Changes in Intestinal Permeability in Symptomatic and Asymptomatic Runners

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Abstract
Gastrointestinal symptoms such as diarrhea, cramping, nausea and gastric pain occur frequently in runners during training and competitions. The mechanisms leading to the distress are not fully understood, nor the reason why some remain asymptomatic. However, hyperthermia induced by exercise elevation of core temperature and oxidative damage due to reduced gastric blood flow have been postulated to affect the intestinal epithelial cells. Both sources of stress disrupt the binding of the epithelial tight junction proteins and increase permeability of the membrane to luminal endotoxins. Endotoxins reaching the blood stream through leaky tight junctions lead to an inflammatory response mediated by cytokines. These mechanisms may underlie the gastrointestinal symptoms often experienced by endurance athletes.

The aim of this study was to measure running-induced changes in intestinal permeability and inflammatory markers and investigate their association with gastrointestinal symptoms. A secondary objective was to inspect possible correlations between gastrointestinal symptom occurrence and intake of certain nutrients. A total of 17 active runners were allocated into a control group (asymptomatic) or a symptomatic group based on a symptom history questionnaire and completed a 90-minute running test. Intestinal permeability at baseline and after the run were assessed via urine recovery of orally administered Iohexol. LPS (endotoxin) and zonulin concentrations were determined from serum samples. Participants kept a food diary for three days before each measurement and filled out a symptom questionnaire after the run.

No significant difference was found in intestinal permeability between symptomatic and asymptomatic runners either at rest or following strenuous exercise. However, both groups experienced a significant increase in intestinal permeability from baseline to after running. LPS concentrations were significantly higher at baseline in the symptomatic group. This may explain the higher symptom occurrence in the symptomatic group. Zonulin levels were higher in control group than symptom group after the run. Zonulin concentration was also higher in the control group after the run compared to baseline. The symptom group reported more stomach pain and stool changes after running compared to controls. Comparison of average intake of various nutrients between the two groups showed no significant differences, indicating an individual predisposition as the cause of symptoms rather than diet alone. The lack of difference in intestinal permeability between the groups combined with the difference in symptom occurrence indicates that intestinal permeability changes alone do not account for symptom development. A possible factor may be individual differences in intestinal mucosa repair ability or some underlying pathology.

Keywords
Intestinal Permeability, Iohexol, LPS, Running

Where deposited

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1. Review of Literature

1.1 Protective role of gastrointestinal barrier

The gastrointestinal epithelium is known for its role in digestion and efficient uptake of nutrients. It forms a selectively permeable barrier, letting certain substances like nutrients, water, and electrolytes permeate while excluding unwanted luminal contents such as bacterial toxins. It is a single-cell layer and forms the largest and most important barrier against the external environment (Groschwitz and Hogan, 2009). The gastrointestinal epithelial barrier is maintained by formation of protein-protein networks that mechanically link together neighboring epithelial cells and prevent free passage of molecules between these cells. The adhesive junctional complexes connecting neighboring cells consist of desmosomes, adherens junctions, and tight junctions and are formed from protein networks of interacting transmembrane proteins (Groschwitz and Hogan, 2009). The transmembrane proteins interact both extracellularly and intracellularly with adaptor proteins that link to the cytoskeleton (Groschwitz and Hogan, 2009). Of these complexes, tight junctions (Figure 1, p.5) are the most important in the context of intestinal permeability.
The intestinal epithelium consists of a single layer of polarized epithelial cells. Intestinal epithelial junctional complexes connecting neighboring cells consist of desmosomes, adherens junctions, and tight junctions. Desmosomes are localized dense plaques that are connected to keratin filaments. Adherens and tight junctions both consist of transcellular proteins connected intracellularly through adaptor proteins to the actin cytoskeleton (Groschwitz and Hogan, 2009).

The luminal contents of the intestine harbor products that can both initiate and sustain inflammatory responses in the intestinal epithelium. Such substances may include food antigens, bile, hydrolytic enzymes, and endotoxin (lipopolysaccharide, LPS) (Lambert, 2009). Several other stresses have been suggested to damage the integrity of the barrier, including psychological stress (Söderholm and Perdue, 2001), heat stress (Moseley et al., 1994), certain medications (Lambert et al., 2007), and exercise (Pals et al., 1997). In addition to enterocyte membranes and tight junctions joining the neighboring epithelial cells and forming the gastrointestinal barrier, mucus and tissue macrophages also contribute to barrier function, or the ability to control the passage of substances from the gut lumen to circulation, by restricting passage of unwanted substances to the circulatory system (Lambert, 2009). Loss of the gastrointestinal barrier integrity leads to increased intestinal permeability which refers to the relative ease of unmediated passage of substances through the epithelial wall into blood circulation. It typically refers to the nonmediated diffusion of large normally restricted particles of >150 Da across the epithelial barrier and into blood circulation (Lambert, 2009). Although a low level of permeability is...
present normally in the intestine, the gastrointestinal barrier and immune system together prevent entry and damage by pathogens (Lambert, 2009).

