Proteomic Analysis Reveals Changes in the Liver Protein Pattern of Rats Exposed to Dietary Folate Deficiency[1]

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ABSTRACT Epidemiologic and experimental studies showed that folate deficiency is associated with increased risk of degenerative diseases by enhancing abnormal one-carbon metabolism. We studied the changes in the proteome of liver, the main tissue of folate storage and metabolism, in a rat model of dietary folate depletion. Four-month-old rats were fed for 4 wk an amino acid–defined diet without folate and compared with pair-fed rats given the same diet adequately supplemented with folic acid. Folate deprivation decreased plasma and hepatic folate concentrations dramatically, while increasing homocysteinemia significantly. Using 2-dimensional electrophoresis and matrix-assisted laser desorption/ionization time-of-flight MS, we identified 9 spots corresponding to differentially expressed proteins in the liver of folate-deficient rats compared with controls. Among those spots, 4 had a significantly increased volume, whereas the volume of the 5 other spots was decreased. Upregulated proteins included glutathione peroxidase (GPx) 1 and peroxiredoxin 6, 2 enzymes involved in the response to oxidative stress, and MAWD binding protein (MAWDBP), which has been associated with cancer. MAWDBP was simultaneously identified as a second spot with a lower isoelectric point (p*I*) that vanished almost completely after folate deficiency. Decreased abundance was also observed for cofilin 1, a protein linked to tumorigenesis, and for the GRP 75 precursor and preproalbumin, both of which are responsive to oxidative stress and/or inflammation. Moreover, an enzyme activity assay and/or Western blot analysis of GPx-1 and MAWDBP confirmed the proteomic findings. Our results show that folate deficiency modifies the abundance of several liver proteins consistently with adaptive tissue responses to oxidative and degenerative processes. J. Nutr. 135: 2524–2529, 2005.

KEY WORDS: • *proteomics* • *antioxidant enzymes* • *homocysteine* • *hepatic proteins* • *folate deprivation*

Folate, a generic term for all compounds that exhibit vitamin activity similar to that of pteroylmonoglutamic acid (folic acid), plays a fundamental physiological role in 1-carbon metabolism (1). Folate nutritional status depends on intake from food and folic acid supplements as well as on the bioavailability of the various ingested forms of this vitamin (2). In developed countries, severe folate deficiency is uncommon, but specific population subgroups, e.g., pregnant or lactating women and elderly subjects, may be at risk for moderate folate deficiency (3,4). Folates are currently under intense scrutiny for their ability to modulate disease risk. Periconceptual voluntary supplementation of women or mandatory fortification of enriched cereal-grain products with folic acid has significantly reduced the incidence of neural tube defects (3,5). Moderate folate deficiency is also associated with an increased risk of age-associated degenerative diseases such as occlusive vascular diseases (6), cognitive and neurological dysfunction (7), and cancers, e.g., colorectal cancer (8). Additionally, genetic polymorphisms of enzymes involved in 1-carbon metabolism were linked to some of these diseases (9).

To date, no causal relation between insufficient folate status and the etiology of degenerative diseases has been demonstrated. It is assumed that this relation involves impaired remethylation of homocysteine (Hcy),[3] transmethylation reactions (e.g., DNA hypomethylation), and nucleic acid synthesis (10). Cross-sectional studies, case-control studies, and meta-analyses suggested that elevated plasma total Hcy (tHcy) concentration in folate-deficient subjects is an independent

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[3] Abbreviations used: AKR1C9, aldo-keto-reductase 1C9; C, control; 2-DE, 2-dimensional electrophoresis; FD, folate-depleted; GPx, glutathione peroxidase; GRP 75, glucose-regulated protein 75; Hcy, homocysteine; 3-α-HSD, 3-α-hydroxysteroid dehydrogenase; IEF, isoelectric focusing; I.N., index number; IPG, immobilized pH gradient; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight MS; MAPK, mitogen-activated protein kinase; MAWD, putative MAPK activator with WD repeats; p*I*, isoelectric point; PMF, peptide mass fingerprinting; Prdx, peroxiredoxin; tHcy, total homocysteine; WD, tryptophan aspartic acid.

