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Fiber-rich diets alter rat intestinal leukocytes metabolism

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This study addressed the following question: What is the effect of fermentable and nonfermentable fiber-rich diets on intestinal immune cells' function and metabolism? For this purpose, weaning rats received, for 8 weeks, two types of fiber-enriched (30%) diets with different fermentable/nonfermentable fiber ratios, that is, oat bran (0.3) and wheat bran (0.14). The results of these two experimental groups were compared with those of the low-fiber control group having a 0.22 fermentable/nonfermentable fiber ratio. The total number and proportion of leukocytes in plasma, total number of cells in the lymphoid organs, lymphocyte proliferative activity and capacity of phagocytosis, hydrogen peroxide production, and adherence of macrophages were investigated. The activities of key enzymes of glycolysis and glutaminolysis, and of the Krebs cycle of lymphocytes from the mesenteric lymph nodes and macrophages from the intraperitoneal cavity were determined. The metabolic response of lymphocytes and macrophages from rats fed the three diets to Bacillus Calmette-Guérin-*stimulus was also investigated. The number of lymphocytes in the mesenteric lymph nodes was lower in both fiber-rich diets than in the control but did not have any difference in the remaining lymphoid organs. Wheat bran caused a significant reduction in the phagocytosis capacity and adherence index of macrophages, whereas oat bran did not have a significant effect. The response of glucose and glutamine metabolism to* Bacillus Calmette-Guérin-*stimulus was not altered by the diets in lymphocytes, whereas in macrophages, the increase in glutaminase and hexokinase activities was abolished.* (J. Nutr. Biochem. 11:555–561, 2000) *© Elsevier Science Inc. 2000. All rights reserved.*

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Introduction

Short-chain fatty acids (SCFA) comprise the major solute fraction of fecal water, being produced by fermentation of water-soluble fiber by anaerobic bacteria normally resident in the large bowel.[1] Acetic, propionic, and butyric acids are the predominant forms of the SCFA in the gastrointestinal tract.[2] The proportion of the short-chain fatty acids produced in the intestine bowel varies with the type of fiber-rich diet given.[3] For instance, wheat bran (WB) produces a lower amount of SCFA than oat bran (OB) in the lumen of the colon.[4]

There has been much interest in the possible influence that fiber-rich diets may exert on immune function. Butyrate downregulates the stimulatory function of peripheral blood-derived antigen-presenting cells,[5] upregulates Kupffer cell PGE_2 production,[6,7] and inhibits B-lymphocyte function.[8] Evidence has been obtained that propionate modulates the proliferation of lymphocytes.[9] In the presence of low concentrations (0.04 and 0.1 mmol/L) of propionate, there is an increase of lymphocyte proliferation; however, at concentrations of propionate above 1 mmol/L, an inhibition of lymphocyte proliferation is observed. It has to be mentioned that the plasma concentrations of SCFA (mmol/L) are 1.03 for acetate, 0.27 for propionate, and 0.30 for butyrate.[10]

Lymphocytes and macrophages utilize glucose and glutamine at high rates but these substrates are only partially oxidized. Glucose is converted to lactate, and glutamine to glutamate, aspartate, and lactate.[11] Pyruvate generated by either substrate is not completely oxidized, and some of it may be converted to acetyl-CoA for lipid synthesis in these cells.[12,13] In fact, lipogenesis has been recognized to play an

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important role for the function of lymphocytes and macrophages. Recently, Cavaglieri Felippe et al.[14] found that fiber-rich diets induce marked changes in the fatty acid composition of intraperitoneal macrophages and mesenteric lymph node lymphocytes.