1.1.1 Paracellular and transcellular transport

Two modes of transport have been established for intestinal permeation as shown in Figure 2 (p.8). These include the transcellular route, where substances cross the epithelial barrier through the cells with transport predominantly regulated by selective transporters for amino acids, electrolytes, short-chain fatty acids, and sugars and the paracellular route, where substances diffuse between neighboring epithelial cells with transport mediated by tight junctions (Groschwitz and Hogan, 2009). In a transcellular pathway, compounds must cross the microvillus membrane of the epithelial cell by active transport or endocytosis (de Punder and Pruimboom, 2015). However, water-soluble substances cannot cross lipid bilayer membranes and therefore the paracellular pathway mediated by tight junctions becomes an important means of transport for water, solutes, and minerals across the intestinal barrier (Kapus and Szászi, 2006; Turner et al., 1997). Tight junctions are an important part of several types of absorptive and secretory epithelia. In the gastrointestinal system, tight junctions act as gatekeepers, forming the permeability barrier by controlling the passage of ions and larger molecules between the neighboring cells. The multi-protein epithelial tight junction complexes are composed of transmembrane proteins, such as claudin, peripheral membrane proteins, such as zona occludens, and regulatory molecules including kinases (Turner, 2009). The tight junction is found on the most apical region of the junction and forms a very tight contact between neighboring cells. Permeation studies using tracers have clearly demonstrated that the tight junction forms the site where paracellular diffusion of particles is restricted (Magnuson et al., 1978). One hypothesis to explain the differences in permeability between substances of different characteristics indicates that smaller molecules find the greatest number of suitable pores in the epithelia and cross the barrier in greater numbers than larger molecules which would be unable to pass through the pores (Meddings, 1997). These small hydrophilic pores are believed to represent integral membrane proteins of the lipid bilayer (Meddings, 1997). Thus, both large and small (<300nm) molecules cross the epithelial barrier by diffusion between cells but only small molecules can take advantage of the transcellular pathway (Meddings, 1997). This has been demonstrated
through permeability experiments of small water-soluble substances across rat small intestine which demonstrated a low abundance pathway through which large particles can move and which is not size dependent (Meddings, 1997). In other words, larger particles can move through a pathway that is not dependent on the size of the molecule while a second pathway characterized in the experiments is a more numerous but size dependent pathway (Meddings, 1997). Thus, smaller molecules can move across the membrane more rapidly as they can take advantage of smaller pores. These pathways correspond to the paracellular and transcellular modes of transit and are supported by other experiments. Maxton et al. (1986) demonstrated that damage to the epithelium through use of detergents led to increased passage through large pathways with no change in small pathway. This supports the notion that damage to epithelial tight junctions increases permeability to large molecules while the diffusion of small molecules across the epithelial cell membranes is unchanged (Meddings, 1997). In turn, the active uptake of glucose, sodium, and water is mediated by sodium-dependent glucose co-transporters (SGLTs) (Turner, 2000). The transcellular absorption of glucose and sodium to the basolateral membrane by transporters opens up the paracellular pathway structure and allows the diffusion of water and small nutrients by creating an osmotic gradient (Turner, 2000). Tight junctions limit solute transit in the paracellular pathway, which is typically more permeable than the transcellular pathway. Therefore, tight junctions become the rate-limiting step in solute transport across the epithelium and the principal determinant of mucosal permeability (Turner, 2009).

Two routes of paracellular transport exist and depend on size and charge selectivity. One route allows permeation of large solutes including proteins and bacterial endotoxins (Van Itallie et al., 2008; Watson et al., 2005). The exact size at which solutes are excluded from this pathway has not yet been determined although it is clear that whole bacteria are too large to pass (Turner, 2009). As might be expected from a pathway which allows large solutes to pass, this pathway is not dependent on charge (Turner, 2009) and cytokines, including interferon-gamma (IFN-γ) and tumor necrosis factor α (TNF-α) may increase passage across the barrier (Clayburgh et al., 2006; Wang et al., 2005; Watson et al., 2005). A second route of paracellular permeation is defined by charge dependent small pores which are thought to be mediated by tight junction claudin proteins which are the primary determinants of charge selectivity (Amasheh et al., 2002; Colegio et al., 2003; Simon et al., 1999). Thus, tight junctions exhibit both size and charge sensitivity and these properties can be influenced by physiological and pathological factors (Turner, 2009). In
addition to their role as gate-keepers in paracellular transport, tight junctions also participate in transcellular transport by maintaining the polarization of the apical and basolateral domains in epithelial cells (Gumbiner and Louvard, 1985; Simons and Fuller, 1985). This polarization allows the diffusion of substrates across the epithelial cell membrane, as transport proteins are located appropriately for their function.

Figure 2. Pathways of epithelial permeability. In transcellular permeation solutes or water move through intestinal epithelial cells. In paracellular permeation solutes move in the intercellular space between epithelial cells and permeation is regulated by tight junctions found at the junction of the apical-lateral membranes (Groschwitz and Hogan, 2009).

1.2 Gastrointestinal complaints in endurance athletes

Gastrointestinal problems are extremely common in endurance athletes and are often reported as the main cause of underperformance in a race. Depending on the methodology, 30-90% of athletes suffer from gastrointestinal problems during exercise (Jeukendrup et al., 2000). However, the mechanisms underlying gastrointestinal symptoms remain unelucidated. Symptoms in runners vary greatly from mild exercise related discomfort or nausea to severe pain
and diarrhea. Ultimately, these symptoms have the potential to hinder both exercise performance and recovery, and disrupt the training progress of an athlete. Currently, there is no standard definition of what constitutes a minor or major symptom in this context, but it has been proposed that a major symptom is one that affects either health or sports performance (Oliveira et al., 2014). No sport specific symptom pattern can be found among runners but instead symptoms are highly individual. However, gastrointestinal disturbances occur more often during running than in sports with less vertical impact (Wright et al., 2011). Gastrointestinal symptoms can usually be classified into either upper or lower gastrointestinal symptoms. Typically lower gastrointestinal symptoms are more severe and more common in runners but even upper gastrointestinal symptoms can be harmful and have severe health consequences (Peters et al., 1999).

1.2.1 Prevalence of gastrointestinal complaints

Symptom occurrence varies greatly between different competitions and individuals. In elite athletes, the prevalence of gastrointestinal complaints was reportedly 70% (Peters et al., 1999) while in an internet-based questionnaire study of 1281 athletes, 45% of runners reported gastrointestinal symptoms during running (Steege et al., 2012). A total of 85% of participants at an ultramarathon experienced gastric bleeding (Baska et al., 1990) and 93% of triathletes competing in difficult conditions experienced gastrointestinal problems such as flatulence, urge to defecate, heartburn, abdominal pressure, nausea, stomach ache, intestinal cramps and urge to vomit (Jeukendrup et al., 2000). Besides the large variation in prevalence of symptoms seen with different methodologies, symptoms also vary largely depending on age, gender, training status, environmental conditions and exercise intensity. Often, gastrointestinal symptoms may be disruptive enough to cause dropping out of the race. In the study by Jeukendrup et al. (2000), 14% of the participating athletes were unable to complete the race and in the study by Stuempfle and Hoffman (2015), 35.6% of non-finishers in the race dropped out due to gastrointestinal distress. Gastrointestinal symptoms also played a major role in the athletes' performance and recovery as nearly half of race finishers reported that gastrointestinal symptoms affected their race performance (Stuempfle and Hoffman, 2015). The gastrointestinal damage incurred by runners may even include mucosal lesions and ischemic colitis which are both commonly observed during running (Choi et al., 2001; Gaudin et al., 1990; Steege et al., 2012) along with
gastric bleeding (Baska et al., 1990). In the study by Gaudin et al. (1990), all runners showed pathological features of gastrointestinal lesions following a 18-50km run. Similarly, in the study by Steege et al. (2012), gastrointestinal ischemia was observed in all athletes following maximum intensity exercise. Despite the common occurrence of symptoms, the etiology of gastrointestinal complaints in endurance athletes remains poorly understood.

