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# Assessment of the Benefits of Live Yogurt: Methods and Markers for *in vivo* Studies of the Physiological Effects of Yogurt Cultures

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This document addresses nutritional and functional changes brought about by heat treatment of yogurt containing live cultures. Several lines of research evidence suggest that these products are not equivalent. Recent research shows that yogurt bacteria are able to survive passage through the human intestine. Yogurt containing viable starter cultures has been shown to improve lactose digestion in lactose-intolerant people; heat treatment of the product diminishes this improvement. There are indications for a role of live yogurt cultures in modulating the immune system of the consumer. Long-term consumption of live yogurt reduces nasal allergies, particularly in young adults, a reduction that is not observed any more after heat treatment. Studies in the growing pig, an accepted model for studying protein digestion in humans, show that nitrogen absorption from live yogurt is higher and more evenly distributed in time than nitrogen absorption from heat-treated fermented milk. Taken together, these findings indicate that heat treatment of yogurt results in relevant nutritional and functional changes which would challenge an assertion of their equivalency. Such differences should be reflected in naming of these products to avoid consumer confusion. Additional health benefits of yogurt include the release of bioactive peptides, impact on gut flora, alleviation of diarrhoea in children, immune system modulation, prevention of infections, inhibition of mutagenesis and carcinogenesis, improved oral health and improved symptoms of collagen-induced arthritis. These effects have not been tested and documented for heat-treated fermented milk. **Key words:** yogurt, fermented milk product, *Lactobacillus*, heat treatment, nutrition, health, lactose digestion, allergy, immunology, nitrogen, protein.

## INTRODUCTION

There is debate about the nutritional and functional relevance of the consequences of heat treatment of yogurt. Whereas some assert that heating of yogurt does not substantially change its qualities, others are convinced that heat-treated fermented milk differs significantly from yogurt with live cultures.

A clear consequence of heat treatment is loss of the viability of the yogurt cultures present in the product. Viable yogurt bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, are each present at levels of at least 10<sup>7</sup> colony forming units per gram (cfu/g). When the yogurt is consumed, the bacteria remain active during their transient stay (they do not colonize the intestine, as observed with the methods used today) and exert important physiological effects.

Heat treatment after fermentation destroys these bacteria. Hargrove and Alford (1) showed that heat treatment for 2 min at 60°C, 65°C and 70°C reduces the bacterial count of the yogurt by 73%, 99.9% and 99.999%,

respectively. Heat treatment also destroys most of the natural body and viscosity of the yogurt. Stabilizers have to be added to protect casein during heat treatment, thus enabling the products to retain their consistency after the treatment. Fermented dairy products intended for heat treatment therefore contain between 0.1 and 0.5% of stabilizing agents (2, 3).

This document presents the physiological effects associated with the consumption of yogurt and its specific live and active cultures, and the consequences of heat treatment after fermentation on these beneficial effects.

## YOGURT BACTERIA SURVIVE IN THE HUMAN INTESTINE

Bianchi-Salvadori et al. (4, 5) first studied the fate of yogurt bacteria in the intestine. Survival was initially assessed by checking the presence of bacteria in faeces of animal or human consumers. Modern and more reliable techniques have shown that yogurt bacteria do indeed survive their passage through the human intestine.

Pochart et al. (6) showed that, after fresh yogurt ingestion, viable starter culture reached the duodenum and still showed  $\beta$ -galactosidase activity.

Brigidi et al. (7) fed five healthy subjects a yogurt-free diet for a washout period of 10 days. Then they fed them a diet containing 250 g yogurt per day for 10 days, followed by another washout period. From the third till the tenth day of the period of yogurt consumption, *S. thermophilus* could be detected by polymerase chain reaction (PCR) in the faecal samples of the subjects ( $5 \times 10^5$ /g faeces). After the end of the this period the *S. thermophilus* count in the faecal samples decreased again. Theoretically, PCR-based techniques do not discriminate between live and dead cells, but they need intact cells. Bacteria that have been inactivated, either by heat treatment or by gastric acid and bile salts would no longer be intact and thus will not be detected by PCR.