The present study was undertaken to investigate the effect of fermentable and nonfermentable fiber-rich diets on glucose and glutamine metabolism of the intestinal immune cells and its possible association with the changes in their functioning. For this purpose, weaning rats were randomized to one of the three diets: low-fiber control, WB and OB supplemented (30%) for 8 weeks. The three groups present different fermentable/nonfermentable ratios, that is, OB (0.3), WB (0.14), and control (0.22). The following parameters were investigated: total number and proportion of leukocytes in plasma, total number of cells in the mesenteric lymph nodes and other lymphoid organs (thymus, spleen, and cervical lymph nodes) for comparison, lymphocyte proliferative activity, and capacity of phagocytosis, hydrogen peroxide production, and adhesivity of macrophages. The effect of fiber-rich diets on maximal activities of key enzymes of glycolysis (hexokinase), penrose-phosphate pathway (glucose-6-phosphate dehydrogenase), glutaminolysis (phosphate-dependent glutaminase), and of the Krebs cycle (citrate synthase) of the lymphocytes from the mesenteric lymph nodes and macrophages from the intraperitoneal cavity and of the thymus and spleen (as nonintestinal lymphoid organs) was determined. The metabolic response of mesenteric lymph node lymphocytes and intraperitoneal macrophages to *Bacillus Calmette-Guérin* (BCG)-stimulus was also investigated. BCG is well known as a potent stimulator of immune cell function and this treatment was used to test the effect of the diets under conditions of immune stimulation.

Materials and methods

Animals and diets

Weaning male Wistar rats weighing 50–60 g were obtained from the Animal's house of the Institute of Biomedical Sciences, University of São Paulo (São Paulo, Brazil). During the experiment, the rats were kept in groups of 5 at 23 ± 2°C with a 12-hr light/dark cycle; lights on at 7:00 AM. The rats were divided into three groups: control diet commercially available (Nuvilab CR1, obtained from Nuvital Nutrients Ltda, Colombo, Paraná, Brazil) presenting a similar composition to that reported by Anderson[15] and shown in (*Table 1*); OB-enriched diet, prepared by addition of 300 g of OB in 1 kg of control diet; and WB-enriched diet, prepared by addition of 300 g of WB in 1 kg of control diet. The fermentable/nonfermentable fiber ratios, as determined by the method of Asp et al.[16] were: 0.22, 0.30, and 0.14 for control, OB, and WB, respectively. In the control group, the main fiber sources were corn and soybean bran. OB-enriched diet, as compared with diet enriched with WB causes a marked increase of SCFA concentrations in the lumen of colon; 52.2% and 36.7% for propionate, and 131.7% and 79.2% for butyrate in the distal and proximal portions, respectively.[4] The rats were fed the respective diets for 8 weeks and, during this time, food disappearance and fecal output per cage of 5 rats and body weight gain were evaluated weekly. After this period, the rats were killed by decapitation, and intraperitoneal macrophages, mesenteric lymph node lymphocytes, thymus, spleen, and cervical lymph nodes were collected. Animals were always killed between 8:00 AM and 11:00 AM. This protocol used is in accordance with Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation and was approved by the Institute of Biomedical Sciences, University of São Paulo Ethical Committee for Animal Research.

Table 1 Composition of control diet

Ingredient	g/kg diet
Protein	
Casein*	180
Fat†	
Mainly polyunsaturated triglycerides soy bean oil	40
Carbohydrates	
Mainly starch	455
Minerals and vitamins‡§	88
Calcium	18
Fiber	
Water-insoluble fiber	180
Water-soluble fiber	39