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risk factor for cardiovascular diseases (6,11) and is associated with cognitive decline and neuropsychiatric disorders such as Alzheimer's disease (12,13). The putative mechanisms of the adverse effects of Hcy on cells include oxido-reduction reactions, activation of proliferation- or apoptosis-signaling pathways, and alteration of gene expression (7,14). Numerous studies have focused on the Hcy-lowering effect of natural folate and its possible protective effects against degenerative diseases (4,6,10,15). Additionally, disruption of DNA integrity through chromosomal breaks and uracil misincorporation, alteration of DNA repair, and/or change in the expression of critical tumor suppressor genes and protooncogenes could increase the risk of cancer in subjects with low folate status (8,16). Nevertheless, more data are required to clearly explain the cellular and molecular mechanisms that underlie metabolism changes associated with folate deficiency.

The present study was designed to improve our understanding of these changes in the liver of rats subjected to dietary folate depletion. Liver constitutes an important tissue for folate metabolism, and decreased folate concentration and disturbed 1-carbon metabolism take place in this tissue during folate deficiency (17,18). However, little is known about the changes that occur concomitantly at the level of abundance of hepatic proteins. These aspects were investigated by a proteomic analysis of the liver of rats fed a diet without folate for 4 wk compared with pair-fed rats given the same diet adequately supplemented with folic acid. Differentially expressed proteins were identified using 2-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS. Possible connections of these proteins with degenerative diseases associated with folate deficiency were discussed.

MATERIALS AND METHODS

Diets, rats, and tissue samplings. Pellets of an L-amino acid-defined diet (19) supplemented with 1.5 or 0 mg folic acid/kg diet, comprising the control (C) or folate-depleted (FD) diet, respectively, were synthesized by the INRA-Unité de Préparation des Aliments Expérimentaux. Both diets contained the antibiotic succinylsulfothiazole (1%) to suppress folate production by the intestinal microflora. Such a treatment was shown to be the most reliable for studying the exclusive effect of dietary folate (19). According to the literature (20,21), the control diet met the rat folate requirement.

Male Wistar rats [$n = 14$, 4 mo old, initial weight (mean ± SD) 483 ± 50 g] were obtained from Charles River Laboratories. They were housed individually in cages placed in a room maintained at 20–23°C, with a normal 12-h dark:light cycle. After a 3-wk acclimation period during which they were fed the C diet, rats were randomly assigned to continue the C diet or to consume the FD diet and pair-fed for an experimental period of 4 wk with free access to water. The rats were weighed 3 times/wk and their food consumption was monitored daily. Among the FD group, 1 healthy rat died during the experiment (for an undetermined reason). At the end of wk 4, the rats were killed under anesthesia (40 mg/kg pentobarbital-sodium i.p.). Blood was quickly sampled from the abdominal artery with a heparinized syringe. After rapid removal, tissues were rinsed with Krebs-Ringer buffer, then immediately frozen in liquid nitrogen and stored at −80°C for further analysis. Hematocrit was measured in an aliquot of blood just after killing using a Hematokrit Centrifuge (Hettich). Blood was centrifuged at 1000 × *g* for 10 min at 4°C and, for folate analysis, plasma aliquots were supplemented with 0.1 volume of 5 mmol/L of sodium ascorbate. All plasma aliquots were stored at −80°C under nitrogen. This study was approved by the Ethical Committee of INRA-Theix Research Center.