Doré et al. (unpublished observations) fed 12 healthy subjects a regular Western European diet with the exclusion of food containing live lactic acid bacteria for a washout period of 7 days. Then their diet was supplemented for 10 days with a fermented milk containing *L. bulgaricus* ( $10^{10}$ /day). At the beginning of this intervention period *L. bulgaricus* DNA was detected by PCR in the faecal samples of only one of the subjects. At day 10 *L. bulgaricus* DNA was found in the faecal samples of all subjects, indicating the presence of  $10^6$  *L. bulgaricus* bacteria/g faeces. After another 10 days on a washout diet, no more *L. bulgaricus* DNA was found in any of the faecal samples, indicating that the consumption of live cultures is directly related to their presence in the faecal samples.

These experiments show that *L. bulgaricus* and *S. thermophilus* can survive during their passage through the human intestine after yogurt ingestion.

The team of Maldonado-Galdeano and Perdigon have experimental evidence that non-viable lactic acid bacteria are rapidly eliminated from the gut (24 h) as opposed to viable bacteria, which might remain up to 72 h (unpublished observations). A study currently ongoing in France, with healthy human volunteers, with classic enumeration techniques, will further substantiate the above (G. Corthier, personal communication).

## YOGURT BACTERIA IMPROVE LACTOSE DIGESTION

Savaiano et al. (8), Kolars et al. (9), Martini et al. (10) and many others have shown that lactose maldigesters can digest lactose in yogurt better than lactose in milk (11). Some of these studies have included heat-treated fermented milks as controls.

A generally accepted marker of lactose maldigestion is elevated exhalation of hydrogen that is produced from the fermentation of non-digested lactose that reaches the colon. The fermentation also results in the production of other gases. The gas production may result in symptoms such as

flatulence, abdominal pain and diarrhoea (12). Lactose maldigestion has to be discriminated from lactose intolerance. About 80% of the world adult population maldigests lactose, especially in Asia, Africa, Australia and South America. By definition, lactose maldigesters do not absorb a sizeable fraction of a given lactose dose in the small bowel, so that this non-absorbed lactose reaches the colon. Lactose intolerance means that this malabsorption leads to discernible symptoms.

Lerebours et al. (13), in a double-blind study in 16 lactase-deficient subjects, confirmed that yogurt enhances lactose digestion, this beneficial effect being destroyed by heat treatment. Moreover, the long-term (8-day) ingestion of either yogurt or heat-treated fermented milk did not modify the results of hydrogen breath tests in comparison with a 24-h ingestion. The mucosal lactase and  $\beta$ -galactosidase activities were not significantly modified by either of the two products. The authors conclude that in lactase-deficient subjects, no adaptation occurs after eating yogurt or heat-treated fermented milk, and that the increased lactose absorption in yogurt must be mainly related to an intraluminal process.

Shermak et al. (14) observed that 14 lactose-malabsorbing children experienced significantly fewer symptoms after consuming yogurt containing active cultures than after consuming milk; and that heat-treated fermented milk had an intermediate effect.

More recently, Hertzler and Clancy (15) expanded on this observation. They gave 20-g lactose portions of either milk (2% fat), plain yogurt or raspberry-flavoured yogurt, to 15 healthy free-living adults with lactose maldigestion, following an overnight (12-h) fast. They monitored the breath hydrogen excretion and lactose intolerance symptoms hourly for 8 h after each test meal. The breath hydrogen area under the curve was  $224 \pm 39$  ppm  $\times$  h for milk, and  $76 \pm 14$  ppm  $\times$  h for both types of yogurt. This difference is statistically significant ( $p < 0.001$ ). The yogurts also reduced the perceived severity of flatulence by 54% to 71% relative to milk. This study illustrates the reality of digestive discomfort experienced by lactose malabsorbers and the interest of live yogurt for this population.

The mechanism explaining this better lactose tolerance with yogurt than with milk has been studied by Drouault et al. (16) and Corthier (17). First the authors showed that in adult gnotobiotic mice, as a model for lactose maldigesting humans, lactose is not digested. Mice inoculated with the yogurt bacterium *S. thermophilus* are able to digest lactose, while mice inoculated with *S. thermophilus* with an inactivated lactase enzyme do not digest lactose (Table I).

