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Effect of Walker 256 tumor growth on intestinal absorption of leucine, methionine and glucose in newly weaned and mature rats

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Abstract

In tumor-bearing rats, most of the serum amino acids are used for synthesis and oxidation processes by the neoplastic tissue. In the present study, the effect of Walker 256 carcinoma growth on the intestinal absorption of leucine, methionine and glucose was investigated in newly weaned and mature rats. Food intake and carcass weight were decreased in newly weaned (NT) and mature (MT) rats bearing Walker 256 tumor in comparison with control animals (NC and MC). The tumor/carcass weight ratio was higher in NT than in MT rats, whereas nitrogen balance was significantly decreased in both as compared to control animals. Glucose absorption was significantly reduced in MT rats (MT = 47.3 ± 4.9 *vs* MC = 99.8 ± 5.3 nmol min^{-1} cm^{-1}, Kruskal-Wallis test, $P<0.05$) but this fact did not hamper the evolution of cancer. There was a significant increase in methionine absorption in both groups (NT = 4.2 ± 0.3 and MT = 2.0 ± 0.1 *vs* NC = 3.7 ± 0.1 and MC = 1.2 ± 0.2 nmol min^{-1} cm^{-1}, Kruskal-Wallis test, $P<0.05$), whereas leucine absorption was increased only in young tumor-bearing rats (NT = 8.6 ± 0.2 *vs* NC = 7.7 ± 0.4 nmol min^{-1} cm^{-1}, Kruskal-Wallis test, $P<0.05$), suggesting that these metabolites are being used for synthesis and oxidation processes by the neoplastic cells, which might ensure their rapid proliferation especially in NT rats.

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- Aging

Cachexia is a poorly understood syndrome characterized by anorexia, generalized host wasting and a variety of metabolic alterations that result in death. Continued neoplastic growth leads to host cachexia and to a negative nitrogen balance. Rats bearing Walker 256 tumor show a decrease in lean body mass with an increase in neoplastic tissue, and this process is accompanied by marked protein degradation in host tissues (1). The decrease in lean body mass occurring in Walker 256 tumor-bearing rats has been assumed to occur due to a reduction in protein synthesis, an increase in protein degradation or both (1). There is a close relationship between tumor growth and muscle proteolysis, and the growing tumor is making a demand on required nutrients, especially essential amino acids (2). Tumors have a characteristically high rate of glycolysis, which is essential for tumor growth or marginal manifestation of malignant transformation. Protein breakdown in skeletal muscle produces branched-chain amino acids, which are oxi-

dized by host tissues and/or utilized for the synthesis process by tumor cells (3). Leucine is an important ketogenic amino acid that provides energy and a substrate for protein synthesis in the skeletal musculature, and leucine utilization by tumor cells is very high compared with normal cells due to the high rate of lipid and protein synthesis (3). In fact, Walker 256 tumor growth causes a marked raise in the daily need for leucine in rats (3). Methionine is an important essential amino acid for the protein synthesis, and the growth of many tumors depends on the exogenous methionine supply (4). One important point, however, remains to be investigated: does Walker 256 tumor growth affect intestinal absorption of amino acids? This is a very important issue to be investigated which certainly could play a key role in the establishment of cachexia under these conditions. The effect of Walker 256 tumor growth on intestinal absorption of methionine and leucine was investigated. The absorption of glucose was also examined for comparison. Knowing that tumors grow faster in young than aged subjects (5), the experiments were performed on newly weaned and mature rats.