Folate and homocysteine assays. Frozen liver pieces were homogenized in ice-cold phosphate extraction buffer [0.1 mol/L of sodium phosphate pH 7, 0.2 mol/L of mercaptoethanol and 2% (wt:v) sodium ascorbate as antioxidants] and then placed into a boiling water bath for 10 min to precipitate the proteins. After cooling over ice, the homogenate was stirred and centrifuged at 12,000 × g for 10 min. Then, a supernatant aliquot was incubated at pH 7 for 3 h in a shaking water-bath at 37°C with 0.2 volumes of chicken pancreas conjugase extract (Difco Labs, BD) (10 g/L) to hydrolyze liver folylpolyglutamates.

Plasma and liver folate concentrations were measured by microbiological assay using *Lactobacillus casei* ATCC 7469 (*L. rhamnosus*; Institut Pasteur) and free folic acid-casei medium (Difco Labs) (22). Plasma tHcy concentrations were determined by HPLC and fluorometric detection (Waters), using the HPLC Reagent Kit from Bio-Rad Laboratories following the supplier's instructions (22).

2-DE of liver proteins. 2-DE was performed as previously described (23,24) with some modifications. Importantly, all 2-DE experiments were carried out simultaneously to optimize the analytical reproducibility. Frozen samples of liver from C or FD rats were homogenized in extraction buffer [5 mol/L of urea, 2 mol/L of thiourea, 4% (wt:v) CHAPS, 40 mmol/L of Tris, 2 mmol/L of tributylphosphine, 0.2% Biolytes], and the homogenate was centrifuged at 100,000 × *g* for 1 h. The protein concentration of the supernatant was determined using the Bio-Rad RC DC protein assay kit.

For immobilized pH gradient (IPG) isoelectric focusing (IEF), 300 or 1000 μg of proteins (for analytical or preparative gels, respectively) were loaded onto 17-cm Bio-Rad ReadyStrips, pH 3–10 nonlinear, by inclusion of an adequate volume of extract in rehydration buffer [9 mol/L of urea, 4% (wt:v) CHAPS, 0.1 mol/L of dithiothreitol, 0.2% biolytes, 0.0002% (wt:v) Bromophenol blue]. Passive rehydration of the strips was carried out over 13 h. The IEF consisted in increasing the voltage gradually from 250 to 5000 V over a 20.5-h period. After equilibration of the IPG strips, SDS-PAGE was performed on 12% polyacrylamide gels. Separate protein spots were visualized on analytical or preparative gels by silver staining or 0.02% (wt:v) colloidal Coomassie blue staining, respectively.

Image analysis. Gel images were acquired and analyzed using the PDQuest software (Bio-Rad) (24). For a given gel, the volume of each protein spot was calculated (ppm) by dividing its raw volume by the sum of the volumes of all valid spots. Normalized volumes between the C ($n = 6$) and FD ($n = 5$) groups were compared using Student's *t* test.

In-gel digestion, desalting, concentration, and MALDI-TOF MS identification of protein spots. All of these steps were performed essentially as described previously (24). Peptide mass fingerprints (PMF) were compared with mammalian databases (NCBI nonredundant and SWISS-PROT) (25,26). The search criteria used were 1 missing trypsin cleavage site, partial carbamidomethylation of cysteine, partial methionine oxidation, and a mass deviation < 30 ppm. Z-scores were defined by comparison of search results against estimated random match population. Z-scores > 1.65 were considered significant ($P < 0.05$). All of the protein identifications were in the expected size range based on position in the gel.

Western blot assays. Liver extracts were prepared in 1 mmol/L of EDTA:0.13 mmol/L of BHT:100 mmol/L of phosphate buffer pH 7.4 (0.5 g of liver in 5 mL of buffer) before centrifuging twice at 10,000 × *g* for 10 min at 4°C. Supernatant proteins (2 μg) were separated by SDS-PAGE on a 15% (v:v) polyacrylamide gel and Western blots were carried out as described previously (27), using rabbit antibody to human glutathione peroxidase 1 (GPx 1; Acris Antibodies) at 1:5000 dilution and the Amersham ECF Western-blotting kit.

