Effect of folate oversupplementation on folate uptake by human intestinal and renal epithelial cells

Balasubramaniem Ashokkumar, Zainab M Mohammed, Nosratola D Vaziri, and Hamid M Said

ABSTRACT

Background: Folic acid plays an essential role in cellular metabolism. Its deficiency can lead to neural tube defects. However, optimization of body folate homeostasis can reduce the incidence of neural tube defects and may decrease the risk of Alzheimer and cardiovascular diseases and cancer. Hence, food fortification and intake of supplemental folate are widespread.

Objective: We examined the effects of long-term folate oversupplementation on the physiologic markers of intestinal and renal folate uptake processes.

Design: Human-derived intestinal Caco-2 and renal HK-2 epithelial cells were maintained (5 generations) in a growth medium over-supplemented (100 μmol folic acid/L) or maintained under sufficient conditions (0.25 and 9 μmol folic acid/L).

Results: Carrier-mediated uptake of 3H-folic acid (2 μmol/L) at buffer pH 5.5 (but not buffer pH 7.4) by Caco-2 and HK-2 cells maintained under the folate-oversupplemented condition was significantly (P < 0.01) and specifically lower than in cells maintained under the folate-sufficient condition. This reduction in folic acid uptake was associated with a significant decrease in the protein and mRNA levels of the human reduced-folate carrier (hRFC) and a decrease in the activity of the hRFC promoter. It was also associated with a decrease in mRNA levels of the proton-coupled folate transporter/heme carrier protein 1 (PCFT/HCP1) and folate receptor (FR).

Conclusions: Long-term oversupplementation with folate leads to a specific and significant down-regulation in intestinal and renal folate uptake, which is associated with a decrease in message levels of hRFC, PCFT/HCP1, and FR. This regulation appears to be mediated via a transcriptional mechanism, at least for the hRFC system.


KEY WORDS Folate oversupplementation, intestinal folate uptake, renal folate uptake, hRFC, human reduced-folate carrier, PCFT/HCP1, proton-coupled folate transporter/heme carrier protein 1, folate receptor

INTRODUCTION

Folates play a fundamental physiologic role in one-carbon metabolism and are essential for the synthesis of precursors of nucleic acids, metabolism of several amino acids (including homocysteine), and initiation of protein synthesis in the mitochondria (1–3). Thus, it is not surprising that deficiency of this micronutrient leads to a variety of clinical abnormalities that range from neural tube defects to megaloblastic anemia (1–3). In contrast to the adverse effects of folate deficiency, optimization of body folate homeostasis was shown to lead to a significant reduction in the incidence of neural tube defects (4, 5) and omphalocele (6). Some epidemiologic studies suggested that benefit may accrue from folate supplements for cardiovascular diseases (7), Alzheimer disease (8), and certain types of cancer [e.g., colorectal cancer (9, 10)]. However, blinded, randomized clinical trials are raising serious doubts about such supplement benefits in some cases such as cardiovascular disease (11). Because of the benefits presumed, both realistically for birth defects and suggested epidemiologically for other medical problems, a mandatory fortification of grain products with folic acid was instituted in many countries, including the United States. Also, all women of childbearing age are advised now to take a daily supplement of folic acid (400 μg/d). Thus, the intake of folic acid from fortified food (≈100–200 μg/d) together with the use of nutritional supplements [that provide an additional 400 μg folic acid/standard multivitamin preparation (12)] and consumption of nutrition bars and drinks [which are often supplemented with 400 μg folic acid/sering (12)] create a state of folate oversupplementation in a significant segment of the population (12). This practice is occurring with little knowledge of the potential safety and physiologic consequences of chronic intake of such high doses of folic acid. A few reports, however, have raised questions about the validity of such practices (13–15). In this study, we examined the effect of long-term oversupplementation with folic acid on the physiology of folate transport in the human intestinal and renal epithelial cells.