Newly weaned (25 days old, N = 33) and mature (90 days old, N = 25) rats were divided into four groups: newly weaned control (NC), mature control (MC), newly weaned tumor-bearing (NT), and mature tumor-bearing (MT) animals. All animals were housed in metabolic cages under normal conditions (22 ± 2°C, 12/12 h light/dark cycle), with free access to a semi-synthetic diet and water (6). Groups NT and MT were subcutaneously implanted with Walker 256 tumor (approximately 0.25 x 10^6 cells in 0.5 ml). Control groups (NC, MC) were injected with 0.5 ml 0.9% NaCl. Thirteen and 19 days after tumor implantation, the newly weaned and mature groups were submitted to intestinal perfusion (7) to estimate leucine, methionine and glucose absorption. Ringer solution containing 138.7 µmol/l glucose, or 167.5 µmol/l DL-methionine or 190.5 µmol/l L-leucine, at 37°C, pH 7.0, was used for intestinal perfusion over a period of 60 min at a flow rate of 0.5 to 1.0 ml/min. Glucose, methionine and leucine content in perfused fluid was determined by spectrophotometric methods (8-10). Kruskal-Wallis one-way analysis (11) was used to assess the comparisons among the groups and the level of significance was set at $P<0.05$.

Anorexia is frequently seen in patients and animals with cancer, leading to cachexia of the host (12). In the present study, we observed that tumor implantation caused a marked reduction in daily food intake by newly weaned and mature rats (62 and 39%, respectively) compared with control animals (Table 1). This difference may indicate that the changes produced by tumor growth in young animals are more pronounced than in adults. In fact, tumors grow faster in young than in aged patients (5). Studies employing CO/COBS rats bearing Walker 256 tumor for 14 days showed a 20% reduction in food intake and a 15% reduction in body weight (12). In our study, body weight decreased by 20% in NT and by 8.3% in MT rats, 13 and 19 days after implantation of neoplastic cells, respectively (Table 1). Decreases in nitrogen balance, calculated by the difference between total nitrogen intake and urinary and fecal excreted nitrogen (13), were 67.7% in NT and 73.3% in MT animals in comparison to the control group. During this period, there was a progressive increase in calculated tumor weight (14), with a greater tumor/carcass weight ratio in NT (11%) than in MT (7.9%), with carcass weight being the difference between body and tumor weights (Table 1). Patients with cancer show a significant increase in proteolysis, i.e., twice that observed in malnourished patients. This wasting of lean body mass reveals the incapacity to conserve body protein (15). Enhanced rates of whole body protein turnover contribute substantially to the protein waste, as pointed out in several reports using tumor-bearing experimental animals (1).

Table 1 - Effect of Walker 256 tumor growth on various parameters of newly weaned and mature rats.

The results are reported as mean ± SEM. N = Number of rats. NC, Newly weaned controls; NT, newly weaned tumor-bearing rats; MC, mature controls; MT, mature tumor-bearing rats. *P<0.05 for comparison of NC or MC *vs* NT or MT. $^{+}$P<0.05 for comparison between NT and MT.

Parameters	Groups			
	NC (N = 15)	NT (N = 18)	MC (N = 12)	MT (N = 13)
Body weight - 1st day (g)	45.6 ± 1.6	45.2 ± 1.4	239 ± 7.2	232 ± 8.7
Body weight - last day (g)	88.5 ± 2.9	70.8 ± 1.8*	290 ± 6.3	266 ± 5.8*
Food intake - 1st day (g)	5.5 ± 0.7	5.6 ± 0.9	21.2 ± 2.3	21.2 ± 5.0
Food intake - last day (g)	7.6 ± 1.7	2.9 ± 2.1*	11.2 ± 1.5	6.8 ± 5.1*
Nitrogen balance - 1st day (mg nitrogen/24 h)	144 ± 8	158 ± 7	509 ± 38	556 ± 44
Nitrogen balance - last day (mg nitrogen/24 h)	266 ± 16	86 ± 17*	195 ± 14	52 ± 28*
Tumor weight (g)	-	7.7 ± 1.7	-	21.2 ± 2.2^{+}
Tumor weight as percentage of carcass weight - last day (%)	-	11.0 ± 2.2	-	7.9 ± 2.9^{+}