1.2.2 Physiological causes of gastrointestinal complaints

Currently it is recognized that while the etiology of exercise-induced gastrointestinal disturbances is likely multifactorial, intestinal ischemia and subsequent alterations in epithelial membrane permeability are ascribed as the main pathophysiological mechanisms for the development of symptoms (Oliveira et al., 2014). Other factors affecting symptom development likely include nutritional and mechanical factors along with a possible genetic influence.

1.2.2.1 Changes in gut motility

Changes in gut motility occur frequently during running and may be observed in the esophagus, stomach, or intestine. Studies have linked decreases in sphincter tone to gastro-esophageal reflux while the effects on gastric emptying are still less clear (Oliveira et al., 2014). One early finding showed no change in gastric emptying at moderate intensity while another showed that high intensity or intermittent exercise may reduce gastric emptying time (Oliveira et al., 2014). However, other studies have shown that exercising in hot conditions or while dehydrated reduces gastric emptying time (Neufer et al., 1989; Rehrer et al., 1990a). Additionally, damage to gastrointestinal mucosa increases in very hot or humid conditions while dehydrated due to the reduction in blood volume and increased ischemia. Damage to intestinal epithelia in turn can lead to more gastrointestinal disturbances. Contrary to the changes in esophageal or gastric motility, effects on the small intestine seem to be small. One study examined small intestinal and colonic transit time in symptomatic and asymptomatic runners at rest and during exercise but failed to see any difference in transit time even though diarrhea was observed in the study (Rao et al., 2004). This would indicate that the symptoms were not caused by changes in transit time.
but by some other mechanism instead. Overall, the effect of exercise on gut motility appears quite small although some changes to gastric emptying time occur at higher intensities.

1.2.2.2 Splanchnic hypoperfusion

1.2.2.2.1 Redistribution of blood flow

Long-duration exercise places an extreme adaptive demand on the body and leads to a redistribution of blood flow. During exercise, blood is diverted to the working muscles and cardiopulmonary system to supply the higher oxygen and nutrient demands. Norepinephrine is released during strenuous exercise in the sympathetic nervous system to trigger splanchnic vasoconstriction and allow greater blood flow to active tissues (Wright et al., 2011). Blood is also diverted to the skin and periphery to increase heat dissipation and cool the body. This demand by the working muscles and active tissues directs blood away from the central tissues including the stomach, intestines and liver. As much as 80% of blood flow to the gut is diverted in humans during exercise (Clausen, 1977). This decreased splanchnic blood flow leads to ischemic damage to the intestinal epithelial cells and disrupts the integrity of the gastrointestinal barrier (Pals et al., 1997; Wijck et al., 2012). This disruption of the gastrointestinal barrier may be the cause of the reported nausea, vomiting, diarrhea, and pain (Oliveira and Burini, 2009, 2011) although direct evidence is still lacking. Dysfunction of the epithelial gastrointestinal barrier can also affect physical performance and post-exercise recovery negatively by causing gastrointestinal distress and impairing uptake of nutrients, fluids, and electrolytes (Oliveira et al., 2014). Intestinal ischemia is often caused by a combination of hyperthermia, dehydration, and exhaustion (Oliveira and Burini, 2009). Further damage to epithelia can also be caused after ischemia by reperfusion of blood due to oxidative stress and inflammation (Grootjans et al., 2010). In an investigation by Schaub et al. (1985), epithelial surface changes known to occur during ischemia were observed in an athlete following a marathon. The ischemia also commonly causes gastrointestinal bleeding and intestinal infarctions in some cases even require surgery (Lucas and Schroy, 1998).
1.2.2.2 Endotoxemia

Severe disruption of epithelial integrity by strenuous exercise may also lead to leakage of bacterial endotoxin into blood circulation, or endotoxemia, causing inflammation (Jeukendrup et al., 2000). The disruption of barrier integrity involves disruption of tight junctions that connect epithelial cells, either by loss of tight junction proteins or disruption of cytoskeleton of epithelial cells. Intestinal ischemia and reperfusion can also lead to Paneth cell apoptosis (Grootjans et al., 2011). These cells produce and secrete antimicrobial proteins to prevent bacterial translocations and inflammation, and are found in the crypts of the intestine. However, damage to Paneth cells during exercise has never been examined but exercise-induced ischemia may contribute to Paneth cell destruction and thereby loss of gastrointestinal barrier function (Wijck et al., 2012). Damage to the intestine is exacerbated through exercising in hot or humid conditions that cause increases in fluid loss through sweating and result in decreased plasma volume and dehydration that further lowers the blood supply to the intestine (Lambert et al., 2008a).