*Veghel–The Netherlands. Division of Campina Melkunie bv.
†Fatty acid composition: lauric acid (not determined; n.d.), myristic acid (0.25 g/kg), palmitic acid (6.81 g/kg), palmitoleic acid (0.20 g/kg), stearic acid (1.05 g/kg), oleic acid (10.64 g/kg), linoleic acid (19.76 g/kg), linoleic acid (1.29 g/kg), and arachidonic acid (n.d.).
‡Vitamin mixture: all-trans-tocopherol (200,000 IU/kg), cholecalciferol (66,000 IU/kg), α-tocopherol (0.30 g/kg), K[a] (0.06 g/kg), vitamin B-12 (0.0001 g/kg), riboflavin (0.08 g/kg), niacin (0.95 g/kg), pantothenic acid (0.24 g/kg), thiamine (0.04 g/kg), choline (20 g/kg), pyridoxine (0.60 g/kg), biotin (0.001 g/kg), and folic acid (0.005 g/kg).
§Trace metals: manganese (0.50 g/kg), iodine (0.02 g/kg), iron (0.65 g/kg), zinc (0.35 g/kg), copper (0.26 g/kg), and Antioxidant (butylated hydroxytoluene-BHT;[b] 1.0 g/kg).
[a]K was purchased from Roche Laboratories.
[b]BHT was obtained from Shell Chemical (Houston, TX USA).

Chemicals and enzymes

All chemicals and enzymes were obtained from Sigma Chemical Co. (St. Louis, MO USA). [2−^{14}C]-Thymidine (2.0 GBq/mmol) was obtained from Amersham Pharmacia Biotech Inc. (Piscataway, NJ USA).

Determination of the number of cells

The total and differential number of leukocytes in the blood was determined by visual analysis using an optic microscope. The cells from cervical and mesenteric lymph nodes, spleen and mesenteric lymph nodes, spleen and thymus were isolated as previously described[17] and counted in an optic microscope using a Neubauer chamber.

Preparation of macrophages and mesenteric lymph node lymphocytes

The rats were killed by decapitation without anesthesia. After laparotomy, macrophages from the intraperitoneal cavity were collected. Resident macrophages were obtained by intraperitoneal lavage with 6 mL of sterile phosphate buffered saline (PBS) at pH 7.2.[18] Mesenteric lymph nodes were dissected from rats and lymphocytes were prepared as previously described.[19] BCG-activated macrophages and lymphocytes were obtained in the same way. However, in this group, the rats were intraperitoneally injected with 25 mg of BCG 7 days prior to the harvest. Cell viability was confirmed by Trypan blue exclusion (>95%).

Incorporation of [2−^{14}C]-thymidine into lymphocytes (cell proliferation).

The rates of incorporation of [2−^{14}C]-thymidine into DNA were evaluated in lymphocytes cultured in the absence or in the presence of concanavalin A (Con A). Lymphocytes were cultured in wells of microtiter culture plates (2×10^5 cells in 200 μL/well) in HEPES-buffered Roswell Park Memorial Institute (RPMI) medium containing 10% fetal calf serum, 1×10^5 U/L streptomycin, 2×10^5 U/L penicillin, and 5 mg/L Con A. Con A is a specific mitogen for T-cell proliferation.[9] The cultures were incubated at 37°C in atmosphere of 5% CO_2/95% air. After incubation for as long as 48 hr, [2−^{14}C]-thymidine (740 Bq/well) was added to each well, and the cells were incubated for a further period of 18 hr. The cells were harvested onto filter mats (Cat. No. 11731, Skatron Combi, Suffolk, UK), which were washed and dried. Scintillation fluid (UniverSol™ ES–ICN Biomedical Research Products, Cat. No. 882480, Costa Mesa, CA USA) was added to the filters and the radioactivity incorporated was measured by scintillation counter (β-counter Beckman LS 6000 IC, Beckman Instruments, Fullerton, CA USA).

Preparation of zymosan (Saccharmoyces cerevisia) for measurements of phagocytosis

Zymosan (35 mg of the extract in 100 mL PBS, Cat. No. Z-7250, Sigma, St. Louis, MO USA) was boiled for 30 min and washed twice with PBS prior to use. Subsequently, the zymosan particles were resuspended at 14 mg of the extract/mL in PBS, and the solution was stored at −20°C. For the opsonization, 0.5 mL of zymosan particles (14 mg of the extract/mL) were mixed with 0.5 mL of rat serum and incubated for 30 min at 37°C. The opsonized zymosan particles were washed and resuspended at 1 mg of the extract/mL in PBS.

