Serum Homocysteine, Cholesterol, Retinol, α-Tocopherol, Glycosylated Hemoglobin and Inflammatory Response during Therapy with Bevacizumab, Oxaliplatin, 5-Fluorouracil and Leucovorin

BOHUSLAV MELICHAR1,6, HANA KALÁBOVÁ1, LENKA KRČMOVÁ2,5, MARKéta KAŠPAROVÁ2,5, EVA MALÍŘOVÁ3, KAROLíNA MELICHAROVÁ2, MIROSŁAV PECKA4, RADOMÍR HYŠPLER2 and DAGMAR SOLICHOVÁ2

Departments of 1Oncology and Radiotherapy, 2Gerontology and Metabolic Care, 3Nuclear Medicine, and 4Hematology, Charles University Medical School and Teaching Hospital, Hradec Králové; 5Department of Analytical Chemistry, Charles University School of Pharmacy, Hradec Králové; 6Department of Oncology, Palacký University Medical School and Teaching Hospital, Olomouc, Czech Republic

Abstract. Background: Targeted agents present with a new spectrum of side-effects, including toxicities that negatively impact the risk of atherosclerosis. The aim of the present study was to evaluate the effect of the combination of targeted therapy and chemotherapy on serum homocysteine and other laboratory parameters of cardiovascular risk in patients with metastatic colorectal carcinoma. Patients and Methods: Thirty-one patients with metastatic colorectal carcinoma treated with the combination of bevacizumab, oxaliplatin, 5-fluorouracil and leucovorin were studied before and during the therapy. Results: Serum homocysteine decreased significantly throughout the course of treatment. Total cholesterol and low-density lipoprotein cholesterol also decreased significantly during the first month of therapy. In contrast, serum retinol significantly increased during the second and third months of treatment. A significant increase in glycosylated hemoglobin was also observed. After an initial rise, serum C-reactive protein (CRP) and carcinoembryonic antigen (CEA) were significantly lower compared to baseline throughout the course of treatment. Serum ferritin increased throughout most of the course of treatment. A significant correlation was observed between CRP and high-density lipoprotein cholesterol, retinol, ferritin, and CEA. CEA correlated with hemoglobin, retinol, and ferritin. Retinol correlated significantly with hemoglobin. Conclusion: Tumor control, reflected in lower CEA, resulted in suppression of the acute phase response and generally in favorable effects on laboratory parameters indicative of risk factors of atherosclerosis, including lower homocysteine concentrations, and lower total and LDL cholesterol.

The prognosis of metastatic colorectal carcinoma has improved substantially during the last decade as a result of the introduction of new effective cytotoxic agents (1) and, more recently, targeted agents, the monoclonal antibodies cetuximab and bevacizumab (2, 3). It has been demonstrated that combination of 5-fluorouracil with either irinotecan or oxaliplatin is, in terms of efficacy, superior to 5-fluorouracil alone (4, 5). The addition of bevacizumab, monoclonal antibody against vascular endothelial growth factor (VEGF), to combination chemotherapy has been shown to increase survival both in the first-line as well as in the second-line setting (2, 6). Although therapy with bevacizumab is, in general, well tolerated, the drug has a peculiar toxicity profile, with the principal side-effects including proteinuria, hypertension and thrombosis. The spectrum of these toxicities is linked to the fact that VEGF is an endothelial growth factor, and VEGF blockade leads to endothelial cell dysfunction (7). These toxicities are usually mild and of little clinical significance in most patients because of their limited life expectancy, but could be of concern in long-term survivors because they are associated with increased risk of atherosclerosis and its complications.

Thus, with the remarkable improvement of long-term prognosis in patients with inoperable metastatic colorectal carcinoma treated with the combination of targeted agents...
and chemotherapy that may, when combined with secondary surgery (8), even result in cure in some patients, chronic effects of treatment on the cardiovascular system may become an important consideration. Risk factors of atherosclerosis are complex, and, along with hypertension or hypercholesterolemia, include high serum concentrations of homocysteine, C-reactive protein (CRP) or parameters of oxidative stress (9). The effect of anticancer therapy on laboratory parameters associated with the risk of atherosclerosis, e.g. serum lipids, has been well defined for hormonal agents (10, 11), but less is known about the effect of targeted agents or chemotherapy on serum cholesterol or other laboratory parameters of cardiovascular risk.

