method of Pogribny et al [19], incorporation of [³H]-dCTP is inversely related to the degree of endogenous methylation. Values are mean \pm SEM (n = 6). *Different from control, $P \leq 0.05$.

Table 1. Real-time RT-PCR primers

Target	Primers (5' → 3')
GNMT	F*: ACA ACA AAG CCC ACA TGG TAA CCC R: AGC CGA AAC TTA CTG AAG CCA GGA
PEMT	F: TGT GCT CTC CAG CTT CTA TGC ACT R: AGG GAA ATG TGG TCA CTC TGG ACT
MS	F: TTG GCC TAC CGG ATG AAC AAA TGC R: AGC CAC AAA CCT CTT GAC TCC TGT
BHMT	F: ATC TGG GCA GAA GGT CAA TGA AGC R: TGA CTC ACA CCT CCT GCA ACC AAT
CBS	F: AAC ATG TTG TCC TCC CTG CTT GCT R: TCG GCT TGA ACT GCT TGT AGA GGA
DNMT1	F: TGT GGC AAG AAG AAA GGT GGC AAG R: TGG ATG GAC TTG TGG GTG TTC TCA
DNMT3a	F: AGA GTG TCT GGA ACA CGG CAG AAT R: TGC TGG TCT TTG CCC TGC TTT ATG
DNMT3b	F: TGC GCC TGC AAG ACT TCT TCA CTA R: TGC AGG AAT CGC TGG GTA CAA CTT
MBD1	F: CCT GCA CCT TTG TGC TGT GAG AAT R: CAG TCT TTG CAC AAT GTC CTG CGT
MBD2	F: TCA GAA GTA AAC CAC AGC TGG CGA R: ACT AGG CAT CAT CTT GCC GCT TCT

1
2
3 MBD3 F: GAA GAA GTT TCG CAG CAA GCC ACA
4
5 R: CAT CTT TCC CGT GCG GAA ATC GAA
6
7
8 MBD4 F: AGC TAA ACC TCA GGA CAC GAA GCA
9
10 R: TTG GAC AGGCTG TTG CTA TCT GGA
11
12
13 MeCP2 F: GCA GCA GCA TCA GAA GGT GTT CAA
14
15 R: TGC TTG GAA AGG CAT CTT GAC GAG
16
17
18 18S F: GAA CCA GAG CGA AAG CAT TTG CCA
19
20 R: ATG GTC GGA ACT ACG ACG GTA TCT
21

22 *F denotes forward primer, R denotes reverse primer.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 2. Tissue-specific activity and abundance of enzymes regulating methyl group and homocysteine metabolism in lean (+/?) and ZDF (*fa/fa*) rats at 12 and 21 wk of age

	Tissue	12 wk		21 wk	
		Lean	ZDF	Lean	ZDF
GNMT Activity (pmol/(min • mg protein))	Liver	305 ± 17	381 ± 7*	273 ± 8	324 ± 13*
	Kidney	45 ± 1	43 ± 3	54 ± 5	73 ± 3*
	Heart	n.d.	n.d.	n.d.	n.d.
GNMT Abundance Relative to control	Liver	1.00 ± 0.14	1.65 ± 0.10*	1.00 ± 0.03	1.08 ± 0.02*
	Kidney	1.00 ± 0.10	0.80 ± 0.11	1.00 ± 0.07	0.98 ± 0.07
	Heart	1.00 ± 0.01	0.99 ± 0.01	1.00 ± 0.04	0.99 ± 0.05
MS activity (pmol/(min • mg protein))	Liver	416 ± 40	498 ± 21*	541 ± 29	562 ± 40
	Kidney	886 ± 47	976 ± 15	809 ± 34	746 ± 22
	Heart	375 ± 13	359 ± 7	286 ± 20	343 ± 22*
CBS Abundance Relative to control	Liver	1.00 ± 0.09	1.27 ± 0.05*	1.00 ± 0.09	0.78 ± 0.06*
	Kidney	1.00 ± 0.11	0.67 ± 0.11*	1.00 ± 0.08	1.02 ± 0.07
	Heart	1.00 ± 0.14	0.81 ± 0.16	1.00 ± 0.03	0.88 ± 0.05*

Data are means ± SEM (n = 4-6). The abbreviation n.d. indicates activity was not detected.