These data strongly suggest that the lactase from the yogurt bacteria is responsible for the lactose digestion. Corthier (17) puts forward two hypotheses to explain this: either the bacteria produce the enzyme while they are alive in the yogurt; after ingestion of the yogurt the bacteria die

Table I

Lactose excretion in the faeces of gnotobiotic mice and of mice inoculated with *S. thermophilus* (16, 17)

Gnotobiotic mice inoculated with	Lactose (4.5%) in drinking water	Lactose intake (g/24 h)	Lactose excreted in faeces (g/24 h)
Not inoculated	No	0.00 ± 0.00	0.00 ± 0.00
Not inoculated	Yes	0.32 ± 0.01	0.31 ± 0.01
<i>S. thermophilus</i>	Yes	0.32 ± 0.01	0.14 ± 0.01
<i>S. thermophilus</i> with inactivated lactase*	Yes	0.32 ± 0.01	0.32 ± 0.01

\**S. thermophilus* with gene for lactase deleted.

off and the lactase is released in the gut; or the bacteria stay alive in the gut and are actively producing the enzyme after ingestion. To discriminate between these two possibilities Corthier used the 'reporter gene' technique. He inserted the gene for the light-producing bacterial enzyme luciferase in the *S. thermophilus* gene for lactase. Whenever the lactase gene is expressed, the luciferase gene will be expressed as well. The amount of light produced is a measure of the activity of the lactase gene.

Corthier inoculated gnotobiotic mice after birth with a 'human' gut flora. He administered *S. thermophilus* carrying the reporter gene to these animals in drinking water. At various points in time after administering the *S. thermophilus* the animals were killed, and the amount of light produced by the luciferase in the ileum was measured. Fig. 1 shows the effect of adding lactose (4.5%) to the drinking water on the production of light. It is clear that the presence of lactose switches on the production of the lactase enzyme of *S. thermophilus* in the gut of the animals.

The results of Drouault et al. and Corthier demonstrate that yogurt bacteria are intact and alive in the digestive tract of mice, and that the bacterial enzymes permease and  $\beta$ -

galactosidase are needed for the digestion of lactose, thus indicating that this digestion takes place within the bacterial cell.

Heat treatment of milk after fermentation not only destroys the yogurt cultures, it also inactivates intracellular and extracellular bacterial lactase, as lactase is a heat-sensitive enzyme (18).

Corthier's conclusion is consistent with that of Pelletier et al. (19) who studied the effect of yogurt ( $10^8$  bacteria/ml), heat-treated fermented milk and milk in 24 lactose-intolerant male subjects. They used a double-blind randomized cross-over design. At each test the subjects received a 25-g lactose dose in one of the products. The subjects reported the occurrence of digestive adverse events. Hydrogen excretion was monitored before and every 30 min after administration for 8 h. Table II shows that the breath hydrogen excretion after ingestion of yogurt with live bacteria is less than half of that after ingestion of heat-treated fermented milk. Table II also shows that although compared with milk, the heat-treated fermented milk offers some protection against the lactose-intolerance symptoms, the protection by yogurt with live bacteria is much better.