Homocysteine is an intermediate in the metabolism of methionine. Increased serum concentration of homocysteine is, in most cases, caused by a deficiency of folate or vitamin B12. Hyperhomocysteinemia has been amply documented in patients with vascular disorders, and increased homocysteine levels are a well-defined risk factor of atherosclerosis and thrombosis (12, 13). Hyperhomocysteinemia has also been documented in cancer patients (14-16).

Patients with advanced cancer have laboratory evidence of activation of the acute phase response. CRP is the parameter most widely used to assess the acute phase response, CRP concentrations are also increased in the serum of patients with atherosclerosis, and elevated serum CRP levels predict the risk of future cardiovascular events (17-19).

Administration of cytotoxic agents or biological agents is associated with oxidative stress, and disorders of antioxidant balance are thought to be involved in the toxicity associated with radiotherapy and chemotherapy. Oxidative stress also plays an important role in atherosclerosis as the atherogenic potential of serum lipids is dependent on oxidation (20) and is affected by liposoluble antioxidants (21). Moreover, antioxidant administration may retard atherosclerosis (22). Vitamin E represents the major antioxidant in the serum (23).

The term vitamin E denotes several naturally occurring tocopherols and tocotrienols, but alpha-tocopherol is responsible for most of the vitamin E activity in animal tissues. Disorders of antioxidant balance involving vitamin E are also thought to be involved in the toxicity associated with radiotherapy (24), and chemotherapy (25). A decrease in serum alpha-tocopherol has been observed during systemic chemotherapy (26-29). Retinol is a major circulating form of vitamin A that has also antioxidant activity and plays an essential role in many physiological functions, including vision, growth, development and differentiation, and immune response (23). Alterations of iron metabolism are also associated with oxidative stress. In some studies, elevated serum ferritin, an indicator of iron stores, was associated with the risk of acute myocardial infarction (30, 31), and progression of atherosclerosis (22). Serum ferritin concentrations are increased in the acute phase response and, along with other indicators of the acute phase response, ferritin is elevated in cancer patients. Recently, we described a marked increase in serum ferritin in breast cancer patients treated with chemotherapy (32). The aim of the present study was to evaluate the effect of combination therapy with bevacizumab, oxaliplatin, 5-fluorouracil and leucovorin on serum homocysteine and some other parameters of cardiovascular risk in patients with metastatic colorectal carcinoma.

Patients and Methods

Patients. Thirty-one patients, 11 women and 20 men, aged 57±10 (range 33-70) years, with histologically verified metastatic colorectal carcinoma were included in the present study. The intravenous regimen consisted in 29 patients of combination of bevacizumab and FOLFOX7 as follows: bevacizumab (5 mg/kg), oxaliplatin (130 mg/m2 every 2 weeks), leucovorin (400 mg/m2) and 5-fluorouracil (400 mg/m2 bolus and 2400 mg/m2 for 46 hour infusion) every 2 weeks. Two patients were treated with a modified regimen of bevacizumab, oxaliplatin, 5-fluorouracil and leucovorin combining systemic bevacizumab and hepatic arterial infusion of oxaliplatin. Twenty-two patients were treated in the first line of therapy, and 9 patients were treated in the second or higher line of therapy. Two patients had diabetes mellitus and 3 patients had hyperlipidemia controlled with therapy. Standard premedication before each cycle included intravenous granisetron or ondansetron and dexamethasone (16 mg). The project was approved by the Institutional Ethical Committee, and the patients signed informed consent.

Measurement of serum homocysteine, lipids and CRP. Blood samples were taken before the start of therapy, after the first cycle, and before each subsequent cycle of combined therapy (maximum 8 cycles). Homocysteine concentration was determined immunochemically (Immulite 2000; Siemens Healthcare Diagnostics, Deerfield, USA). Serum cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and CRP were determined using commercially available kits on a MODULAR analyzer (Hoffmann-La Roche, Basel, Switzerland).