Patients with major trauma, sepsis, or shock exhibit similar changes in blood redistribution as can be caused by extreme exercise (Jeukendrup et al., 2000). In these cases, the hypoperfusion of the gut causes shock-induced mucosal damage and permits the leakage of gram-negative bacteria or their endotoxins such as LPS into blood circulation. Once in circulation, endotoxins lead to various symptoms including fever, nausea, shivering, dizziness, gastrointestinal disturbances such as diarrhea and vomiting, and eventually sepsis (Brock-Utne, 1988). These symptoms and especially gastrointestinal disturbances are commonly experienced by endurance athletes as studies point to a symptom prevalence of 30-50% in marathon runners and gastric ischemia has been shown to occur frequently even in asymptomatic athletes (Nielsen et al., 1995). The occurrence of ischemia in asymptomatic athletes may imply that symptomatic athletes are more susceptible to the effects of exercise induced ischemia. This is supported by the occurrence of the same phenomenon with other gastrointestinal disorders, namely irritable bowel syndrome and gastro-esophageal reflux disease (Drossman et al., 2002). Exercise-induced ischemic damage to the intestines can also induce mucosal erosions and gastrointestinal bleeding in athletes surveyed by endoscopy (Øktedalen et al., 1992). Other methods such as fecal occult blood tests have also indicated fecal blood loss in otherwise healthy athletes (Bi and Triadafilopoulos, 2003) and
examination of 450 trained endurance athletes in cycling, triathlon, and running revealed running to be most strongly associated with gastrointestinal problems (Peters et al., 1999). This may be due to the aforementioned ischemic and mechanical stresses imposed by running at a greater degree than lesser-impact sports. While gastrointestinal bleeding induced by exercise does not usually merit immediate medical attention, it can affect training and performance and lead to more serious harm to the athlete. Studies suggest that up to one quarter of athletes may experience gastrointestinal bleeding during intense or prolonged exercise (McCabe et al., 1986). Gastrointestinal bleeding may also contribute to the commonly occurring anemia or low iron and/or ferritin encountered among runners (McMahon et al., 1984). In a study by Stewart et al. (Stewart et al., 1984), 20 out of 24 runners surveyed after a race of 10 to 42.2km showed increased fecal hemoglobin levels, indicating a very high occurrence of gastrointestinal bleeding among runners. A cause for the variation in perceived symptoms among athletes may simply be overreaching of the adaptive capacity of the gastrointestinal tract. Because while exercise induced ischemia has been demonstrated to damage intestinal epithelia, there is also evidence of adaptive capability to exercise through improved gastrointestinal barrier function and increased gastric emptying (Gisolfi, 2000). This might indicate that the intestinal mucosa adapts to better withstand the exercise-induced damage or that its recovery capabilities improve after damage is already sustained. Indeed, after one study symptomatic athletes reduced their training intensity for a few months and, after slowly building up their training, were able to resume exercise at the original intensity (Steege et al., 2012). As one adaptation, blood flow to the intestines is generally better maintained in trained than untrained subjects (Gisolfi, 2000). Also, results from an animal study indicate that the difference in heat tolerance between sedentary and physically fit animals is in part attributable to endotoxin leaving the gut (Gisolfi, 2000). In the study, the rise in core temperature during exercise in untrained animals was significantly greater than in trained animals. However, injection of indomethacin which blocks endotoxin-induced fever lowered the core temperature of the untrained animals to the level of the trained animals (Gisolfi, 2000). Additionally, the gastrointestinal system has a capacity to regenerate, with epithelial cells having an average lifespan of only four to five days (Gisolfi, 2000). Even in cases where gastric bleeding occurs, it is generally no longer detected by esophagogastroduodenoscopy and colonoscopy more than 48 hours after exercise (Schwartz et al., 1990).
1.2.2.3 Intestinal permeability and absorption

Related to the splanchnic hypoperfusion and subsequent damage to intestinal epithelia is the increase in intestinal permeability. Gastrointestinal symptoms during distance running have been previously associated with changes to the small intestinal permeability (Øktedalen et al., 1992). A vital function of the intestinal barrier is to prevent the penetration of carcinogenic, toxic, or antigenic substances into the interstitial fluid and systemic circulation. Entry of pathogens through a damaged epithelial barrier with increased permeability provokes a systemic inflammatory response that may lead to the gastrointestinal symptoms experienced during running (Øktedalen et al., 1992). As mentioned earlier in the review of the gastrointestinal barrier function, particles can cross the epithelial barrier through either transcellular or paracellular transport. Tight junctions function mainly in paracellular transport but also maintain cell polarization in epithelial cells and thus affect transcellular transport. Damage to and loosening of tight junctions caused by medications, stress, exercise (Hollander, 1992), disease, alcohol (Napolitano, 1995), or thermal stress (Napolitano, 1995) leads to increased permeability of the gut to larger particles. Pals and coworkers (1997) were the first to demonstrate increased intestinal permeability at high intensity exercise but no changes at lower intensity. In their study, six healthy test subjects experienced significant increases in intestinal permeability following a 60 minute treadmill run at 80% of maximal oxygen uptake (peak VO₂) compared to 40% or 60% peak VO₂ (Pals et al., 1997). This indicates that permeability changes during exercise may be intensity dependent. Moses (1991) also observed increased intestinal permeability during high intensity running with subjects running for 90 minutes at 60 and 85% maximal O₂ uptake with 30 second sprints every 15 minutes. Likewise, Øktedalen et al. (1992) demonstrated increased permeability after a half-marathon or marathon race. Although the link of increased intestinal permeability to gastrointestinal symptoms has not been proven, van Nieuwenhoven et al. (2003) demonstrated that symptomatic athletes have increased intestinal permeability following running or cycling compared to asymptomatic controls. In the study, intestinal permeability, orocaecal transit time, gastric reflux, gastric emptying and glucose absorption were measured in 10 symptomatic and 10 asymptomatic runners at rest and following running or biking. No significant differences were found between the groups during rest. No difference was found in
gastric emptying or intestinal glucose absorption either, but orocaecal transit time, gastric reflux, and intestinal permeability increased significantly more after exercise in symptomatic runners (Nieuwenhoven et al., 2003).

In summary, exercise can affect the gastrointestinal tract in various ways, many of which are intensity dependent. Intense or long-duration exercise can reduce gastric emptying time, cause ischemic damage via reduction of blood flow to the intestine, and increase intestinal permeability.