Macrophage phagocytosis

Macrophages were incubated with 10 mL PBS containing opsonized zymosan for 30 min at 37°C. The phagocytosis was interrupted by placing the flasks on ice for 10 min. The percentage of phagocytosis could be determined by counting (in a Neubauer chamber) the number of cells that had phagocytosed three or more particles of zymosan.

Hydrogen peroxide production

This method for determining the production of H_2O_2[20] is based on the horseradish peroxidase (HRPO)-dependent conversion of phenol red by H_2O_2 into a colored compound. The cells were incubated in siliconized flasks in 1 mL of PBS and the solution of phenol red and HRPO in the presence of phorbol-myristate-acetate (PMA); 20 nmol/L) and glucose (5 mmol/L), under atmosphere of 5% CO_2/95% air at 37°C. After 1-hr incubation, the reaction was interrupted by using 10 μL of 1 mol/L NaOH solution, and the amount of H_2O_2 formed was measured spectrophotometrically at 620 nm (EL 311 SX, Bio-Tek Instrument, Winooski, VT USA). The production of H_2O_2 in the absence of PMA was low and did not differ among the groups.

Adherence index

The adherence to a smooth plastic surface, which resembles adherence to animal tissues[21] was measured. The method was carried out as previously described by De La Fuente et al.[22] Aliquots of 200 μl of peritoneal suspension were placed in eppendorf tubes. After 60 min of incubation at 37°C, aliquots of 10 μL from each sample were removed after gently shaking to resuspend the sedimented cells, and the number of nonadhered macrophages was determined using Neubauer chambers and an optical microscope. The adherence index (AI) was calculated according to the following equation:

$$AI = 100 - \frac{\text{macrophages/mL supernatant}}{\text{macrophages/mL original sample}} \times 100$$

Assays of hexokinase, citrate synthase, and glucose-6-phosphate dehydrogenase

For enzyme assays, the spleen, thymus, and separated lymphocytes and macrophages were previously frozen in liquid nitrogen. The spleen and thymus were homogenized using polytron (PCU-2) in a proportion of 1:10 with the appropriate extraction medium for each enzyme. The cell homogenate was obtained by using a glass manual homogenizer. The activities of hexokinase (EC 2.7.1.1), citrate synthase (EC 4.1.3.7), and glucose-6-phosphate dehydrogenase (EC 4.1.3.7) were determined as previously described.[23,24] The extraction medium for hexokinase contained 50 mmol/L Tris HCl, 1 mmol/L EDTA, 30 mmol/L $MgCl_2$ and 20 mmol/L β-mercaptoethanol at pH 7.4. The extraction medium for citrate synthase and glucose-6-phosphate dehydrogenase contained 50 mmol/L Tris HCl and 1 mmol/L EDTA; the final pH values were 7.4 and 8.0, respectively. For all enzyme assays, 0.05% (v/v) Triton X-100 was added to the assay system to complete the extraction of the enzymes.

The final volume of the assay mixture in all cases was 1 mL. Citrate synthase was assayed by following the rate of change at 412 nm, and the other enzymes were assayed by following the changes at 340 nm. All spectrophotometric measurements were performed in a Gilford Response recording spectrophotometer at 25°C. For all enzymes studied, preliminary experiments established that extraction and assay procedures produced maximum enzyme activities, as described by Crabtree et al.[25]

Glutaminase assay

For the measurement of phosphate-dependent glutaminase activity in lymphocytes, the cells were obtained by centrifugation at 4°C and homogenized in an extraction medium containing 150 mmol/L potassium phosphate, 1 mmol/L EDTA, and 50 mmol/L Tris HCl at pH 8.6. Phosphate-dependent glutaminase (EC 3.5.1.2) was assayed as described by Curthoys and Lowry[26] and Pithon-Curi et al.[27] The assay medium consisted of 50 mmol/L phosphate buffer, 0.2 mmol/L EDTA, 50 mmol/L Tris HCl, 20 mmol/L glutamine, and 0.05% (v/v) Triton X-100 at pH 8.6, to which 0.1 mL of homogenate was added. The total volume was 1 mL. Assay media, in duplicate, were incubated at 37°C. The reaction was stopped by addition of 0.2 mL of 250 g/L perchloric acid solution, and then neutralized. The amount of glutamate was determined as described by Bernt and Bergmeyer[28] at 340 nm in a Gilford Response spectrophotometer.

