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Reduction in heat-induced gastrointestinal hyperpermeability in rats by bovine colostrum and goat milk powders

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Prosser, C., K. Stelwagen, R. Cummins, P. Guerin, N. Gill, and C. Milne. Reduction in heat-induced gastrointestinal hyperpermeability in rats by bovine colostrum and goat milk powders. *J Appl Physiol* 96: 650–654, 2004. First published October 3, 2003; 10.1152/japplphysiol.00295.2003.—Male Sprague-Dawley rats were assigned to one of three dietary groups [standard diet (Cont; $n = 8$), standard diet plus bovine colostrum powder (BColost 1.7 g/kg; $n = 8$), or goat milk powder (GMilk 1.7 g/kg; $n = 8$)] to determine the ability of these supplements to reduce gastrointestinal hyperpermeability induced by heat. Raising core body temperature of rats to 41.5°C increased transfer of ^{51}Cr-EDTA from gut into blood 34-fold relative to the ambient temperature value ($P < 0.05$) in the Cont group of rats, indicative of increased gastrointestinal permeability. Significantly less ($P < 0.01$) ^{51}Cr-EDTA was transferred into the blood of rats in either the BColost (27% of Cont) or GMilk group (10% of Cont) after heating, showing that prior supplementation with either bovine colostrum or goat milk powder significantly reduced the impact of heat stress on gastrointestinal permeability. The changes in the BColost group were not significantly different than those of the GMilk group. The potential mechanism of the protective effect of bovine colostrum and goat milk powders may involve modulation of tight junction permeability, because both powders were able to maintain transepithelial resistance in Madin Darby canine kidney cells challenged with EGTA compared with cells maintained in media only. The results show that bovine colostrum powder can partially alleviate the effects of hyperthermia on gastrointestinal permeability in the intact animal. Moreover, goat milk powder was equally as effective as bovine colostrum powder, and both may be of benefit in other situations where gastrointestinal barrier function is compromised.

heat; gastrointestinal permeability; colostrum; goat milk

GASTROINTESTINAL DISCOMFORT is a common side effect of strenuous exercise, although its aetiology is unclear (5). Oktedalen et al. (18) and Pals et al. (19) have linked an increase in intestinal permeability to gastrointestinal symptoms after long-distance or prolonged high-intensity running. Moseley and Gisolfi (16) hypothesized that exercise in the heat can result in gut hyperpermeability due to combined thermal and ischemic injury to the gut. Heat stress has been shown to result in intestinal injury (4, 27), and heating rats to 41.5–42°C was found to induce a marked increase in intestinal epithelial damage and permeability (13).

The intestinal epithelium provides a physical barrier between the luminal contents and the interior environment of the body and protects the body against entry of bacteria, bacterial toxins, and other unwanted macromolecules (1). Moseley and Gisolfi (16) suggested that an increase in gastrointestinal permeability resulting from exercise and heat could lead to endotoxemia and release of inflammatory cytokines such as tumor necrosis factor. It is known that pro- and anti-inflammatory cytokines are released in response to physical activity (14) and that overheating induces acute-phase proteins in plasma, indicative of inflammatory activity, and symptoms of multiple organ failure or shock (4).

Combined, the above studies suggest that increased gastrointestinal permeability, perhaps resulting in release of toxic levels of cytokines, is a key factor in producing symptoms of heatstroke. Therefore, agents that can reduce or prevent gastrointestinal hyperpermeability would offer a significant benefit by reducing the impact of heat stress on individuals.

Gastrointestinal hyperpermeability is induced by many other stimuli including nonsteroidal anti-inflammatory drugs (2). Bovine colostrum has been shown to prevent indomethacin-induced gastrointestinal injury and ulceration in mice (20), and intestinal hyperpermeability in humans (21). The authors of these studies hypothesized that colostrum acted by first stimulating the movement of healthy cells into the site of damage and second by actually stimulating the growth of new cells. In support of this hypothesis, bovine colostrum contains several factors that can stimulate growth of epithelial cells, at least in culture (17, 22, 25). Colostrum also contains antimicrobial and antiviral agents that reduce the impact of bacterial or viral pathogens on intestinal epithelium (9). Thus a colostrum supplement could act as a prophylactic substance to prevent or reduce the impact of agents that cause mucosal damage or, alternatively, as a therapeutic aid for restoration of mucosal barrier function by stimulating epithelial cell growth. In contrast to colostrum, bovine milk was much less effective in preventing indomethacin-induced gastric or intestinal damage in mice (20). However, in a preliminary study, we have found that New Zealand goat milk powder had a similar effect as bovine colostrum in reducing indomethacin-induced damage in the rat intestinal epithelium (24).