The intestine plays a central role in regulating body folate homeostasis because the vitamin cannot be synthesized in the body and must be obtained from exogenous sources. Similarly, the kidneys play a pivotal role in regulating body folate homeostasis by reabsorbing the filtered vitamin, thus preventing its losses in the urine. Intestinal absorption of folate occurs via a specialized, acidic pH–dependent carrier-mediated process that involves the reduced folate carrier (RFC) (16–18) as well as a...
recently described proton-coupled folate transporter/heme carrier protein 1 (PCFT/HCP1) (19). The renal folate uptake process is also specialized and saturable and, depending on the prevailing folate concentration, appears to involve the folate receptor (FR) and RFC and possibly the PCFT/HCP1 systems (19–24). To achieve our stated goals of examining the effect of long-term oversupplementation with folic acid on intestinal and renal folate uptake process, we used Caco-2 and HK-2 cells as models.

MATERIALS AND METHODS

[1^H]-folic acid (specific activity: 20 Ci/mmol; radiochemical purity: 98%) was obtained from Moravek Biochemicals (Brea, CA). TRIzol reagent and Lipofectamine were purchased from Life Technologies (Rockville, MD). DNA oligonucleotide primers were from Sigma Genosys (The Woodlands, TX). Routine biochemicals, enzymes, fetal bovine serum, and cell culture reagents were all of molecular biology quality and were purchased from either Fisher Scientific (Tustin, CA) or Sigma (St Louis, MO).

Cell culture and uptake studies

The human-derived intestinal epithelial Caco-2 cells and the human-derived renal proximal tubular epithelial HK-2 cells (ATCC, Manassas, VA) were used in this investigation. These cells were chosen because they have proven to be excellent models in such physiologic investigations and to yield data similar to those found with native intestinal and renal epithelial cells (25, 26). Caco-2 cells, a human colon carcinoma cell line, differentiate spontaneously in the culture to become small intestinal villus-like absorptive cells (27–29). HK-2 cells are an immortalized proximal tubule epithelial cell line from normal adult human kidney. Cells were grown and subcultured in custom-made Dulbecco’s modified Eagle’s medium containing 5% fetal bovine serum (Hyclone, Logan, UT), glutamine (0.29 g/L), sodium bicarbonate (2.2 g/L), penicillin (100 000 U/L), and streptomycin (10 mg/L) in an atmosphere of 5% CO2-95% air at 37 °C. For experiments, cells were maintained for 5 generations in a growth medium oversupplemented with folate (10 μmol/L) or maintained under folate-sufficient condition (0.25 μmol/L). Another group of cells was maintained in the presence of an intermediated folate concentration of 9 μmol/L.

Folic acid uptake was performed with the use of well-washed confluent monolayers (3–4 d after confluence) of Caco-2 (passages between 10 and 16) and HK-2 (passages between 8 and 16) cells. Uptake was measured at 37 °C in Krebs-Ringer solution containing 5% CO2, 95% air at 37 °C. Cells were then rinsed twice with ice-cold buffer, digested in sterile water, and 2 different dilutions were used for each polymerase chain reaction analysis of human reduced-folate carrier (hRFC), proton-coupled folate transporter/heme carrier protein 1 (PCFT/HCP1), and folate receptor (FR) mRNA in Caco-2 and HK-2 cells.

| Gene         | Primer sequence (5’–3’)       | Ampli
con     |
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<tr>
<td>PCFT/HCP1</td>
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<tr>
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<td>750</td>
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<tr>
<td>β-Actin 2</td>
<td>CAAAGTCAGCACTACAGGAG</td>
<td>116</td>
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<tr>
<td>PCFT/HCP1</td>
<td>CAAGTGAGCTCTTTGGT</td>
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<td>FR</td>
<td>GAGCCACATAGGCTGGAC</td>
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Semiquantitative reverse transcription–polymerase chain reaction analysis of hRFC, PCFT/HCP1, and FR mRNA in Caco-2 and HK-2 cells maintained in the presence of different concentrations of extracellular folate

Total RNA was prepared with the use of the TRIzol reagent from confluent Caco-2 and HK-2 cells that were maintained in growth media containing sufficient and oversupplemented concentrations of folate. Total RNA (3 μg) was reverse transcribed with oligo (dT) primers with the use of Superscript II (Life Technologies) following the manufacturer’s procedures. After the reverse transcription (RT), all samples were diluted with sterile water, and 2 different dilutions were used for each polymerase chain reaction (PCR) with primer pairs specific for the hRFC, PCFT/HCP1, FR (both α and β isoforms), and the housekeeping gene β-actin to accurately determine their level of expression in the Caco-2 and HK-2 cells. Gene-specific primers corresponding to the PCR targets were designed by using the specifications given by the vendors (Bio-Rad Laboratories) and shown in Table 1. The conditions for semiquantitative RT-PCR were 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min (33 cycles). The products were analyzed on 2% agarose gels, the images were captured with the use of an Eagle Eye II system (Stratagene, LA Jolla, CA), and the amplified RT-PCR products were normalized to amplified β-actin gene controls as described previously (17).