Protein determination

Protein content of the cell and organ homogenates was measured by the method of Lowry et al.[29] using bovine serum albumin as standard.

Expression of results

Hydrogen peroxide production and enzyme activities are expressed as nmol/min.mg protein.

Table 2 Total and differential number of leukocytes from the blood of rats fed control, oat bran (OB), and wheat bran (WB) diets for 8 weeks†

Leukocytes	Control	OB	WB
Total number/mL	9180 ± 106.5[b]	8550 ± 31.5[c]	9760 ± 48.6[a]
Differential counts (%)			
Neutrophils	11.0 ± 1.70	9.00 ± 1.65	14.6 ± 1.33
Eosinophils	2.00 ± 1.05	1.25 ± 0.25	1.40 ± 0.24
Basophils	1.00 ± 0.31	0.25 ± 0.25	1.20 ± 0.37
Monocytes	8.60 ± 0.40[a]	6.75 ± 0.25[b]	5.00 ± 1.00[b]
Lymphocytes	77.4 ± 1.78	82.8 ± 2.13	77.8 ± 2.13

*Values are presented as mean ± SEM of 5 rats per group.
[a,b,c]$P < 0.05$.

Statistical analysis

Analysis of the differences among the groups in all cases was assessed by using analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test (GraphPad InStat tm V2.05a). The level of significance was set at $P < 0.05$ in all cases.

Results

Food disappearance (an average of 15.1 g/day in 8 weeks from 4 cages) and fecal output (3.26 g/day in 8 weeks from 4 cages) per cage of 5 rats and body weight gain of 236.6 g in 8 weeks to each rat did not markedly differ among the three groups as indicated by ANOVA. In spite of this, however, rats fed fiber-rich diets presented a higher, but not significant, increase in food intake as compared to controls: 1.0–3.0 g per day. This increase in food intake was probably enough to compensate for the lesser intake of vitamins and minerals in the fiber-enriched groups and may explain the same rate of body weight gain observed. Plasma total leukocytes number was higher in the WB (6.3%) and lower in the OB (6.9%), as compared with the control group (*Table 2*). However, both fiber-rich diets reduced the proportion of monocytes: 42% in the WB and 22% in the OB.

The number of cells (as determined in the whole organ) of the cervical lymph nodes, spleen and thymus was not affected by either fiber-rich diet (1.02×10^7 cells in the cervical lymph nodes, 2.24×10^7/spleen, and 9.26×10^6/thymus). The total number of lymphocytes in the mesenteric lymph nodes, however was significantly greater in rats fed both fiber-rich diets. The values of 5 rats (1×10^7 cells) were 3.74 ± 0.21 for control, 6.72 ± 0.29 for WB, and 5.05 ± 0.37 for OB ($P < 0.05$). This effect of the fiber-rich diets was significantly greater in BCG-injected rats: 6.04 ± 0.54 for control, 13.41 ± 0.74 for WB, and 9.87 ± 0.42 for OB ($P < 0.05$).

The proliferation of lymphocytes obtained from the 3 groups, as measured by incorporation of [2−^{14}C]-thymidine, did not differ in the presence and absence of Con A (data not shown).

Both fiber-rich diets did not alter the number of cells in the intraperitoneal cavity (*Table 3*). Also, cell migration in response to BCG stimulus was not affected by the diets given. However, rats treated with WB had a lower ($P < 0.05$) capacity of phagocytosis (37%) and adherence (22%) of macrophages, whereas OB did not have a significant effect. The production of H_2O_2 in the presence of PMA was not different among the groups.