Asterisks (*) indicate a significant difference from control, $P \leq 0.05$.

Table 3. Hepatic mRNA abundance of enzymes regulating methyl group supply and utilization in lean (+/?) and ZDF (*fa/fa*) rats at 12 and 21 wk of age.

Target	12 weeks, mean fold induction		21 weeks, mean fold induction	
	Lean	ZDF	Lean	ZDF
GNMT	1.00 ± 0.55	1.03 ± 0.23	1.00 ± 0.13	1.36 ± 0.16*
PEMT	1.00 ± 0.40	3.31 ± 1.20	1.00 ± 0.18	1.73 ± 0.21*
MS	1.00 ± 0.15	1.91 ± 0.58	1.00 ± 0.27	1.30 ± 0.24
BHMT	1.00 ± 0.49	1.64 ± 0.47	1.00 ± 0.22	2.06 ± 0.54*
CBS	1.00 ± 0.54	0.77 ± 0.29	1.00 ± 0.28	1.10 ± 0.20
DNMT1	1.00 ± 0.23	0.97 ± 0.33	1.00 ± 0.29	1.95 ± 0.37*

Data are means ± SEM (n = 4-6). Asterisks (*) indicate a significant difference from control, $P \leq 0.05$.

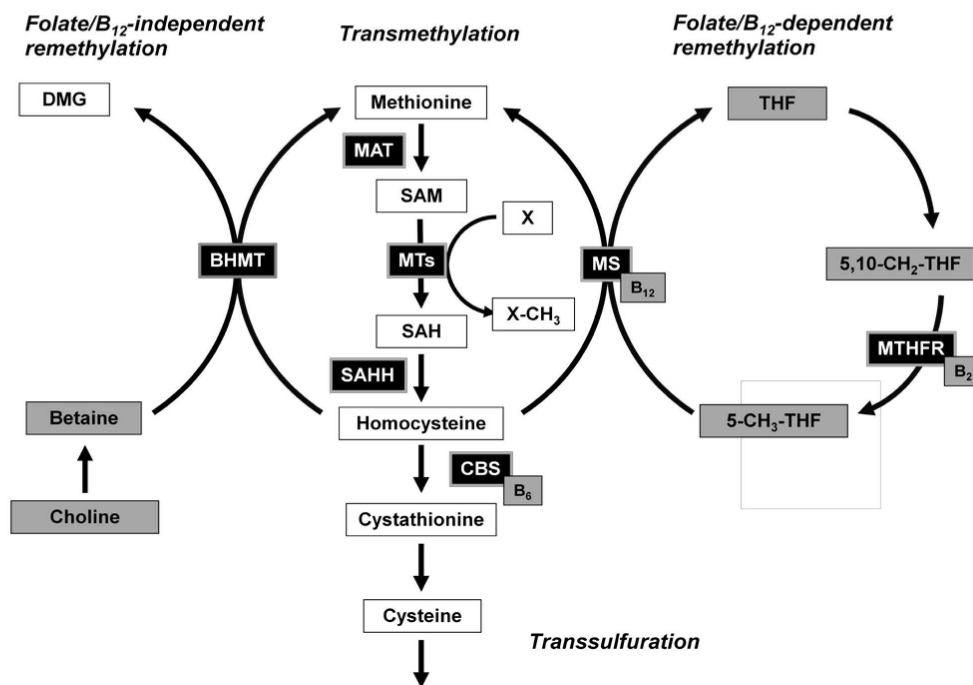
Table 4. Fasted plasma and tissue concentrations of total homocysteine in lean (+/?) and ZDF (*fa/fa*) rats.