Western blot analysis of hRFC and FR in Caco-2 and HK-2 cells maintained in growth media with different folate concentrations

Western blot analysis was performed with the use of the membranous fractions of Caco-2 and HK-2 cells maintained in varying concentrations of folate. The membranous fractions were isolated by homogenizing the cells in a buffer containing 300 mmol mannitol/L, 5 mmol EGTA/L, and 12 mmol Tris-HCl/L.
well as a cocktail of protease inhibitors (1 mmol phenylmethylsulfonyl fluoride/L, 1 μg aprotinin/mL, and 0.5 μg leupeptin/mL) (30). Protein (100 μg) samples were treated with Laemmli sample buffer and resolved on an 8% sodium dodecyl sulfate–polyacrylamide gel. After electrophoresis, the proteins were electroblotted on Hybond electrochemiluminescence nitrocellulose membrane (Amersham Pharmacia Biotech, Piscataway, NJ), washed twice with phosphate-buffered saline (PBS)–TWEEN 20 for 10 min, and blocked with 5% dried milk in PBS-TWEEN 20. Blots were probed with previously validated rabbit anti-hRFC polyclonal antibodies (1:1000 in PBS-TWEEN 20) (30). For FR, blots were probed with polyclonal antibodies generated against FR epitope corresponding to amino acids 1–257 (Santa Cruz Biotechnology, Santa Cruz, CA). Immunodetection was performed with goat anti-rabbit immunoglobin G conjugated to horseradish peroxidase (1:1000 in PBS-Tween 20). Specific bands were measured by using the Eagle Eye II system (Stratagene). Western blotting for β-actin was performed as loading control. Blots were incubated with a 1:1000 dilution of a goat anti-β-actin antibody (Santa Cruz Biotechnology) and developed as described here earlier.

**hRFC promoter activity: transfection and reporter gene assay**

A fusion construct of the full-length hRFC promoter B (pB) with the luciferase reporter gene (hRFC-pB-luciferase) prepared in pGL3-basic vector, kindly provided by Dr. Larry H Matherly of the Wayne State University School of Medicine, Detroit, MI, was used in this investigation (31). The hRFC-pB-luciferase construct was transfected into Caco-2 and HK-2 cells as described previously (30). Cells were cotransfected in 12-well plates at ≈70–75% confluency with 2 μg of each test construct and 100 ng of the Renilla transfection control plasmid Renilla luciferase-thymidine kinase (pRL-TK; Promega, Madison, WI). Transfection was performed with Lipofectamine reagent (Invitrogen, San Diego, CA) according to the manufacturer’s instructions. Cells were then harvested at 3–4 d after transfection (confluence), and Renilla-normalized firefly luciferase activity was determined by using the Dual Luciferase Assay system (Promega). Firefly luciferase activity was normalized relative to the Renilla luciferase activity in the same cell extracts. Data are presented as means ± SEMs of at least 3 independent experiments and given as folds over pGL3-Basic expression, which was set arbitrarily at 1.

**Data presentation and statistical analysis**

Transport data presented in this study are mean ± SEM of multiple separate uptake determinations and are expressed in picomoles or femtomoles per milligram protein per 7 min. Data were analyzed with the use of analysis of variance (http://faculty.vassar.edu/lowry/anova1u.html), with statistical significance set at 0.05. All semiquantitative RT-PCR and Western blot analyses were performed on at least 3 separate occasions with comparable results, and data presented are from representative sets of experiments.
The results show the level of the hRFC mRNA (normalized to β-actin mRNA) to be significantly (P < 0.01) lower in cells maintained under folate-oversupplemented condition than in cells maintained under folate-sufficient condition.