Analysis of alpha-tocopherol and retinol. Serum alpha-tocopherol and retinol were determined before and during the therapy by high-performance liquid chromatography as described elsewhere (33). Blood samples were drawn from a peripheral vein after an overnight fast. The samples were transferred immediately to the laboratory, centrifuged (1600g for 10 minutes at 16°C), the serum was separated and then frozen at –20°C until analysis. In the liquid-liquid extraction procedure, 500 μl of serum were deproteinized by cool ethanol denatured with 5% methanol (500 μl for 5 minutes at 4°C). Subsequently, 2500 μl of n-hexane were added to this mixture and extracted for 5 minutes by a vortex apparatus. After centrifugation (1600g for 10 minutes at 0°C), the aliquot (2000 μl) of the clean extract was separated and evaporated under nitrogen (60°C). The residue was dissolved in 400 μl methanol and analyzed by reversed-phase high performance liquid chromatography using external standard calibration. The analyses were performed using a Perkin Elmer high-performance liquid chromatography set (Norwalk, USA).
Results

As shown in Table I, serum homocysteine decreased significantly throughout the course of treatment. Serum total cholesterol also decreased significantly during the first month of therapy. This decrease affected LDL cholesterol. After an initial mild decrease, HDL cholesterol significantly increased late during the course of treatment. The decrease of total cholesterol during the first month of therapy was accompanied by a reduction in alpha-tocopherol. In contrast, serum retinol significantly increased during the second and third months of treatment. A significant increase in glycosylated hemoglobin was also observed at several visits. After an initial significant increase, CRP significantly decreased compared to baseline throughout the course of treatment. Hemoglobin concentration remained relatively stable, but serum ferritin increased throughout most of the course of treatment. The course of CEA mirrored concentrations of CRP. After an initial rise, that was, however, not significant, a significant decrease of serum CEA was observed starting from the second month of therapy.

Significant negative correlations were observed between CRP and HDL cholesterol ($r_s = -0.37$, $p<0.05$), retinol ($r_s = -0.70$, $p<0.0001$; Figure 1) and positive correlations were noted between CRP and ferritin ($r_s = 0.52$, $p<0.01$), and CEA ($r_s = 0.60$, $p<0.001$). CEA correlated inversely with hemoglobin ($r_s = -0.36$, $p<0.05$), and with retinol ($r_s = -0.60$, $p<0.001$), and positively with ferritin ($r_s = 0.42$, $p<0.05$). Retinol correlated significantly with hemoglobin ($r_s = 0.77$, $p<0.00001$). Alpha-tocopherol correlated significantly with total cholesterol ($r_s = 0.48$, $p<0.01$) and LDL cholesterol ($r_s = 0.42$, $p<0.05$), and total cholesterol correlated significantly with LDL cholesterol ($r_s = 0.95$, $p<0.00001$).

Discussion

The present data demonstrate that bevacizumab administered with combination chemotherapy has a complex short-term effect on laboratory measured risk factors of atherosclerosis that includes changes both favorable and unfavorable for the long-term risk of atherosclerosis. Some of these changes are probably due to the effect of combined therapy on metastatic tumor. Reduced tumor burden results in lower serum CEA and CRP concentrations. Suppression of the systemic inflammatory response also results in high retinol and, possibly, lower homocysteine concentrations.

The serum concentrations of cholesterol and LDL cholesterol decreased markedly during the first cycle of therapy. Non-fasting samples were used for lipid analyses in the present study, similarly to some earlier reports that examined the effect of anticancer agents (36). In cancer patients receiving emetogenic chemotherapy, it is difficult to require an overnight fast before chemotherapy repeatedly. The decrease of serum total and LDL cholesterol was evident mostly during the first month of therapy. Later in the course of treatment, an increase of HDL cholesterol was observed that could also have a favorable impact on the progression of atherosclerosis.
With some limitations, serum CEA concentrations may reflect tumor burden (37). With a latency, after an initial rise that was, however, not significant, serum CEA decreased, indicating reduced tumor mass resulting from the effective anticancer therapy. An initial surge of serum CEA concentrations after the start of treatment that is indicative of therapeutic response has been described in patients with metastatic colorectal carcinoma (38, 39).