Our hypothesis was that supplementing the diet with either bovine colostrum or goat milk powder would reduce heat-induced gastrointestinal hyperpermeability. To test this hypothesis, we supplemented the diet of rats for 7 days with bovine colostrum or goat milk powder and then measured the gastrointestinal permeability by using ^{51}Cr-labeled EDTA during the time when the core body temperature of the rats was raised to 41.5°C. In addition, the effect of these supplements on epithelial permeability was measured in vitro to determine whether they had a direct effect on the epithelium.

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Table 1. *Composition of standard diet provided to rats*

%Dry matter	88
%Crude protein	18
%Crude fat	3.1
%Ash	6.2
%Fiber	22
%Carbohydrate	35

MATERIALS AND METHODS

Animals and diets. Twenty-four male Sprague-Dawley rats (200–250 g) were randomly assigned to one of three dietary groups: standard diet (Cont; $n = 8$), standard diet supplemented with bovine colostrum powder (BColost; 1.7 g/kg, $n = 8$), and standard diet supplemented with goat milk powder (GMilk; 1.7 g/kg, $n = 8$). The composition of the standard diet, which consisted of dry pellets, is listed in Table 1. The supplement (0.4 g) was mixed with 3.0 g of gelatine and fed once in the morning for 7 days. All rats readily consumed the powder/gelatine supplement. The Cont group was fed gelatine without supplement. The amount of protein or carbohydrate in the supplement was estimated to provide no more than 15% of the rats' daily intake. The standard diet and water were available ad libitum to all groups.

Rats were maintained in a regulated environment at a constant temperature of 22°C and relative humidity of 44%. Animal manipulations were conducted in compliance with the Code of Ethical Conduct for Animal Experimentation and approved by AgResearch, Ruakura Animal Ethics Committee.

Gastrointestinal permeability. Gastrointestinal permeability was determined by measuring the amount of ^{51}Cr-EDTA (New England Nuclear Life Sciences, Auckland) transferred into blood 90 min after 0.5 ml of 10 μCi/ml ^{51}Cr-EDTA was given orally. Blood (~0.3 ml) was sampled from the hindlimb by using the procedure described by Hem et al. (8). Briefly, the rat was restrained in a plastic container, the hind leg was shaved, and the saphenous vein was punctured with a fine needle. The blood was collected into tubes held under the leg. The amount of ^{51}Cr-EDTA was quantified by using a γ-counter (Wallac). Rats were fasted overnight on each of the test days but were offered water ad libitum.

Baseline gastrointestinal permeability was measured in half the rats at ambient temperature (22°C) 5 days after the diet was started and in all rats after heat treatment. There was a minimum of 2 days separating the assessment of gastrointestinal permeability at ambient temperature and heating. A preliminary study showed that the level of radioactivity in blood after 2 days was no different from the background level, suggesting that 2 days was sufficient time to allow clearance of any residual ^{51}Cr-EDTA in blood (data not shown). Rats were given ^{51}Cr-EDTA at least 5 min before the heating episode. The blood was collected 90 min after the oral dosing with ^{51}Cr-EDTA, representing 70–80 min after the rats had reached maximum core body temperature.

Heat treatment. Immediately after administration of ^{51}Cr-EDTA, a thermocouple temperature probe (World Precision Instruments) was placed into the rat's rectum to continuously record internal body temperature. After a further 5 min to obtain a stable recording of body temperature, the rat was placed into an enclosure heated to 40–55°C by means of an infrared heating lamp fixed above the enclosure. The rat was allowed free movement within the enclosure so that it was not directly under the lamp. As soon as core body temperature reached 41.5°C, the rat was removed from the enclosure and left to cool at ambient temperature (22°C). The time taken to reach 41.5°C was recorded, as was the time taken to then return to baseline temperature.