Data on the effect of folate oversupplementation on hRFC protein expression is shown in Figure 2B. Western analysis was performed with the use of specific polyclonal anti-hRFC antibodies as described in "Methods." The results showed a
The PCFT/HCP1 is a recently identified folate transporter that is believed to play a role in normal folate uptake by human intestinal epithelial cells (19). In this study we examined the effect of maintaining Caco-2 cells in folate-oversupplemented growth medium on hRFC mRNA expression. Semiquantitative RT-PCR was performed with the use of RNA isolated from cells grown under folate-oversupplemented and -sufficient conditions and specific primers for the hRFC (Table 1). The results showed the expression of the hRFC mRNA to be significantly ($P < 0.01$) lower in cells maintained in folate-oversupplemented growth medium than in cells maintained in folate-sufficient condition (Figure 2D).

Effect of folate oversupplementation on renal folate uptake

The results on the effect of maintaining the human-derived renal epithelial HK-2 cells in folate-oversupplemented growth medium on folic acid (2 µmol/L) uptake at buffer pH 5.5 are shown in Figure 3. As can be seen, uptake by cells grown in folate-oversupplemented growth medium (100 µmol/L) was significantly ($P < 0.01$) lower than uptake by cells maintained in folate-sufficient growth medium (0.25 µmol/L); uptake by cells maintained in the presence of 9 µmol/L folate fall in between. However, uptake of the unrelated biotin (7.9 nmol/L) was similar under the different folate conditions (21.3 ± 1.9, 22.4 ± 0.8, and 22.2 ± 2.9 nmol·mg protein$^{-1}$·min$^{-1}$ in the cells maintained in the presence of 100, 9, and 0.25 µmol folate/L, respectively). When uptake of folic acid (2 µmol/L) was examined at buffer pH 7.4, the uptake was found to be similar under the different folate conditions (Figure 3). As reported previously for renal folate uptake (23), uptake of folic acid by HK-2 cells maintained in folate-sufficient growth medium was significantly ($P < 0.01$) higher at buffer pH 5.5 than at buffer pH 7.4 (0.53 ± 0.06 and 0.12 ± 0.01 pmol·mg protein$^{-1}$·min$^{-1}$, respectively).

Effect of maintaining HK-2 cells in folate-oversupplemented growth medium on hRFC steady state mRNA and protein and on hRFC promoter activity

The normal kidney expresses FR, RFC, and PCFT/HCP1 systems (19–24). The FR functions in the nanomolar range (20–24), whereas the hRFC and the PCFT/HCP1 systems function in the micromolar range (16, 18, 19). In this study, we determined the effect of maintaining the HK-2 cells in folate-oversupplemented growth medium on the mRNA expressions of hRFC, the PCFT/HCP1 systems, and FR. Semiquantitative RT-PCR was performed on RNA isolated from cells grown under folate-oversupplemented and -sufficient conditions using specific primers for hRFC, PCFT/HCP1, and FR (Table 1). The results (Figure 4, A, B, and C) showed the mRNA of the hRFC, PCFT/HCP1, and FR (normalized to β-actin gene) to be significantly ($P < 0.01$ for all) lower in cells maintained in folate-oversupplemented growth medium than in cells maintained in folate-sufficient medium.

Effect of maintaining HK-2 cells in folate-oversupplemented growth medium on protein expressions of hRFC and FR was also examined by Western analysis with the use of specific polyclonal antibodies as described in "Methods." The results showed a significant ($P < 0.01$) reduction in the levels of both proteins in cells maintained under folate-oversupplemented conditions than in cells maintained under folate-sufficient conditions (Figure 4, D and E).

We also examined the effect of maintaining HK-2 cells in folate-oversupplemented growth medium on act on FR1C promoter activity of the hRFC gene (a prominent hRFC promoter in a variety of tissues (31, 34)). The results (Figure 4F) showed activity of the hRFC promoter to be significantly ($P < 0.01$) lower in cells maintained in folate-oversupplemented growth medium than in cells maintained in folate-sufficient growth medium.