For the putative mitogen-activated protein kinase (MAPK) activator with tryptophan aspartic acid (WD) repeats (MAWD) binding protein (MAWDBP) assay, Western blots were performed on liver protein extracts resolved by 2-DE gels, as explained above. On the basis of the proteomic data, an area of ~50 cm^2 around the MAWDBP protein spots was cut from the 2-DE gels and processed for Western blot analysis using rabbit antibody to rat MAWDBP (a kind gift from Dr. A. Pawlak, INSERM U581, Créteil, France) at 1:4000 dilution.

Assay of liver GPx activity. GPx specific activity (U/mg protein) was determined in liver extracts according to previously published procedures (28,29). Protein concentration of liver extracts was determined with a Bradford assay (Bio-Rad) following the supplier's instructions.

Statistics. Results were expressed as means ± SD. Statistical analyses were performed using V3.00 GraphPad InStat (GraphPad Software). The statistical significance of differences between means of FD and C rat groups was assessed using the 2-tailed Student's *t* test or Mann-Whitney test, with $P < 0.05$ considered significant. Correlations were assessed using the Pearson's correlation coefficient (r).

RESULTS

Biological and biochemical characteristics of folate-depleted adult rats. Compared with the pair-fed controls (C), body weight (515 ± 40 and 507 ± 17 g), liver weight (11.7 ± 1.7 and 12.1 ± 1.5 g), and hematocrit (42.2 ± 2.8 and 39.2 ± 3.0%), of rats subjected to dietary folate depletion for 4 wk (FD) were not affected. On the other hand, folate depletion caused a 91 and 80% decrease in plasma and liver folate concentrations, respectively (**Table 1**). Folate deficiency was also accompanied by a 3.6-fold increase in the plasma tHcy concentration of the rats. When the individual data of rats of both groups (n = 13) were pooled, plasma folate and tHcy concentrations ($r = -0.82$, $P = 0.0005$) and hepatic folate and plasma tHcy concentrations ($r = -0.81$, $P = 0.0007$) were negatively correlated (data not illustrated).

Changes in the liver proteome of folate-depleted adult rats. In preliminary experiments, for each group, 2-DE gels were done in triplicate with a mixture of liver protein extracts obtained from 3 different rats. Triplicate gels obtained from a given protein mixture did not differ in terms of spot number and volume (not illustrated). Then, additional 2-DE gels were run in parallel with liver protein extracts from individual C (n = 6) and FD (n = 5) rats. Representative silver-stained 2-DE gels of the liver proteome of C and FD rats are presented in **Figure 1***A* and *B*, respectively. Under our experimental conditions, ~560 protein spots were detected per gel. The volumes of 9 protein spots changed significantly after folate deficiency (**Table 2**). Their position and index number (I.N.) are shown in Figure 1*A* and *B*. Some of the changes observed are magnified in **Figure 2** and their magnitude is given in Table 2. In particular, the spot numbered 4117 was markedly absent in "FD gels" in contrast to "C gels."

All of the 9 protein spots could be attributed to known proteins using PMF (**Tables** 2 and **3**). Four spots presenting significantly increased volumes under FD conditions were identified as peroxiredoxin 6 (Prdx 6, I.N. = 3103), MAWDBP (I.N. = 4112), cytosolic GPx 1 (I.N. = 5012), and 3-α-hydroxysteroid dehydrogenase (3-α-HSD; I.N. = 6230). The ratios of the volumes (FD:C) were 1.2, 1.55, 1.34, and 1.48, respectively. Five protein spots with significantly lower volumes in FD rats were identified as 3 (2),5-bisphosphate nucleotidase 1 (I.N. = 2323), DNAk-type molecular chaperone grp 75 precursor (I.N. = 2735), preproalbumin (I.N. = 3739), MAWDBP (I.N. = 4117), and cofilin 1 (I.N. = 8003).