The rate of increase in temperature was calculated from the rise in temperature per minute from baseline to maximum temperature. Thermal stress (°C · min) was quantified in accordance with Hubbard et al. (10): (maximum temperature − 40.4°C) × heated time, where heated time is the total time during which core body temperature was above 40.4°C.

Cell culture. The effect of bovine colostrum and goat milk powders on tight junction permeability was measured in MDCK cells (Madin Darby canine kidney cell line from American Type Culture Collection) by the transepithelial electrical resistance (TER) model described by Stelwagen and Ormrod (28). MDCK cells were grown to confluence in Dulbecco's modified Eagle's media (Life Technologies, Auckland, New Zealand) on 12-mm-diameter inserts, each of which contained a permeable membrane (Nunc, Auckland, New Zealand). TER was measured by using a voltohmmeter in an Endohm 12 chamber (World Precision Instruments). Bovine colostrum and goat milk powders were reconstituted to 10% (wt/vol) in water then centrifuged at 50,000 *g* for 2 h to clarify the sample by removing the fat and casein. This was necessary to reduce the variability in measuring TER with the Enhdohm 12 chamber caused by the presence of fat or casein. The supernatant, containing whey components, was added to cells at 10% (vol/vol) and left for 24 h as a pretreatment. Control wells contained Dulbecco's modified Eagle's media only. TER was measured just before addition of 1 mM EGTA to the inserts and again 2 h after EGTA challenge and was expressed as percent change from the TER value measured before EGTA addition. Both powders were tested in four separate cultures.

A dose-response curve was also generated by adding either the supernatant fraction of bovine colostrum or goat milk at 5, 10, or 20% (vol/vol) before challenge with 1 mM EGTA.

Statistical analysis. The statistical significance of differences between Cont and BColost and GMilk was tested by analysis of variance with Dunnet's method of comparison with Cont. Analysis of variance was also used to compare the effect of heating within groups.

RESULTS

Gastrointestinal permeability at ambient temperture. The amount of ^{51}Cr-EDTA transferred into blood of rats maintained at ambient temperature (22°C) was only just detectable. The values were 1.5 ± 0.9, 4 ± 4, and 0.8 ± 0.5 counts per minute (cpm) per milliliter (means ± SE, $n = 4$) for Cont, BColost, and GMilk groups, respectively. There was no significant effect of dietary supplementation on the amount of ^{51}Cr-EDTA transferred, indicative of a similar degree of gastrointestinal permeability in all groups of rats when kept at ambient temperature.

Heat treatment. The maximum temperature obtained, the level of thermal stress experienced, and the heating rate applied

Table 2. *Measures of heat stress applied to the 3 groups of rats*

	T_{min}, °C	T_{max}, °C	Thermal Stress, °C·min	Heating Rate, °C/min	Time to Return to Baseline, min
Cont	37.8±0.2	41.7±0.05	15.2±0.4	0.30±0.03	29±3
BColost	37.9±0.1	42.0±0.12	17.9±1.3	0.35±0.04	20±2
GMilk	37.8±0.2	41.8±0.05	16.2±1.4	0.34±0.05	24±2

Values are means ± SE; $n = 8$/group. T_{min}, baseline core body temperature; T_{max}, maximum core body temperature; Cont, rats fed standard diet; BColost, rats fed a diet supplemented with bovine colostrum powder; GMilk, rats fed a diet supplemented with goat milk powder.

to each of the three groups of rats are indicated in Table 2. There was no significant difference ($P > 0.05$) among the three groups for any of these parameters, and all rats survived the heating episode.

Gastrointestinal permeability under heated conditions. Elevation of core body temperature of rats in the Cont group to 41.5°C increased the concentration of ^{51}Cr-EDTA in blood 34-fold compared with the concentrations in blood of the rats maintained at ambient (22°C) temperature (Fig. 1). These data are consistent with there being greater transfer of ^{51}Cr-EDTA from gut into blood due to increased permeability of the small intestine after heat stress.