DISCUSSION

Our aims in these investigations were to examine the effects of long-term oversupplementation with folic acid on human intestinal and renal folate uptake. The physiologic and nutritional importance of such investigations relate because chronic supplementation with high concentrations of folic acid is widespread in the general population and because no study has investigated the possible effect of such long-term practice on the physiologic markers of folate, including its intestinal and renal uptake processes. Therefore, the present study was designed to address these issues with the intestinal epithelial Caco-2 cells and the renal epithelial HK-2 cells as models. Both of these human-derived epithelial cell lines have been shown to be excellent in...
FIGURE 4. A: Effect of folic acid oversupplementation on human reduced folate carrier (hRFC) mRNA level in HK-2 cells. Semiquantitative reverse transcription–polymerase chain reaction (RT-PCR) analysis was performed with the use of mRNA from HK-2 cells maintained in growth media oversupplemented (100 μmol/L) or sufficient (0.25 μmol/L) in folate (as well as in the presence of an intermediate folic acid concentration of 9 μmol/L) and the hRFC specific primers as shown in Table 1. All experiments were run on at least 3 separate occasions. Results of a representative experiment are shown. Values are mean ± SEM. *P < 0.05; **P < 0.01. B: Effect of folic acid oversupplementation on folate receptor (FR) mRNA in HK-2 cells. Semiquantitative RT-PCR analysis was performed with the use of mRNA from HK-2 cells maintained in growth media oversupplemented (100 μmol/L) or sufficient (0.25 μmol/L) in folate (as well as in the presence of an intermediate folic acid concentration of 9 μmol/L) and the FR specific primers as shown in Table 1. All experiments were run on at least 3 separate occasions. Results of a representative experiment are shown. Values are mean ± SEM. *P < 0.05; **P < 0.01. C: Effect of folic acid oversupplementation on proton-coupled folate transporter/heme carrier protein 1 (PCFT/HCP1) mRNA in HK-2 cells. Semiquantitative RT-PCR analysis was performed with the use of mRNA from HK-2 cells maintained in growth media oversupplemented (100 μmol/L) or sufficient (0.25 μmol/L) in folate (as well as in the presence of an intermediate folic acid concentration of 9 μmol/L) and the PCFT/HCP1 specific primers as shown in Table 1. All experiments were run on at least 3 separate occasions. Results of a representative experiment are shown. Values are mean ± SEM. *P < 0.01. D: Effect of folic acid oversupplementation on the level of hRFC protein in HK-2 cells. Western blot analysis was performed with the use of membranous fractions of HK-2 cells maintained in growth media oversupplemented (100 μmol/L) or sufficient (0.25 μmol/L) in folate (as well as in the presence of an intermediate folic acid concentration of 9 μmol/L) and specific anti-hRFC polyclonal antibodies. Image and data shown are representative of 3 separate sets of experiments. Values are mean ± SEM. *P < 0.01. E: Effect of folic acid oversupplementation on the level of FR protein in HK-2 cells. Western blot analysis was performed with the use of membranous fractions of HK-2 cells maintained in growth media oversupplemented (100 μmol/L) or sufficient (0.25 μmol/L) in folate (as well as in the presence of an intermediate folic acid concentration of 9 μmol/L) and specific anti-FR polyclonal antibodies. Image and data shown are representative of 3 separate sets of experiments. Values are mean ± SEM. *P < 0.05; **P < 0.01. F: Effect of folic acid oversupplementation on the activity of hRFC–promoter B (pB) promoter B (pB) in HK-2 cells. Cells maintained in growth media oversupplemented (100 μmol/L) or sufficient (0.25 μmol/L) in folate (as well as in the presence of an intermediate folic acid concentration of 9 μmol/L) were cotransfected with hRFC–pB promoter: luciferase reporter plasmids and a control pGL3-basic vector as described in Subjects and Methods. Values are mean ± SEM; n = 3. Firefly luciferase activity was normalized relative to the activity of simultaneously expressed Renilla luciferase. *P < 0.05; **P < 0.01.
vitro models for investigating physiologic and molecular aspects of nutrient transport with results that are similar to those found in native human intestinal and renal epithelia (25, 26).