TABLE 1

Changes in folate status and homocysteinemia after folate depletion in adult rats[1]

	Control	Folate-depleted
n	7	6
Plasma folate, *nmol/L*	159.6 ± 12.7	14.1 ± 1.1*
Liver folate, *nmol/g wet tissue*	20.5 ± 2.2	4.0 ± 1.0*
Plasma homocysteine, *μmol/L*	6.4 ± 1.8	22.9 ± 8.6*

[1] Values are means ± SD. * Different from control according to Mann-Whitney test ($P < 0.05$).

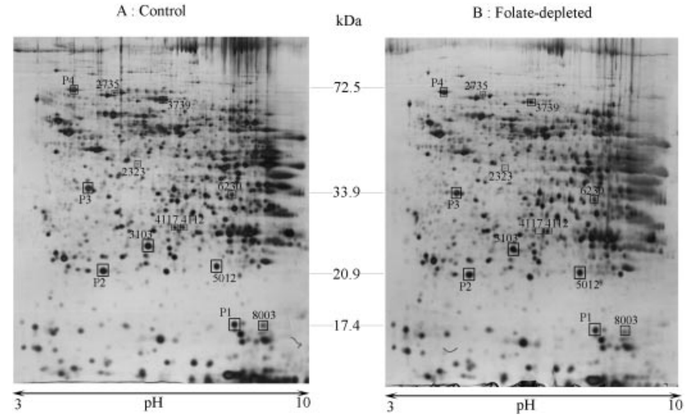

FIGURE 1 Densitometry scanning of typical silver-stained 2-DE gels of liver total proteins from adult rats fed (*A*) a control or (*B*) a folate-depleted diet. *X*-axis: isoelectric point (p*I*) from 3 to 10. *Y*-axis: molecular weight (MW). The squared areas mark 9 protein spots exhibiting significantly different volumes between the 2 conditions. The numbers correspond to the index (I.N.) assigned to the proteins in the gel chosen as the reference in PDQuest analysis. P1, P2, P3, and P4 stand for proteins found that were identical to proteins identified under similar conditions of analysis in previously published 2-DE gels of the rat liver proteome (30): nucleoside diphosphate kinase B (p*I* = 7.5 and MW = 17.4 kDa), phosphatidylethanolamine-binding protein (p*I* = 5.6 and MW = 20.9 kDa), senescence marker protein-30 (p*I* = 5.3 and MW = 33.9 kDa) and 78-kDa glucose-regulated protein (p*I* = 4.9 and MW = 72.5 kDa).

The magnitude of the decreases [(C spot volume − FD spot volume) × 100/C spot volume] observed was 40.8, 33.8, 38.9, ~100, and 47.4%, respectively.

Confirmation of proteomic data by Western blot analysis and enzyme activity assay. To verify the accuracy of the proteomics results, the amounts of GPx 1 protein in liver homogenates from FD and C rats (n = 4 rats/group) were compared using Western blot analysis with a polyclonal antibody specific to GPx 1 (**Fig. 3***A*). Fluorescence quantification of the immunoreactive protein confirmed that GPx 1 protein abundance was increased 1.43 fold ($P = 0.035$) in the liver of FD rats [1.88 ± 0.33 arbitrary U ($\times 10^6$) vs. 1.32 ± 0.25 arbitrary U (x 10^6) in controls]. Additionally, measurement of enzyme activity in the same homogenates indicated that dietary folate deficiency increased ($P = 0.0019$) the specific activity of liver GPx from 1.01 ± 0.07 (C rats, n = 4) to 1.41 ± 0.14 U/mg proteins (FD rats, n = 4) (data not illustrated). Finally, probing 2-DE-separated liver homogenates by Western blotting with a rabbit polyclonal antibody specific to human MAWDBP confirmed the identity of the 2 protein spots 4112 and 4117 (Fig. 3*B*). It also showed that spot 4117 was no longer evident in samples from the liver of FD rats, in accordance with the result of silver-stained 2-DE gels (Fig. 2*D*).