Serum CEA concentrations correlated significantly with serum CRP, an indicator of the acute phase response. An increase in laboratory parameters of the acute phase response has been well described in patients with metastatic colorectal carcinoma (40). The kinetics of CRP was similar to CEA, including the initial surge. The decrease of CRP concentration was linked to the lowering of the circulating homocysteine concentration, suggesting an association between inflammatory response and homocysteine that has been postulated earlier (14, 41). As CRP is predictive of cardiovascular event risk (9, 17-19, 42), a marked decrease of CRP after combined therapy could have a potentially favorable effect on the risk of cardiovascular events.

In association with the acute phase response, serum ferritin concentrations increased significantly after the start of treatment, but remained increase compared to baseline. This observation is in agreement with our earlier observation of increased ferritin in breast carcinoma patients treated with neoadjuvant chemotherapy (32). While the early marked increase in serum ferritin may reflect the activity of the acute phase response, sustained elevated ferritin levels may be the result of transient fluctuations of serum iron concentrations that accompany the administration of chemotherapy (32, 43-45).

Homocysteine concentrations decreased significantly throughout the course of therapy. Homocysteine may be produced by tumor cells (15), and the decrease of serum homocysteine after combined therapy may be explained by inhibition of tumor growth resulting from the treatment (16). The published information on homocysteine in patients treated with chemotherapy or targeted therapy is limited. A decrease in serum homocysteine has been documented after the administration of ifosfamide/mesna (46), but an increase was observed after methotrexate administration (47). A decrease of circulating homocysteine concentrations has also been described after leucovorin administration (48). The changes of circulating homocysteine concentrations observed in the studies mentioned above are a result of the effect of anticancer agents on homocysteine metabolism, rather than an increase in circulating homocysteine concentrations observed in the studies mentioned above as result of the effect of anticancer agents on circulating homocysteine (49).

Table I. Laboratory parameters during therapy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
<th>Visit 5</th>
<th>Visit 6</th>
<th>Visit 7</th>
<th>Visit 8</th>
<th>Visit 9</th>
<th>Visit 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time from the start of treatment</td>
<td>–2±4</td>
<td>7±1</td>
<td>15±4</td>
<td>35±7</td>
<td>52±14</td>
<td>65±11</td>
<td>81±15</td>
<td>101±14</td>
<td>114±14</td>
<td>135±16</td>
</tr>
<tr>
<td>n</td>
<td>31</td>
<td>30</td>
<td>31</td>
<td>31</td>
<td>30</td>
<td>28</td>
<td>27</td>
<td>22</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Homocysteine (μmol/l)</td>
<td>12.9±6.1</td>
<td>9.0±3.1</td>
<td>9.0±3.1</td>
<td>11.0±4.0</td>
<td>9.9±3.7</td>
<td>9.2±3.4</td>
<td>9.1±2.5</td>
<td>9.8±2.9</td>
<td>8.5±2.0</td>
<td>8.9±2.0</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.6±1.7</td>
<td>4.7±1.3</td>
<td>4.8±1.3</td>
<td>5.2±1.4</td>
<td>5.1±1.0</td>
<td>5.5±1.5</td>
<td>5.4±1.0</td>
<td>5.3±1.2</td>
<td>5.2±0.8</td>
<td>4.8±0.8</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.8±1.4</td>
<td>2.8±1.1</td>
<td>3.1±1.0</td>
<td>3.3±1.3</td>
<td>3.2±0.9</td>
<td>3.4±1.4</td>
<td>3.4±1.1</td>
<td>3.2±0.9</td>
<td>3.3±0.6</td>
<td>2.8±0.6</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2±0.37</td>
<td>1.1±0.41</td>
<td>1.2±0.34</td>
<td>1.3±0.44</td>
<td>1.4±0.45</td>
<td>1.5±0.42</td>
<td>1.3±0.39</td>
<td>1.3±0.36</td>
<td>1.3±0.39</td>
<td>1.3±0.36</td>
</tr>
<tr>
<td>Retinol (μmol/l)</td>
<td>1.29±0.47</td>
<td>1.3±0.50</td>
<td>1.3±0.48</td>
<td>1.4±0.46</td>
<td>1.4±0.47</td>
<td>1.5±0.50</td>
<td>1.3±0.37</td>
<td>1.3±0.36</td>
<td>1.3±0.37</td>
<td>1.3±0.37</td>
</tr>
<tr>
<td>Alpha-tocopherol (μmol/l)</td>
<td>25.7±5.5</td>
<td>21.5±5.3</td>
<td>24.5±5.3</td>
<td>24.5±5.3</td>
<td>25.2±5.1</td>
<td>26.5±5.0</td>
<td>25.3±5.0</td>
<td>24.2±6.1</td>
<td>25.3±7.0</td>
<td>21.8±5.7</td>
</tr>
<tr>
<td>Glycosylated hemoglobin (%)</td>
<td>3.6±0.8</td>
<td>3.7±0.9</td>
<td>3.8±0.9</td>
<td>3.8±1.0</td>
<td>3.8±1.0</td>
<td>3.8±1.0</td>
<td>3.8±1.0</td>
<td>3.8±1.0</td>
<td>3.8±1.0</td>
<td>3.8±1.0</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>35±49</td>
<td>51±54</td>
<td>19±25</td>
<td>19±25</td>
<td>16±19</td>
<td>17±25</td>
<td>15±27</td>
<td>10±31</td>
<td>10±11</td>
<td>22±26</td>
</tr>
<tr>
<td>Hemoglobin (g/l)</td>
<td>12±16</td>
<td>12±17</td>
<td>12±17</td>
<td>12±17</td>
<td>12±14</td>
<td>119±16</td>
<td>121±17</td>
<td>119±21</td>
<td>117±18</td>
<td>119±14</td>
</tr>
<tr>
<td>Ferritin (μg/l)</td>
<td>150.3±197.6</td>
<td>332.9±335.1</td>
<td>171.8±169.3</td>
<td>177.1±159.6</td>
<td>152.6±139.5</td>
<td>182.7±151.4</td>
<td>190.0±164.8</td>
<td>393.9±861.9</td>
<td>385.6±773.1</td>
<td>196.1±131.6</td>
</tr>
<tr>
<td>Carcinoembryonic antigen (μg/l)</td>
<td>565.2±1071.4</td>
<td>957.3±2122.8</td>
<td>933.8±2194.9</td>
<td>553.8±1264.0</td>
<td>389.1±775.0</td>
<td>401.3±774.9</td>
<td>285.9±724.5</td>
<td>119.2±230.1</td>
<td>175.0±284.1</td>
<td>234.4±375.8</td>
</tr>
</tbody>
</table>