Sample source	Age (wk)	Total Homocysteine (μM plasma or $\mu\text{mol/g}$ tissue)	
		Lean	ZDF
Plasma	12	6.8 ± 0.3	$2.2 \pm 0.1^*$
	21	3.6 ± 0.3	$1.6 \pm 0.1^*$
Liver	12	155 ± 22	166 ± 10
	21	168 ± 7	203 ± 20
Kidney	12	11.0 ± 4.1	$4.1 \pm 0.7^*$
	21	6.3 ± 0.9	$3.2 \pm 1.1^*$

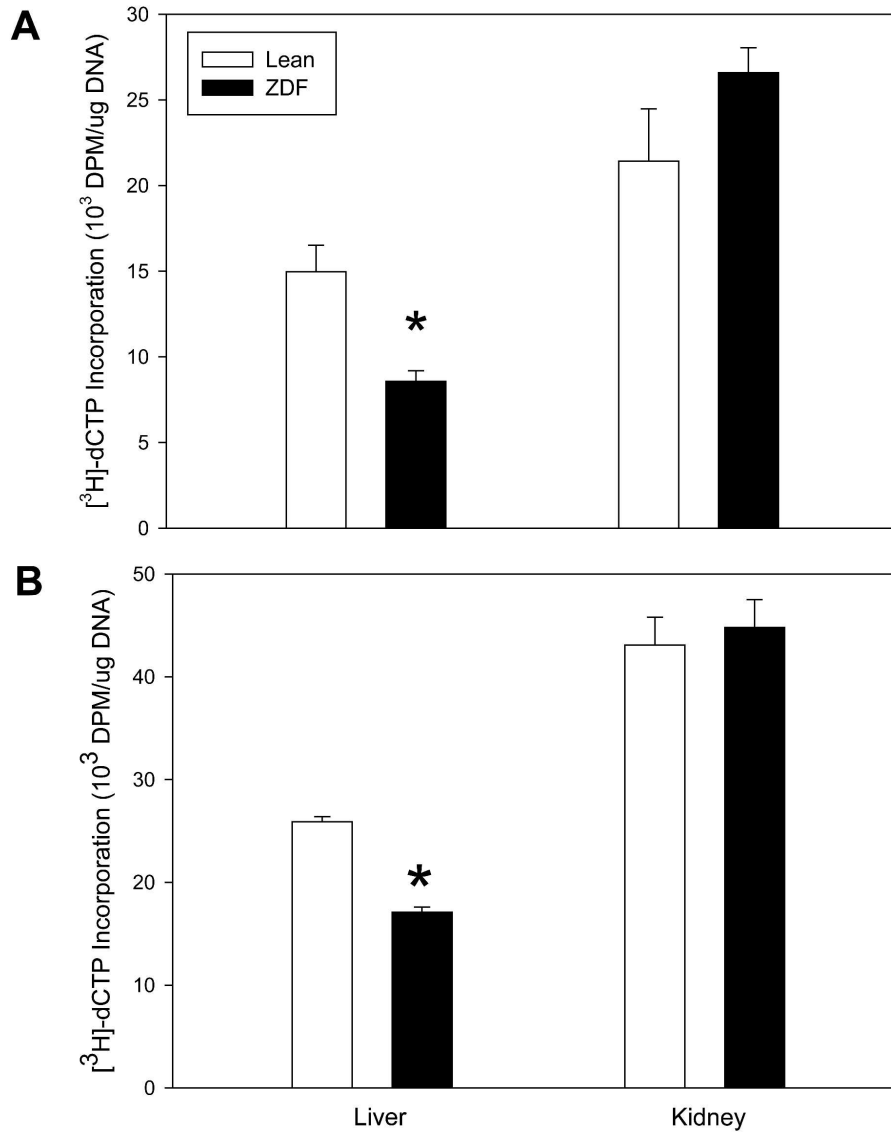
Data are means \pm SEM (n = 4-6). Asterisks (*) indicate a significant difference from control, $P \leq 0.05$.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 1



169x155mm (600 x 600 DPI)



169x208mm (600 x 600 DPI)