DISCUSSION

Liver is an important tissue in folate metabolism, and long-term folate deficiency was shown to disturb 1-carbon metabolism (14,17,18,31). The aim of the present study was to determine the changes in the liver proteome of adult rats after 4 wk of consuming a folate-free diet. Such a nutritional condition is sufficient to cause a substantial decrease in the folate content of plasma and liver concurrently with an increase in plasma tHcy; for the first time, we showed that this leads to

TABLE 2

Changes in abundance of hepatic proteins in folate-deficient rats[1]

Protein index number (I.N.)	Protein name	Normalized volume (ppm) Control	Folate-depleted	Change in protein abundance[2]	*P*-value
3103	Prdx 6	9563 ± 1052	11458 ± 1525	↑ 1.20×	0.038
4112	MAWDBP	3174 ± 534	4921 ± 1351	↑ 1.55×	0.028
5012	GPx 1	7945 ± 736	10631 ± 2148	↑ 1.34×	0.018
6230	3-α-HSD	3387 ± 466	5021 ± 1426	↑ 1.48×	0.026
2323	3(2),5-bisphosphate nucleotidase 1	487 ± 175	288 ± 70	↓ 40.8%	0.042
2735	DNAk-type molecular chaperone grp 75 precursor	1503 ± 291	994 ± 278	↓ 33.8%	0.016
3739	Preproalbumin, 608 aa	19890 ± 5508	12159 ± 3542	↓ 38.9%	0.025
4117	MAWDBP	3562 ± 622	1.2 ± 0.6	↓ 99.9%	<0.001
8003	Cofilin 1	2721 ± 858	1431 ± 499	↓ 47.4%	0.016

[1] Values are means ± SD (n = 6 and 5 in the control and folate-depleted group, respectively). *P* values derived from statistical analysis using Student's *t* test.

[2] Fold increase (with up arrow) or percent of decrease (with down arrow) in the normalized volume of a given protein spot in the liver of folate-depleted rats vs. control rats.

significant variations in the abundance of several hepatic proteins.

Using 2-DE and MALDI-TOF MS, we were able to identify 9 proteins exhibiting differential abundance in the liver of FD rats compared with their C counterparts. Two of these proteins involved in the control of oxidative stress, i.e., GPx 1 and Prdx 6, were upregulated in the liver of FD rats. For GPx 1, this finding was confirmed by Western immunoblotting using an antibody specific to this enzyme and by measurement of specific activity of liver total GPx. This agrees with the fact that GPx 1 is the major GPx in the liver (32). GPx 1 is a selenium-dependent, cytosolic enzyme that can reduce soluble hydroperoxides such as H_2O_2 as well as organic hydroperoxides at the expense of glutathione. Prdx 6 belongs to another family of antioxidant enzymes, also named thioredoxin peroxidases, and is abundantly expressed in hepatocytes (33). Prdx can reduce H_2O_2 and alkyl hydroperoxides at the expense of thiols. Moreover, GPx and Prdx likely play cell- and tissue-specific roles in metabolic regulation including cytokine signaling, transcriptional regulation, and/or apoptosis (32,33). Upregulation of GPx 1 and Prdx 6 in the liver of FD rats is indicative of a tissue response to an oxidative stress induced by

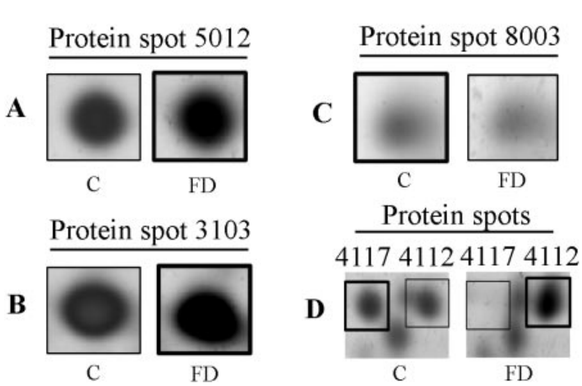

FIGURE 2 Differentially expressed protein spots in the liver proteome of adult rats fed the FD or C diet. Bold-framing of spots in expanded images indicates significantly higher volumes when comparing the 2 experimental conditions (see Table 2).