Significantly less ($P < 0.01$) ^{51}Cr-EDTA was transferred into blood in rats in either the BColost group (27% of Cont) or GMilk group (10% of Cont) after heating, but this was still significantly ($P < 0.01$) higher than that transferred at ambient temperature. The amount of ^{51}Cr-EDTA transferred into blood of rats in the BColost group was not significantly different ($P > 0.05$) from that of the GMilk group.

Cell culture. MDCK cells in culture exposed to reconstituted bovine colostrum or goat milk powders had very similar baseline TER (Table 3) to cells with media only (Cont). The TER fell to 60% of baseline in Cont wells 2 h after addition of EGTA, indicating a breakdown in the epithelial barrier. Cells cultured with bovine colostrum or goat milk, however, maintained TER after EGTA challenge, indicating maintenance of barrier function by whey factors.

The response of MDCK cells to different doses of bovine colostrum and goat milk is shown in Fig. 2. Although bovine colostrum achieved maximal protection against EGTA challenge at 10%, response to goat milk continued to increase out to 20%.

Table 3. *TER measurements for MDCK cells exposed to 1 mM EGTA*

	Baseline TER, Ω·cm^2	TER After EGTA, % of baseline
Cont	8,800±713	60±8
BColost	8,923±979	106±8*
GMilk	7,886±966	81±11†

Values are means ± SE; n = 4/group. TER, transepithelial electrical resistance. *$P < 0.001$, †$P < 0.05$ compared with Cont.

DISCUSSION

The present data demonstrate an increase in gastrointestinal permeability in rats subjected to heat and are in keeping with the reports of the hyperthermia-induced increase in transfer of intestinal endotoxin (27) and FITC-labeled dextran (13) to blood of rats. It is known that the severity of heat stress in rats is dependent on both the intensity and duration of exposure to temperatures above 40.4°C (10). Furthermore, the extent of intestinal damage, contributing to a breakdown in intestinal barrier function, increases with higher thermal load (13). The thermal load we achieved ranged from 11 to 24°C · min, with an overall average of 16°C · min for all rats. This level did not result in mortality in any of the rats, in keeping with Damanhouri and Tayeb (3). Nevertheless, this still resulted in a dramatic increase in gastrointestinal permeability in the rats.

Supplementation with bovine colostrum powder significantly reduced the amount of ^{51}Cr-EDTA transferred from gut to blood in rats after heat exposure, consistent with its ability to prevent gastrointestinal epithelial barrier dysfunction induced by indomethacin (21). In addition, this study has shown that supplementation with goat milk powder produces a protective outcome similar to that of bovine colostrum powder, at least with respect to increases in gastrointestinal permeability caused by heat stress.

The mechanism underlying the heat-induced changes in gastrointestinal permeability in the rat most likely relates to hypoxia. This would arise from redistribution of cardiac output from viscera to cutaneous regions to dissipate heat (12, 32). In support of this hypothesis, hyperthermia reduced splanchnic blood flow by 40% in rats (7) and produced both metabolic stress and cellular hypoxia in the splanchnic tissues (6). Clinical and experimental evidence suggest that ischemia and subsequent reperfusion are very closely linked to gut injury, initially mediated by reactive oxygen metabolites (11).

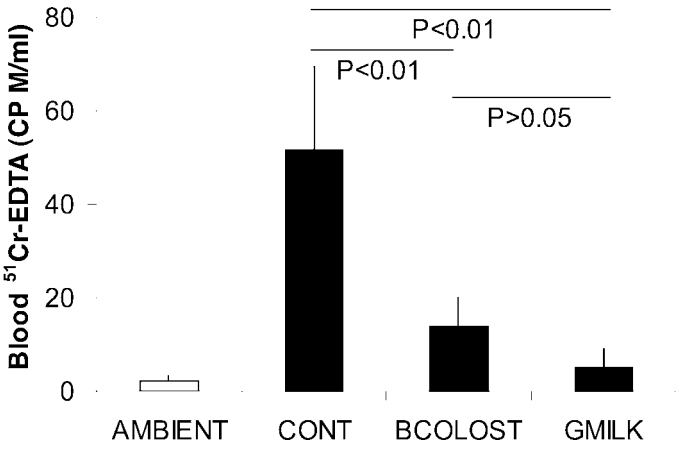

Fig. 1. Gastrointestinal permeability in rats at ambient temperature (22°C) or after in rats heating (closed bars) fed a standard diet alone (Cont) or a diet supplemented with bovine colostrum powder (BColost) or goat milk powder (GMilk). Blood was sampled 90 min after oral dose with ^{51}Cr-EDTA, 70–80 min after maximal core body temperature in the rats. Data are means ± SE; n = 8 rats/group, except for ambient data, where data for all groups were pooled (n = 12). Statistical significance of differences between individual groups is indicated under the lines connecting the groups.