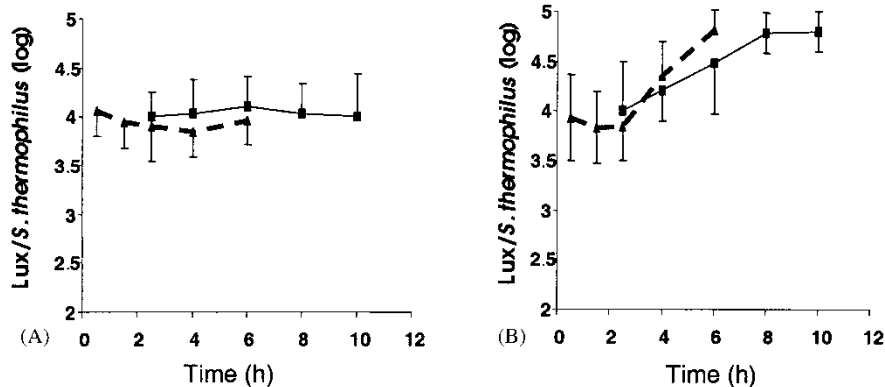


Fig. 1. Effect of water (A) or water containing 4.5% lactose (B) on the activity of *S. thermophilus* lactase in the intestine of gnotobiotic mice inoculated at birth with a human-type flora (17). Regulation in the ileal lumen of the  $\beta$ -galactosidase promoter of *Streptococcus thermophilus* when the strain is given to axenic or human flora-associated mice. The gene for the light-producing bacterial enzyme, luciferase, is inserted in the *S. thermophilus* gene for lactase. Whenever the lactase gene is expressed, the luciferase gene is expressed as well. The amount of light produced is a measure of the activity of the lactase gene. Animals were inoculated intragastrically with *S. thermophilus*, killed, and the digestive contents were collected at different time points. Measurements of luciferase activity and bacterial counts were performed. Luciferase activity was calculated per bacterial cell. Mice drank either water (A) or a 4.5% lactose solution (B). Dotted lines, mice associated with human flora; continuous lines, axenic mice.

Table II

Effect of consumption of yogurt, heat-treated fermented milk or milk on breath hydrogen excretion and digestive adverse events in 24 male lactose malabsorbers\* (19)

	Yogurt ( $10^8$ bacteria/ml)	Heat-treated yogurt (15 bacteria/ml)	Milk	<i>p</i> value
AUC (ppm/h)	$2445 \pm 709$	$11498 \pm 1494$	$23204 \pm 2650$	0.001
Digestive adverse events	10	18	35	0.02

\*Values are means  $\pm$  (SEM).

AUC, incremental area under the curve.

Labayen et al. (20) did a comparable study, also showing that yogurt with live bacteria resulted in lower breath hydrogen excretion and less severe gastrointestinal symptoms than heat-treated (pasteurized) fermented milk. This study also demonstrated that the oro-caecal transit time was shorter after ingestion of pasteurized fermented milk ( $10.5 \pm 0.6$  h) than after ingestion of 'live' yogurt ( $12.1 \pm 0.5$  h). This difference might explain, in part, the complaints-reducing effect of the yogurt with live cultures.

Rizkalla et al. (21) compared the effects of yogurt and heat-treated fermented milk (500 g/day for 15 days) in 12 healthy men with and 12 healthy men without lactose malabsorption. The study was designed as a cross-over design, separated by a washout period of 15 days. In men with lactose malabsorption, the breath hydrogen excretion was lower after fresh yogurt consumption than after heat-treated fermented milk consumption ( $p < 0.01$ ).

Taken together, these studies show that yogurt, due to its live cultures, improves lactose digestion in people with lactose malabsorption and alleviates gastrointestinal symptoms. Consequently, this benefit is dramatically reduced by heat treatment.

Many lactose maldigesters avoid milk and dairy products because of intestinal discomfort. The consumption of yogurt allows them to benefit from a nutritionally dense food rich in calcium, proteins and vitamins, with fewer symptoms. They will not have the same comfort with heat-treated fermented milk.

The World Health Organization (WHO) recommends replacement of animal milk by yogurt in case of diarrhoea in children (22).

### YOGURT BACTERIA MODULATE IMMUNE FUNCTIONS AND REDUCE ALLERGIC MANIFESTATIONS

Some of the health claims of lactic acid bacteria in functional foods are based on the presumed capacity of these microorganisms to modulate the host immune system. Perdigon et al. (23) showed that the yogurt bacteria *S. thermophilus* and *L. bulgaricus* do have this capacity. They administered the bacteria ( $10^9$  cfu/day) or sterile milk (10% in drinking water) as a control to mice (6 weeks of age) for 2, 5 or 7 consecutive days. The yogurt bacteria induced the expression of high levels of Bcl2 protein (a protein inhibiting apoptosis) in the mucosa intestinal immune cells. The increase of the protein was dose-dependent. The bacteria also caused a dose-dependent increase in the expression of cytokines (TNF- $\alpha$ , INF- $\gamma$ , IL-10 and IL-4).