Our results with the intestinal Caco-2 cells showed that maintaining these cells in a folic acid–oversupplemented growth medium leads to a significant down-regulation in folate uptake at pH 5.5 but not 7.4 compared with uptake by cells maintained under a folate-sufficient condition. The observed down-regulation was specific for folate because uptake of the unrelated water-soluble vitamin, biotin, was not affected by maintaining the cells under different folate conditions. The down-regulation in folate uptake in the folate-oversupplemented cells was associated with a decrease in the mRNA and protein levels of the hRFC system; it was also associated with a decrease in message level of the PCFT/HCP1 system. Both of these transport systems are believed to be involved in the intestinal folate uptake process (17, 19). The observed reductions of mRNA abundance of these transporters raise the possibility of involvement (at least in part) of transcriptional regulatory mechanism(s). This assumption was confirmed, at least for the hRFC system, by showing a significant reduction in the activity of the hRFC system /b/pB in Caco-2 cells maintained in folate-oversupplemented growth medium compared with cells maintained in folate-sufficient growth medium.

About the effect of folate oversupplementation on renal folate uptake, our findings with HK-2 cells showed that maintaining these cells in a folic acid–oversupplemented growth medium leads to a significant down-regulation of folic acid uptake at buffer pH 5.5 compared with cells maintained in folate-sufficient growth medium. No such down-regulation was observed when folic acid uptake was examined at buffer pH 7.4. Again the down-regulation in folic acid uptake by folate oversupplementation was specific for folate, because uptake of the unrelated biotin was not affected by the folate concentration in the growth medium. Unlike the intestine, the normal kidneys express the FR, the RFC, and the PCFT/HCP1 system (19–24), all of which are shown or believed to play a role in renal folate uptake process (19–24). Note that the human renal FR has an apparent K<sub>m</sub> for folic acid in the nanomolar range [≈10 nM (23)], whereas the apparent K<sub>m</sub> of the hRFC and the PCFT/HCP1 is in the micromolar range (16, 18, 19). It is, therefore, reasonable to assume that under our experimental conditions with 2 μmol/L, H-folic acid, uptake is mainly mediated via the hRFC and PCFT/HCP1 systems and that the observed down-regulation in folate uptake is mainly due to changes in the activity of these systems. Nevertheless, we have determined the effect of maintaining the HK-2 cells in folate-oversupplemented growth medium on the steady state mRNA levels of the hRFC system, the PCFT/HCP1 system, and the FR and found a significant reduction in all cases compared with cells maintained under folate-sufficient growth medium. The level of the hRFC and the FR proteins were also reduced in cells maintained under folate-oversupplemented condition compared with cells incubated under folate-sufficient condition. The reduction in mRNA abundance of these transporters raises the possibility that a transcriptional regulatory mechanism(s) may be involved. This assumption was confirmed, at least, for the hRFC system by showing a significant reduction of the hRFC pB activity in HK-2 cells maintained in folate-oversupplemented medium compared with cells maintained in folate-sufficient medium.

From the above discussion, it is clear that both the intestinal and the renal folate uptake processes are down-regulated on long-term oversupplementation with folic acid. This phenomenon may have significant clinical relevance when persons consuming high doses of folate experience serious acute illnesses that lead to abrupt cessation of food intake. In such circumstances down-regulation of renal tubular folate reabsorption in the absence of its continued intake can lead to precipitous depletion of this important essential nutrient at a time when its adequate supplies are critical to meet the heightened metabolic demands and reparative processes. Notable among such cases are persons experiencing catastrophic accidents, stroke, acute gastrointestinal disorders (eg, obstruction, infarction, severe gastroenteritis, acute abdominal events, etc), and fulminant infections, among others. In such cases parenteral administration of folate should be considered to avoid precipitous development of a serious deficiency state. Clinical investigations are needed to test whether this scenario does indeed occur in such patients who have been on folate supplements and to determine the time frame required for restoration of the normal intestinal and renal folate uptake processes.

In summary, results of these investigations show that long-term oversupplementation with folic acid leads to a specific and significant down-regulation of both intestinal and renal folate uptake processes. The observed down-regulation folate uptake was associated with significant reductions of hRFC, PCFT/HCP1, and FR expressions. Furthermore, at least for the hRFC system, the down-regulation appeared to be mediated (in part) via transcriptional regulatory mechanism(s).

We thank the National Institutes of Health and the Department of Veterans Affairs for their financial support.

The author’s responsibilities were as follows—HMS and NDV: planning, data analysis, and writing of the manuscript; BS and ZMM: executed the study, planning, and data analysis. None of the authors had a conflict of interest to declare.

REFERENCES