Walker 256 tumor-bearing rats present severe hypoinsulinemia (16), which favors the catabolic processes (16). In contrast, food deprivation or starvation rapidly increases the ratio of transporting to nontransporting rat small intestinal cells, leading to an increase in glucose transport per milligram (17). We observed only in MT rats a decrease in glucose intestinal absorption (52.6%) despite a reduction in food intake observed in both groups (Figure 1). This decrease in intestinal glucose absorption observed in mature tumor-bearing rats does not seem to affect tumor growth. It is possible that processes like hepatic gluconeogenesis might supply enough glucose for tumor growth (2). The intestinal absorption of amino acids decreased significantly with the animal age (Figure 1). The reason for this could be a decrease in the number and/or size of microvilli, or a decrease in the number of enterocytes and/or brush border carriers (18). In addition, a reduced free amino acids (FAA) concentration in the plasma of tumor-bearing rats (1) may also affect the process of absorption. It is of considerable importance to understand the mechanisms

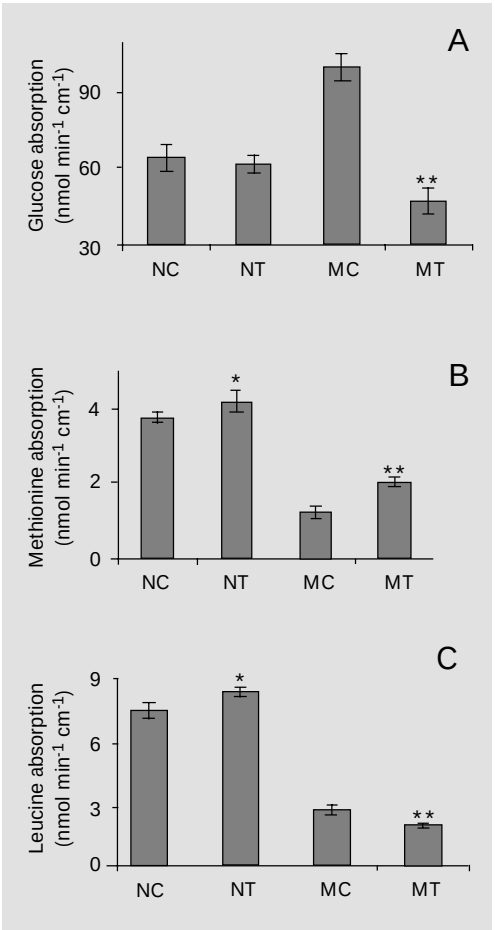

Figure 1 - Effect of Walker 256 carcinoma growth on intestinal absorption of glucose (A), methionine (B) and leucine (C) in newly weaned and mature rats. Data are reported as means ± SEM. NC, Newly weaned control rats (N = 15); NT, newly weaned tumor-bearing rats (N = 18); MC, mature control rats (N = 12); MT, mature tumor-bearing rats (N = 13). *P<0.05 compared to NC; **P<0.05 compared to MC (Kruskal-Wallis one-way analysis).

underlying the adaptive changes in intestinal function in tumor-bearing patients. In fact, the absorptive capacity of the small intestine may change in response to a number of physiological and pathological conditions. With a low dietary protein level, the transport of essential amino acids was maintained or slightly increased by Na^+-dependent and Na^+-independent carriers (19). In this respect, we observed a significant increase in methionine absorption in NT as well as in MT rats, whereas leucine absorption increased only in NT rats (Figure 1). Neutral amino acids transport in the gut may also have an important role in the negative skeletal muscle nitrogen balance (20). In young tumor-bearing animals, the elevation in intestinal transport of methionine and leucine could be associated with their mobilization from host tissues to the neoplastic cells, where amino acids are being used for the synthesis and oxidation processes by these neoplastic cells, which might ensure their rapid proliferation under these conditions. Further studies are needed to find out whether the absorption of other amino acids such as alanine and glutamine is also altered due to the presence of the tumor.

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