Several other studies showed an effect of yogurt and yogurt bacteria on the immune system of animals (24–27) and humans (28–30). Some of these studies failed to show an effect of heat treatment, because of the choice of non-discriminant markers. This stresses the fact that not all the markers are relevant to demonstrate the deleterious effect of heat treatment.

Van de Water et al. (28) did a long-term (1-year) study, in which they gave young adults (20–40 years) or senior adults (55–70 years) either 200 g/day of live yogurt, or 200 g/day of pasteurized fermented milk, or no fermented milk product at all. Fig. 2 shows that, compared with no yogurt and pasteurized fermented milk, the live yogurt reduced nasal allergies, especially in the young adult population.

Trapp et al. (31) found a decrease in days of allergic rhinitis symptoms in live active bacteria-consuming indivi-

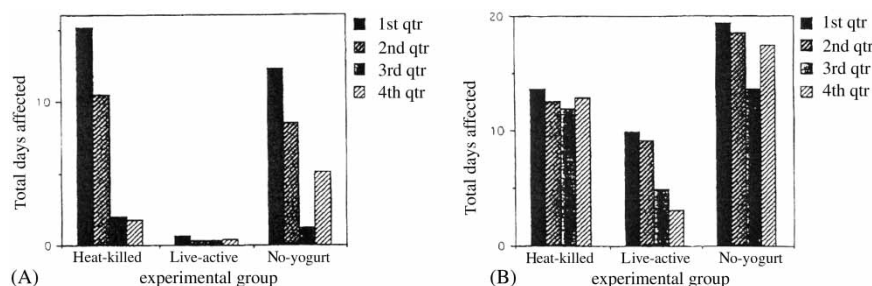


Fig. 2. Influence of chronic consumption of live yogurt or heat-treated fermented milk on nasal allergies over the course of 1 year in a young adult (A) and a senior adult (B) population (28).

**Table III**

*Criteria for the study of physiological effects of yogurt and the deleterious effects of heat treatment*

Criteria	Pasteurized milk	Yogurt and its live cultures	Effect of heat treatment on yogurt
Protein	<p>The proteins in milk are of excellent biological quality and both the caseins and whey proteins are well endowed with essential amino acids</p> <p>The nitrogen absorption from milk is highest during the first hour after ingestion</p>	<p>→ The nutritional value of milk protein is preserved during fermentation and yogurt is a good product for humans with regard to nitrogen intestinal availability (93%)</p> <p>↑ The proteolytic activity of yogurt bacteria results in a breakdown of only 1–2% of milk protein, leading to a higher content of peptides and free amino acids in yogurt than in milk</p> <p>↑ Consumption of yogurt is followed by a more regular release of nitrogen through the intestine, which could result in a more gradual distribution and then absorption of dietary nitrogen components in the body</p> <p>The nitrogen absorption from yogurt is higher and more evenly distributed in time than from milk or heat-treated fermented milk (33, 35)</p>	<p>→ Global absorption is similar to yogurt, but there are differences in kinetics and size of peptides</p> <p>↓ Less nitrogen is absorbed from heat-treated fermented milk than from live yogurt (33, 35)</p>
Bioactive peptides	Milk proteins contain numerous bioactive peptides	↑ During fermentation some bioactive peptides are released from milk proteins (36)	N/A
Vitamins	?	↑ During fermentation the yogurt cultures produce and excrete vitamins. Yogurt contains vitamins especially vitamin B2, B12 and folic acid	↓ Heat treatment has an effect on the concentration of the more labile water-soluble vitamins, especially vitamin B12 (37)
Minerals/calcium	Calcium in milk is bioavailable	→ Calcium in yogurt is bioavailable	↓ During the heating process, a large number of bacterial proteins are denatured, then aggregate and non-specifically bind calcium. This bound calcium is then unavailable for absorption (38)
Presence of live active cultures Capacity to restart fermentation	No live active cultures	<p>↑ Live active cultures are numerous in yogurt (<math>10^8</math>/g)</p> <p>One spoon of yogurt is sufficient to turn milk into yogurt</p>	<p>↓ Heat treatment reduces the viability and activity of cultures</p> <p>The concentration of living bacteria in a fresh yogurt heat-treated above 65°C for &gt; 3 min is reduced from <math>10^9</math> to &lt; <math>10^6</math>/g</p> <p>Heat-treated products are unable to restart a new fermentation</p>
'Antiseptic' effect against contamination of the product	No effect	↑ Yes (acid pH + bacteriocins + mere competition)	→ ↓ Partly impaired by heat treatment Some bacteriocins are thermolabile, some are thermostable