1.2.3 Mechanical causes

Another issue to consider regarding gastrointestinal symptoms in endurance athletes is mechanical damage that is related mainly to impact or posture during sports. In a study measuring gastroesophageal reflux during cycling or running, running caused significantly longer gastroesophageal reflux episodes than biking along with reported heartburn and chest pain in some athletes (Peters et al., 2000). This could likely be because of the repetitive impact caused by running that could subsequently damage the intestinal epithelia and generate the symptoms. The high percentage of blood loss to feces in triathletes following intense training, pre-race, and post-race measurements as well as a significant relationship between athlete blood hemoglobin level and training run intensity in regression analysis (Rudzki et al., 1995) supports this hypothesis of mechanical damage as mucosal damage would lead to gastrointestinal bleeding. In 20 triathletes, 80% exhibited exercise intensity dependent blood loss to feces (Rudzki et al., 1995). This intestinal bleeding is likely caused by the combination of mechanical jostling of the intestines and the exercise-induced gut ischemia (Moses, 1991; Oliveira and Burini, 2009). A lower incidence of gastrointestinal bleeding in cyclists and the absence of blood in feces of walkers support the theory that mechanical trauma in runners contributes to occult blood loss (Oliveira and Burini, 2009). The bouncing of the intestines may also contribute to other symptoms commonly observed in runners, including diarrhea, pain, and urgency (Keeffe et al., 1984). In a study performed on triathletes by Rehrer et al. (1992), all gastrointestinal symptoms reported were found to be more prevalent during the run portion of the triathlon which again may stem from the higher impact and mechanical pressure to the gut. Additionally, posture may play a role in the development of specific symptoms. While lower gastrointestinal symptoms are more
prevalent in runners, cyclists often experience more upper gastrointestinal disturbances (Peters et al., 1999). This may be caused by cyclists bending over the bike handlebars, thus creating increased abdominal pressure (Peters et al., 1999). Intake of fluids during exercise can also lead to “swallowing of air” that can trigger gastrointestinal symptoms. However, it appears that higher levels of training may decrease these gastrointestinal symptoms (Waterman and Kapur, 2012).

1.2.4 Nutritional causes

1.2.4.1 Fluids

Nutrient and fluid ingestion before, during, and after running plays an important role in an athlete’s performance and training progression. Poorly timed or insufficient nutrition and fluids can cause underperformance and impair recovery (Peters et al., 2012). Similarly, certain foods or nutrients such as greasy foods, protein, fructose, and fiber have been linked to increased gastrointestinal distress while running (Rehrer et al., 1992). For example, gastrointestinal symptoms reported by triathlon participants were associated with certain nutritional factors including ingestion of fat, protein, fiber, or concentrated carbohydrate drinks (Rehrer et al., 1992). Particularly osmolalities greater than 500 mOsm/L were found to cause gastrointestinal distress (Rehrer et al., 1992). It appears that foods or drinks that draw fluid into the intestines may be a major cause of some gastrointestinal symptoms. The concentrated carbohydrate drinks are one example of such substances that can cause fluid accumulation. The effects of carbohydrate intake on gastrointestinal symptoms overall are still less clear. In a study by Pfeiffer et al. (2009) conducted on 26 men and 8 women, high carbohydrate (CHO) intakes of 1.4g CHO per minute compared to moderate intake of 1.0g CHO per minute did not produce any increase in gastrointestinal symptoms during a 16km run and both were well tolerated. In a second branch of the randomized double-blind study, 34 men and 14 women were allocated to receive either a glucose or glucose + fructose gel providing 1.4g CHO per minute. Again, no difference was found between groups indicating that the composition of the carbohydrate blend did not play a role in the development of gastrointestinal symptoms (Pfeiffer et al., 2009). However, in a study by Wallis et al. (2007) performed on 8 endurance-trained women, increasing
carbohydrate consumed during cycling led to an increase in gastrointestinal symptoms. In this study the sample size was not large enough to perform statistical analyses on gastrointestinal symptom reports so the results must be taken as descriptive only. The study used water and three different glucose concentrations (0.5g/min, 1.0g/min, 1.5g/min) to test effects on glucose oxidation and endogenous glucose sparing and included a questionnaire on gastrointestinal symptoms (Wallis et al., 2007). Although the greatest symptom occurrence was reported at the high concentration of 1.5g glucose per minute, the highest rate of glucose oxidation and greatest endogenous glucose sparing occurred at the moderate level of 1.0g per minute carbohydrate indicating a race performance advantage at this level of ingestion (Wallis et al., 2007). In a larger study performed on 221 endurance athletes, higher carbohydrate intakes during competition were associated with increased nausea and flatulence, but also with decreased race finish time (Pfeiffer et al., 2012). This indicates that while higher rates of carbohydrate intake may be associated to certain gastrointestinal symptoms, they also contribute to faster finishing times and improved performance. Considering the faster finishing times, the nausea caused by ingestion of gels was likely not severe. From these studies it may be postulated that carbohydrate intake itself is not necessarily the cause of gastrointestinal symptoms but that the response may vary depending on the concentration or osmolality of the gel or beverage (Oliveira et al., 2014).

Another important factor in running performance is fluid ingestion and especially avoiding dehydration. There appears to be link between dehydration during exercise and subsequent gastrointestinal distress. In a field study conducted on 44 runners participating in a marathon, body weight losses of about 4-5% were associated with increased incidence of gastrointestinal disorders (Rehrer et al., 1989). Of runners who lost more than 4% of their body weight, 80% experienced gastrointestinal disturbances which likely were caused by a combination of factors including fluid loss, intestinal ischemia, and elevated core body temperature (Rehrer et al., 1989). Further studies by Rehrer et al. (Rehrer et al., 1989, 1990b) in the laboratory demonstrated that ingestion of fluids during rest, running or biking did not cause any gastrointestinal symptoms. The discrepancy between symptom reports during races and the laboratory findings may be explained by the fact that those reporting symptoms after drinking are likely already dehydrated as it has been demonstrated that voluntary consumption of fluids during exercise is not enough to prevent dehydration (Kristal-Boneh et al., 1988; Strydom et al., 1966). Another factor may be experience level in the use of fluids during practice runs. If one
has trained the intake of fluids during practices then symptoms during competition are less likely to occur (Lambert et al., 2008b). Perhaps the exercise intensity during races is also higher than in training or the psychological stress of a race may affect the development of gastrointestinal symptoms.