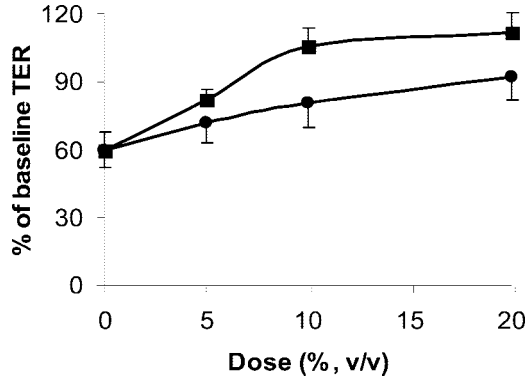

Fig. 2. Effect of different doses of BColost (■) and GMilk (●) on transepithelial electrical resistance (TER) in Madin Darby canine kidney cells after EGTA challenge. TER was measured before and after 2 h of exposure of cells to 1 mM EGTA. Results are expressed as % of baseline (TER before EGTA challenge) and are means ± SE; n = 4/dose.

Exogenous or endogenously reactive oxygen metabolites, generated by hypoxia and reoxygenation, decreased TER in intestinal epithelial cells in culture (30, 31), suggesting a direct effect of reactive oxygen metabolites on tight junctions between epithelial cells. In addition, Moseley et al. (15) observed that epithelial cells grown in culture and then exposed to thermal stress increased transepithelial electrical conductance due to increased paracellular permeability. This increase was reversible, implying that it is possible to impose a direct action on tight junction formation in epithelial cells by heating.

A potential mechanism for the protective effect of bovine colostrum or goat milk powders on heat-induced gastrointestinal permeability may likewise be via a direct effect on maintenance of tight junctions in epithelial cells. TER was reduced in confluent MDCK cells after challenge with EGTA but was maintained in cells cultured with either bovine colostrum or goat milk powder. TER is a measure of the barrier function in epithelia (23) and reflects the formation of tight junctions between epithelial cells (26, 29). Although a kidney cell line was used and not an intestinal cell line, tight junctions are a common feature of all epithelial cells, including those of the intestine. Regulation of tight junction function is also likely to be similar in all tissues, as evidenced by the observation of Stelwagen and Ormrod (28) that tight junctions in kidney and mammary epithelial cells behave similarly in response to a milk-derived factor. Thus the data are consistent with the ability of bovine colostrum or goat milk to protect against breakdown of epithelial permeability in the intact animal and would suggest their direct action on the epithelium, as opposed to an indirect one via buffering capacity or reduction in intestinal microbial load for instance.

The factor, or factors, in bovine colostrum or goat milk powder that maintains the epithelial barrier function is not known. It is clearly present in the whey fraction, because the fat and casein components were removed before testing in vitro. This is similar to the hyperimmune milk factor described by Stelwagen and Ormrod (28) that also maintains tight junction integrity in epithelial cells. Analysis of dose response showed maximal protection with the addition of 10% colostrum, whereas goat milk tended to be less active even at 20%. This implies that goat milk contains a lower concentration of the active factor, or factors, but was nevertheless still effective.

The present results provide strong support for our hypothesis that bovine colostrum powder and goat milk powder can help reduce breakdown of gastrointestinal barrier function that may arise from overheating and therefore may be a useful nutraceutical intervention to reduce heat stress. Heat stroke is a recognized hazard for those people who participate in vigorous sports, particularly in hot, humid conditions, and several authors have implicated gut injury in the pathogenesis of heatstroke (4, 27). However, the degree of heating that induces gastrointestinal hyperpermeability in humans remains to be clarified, as does the potential protective benefits of either bovine colostrum or goat milk under these circumstances.

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GRANTS

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