Table III (Continued)

Criteria	Pasteurized milk	Yogurt and its live cultures	Effect of heat treatment on yogurt
Survival of cultures in the gut	No effect	↑ Yes Live yogurt cultures have been isolated from different regions in the gut <i>L. bulgaricus</i> and <i>S. thermophilus</i> have been demonstrated in faeces (10 <sup>6</sup> /g) (7, and J. Doré, personal communication)	↓ No living lactic cultures are found in the gut after ingestion of heat-treated products
Effect on gut flora	?	↑ Yes Effect on <i>Clostridium</i> diarrhoea (39) Effect on intestinal environment in the elderly (40, 41)	N/A
Interaction with the immune system: 1) adjuvant effect	No effect	↑ Yes	→ Yes The adjuvant effect of killed bacterial cells is well known
Interaction with the immune system: 2) immunomodulatory effect	No effect	↑ Yes	N/A
Prevention of allergy	No effect	↑ Yes (28)	↓ Pasteurized fermented milk is less effective in nasal allergy patients
Protection against infections	?	↑ Protection against <i>Pseudomonas</i> infection in mice (42)	N/A
Lactose digestibility	Lactose is the major carbohydrate in milk (98% of total carbohydrates)  Lactose is, depending on the geographic area, often maldigested by a great part of adult population, with the exception of lactase-sufficient Caucasians Small intestinal transit is very quick in lactose malabsorbers	→ Amount of lactose: during fermentation, yogurt lactic acid bacteria digest 20–30% of initial lactose content. Because of addition of dry matter before fermentation, the final amount of lactose is about the same in yogurt as in milk. Lactose breakdown results in the release of galactose (final content of 1.0–1.5%), and glucose metabolized in lactic acid (final content of 0.7–1.2%)  ↑ Digestion of lactose: during fermentation and during transit through the intestine of the consumer, yogurt lactic acid bacteria produce lactase, thereby reducing symptoms of lactose intolerance (43)  ↑ Yogurt normalizes the small intestinal transit in lactose malabsorbers	→ Amount of lactose: similar to yogurt  ↓ Digestion of lactose: impaired for two reasons 1) Destruction of live bacteria 2) Lactase is a heat-labile enzyme. It is inactivated by heat treatment (8) Heat-treated fermented milk products lack the improving effect on symptoms of lactose intolerance  ↓ Heat treatment impairs the normalization of small intestinal transit in lactase-deficient subjects (44)
Gastric emptying	Irregular and diphasic	↑ Regular and monophasic (45)	→ Similar to yogurt

Table III (Continued)

Criteria	Pasteurized milk	Yogurt and its live cultures	Effect of heat treatment on yogurt
Improvement of diarrhoea symptoms in children	No effect	↑ Yes	N/A
Anti mutagenic and anti-carcinogenic properties in experimental models	?	↑ Indications for a role of yogurt in prevention of colon cancer (46)	N/A
Effect on oral health	?	↑ Fermenting dairy strains inhibit growth of cariogenic bacteria (model study, (47))	N/A
Prevention of collagen-induced arthritis	No effect	↑ <i>L. bulgaricus</i> prevents collagen-induced arthritis (in mice, (48))	N/A
Promotion of growth	?	↑ Yes → indicates equal to milk; ↑ indicates superior to milk	↓ Heat treatment reduces the feed efficiency of yogurt as measured by rat growth rate (49) → indicates no change with heat treatment; ↓ indicates impaired by heat treatment; N/A, indicates that no studies are available on yogurt versus heat-treated fermented milk

(?) means not documented.

duals compared with those consuming a normal diet or pasteurized fermented milk when the consumption was carried out for a full year. The improvement was stepwise and continued throughout the entire year.