1.2.4.2 Carbohydrate and fats

Since carbohydrate consumption and hydration has been shown to benefit performance, it has been postulated that the gut may adapt to allow the ingestion of fluids and carbohydrate during exercise (Oliveira et al., 2014). It has been shown that athletes not accustomed to ingesting foods and fluids during exercise have a twofold increase in the risk of developing gastrointestinal symptoms during exercise (Steege et al., 2008). Cox et al. (2010) demonstrated that the gut can be trained to adapt to increased carbohydrate consumption during exercise. In their study, 16 triathletes or cyclists were pair matched and randomly assigned to iso-energetic groups of either high carbohydrate or low carbohydrate content for 28 days. After 28 days, exogenous glucose oxidation rates were significantly higher in the high carbohydrate group, indicating adaptation to the carbohydrate ingested during training. However, this increase in glucose oxidation did not translate to increases in training-induced improvements in exercise performance (Cox et al., 2010). Lambert et al. (2008b) assessed adaptation to fluids with repeated sessions of fluid ingestion during running. A total of seven endurance-trained subjects participated in six 90 minute treadmill running trials while drinking a glucose-electrolyte solution. In the first trial subjects drank *ad libitum* and in subsequent trials subjects were given fluid according to the rate of fluid loss in sweat determined during the first trial. Gastrointestinal symptoms decreased during repeated trials even though gastric emptying rate did not change (Lambert et al., 2008b). Thus, studies suggest that the gastrointestinal system can adapt to fluid and food intake during exercise but more studies are warranted to investigate the extent and mechanisms underlying such adaptations.

In addition to fluid and carbohydrate intake, other diet components affect intestinal permeability (Kelly et al., 2015) although their role in causing gastrointestinal symptoms during exercise is not well established. Especially high-fat diets have been implicated with increased translocation
of LPS across the gut wall (Moreira et al., 2012). High fat diets increase plasma and fecal endotoxin and lead to reduced expression of the tight junction proteins claudin-1 and occludin (Kim et al., 2012). Foods high in fiber and vegetables have been shown to decrease the high fat or high carbohydrate induced increase in LPS translocation (Ghanim et al., 2009). However, fiber has also been linked to increased gastrointestinal symptoms in athletes (Rehrer et al., 1992).

1.2.4.3 Probiotics

Probiotics and prebiotics have also been shown to affect intestinal permeability. In a double-blinded placebo-controlled cross-over study, lactobacilli probiotics were administered for six weeks to children with moderate and severe atopic dermatitis (Rosenfeldt et al., 2004). The probiotic supplementation significantly decreased gastrointestinal symptoms in the children and resulted in stabilization of the intestinal barrier function (Rosenfeldt et al., 2004). In another study, lactobacilli probiotics administered to healthy adults significantly increased zonula occludens (ZO)-1 and transmembrane protein occludin in the vicinity of the tight-junctions (Karczewski et al., 2010). In the in vitro arm of the study, probiotics induced translocation of zonula occludens to the tight junctions in human epithelium. Additionally, in a randomized double-blind placebo-controlled study, 14 weeks of multi-species probiotic supplementation in athletes decreased fecal zonulin following intense exercise compared to controls when measured at baseline and after 14 weeks (Lamprecht et al., 2012). Zonulin is a protein known to reversibly regulate intestinal permeability by modulating intercellular tight junctions (Fasano, 2011). It can be used as a biomarker of impaired gut barrier function (Fasano, 2012). Some environmental stimuli such as intestinal exposure to bacteria or to gluten lead to zonulin release (Fasano, 2011). Zonulin-dependent loss of barrier integrity is also implicated in a number of autoimmune diseases including celiac disease (Fasano, 2009) and type I diabetes (Fasano, 2012; Sapone et al., 2006). Thus, the reduction in fecal zonulin after probiotic supplementation indicates improved gastrointestinal barrier integrity.

1.2.5 Non-Steroidal Anti-Inflammatory medications

In addition to nutritional causes, gastrointestinal disturbances may arise from the use of non-steroidal anti-inflammatory medications (NSAIDs). In a questionnaire based study, a large
number of athletes reported using NSAIDs before or during races either for treatment of injuries or for anticipated pain reduction (Gorski et al., 2011). However, they were mostly unaware of any adverse effects, which were thoroughly investigated by Gabriel et al. (1991) and included a three times greater relative risk for developing severe adverse gastrointestinal events such as mucosal bleeding or perforation. A study conducted on the Chicago marathon runners investigated the effects of ibuprofen and aspirin ingestion during prolonged exercise and found that ibuprofen was associated with increased intestinal permeability and gastrointestinal symptoms (Smetanka et al., 1999). Similar results were found by Van Wijck et al. (2012) who studied the effects of ibuprofen intake during cycling. Ibuprofen consumption resulted in increased plasma intestinal fatty acid binding protein (I-FABP) which was used to measure the degree of small intestinal damage. Consequently, small intestinal permeability also increased with ibuprofen ingestion and the extent of small intestinal injury and barrier disruption correlated significantly (Van Wijck et al., 2012). Thus, the use of NSAIDs in athletes is strongly discouraged.

1.3 Exercise related endotoxemia

1.3.1 Endotoxemia mechanism

Ischemic damage, mechanical trauma, and heat stress can all lead to damaging of the intestinal endothelial cells and breakdown of the gastrointestinal protective barrier (Gisolfi, 2000). This allows gram-negative bacteria and their endotoxins such as LPS to penetrate through the loosened tight junctions and enter the blood circulation and lymph nodes (Jeukendrup et al., 2000), overwhelming the ability of the liver to clear the endotoxin via the reticulo-endothelial system. The symptoms often experienced by runners including nausea, vomiting, dizziness, high body temperature, and even renal failure are very similar to those provoked by LPS (Jeukendrup et al., 2000). Endotoxins trigger the host’s immune response via incitation of the cytokine network (Jeukendrup et al., 2000). The central inflammatory mediator is proposed to be TNF-α which is produced by both macrophages and monocytes (Jeukendrup et al., 2000). The role of TNF-α is to stimulate production of other cytokines by monocytes or other cells such as endothelial cells. TNF-α also participates in triggering of the acute-phase response along with the other inflammatory cytokines interleukin-1 (IL-1) and interleukin-2 (IL-6) (Deventer et al.,
The acute phase response provoked involves profound changes in plasma protein concentrations that prepare the body for defense against harm such as an inflammatory agent or wound (Jeukendrup et al., 2000).