Mean±standard deviation of the respective parameters are shown. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 and *****p<0.00001 compared to baseline.
concentrations observed in the present study was similar to the
decrease observed in an earlier study in patients treated with
5-fluorouracil/leucovorin (48). With the exception of the initial
surge, serum homocysteine concentrations followed CEA
levels. Homocysteine may have different dynamics from other
primary markers as its concentrations reflect tumor cell
proliferation and the number of live cells (15).

Administration of chemotherapy is associated with oxidative
stress that results in lower concentrations of circulating
antioxidants. Moreover, administration of chemotherapy is
associated with small bowel dysfunction, and disturbances of
the small bowel function are accompanied by a low serum
concentration of retinol (49-51). The present observation of a
drop in alpha-tocopherol is in agreement with earlier
reports of decreased alpha-tocopherol concentrations during
systemic chemotherapy that were associated with oxidative
stress induced by the therapy (26-29). However, in a recent
study we observed increased alpha-tocopherol and retinol
concentrations during paclitaxel/carboplatin combination
chemotherapy (52) and hypothesized that these increases are
probably linked to the suppression of the systemic
inflammatory response by chemotherapy. Serum concentrations
of alpha-tocopherol and retinol are significantly decreased in
patients with advanced cancer (53, 54), and this decrease
correlates with the systemic inflammatory response (53, 55).
In the present cohort of patients, we did not demonstrate an
increase in serum alpha-tocopherol, but an increase in serum
retinol was evident. Significant negative correlation was
observed between CRP and retinol concentrations. The
suppression of the systemic inflammatory response associated
with tumor control could thus explain the increase of serum
retinol observed later during the course of treatment in the
present study.