The team of Maldonado-Galdeano and Perdigon have evidence that the modulation of the intestinal mucosal immune system is completely different using dead or viable lactic acid bacteria (unpublished observations).

These results indicate a role of live yogurt cultures in modulating the immune system of the consumer. In a review of immunological effects of yogurt Meydani and Ha (32) concluded that 'studies provide a strong rationale for the hypothesis that increased yogurt consumption, particularly in immunocompromised populations such as the elderly, may enhance the immune response, which could in turn increase resistance to immune-related diseases'.

#### IMPROVED PORTAL NITROGEN ABSORPTION FROM YOGURT COMPARED WITH MILK – EFFECT OF HEAT TREATMENT

Rychen et al. (33) studied the portal absorption of nitrogen from yogurt compared to milk, and the effect of heat treatment, in the growing pig, a validated model for studying protein digestion in humans (34). To be able to discriminate between exogenous and endogenous protein fractions in the intestinal lumen they used products with <sup>15</sup>N-enriched milk proteins.

The pigs were fitted with two catheters, one placed in the portal vein and one in the brachiocephalic artery. A few days after the placement of the catheters the animals recovered their normal growth rate (400 g/day). At 10, 15 and 20 days after the placement of the catheters the animals were fed 1000 ml of milk, yogurt or heat-treated fermented milk orally, in random orders for different animals on different days.

Portal and arterial blood samples (10 ml) were collected before the products were given and at various time points till 3 h after ingestion. The differences in <sup>15</sup>N between the portal and the arterial blood (porto-arterial differences, PAD) are a measure of the nitrogen absorption from the three products, and Rychen et al. observed that this absorption was high for each product, of the order of 70%. Nitrogen from milk and heat-treated fermented milk is mainly absorbed within 120 min of ingestion, whereas nitrogen absorption from yogurt is more spread over the 4-h post-prandial period.

Mpassi et al. (35) completed the Rychen study. They included a study of the effects of a live yogurt stored for 21 days at 4°C. They found analytical differences in the effect on nitrogen absorption between 'fresh cultured yogurt' (Y0) and '21 days stored yogurt' (Y21), but without detectable physiological consequences. Y21 and Y0 had similar effects, but their effects differed from those of milk or heat-treated fermented milk.



The conclusion that the nitrogen absorption from yogurt is higher than the absorption from heat-treated fermented milk is in line with the results of a classic study by Hargrove and Alford (1), showing that the growth of rats with fresh yogurt as a nitrogen source was better than with heat-treated fermented milk: the weight gain with fresh yogurt was 20% higher than with heat-treated fermented milk as a nitrogen source.

## CONCLUSIONS

From the studies mentioned above it is clear that live yogurt is a source of bacteria surviving in the human intestine. Live yogurt reduces the symptoms of lactose maldigestion. Live yogurt is a good source of absorbable nitrogen. Heat-treatment of the product destroys or diminishes these benefits. Furthermore there are indications for differences between live yogurt and heat-treated fermented milk with respect to prevention of allergies.

These results show that, seen from a nutritional/functional point of view, heat treatment dramatically alters some intrinsic beneficial properties of yogurt.

There is emerging evidence that fermentation of milk has positive effects on the release of bioactive peptides, and that consumption of yogurt has beneficial effects on the gut equilibrium, on the immune system, on prevention of infections, on mutagenesis and carcinogenesis, on oral health and (in animals) on prevention of collagen-induced arthritis. These beneficial effects have not been shown for heat-treated fermented milk.

Table III gives an overview of relevant criteria for the study of the deleterious effect of heat treatment on yogurt (7, 33, 35, 37–49, and J. Doré, personal communication). Some markers will not allow differentiation between live and heat-treated products, for instance gastric emptying, or markers that are linked to homeostasis, since these are very tightly regulated. However, as Table III shows, heat treatment changes yogurt dramatically on a number of relevant parameters, leading to a very different product.

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