Fiber-rich diets did not cause significant difference in the enzyme activities of the lymphoid organs, except for the fact that in the OB group, citrate synthase activity of the spleen and thymus was 350% and 110% greater than in the control, respectively. The values expressed as mean ± SEM of 5 rats in the spleen were 67.29 ± 3.08 for control, 61.19 ± 4.75 for WB, and 305.45 ± 73.70 for OB ($P < 0.05$), and values for the thymus were 102.73 ± 11.72 for control, 81.37 ± 6.24 for WB, and 217.54 ± 22.94 for OB ($P < 0.05$). Lymphocytes isolated from the mesenteric lymph nodes of untreated rats also did not present significant changes in the enzyme activities (*Table 4*). However, under BCG treatment, OB caused significant increase of glucose-6-phosphate dehydrogenase activity (37%), whereas WB decreased (40%) citrate synthase activity. Glutaminase and hexokinase activities were not altered by the fiber-rich diets. BCG administration enhanced the activity of glutaminase (25%), hexokinase (130%), and glucose-6-phosphate dehydrogenase (180%) in lymphocytes from control rats. The fiber-rich diets did not modify the response observed in the control group, except for glutaminase activity, which was not increased in WB. Similarly to lymphocytes, the enzyme activities of intraperitoneal resident macrophages were not modified due to the fiber-rich diets given (*Table 5*). However, macrophages obtained from rats fed both fiber-rich diets and submitted to BCG treatment presented marked changes in some enzyme activities. Hexokinase activity was

Table 3 Total number of cells in the intraperitoneal cavity, percentage of macrophages phagocytosis, hydrogen peroxide production, and adhesivity capacity of macrophages obtained from rats fed control, oat bran (OB), and wheat bran (WB) diets for 8 weeks*

	Control	OB	WB
Peritoneal macrophages ($\times 10^7$)			
Quiescent	1.10 ± 0.10[e]	0.94 ± 0.09[e]	0.88 ± 0.08[e]
BCG-stimulated	2.40 ± 0.15[d]	2.23 ± 0.11[d]	2.17 ± 0.10[d]
Phagocytosis (%)	29.5 ± 3.00[a]	26.3 ± 2.00[a]	18.7 ± 2.40[b]
H_2O_2 production (nmol/min · mg of protein) with PMA	0.29 ± 1.30[d]	0.26 ± 0.83[d]	0.24 ± 0.71[d]
Adherence index†	68.0 ± 1.50[a]	63.0 ± 3.70[a]	53.0 ± 2.60[b]

*Values are presented as mean ± SEM of 5 rats per group.
†AI = 100 macrophages/mL supernatant/macrophages/mL original sample × 100.
[a,b] in the lines = $P < 0.05$,[d,e] in the columns = $P < 0.05$.
PMA–phorbol-myristate-acetate. BCG–*Bacillus Calmette-Guérin*.

Table 4 Maximal activities of phosphate-dependent glutaminase, hexokinase, citrate synthase, and glucose-6-phosphate dehydrogenase of mesenteric lymph nodes lymphocytes from rats fed control, oat bran (OB), and wheat bran (WB)-enriched diets*

Maximal enzyme activities (nmol/min.mg of protein)	Control	OB	WB
Glutaminase			
Quiescent	54.5 ± 4.54[e]	62.3 ± 2.47[e]	68.6 ± 4.57[d]
BCG-stimulated	68.0 ± 3.20[d]	84.4 ± 3.70[d]	76.2 ± 4.40[d]
Glucose-6-phosphate dehydrogenase			
Quiescent	29.7 ± 3.92[e]	34.8 ± 3.52[e]	31.3 ± 3.00[e]
BCG-stimulated	82.3 ± 4.30[b,d]	113 ± 8.76[a,d]	103 ± 9.30[b,d]
Hexokinase			
Quiescent	24.7 ± 0.90[e]	25.9 ± 1.14[e]	26.3 ± 2.10[e]
BCG-stimulated	55.7 ± 6.80[d]	65.8 ± 4.21[d]	64.0 ± 3.10[d]
Citrate synthase			
Quiescent	157 ± 16.8	167 ± 15.31	166 ± 14.8
BCG-stimulated	193 ± 8.40[a]	188. ± 7.90[a]	115 ± 8.20[b]