### 1.3.2 Endotoxemia and LPS following strenuous exercise

Endotoxemia has been reported in some studies after strenuous exercise although the findings are not consistent. In a study by Brock-Utne et al. (1988) the plasma increase in endotoxin levels was measured in randomly selected participants in an ultramarathon event. A total of 89 subjects were selected from runners entering the medical tent and 81% of the study participants had elevated LPS endotoxin levels (above 0.1 ng/ml) with 2% over the reported lethal limit in humans (1ng/ml) (Brock-Utne, 1988). Additionally, runners completing the race in less than eight hours had significantly lower mean plasma endotoxin levels than those taking over eight hours (Brock-Utne, 1988). This may be due to longer duration of mechanical, ischemic and heat damage to intestine in those runners taking more time to finish the race, ultimately causing a greater disruption in intestinal barrier regulation (Brock-Utne, 1988). Of the runners with high endotoxin levels (0,329 ± 0,026 ng/ml), 80.6% experienced gastrointestinal symptoms including vomiting, diarrhea and nausea while only 17.7% of those with low endotoxin levels (0,075 ± 0,005 ng/ml) experienced symptoms (Brock-Utne, 1988). These findings indicate that increased intestinal permeability and subsequent endotoxemia may be contributing to the high gastrointestinal symptom prevalence among runners. Interestingly, plasma endotoxin concentrations and anti-endotoxin IgG concentrations showed a negative correlation (Brock-Utne, 1988). High endotoxin concentrations in the study fell back to normal range after 1-3 weeks in 32 randomly selected runners with endotoxemia and anti-LPS levels were significantly higher than immediately after the run (Brock-Utne, 1988). Conversely, no increases in endotoxin concentrations were found in runners participating in a shorter half-marathon event but anti-endotoxin IgG levels were higher than in those completing the ultramarathon, possibly because of previous exposure and development of antibodies following a marathon three weeks before the measurements (Brock-Utne, 1988). The lack of plasma endotoxin concentration increases could also be caused by less exhaustion in the shorter event, or perhaps by cooler temperatures (Brock-Utne, 1988). Similar results were obtained in a study by Bosenberg et al. (1988) where
endotoxin and anti-endotoxin IgG concentrations were tested before and after an ultradistance triathlon. As in the study performed by Brock-Utne et al. (1988) plasma LPS levels showed significant increase following the race whereas plasma anti-LPS IgG levels decreased significantly (Bosenberg et al., 1988). Interestingly, both LPS and anti-LPS IgG concentrations were directly related to the subject’s training intensity (Bosenberg et al., 1988). It is possible that training-induced damage to the epithelium leads to leakage of endotoxin into circulation and allows the formation of anti-LPS antibodies, possibly attenuating symptoms (Bosenberg et al., 1988). Thus, LPS leakage from the intestine to blood circulation during a race would be attenuated by greater numbers of anti-LPS binding in trained subjects. In a recent study, endotoxin and C-reactive protein (CRP) concentrations increased in ultra-endurance runners compared to controls during a race (Gill et al., 2015). Gastrointestinal symptoms were reported in 75% of Ultra-endurance runners with no symptoms reported in the controls.

However, contrasting studies to those linking increased permeability and bacterial translocation to exercise induced gastrointestinal symptoms exist. Moore et al. (1995) investigated whether endotoxemia was linked to exercise-associated collapse in cyclists participating in a 100-mile race but found no significant difference in endotoxin concentrations between symptomatic athletes and controls. It may be that the bike ride was not strenuous enough to produce the same level of damage to intestinal epithelia as an ultradistance run or triathlon (Moore et al., 1995). In addition, a study by Camus et al. (1997) conducted on marathon participants found a moderate and transient increase in endotoxin levels and slight decrease in anti-endotoxin IgG levels but could not establish a correlation between either of these factors and the observed increase in inflammatory cytokines such as TNF-α and interleukin 6 (IL-6). However, the study authors agree that the measurement of LPS may not have been inclusive enough to show the true amount of bacterial translocation occurring during the marathon or later during the recovery period as measurements were made at 0 h, 1 h, and 24 h post race (Camus et al., 1997). Another factor contributing to this under-evaluation could be the binding of endotoxin to ligands, such as CD14 receptors, and LPS-binding protein (Camus et al., 1997). The authors also agree that further studies about the effect of increased permeability and bacterial translocation, and exercise induced symptoms are warranted (Camus et al., 1997).
1.3.3 Inflammatory cytokines

Another marker of endotoxin leakage is anti-LPS IgG for which a change may be easier to detect as the potential decrease in the amount of antibodies occurs over a longer period of time than the transient increase in LPS concentration which may be difficult to measure (Jeukendrup et al., 2000). The persistence of the antibody decrease occurs due to formation and clearance of LPS-anti-LPS complexes (Jeukendrup et al., 2000). Additionally, decreases in anti-LPS levels can also be seen in the systemic circulation even when LPS concentration increase only occurs in the intestine or portal circulation (Jeukendrup et al., 2000). This marker of endotoxin leakage was used by Jeukendrup et al. (2000) who measured anti-LPS IgG, LPS, and inflammatory cytokines. In total, 93% of study participants reported gastrointestinal symptoms and a mild increase in LPS concentration and a corresponding decrease in anti-LPS concentration was detected following the race (Jeukendrup et al., 2000). Additionally, 40% lower anti-LPS IgG concentrations were detected in trained subjects than in untrained controls, supporting the findings of previous studies and indicating leakage of endotoxin and subsequent antibody production during training (Jeukendrup et al., 2000). The increased level of anti-LPS antibodies can then bind plasma LPS after damage to intestinal barrier and possibly minimize development of gastrointestinal symptoms. Measures of inflammatory cytokines revealed no increase in TNF-α, contrary to the study by Camus et al. (1997). However, the finding is not surprising when taking into account that TNF-α was detected in only 5-54% of patients with sepsis in various studies (Hack et al., 1997). This may be because of the rapid clearance time of TNF-α and perhaps the sensitivity of assays in use at that time. Increased IL-6 levels were, however, reported in the study by Jeukendrup et al. (2000). This points to connection with increased endotoxin concentrations as LPS can stimulate the production of pro-inflammatory cytokines including IL-6, TNF-α, and CRP (de Punder and Pruimboom, 2015). However, increased IL-6 could also be a product of muscle damage or the exercise itself as Bruunsgaard et al. (1997) demonstrated that IL-6 concentrations increased significantly after 2 h of eccentric exercise and that the IL-6 concentration was significantly correlated with the creatinine kinase concentration as a parameter of muscle damage. The levels of positive acute-phase reaction protein CRP were also found to increase 20-fold 16 hours post-race (Bruunsgaard et al., 1997). This is in agreement with other studies including Dufaux et al. (1984) and Liesen et al. (1977) where CRP levels were measured.
the day after two hour or three hour run respectively, and showed a six-fold increase. Liesen et al. (1977) also demonstrated decreased acute-phase response and CRP in trained individuals. Fecal calprotectin can also be used for assessment of intestinal damage. Calprotectin can inhibit zinc-dependent enzymes which are needed to activate cytokines like TNF-α and as such participate in the destruction of microbes (Steinbakk et al., 1990). However large concentrations of the protein may induce cell and tissue damage (Aadland and Fagerhol, 2002). Calprotectin has been shown in many studies to act as a reliable marker of inflammation or damage to the gastrointestinal tract (Aadland and Fagerhol, 2002). In a study by van Wijk et al. (2011), significantly increased fecal calprotectin levels were found after subjecting healthy males to 60-minutes of moderate to high intensity exercise along with increases in small intestinal permeability and small intestinal injury. Figure 3 (p.25) provides a summary of the effects of exercise-induced endotoxemia on intestinal permeability changes.
Figure 3. Schematic diagram illustrating proposed mechanisms of increased intestinal permeability and gut-barrier impairment caused by exercise. Mechanisms with experimental evidence are capitalized. Exercise, especially in combination with heat, dehydration, or NSAIDs increases core body temperature. Splanchnic vasoconstriction occurs to make more blood available to exercising muscles but splanchnic ischemia, cellular hypoxia, and nitric