In earlier studies, low vitamin E intake was associated with
toxicity of chemotherapy in children with acute lymphoblastic
leukemia (56). The administration of vitamin E has been shown
to alleviate some side-effects of radiotherapy (24), and
chemotherapy (25). Although serial monitoring of vitamin E
levels may be a necessary prerequisite for any therapeutic use
of this antioxidant vitamin, serum vitamin E is not being
routinely measured in cancer patients, and, in most reports
published so far, alpha-tocopherol has been investigated in
epidemiological studies in relation to cancer risk. In the present
study, low serum alpha-tocopherol was associated with a
decreased cholesterol concentration and could be linked to the
toxicity of chemotherapy and resulting lower dietary intake.
Lower alpha-tocopherol concentration may have unfavorable
long-term effects on the progression of atherosclerosis. In
contrast to cholesterol or homocysteine, glycosylated
hemoglobin increased significantly during the treatment. An
increase in circulating VEGF has been described in patients
with diabetes mellitus that has been associated with the
presence of complications (57-59). The moderate increase of
glycosylated hemoglobin may be caused by the administration
dexamethasone as part of premedication or the effect of
chemotherapy rather than bevacizumab.

Thus, the administration of bevacizumab and combined
chemotherapy has effects on laboratory parameters that both
increase and decrease the risk of atherosclerosis. Along with
hypertension (7), VEGF inhibition causes proteinuria, another
risk factor of cardiovascular mortality (60). Data from the
present cohort also indicate that the administration of
combined treatment may be associated with impaired glucose
tolerance. The concentrations of alpha-tocopherol, the major
liposoluble antioxidant, decreased during the treatment, but
retinol concentrations increased. Alterations in iron
metabolism, reflected in increased ferritin concentrations, and
associated oxidative stress may also accelerate the progression
of atherosclerosis. On the other hand, therapy led to lower
cholesterol and homocysteine concentration and a decrease in
systemic inflammation reflected in lower CRP levels. The
suppression of the systemic inflammatory response could have
also resulted in increased HDL-cholesterol levels. Most of
these unfavorable changes were moderate in magnitude and
of limited duration, so it cannot be expected that these changes
would have a dramatic effect on the progression of
atherosclerosis.

In the past, the long-term risk of atherosclerosis was of little
concern in patients with metastatic cancer. In the last decade,
data have emerged indicating a significant rise of the incidence
of complications of atherosclerosis in survivors of metastatic
cancer curable with chemotherapy, e.g. germ cell tumors (61-63).
Although the cure rate of metastatic colorectal carcinoma is
much lower compared, for example, to metastatic testicular
carcinoma, the survival of patients with metastatic colorectal
carcinoma has improved dramatically over the past two
decades (1), with median survival currently being close to 2
years (64). In addition, a significant proportion of patients with
initially unresectable tumors become candidates for secondary
resection. In a recently published report, the 5- and 10-year
survival of patients undergoing secondary resection was 33%
and 27%, respectively (8). Thus, a significant proportion of
metastatic colorectal carcinoma patients survive, under therapy,
more than 5 years, and the question of how chronic therapy
affects the long-term risk of cardiovascular disorders is of
legitimate concern. Thus, the observation that the combination
of VEGF-targeted therapy and chemotherapy has, besides
unfavorable effects on hypertension and proteinuria, also
favorable effects on laboratory risk factors of atherosclerosis is
reassuring from the point of view of clinical practice.

In conclusion, combination therapy with bevacizumab,
oxaliplatin, 5-fluorouracil and leucovorin is an effective therapy
that results in marked decrease of serum CEA. Tumor control
also resulted in suppression of the acute phase response,
reflected in CRP concentrations. CRP correlated negatively with
retinol and HDL-cholesterol, and positively with CEA and
ferritin. Tumor control after administration of combined therapy resulted in generally favorable effects on laboratory parameters indicative of risk factors of atherosclerosis, including decreased homocysteine concentrations, lower total and LDL cholesterol, but some changes associated with the treatment, including increased ferritin and increased glycosylated hemoglobin might have a negative impact on the risk of atherosclerosis.

Acknowledgements

The study was supported by a grant of the Internal Grant Agency of the Ministry of Health of the Czech Republic (NR9096-4).

References


Received June 19, 2009
Revised October 8, 2009
Accepted October 13, 2009