*Values are presented as mean ± SEM of 5 rats per group.
[a,b] in the lines = $P < 0.05$, [d,e] in the columns = $P < 0.05$.
BCG–*Bacillus Calmette-Guérin*.

reduced by 46% in the OB and by 50% in the WB groups, whereas citrate synthase activity was lowered by 13% in OB rats. In response to BCG administration, a marked increase of the activities of glutaminase (49%), glucose-6-phosphate dehydrogenase (74%), hexokinase (110%), and citrate synthase (66%) occurred in macrophages from the control group. The effect of BCG on glucose-6-phosphate dehydrogenase and citrate synthase activities was basically maintained in rats fed both fiber-rich diets. However, the increase in the activities of glutaminase and hexokinase of macrophages due to BCG was abolished by both fiber-rich diets.

Discussion

Recent studies pointed out the possible effect of the fibers on the functioning of immune system being able to regulate (by releasing local mediators[30,31]) differentiation and proliferation of intestinal epithelial cells.[32,33] For instance, Tappenden et al.[34] showed that SCFA increase the number of T-cells in the gastrointestinal tract of rats. Lim et al.[35] found a significant effect of the fiber-rich diets on concentrations of immunoglobulins, interferon-γ and tumor necrosis factor-α of the mesenteric lymph nodes. In the present study, fiber-rich diets given for 8 weeks did not alter the number of cells of the spleen, thymus, and cervical lymph nodes but caused a significant increase in the total number of cells of the mesenteric lymph nodes—a lymphoid organ attached to the intestine that consists as part of the intestinal immune system. In addition, several functional aspects and the metabolism of glucose and glutamine in mesenteric lymph nodes lymphocytes and intraperitoneal macrophages were investigated. In a recent study, we found that fiber-rich diets alter the composition of fatty acids of these cells,[14] which could lead to alteration of their function and metabolism.[36,37]

The activity of the key enzymes of glucose and glutamine metabolism of lymphocytes from untreated rats was

Table 5 Maximal activities of phosphate-dependent glutaminase, hexokinase, citrate synthase, and glucose-6-phosphate dehydrogenase of intraperitonial macrophages from rats fed control, oat bran (OB)- and wheat bran (WB)-enriched diets*

Maximal enzyme activities (nmol/min.mg of protein)	Control	OB	WB
Glutaminase			
Quiescent	142 ± 9.80[e]	149 ± 7.40[e]	146 ± 9.52[e]
BCG-stimulated	212 ± 9.30[d]	182 ± 11.40[e]	180 ± 13.8[e]
Glucose-6-phosphate dehydrogenase			
Quiescent	11.3 ± 0.91[e]	14.2 ± 0.54[e]	13.8 ± 0.87[e]
BCG-stimulated	19.7 ± 1.27[d]	19.1 ± 0.73[d]	19.3 ± 0.84[d]
Hexokinase			
Quiescent	283 ± 13.20[e]	268 ± 18.20[e]	277 ± 15.80[e]
BCG-stimulated	582 ± 25.40[a,d]	316 ± 23.40[b,e]	289 ± 21.90[b,e]
Citrate synthase			
Quiescent	29.4 ± 1.20[e]	25.4 ± 1.02[e]	27.3 ± 1.13[e]
BCG-stimulated	48.8 ± 0.83[a,d]	42.3 ± 1.37[b,d]	46.5 ± 1.53[a,d]