folate depletion. Oxidative stress was observed previously in folate-deficient patients (15,34,35), weaning rats (31,36), and cultured cells (37). However, liver total GPx activity was unchanged in weaning rats fed a FD diet compared with age-paired rats fed a diet containing 2 mg of folic acid/kg (31); the latter nutritional condition is similar to that used here as the control condition. Age-dependent regulation of GPx (38) could explain the different responses of this antioxidant enzyme to the oxidative stress induced by folate deficiency in adult and weaning rats.

In the present study, plasma tHcy concentration rose from 6.4 μmol/L in C rats to 22.9 μmol/L in FD rats concomitantly with an increased abundance of hepatic GPx 1 and Prdx 6. Such a variation in plasma tHcy concentration is in the range of that reported for mild hyperhomocysteinemia in humans (13). Moat et al. (39) observed previously that plasma tHcy concentrations $\geq$ 20 μmol/L in humans are associated with increased activity of circulating antioxidant enzymes including plasma GPx. This was attributed, at least in part, to an increase in the amount of enzyme protein, perhaps due to a protective reducing effect of thiols on GPx. Moreover, dietary folate deficiency in mice was reported to result in increased glutathione levels in brain tissue (40). Overall, the last-mentioned 2 studies and the present one support the in vivo existence of compensatory mechanisms to counteract the oxidative stress generated by folate deficiency. Also possibly related to the cellular effects of Hcy is the precursor of DNAk-type molecular chaperone glucose-regulated protein 75 (GRP75), which is one of the proteins presently identified as having a decreased abundance in the liver of FD rats. GRP75 is also known as mitochondrial heat shock protein 70 (HSP70) (41). In 2 populations with frequently elevated homocysteinemia and/or unbalanced redox status, i.e., older subjects and patients with Alzheimer's disease, decreased HSP70 expression was observed in olfactory receptor neurons or mononuclear blood cells, respectively (42,43). Whether mild hyperhomocysteinemia or oxidative stress in FD rats may contribute to the decreased abundance of liver GRP75 precursor remains to be determined. Moreover, the in vivo contribution of Hcy-independent mechanisms, e.g., deficiency of supposedly anti-

TABLE 3

Summary of differentially abundant proteins identified by proteomics in rat liver in response to dietary folate depletion[1]

Identified protein	gi accession number	MW (kDa)/p*I*[2]	Number of peptides matched	% Coverage of matched peptides	Z-score[3]
Prdx 6	16758348	25/5.6	12	63	2.38
MAWDBP (spot 4112)	19924063	32/6.5	12	44	2.34
GPx 1	121668	22/7.7	8	61	2.25
3-α-HSD	19924087	37/6.7	17	60	2.37
3(2),5-bisphosphate nucleotidase 1	25282455	33/5.6	7	30	2.16
DNAk-type molecular chaperone grp 75 precursor	2119726	74/5.9	14	25	2.28
Pre-proalbumin, 608 aa	19705431	69/6.1	33	50	2.40
MAWDBP (spot 4117)	19924063	32/6.5	9	34	2.24
Cofilin 1	8393101	19/8.5	6	33	2.09

[1] The proteins are designated with their gi accession number of the NCBI database.
[2] Theoretical MW and p*I* obtained from the database entry without any processing.
[3] In general, a Z-score ≥ 1.65 indicates a statistically correct match.

oxidant folate molecules (35,44) per se, to the oxidative stress induced by folate depletion is presently unknown.

Folate deficiency has been associated with increased risk of cancers (8,16). Decreased expression of adhesion molecules and increased expression of urokinase occur in the colon mucosa of folate-deficient rats, suggesting that cell detachment and migration, 2 cancer-related processes, may be modulated by folate status (45). We report here that the liver abundance of cofilin 1, a protein downregulated in highly metastatic hepatocellular carcinoma cells (46), was decreased in FD rats. Cofilin 1 acts as an actin-depolymerizing factor that can control actin-based motility by reversible phosphorylation, generate cell surface protrusions, and set the direction of cell migration (47). Another protein possibly linked to cancer, namely, MAWDBP, was identified in the present study as 2 separate spots with the same molecular weight but different isoelectric point (p*I*) values, and regulated by folate status. The volume of the protein spot with the highest p*I* was increased, whereas that of the other spot was almost nil in "FD gels," compared with "C gels." This result obtained on silver-stained 2-DE gels was confirmed by Western blotting of similarly 2-DE-separated liver protein extracts. The 2-spots/1-spot pattern may be indicative of folate-dependent, post-translational modification (e.g., phosphorylation) of this protein. However, current information on the regulation of MAWDBP expression is scarce. Interestingly, it could interact with MAWD, a protein containing WD-40 repeats that contribute to protein/protein interactions in various cellular processes (48). Overexpression of the *MAWD* gene in cultured cells causes activation of MAPK, disruption of contact inhibition and anchorage-independent growth (48). The possible existence of 2 phosphorylation states for MAWDBP would be coherent with such a relation between MAWD and MAPK. Folate depletion also gave rise to a 1.5-fold augmentation in the volume of another protein spot that was identified as 3α-HSD, also known as aldo-keto-reductase 1C9 (AKR1C9). This change might be related to possible transcriptional activation of the *AKR1C* gene by reactive oxygen species via an antioxidant responsive element, as observed previously in HepG2 hepatoma cells (49). Such an induction was suggested to exacerbate cellular damage mediated by various xenobiotics and to play a significant role in carcinogenesis.

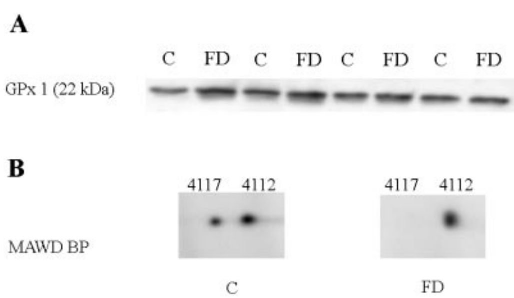

FIGURE 3 Confirmation of proteomic results by Western blot analysis of hepatic GPx 1 and MAWDBP in FD and C adult rats. (*A*) Typical immunoblots of liver homogenates from 4 different C and FD rats using rabbit antibody to human GPx 1. (*B*) Typical immunoblot of 2-DE-resolved FD or C liver homogenates probed with a rabbit antibody to rat MAWDBP. Each immunoblot was repeated 3 times.

A reduced abundance of hepatic preproalbumin was also observed in FD rats, suggesting diminished tissue biosynthesis of this protein and subsequent hypoalbuminemia. Hypoalbuminemia is a frequent feature of cachectic patients afflicted with chronic diseases, including cancer and inflammatory disorders (50). Finally, a decreased abundance of 3(2),5-bisphosphate nucleotidase 1 was also determined in the liver of FD rats. This enzyme is important in RNA processing, sulfation-dependent hepatic detoxification, and phosphoinositide-signaling pathway (51).

In conclusion, the present proteomic analysis shows that in adult rats, dietary folate depletion leads to significant changes in the abundance of several liver proteins concurrently with altered folate status and increased homocysteinemia. The results were validated by activity assay and/or Western blot analysis of both GPx 1 and MAWDBP. Most of the proteins identified are related to the control of oxidative stress, inflammatory response, or cancer-related processes, and the changes in their abundance are consistent with the literature. Future studies will aim at determining the regulatory mechanisms, particularly at the level of protein synthesis and degradation, that link the identified proteins to the redox state and the folate status of individuals.

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