Values are presented as mean ± SEM of 5 rats per group.
[a,b] in the lines = $P < 0.05$, [d,e] in the columns = $P < 0.05$.
BCG–*Bacillus Calmette-Guérin*.

not modified by fermentable and nonfermentable fiber-rich diets given. However, some differences appeared in cells obtained from BCG-injected rats. Glucose and glutamine metabolism plays an important role for lymphocytes proliferation.[38] Glycolysis and glutaminolysis generate precursors for the biosynthesis of macromolecules such as RNA, DNA and lipids (cholesterol and phospholipids) for the process of cell division.[13,39] OB raised the activity of glucose-6-phosphate dehydrogenase in BCG lymphocytes, whereas citrate synthase activity was reduced in WB. These findings, combined, support the proposition that a fermentable fiber-enriched diet may stimulate the pentose-phosphate pathway, and a nonfermentable fiber-rich diet may inhibit the Krebs cycle activity in BCG lymphocytes. These metabolic changes do not seem to be the major cause for the alterations in the number of cells of the mesenteric lymph nodes described.

WB-enriched diets caused a clear reduction of macrophage functioning as indicated by the results of phagocytosis and adherence. Two questions were then raised: What is the significance of these findings? and What is the mechanism involved? As a consequence of the changes described, rats fed a nonfermentable fiber-rich diet may be more susceptible to infections, presenting also a low inflammatory response;[40,41] however, further studies might be carried out to address this point. The difference in the effects of WB and OB is not a surprising finding. Zoran et al.[4] have shown that WB-enriched diets reduce tumor incidence in a rat model of colon cancer independent of butyrate. Diet enriched with WB provokes a significant increase in the mucosal height, whereas OB does not cause any effect.[42] In fact, nonfermentable fibers can be very active in intestinal functioning by various mechanisms such as physical stress,[43] increase in the motility,[44] blood flow of the intestine,[45] and secretion of entero-hormones.[46] The mechanism(s) to explain our findings remains to be fully elucidated, however. The metabolism of glucose and glutamine in quiescent macrophages was not altered by either WB or OB. BCG cells from both OB and WB presented reduced hexokinase activity, whereas lower citrate synthase activity was found in OB only. Therefore, these metabolic alterations cannot be regarded as being the major cause for the impairment of macrophage functioning observed in WB.

The increase in the enzyme activities of glucose and glutamine metabolism of macrophages from control rats due to BCG administration is quite similar to that previously reported.[47] However, few differences occurred between lymphocytes and macrophages. The response of glucose-6-phosphate dehydrogenase activity to BCG injection was still more pronounced in lymphocytes than in macrophages (280% against 74% increase), whereas citrate synthase activity remained unchanged in lymphocytes. The response of control lymphocytes to BCG stimulus was basically maintained when rats were fed both fiber-rich diets, except for glutaminase activity in the WB. In contrast, the macrophage response to BCG stimulus was markedly modified by both fiber-rich diets. OB and WB abolished the response of glutaminase and hexokinase activities. Therefore, the modulating effect of both fiber-rich diets on metabolism of glucose and glutamine in response to BCG is clearly more pronounced on macrophages.

Taken as a whole, the higher number of lymphocytes in the mesenteric lymph nodes of both fiber-rich diets than in the control does not seem to be associated with a stimulation of glycolysis and glutaminolysis. Macrophages functioning was altered differently according to the type of fiber given. Nonfermentable fiber-rich diet (WB) caused a significant reduction in the phagocytosis capacity and macrophages adherence, whereas fermentable fiber-rich diet (OB) did not affect macrophage function. The response of glucose and glutamine metabolism to BCG stimulus was not altered by the fiber-rich diets in lymphocytes, whereas in macrophages, the increase in glutaminase and hexokinase activities was abolished by both fermentable and nonfermentable fibers. Further studies are now being developed to examine the possible anti-inflammatory effect of both fiber-rich diets.

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