oxide (NO•) production cause biochemical effects that can lead to increased intestinal permeability. The increased permeability leads to secretion of interferon-γ (INF-γ), which further reduces gut-barrier function. Constitutive NO• may protect intestinal barrier function by inhibition of inflammatory cells or neutralization of reactive oxygen species. However, large amounts of NO• lead to reduced gut-barrier function and to endotoxin leak, production of inflammatory mediators, hypotension, and circulatory shock/heat stroke. Heat shock protein (HSP) production in response to high core body temperature can attenuate effects of NO• (Gisolfi, 2000).

### 1.4 Measuring intestinal permeability

Intestinal permeability is a measure of intestinal barrier function and relates to the paracellular space between neighboring enterocytes and their adhesive junctional complexes (Turner, 2009). Intestinal permeability can be measured by passive movement of different-sized molecules across the gastrointestinal epithelial barrier. Among the most commonly used molecules are mono- and disaccharide probes, chromium-labeled Ethylenediaminetetraacetic acid (EDTA), and different weight polyethylene glycols (PEGs). The use of oligosaccharides for measuring intestinal permeability was introduced by Menzies and marked the creation of the principle of differential urinary excretion of orally administered probes which is commonly used for the measurement of intestinal permeability (Menzies, 1974). Intestinal permeability is measured in vivo via these non-invasive test substances. Initially, single test substances such as lactulose, Cr-EDTA, technetium-labeled diethylenetriaminopentaacetare (Tc-DTPA), and PEG were used but results of the permeability studies were influenced not only by permeability changes but also any other pre- or post-mucosal changes (Bjarnason et al., 1995). This led to the use of differential urinary excretion of test substances, the most common of which is the use of a di- and monosaccharide (Bjarnason et al., 1995). An ideal permeability probe is one that is water soluble, shows first order kinetics, is nondegradable, nontoxic, and not metabolized in the intestine (Bjarnason et al., 1995). The probe should also not be present naturally in urine or in circulation if measured from plasma (Bjarnason et al., 1995).

#### 1.4.1 Factors affecting permeability measurements

Hyperosmolar stress is one factor that can affect the permeability of the small intestine and potentially lead to the development of gastrointestinal symptoms. For example, administration of
hypertonic lactulose will lead to increased permeability (Laker and Menzies, 1977). An increase in sucrosuria was shown early on after increasing concentration of sucrose administered to subjects and later increased permeation of the oligosaccharide probe lactulose was demonstrated when osmolarity of test substance is increased beyond 1500 mOsm/L (Laker and Menzies, 1977; Menzies, 1974). Osmotic fluid retention of poorly permeating solutes also leads to faster intestinal transit times which reduce sensitivity of measurements.

Another factor that must be considered is the pathway the probes take during permeation. Based on their physiochemical properties, it might be expected that the probes have similar modes of passage across the epithelial barrier but results showing consistent differences between the probes challenge that idea (Bjarnason et al., 1995). Indeed, three different pathways of action were proposed based on work by Maxton et al. (1986) where healthy volunteers were given a test solution of iso-osmotic, hyperosmotic, or cetrimide containing PEG 400, lactulose, L-rhamnose, and Cr-EDTA. Cetrimide was used as a detergent to disrupt the intestinal barrier. Urinary recovery of the probes was measured and findings showed low intestinal permeation of ingested lactulose and Cr-EDTA but 45-fold permeation of L-rhamnose and 100-fold permeation of PEG 400, compared to lactulose (Maxton et al., 1986). Passage of lactulose and Cr-EDTA was substantially increased by cetrimide and hyperosmolar stress, while permeation of L-rhamnose showed little change. PEG 400 was not affected by cetrimide, but the permeability was slightly increased by hyperosmolar stress (Maxton et al., 1986). Based on these findings and as shown in the three-pathway model in Figure 4 (p.28), Cr-EDTA and lactulose were described as using the paracellular mode of transport while rhamnose also permeates via the transcellular pathway through pores and PEG 400 via transcellular pathway and lipid membranes (Bjarnason et al., 1995).
The three-pathway model for intestinal permeability predicts that lactulose and Cr-EDTA diffuse through the paracellular route across tight junctions while rhamnose and PEG 400 also utilize the tracellular pathway via pores and lipid membranes respectively (Bjarnason et al., 1995).

The three-pathway proposal explains quite well the behavior of the probes but some points of contention remain. For one, the direct evidence for these pathways is still lacking because most localizing techniques require binding of the markers to cell components which permeability probes lack. Also, the permeation of monosaccharides is still controversial as, for example, mannitol is used to osmotically shrink membrane vesicles, a feat which would not be possible if its permeability was unrestricted (Kessler et al., 1978). An alternative to these proposed pathways is the single paracellular model shown in Figure 5 (p.29) whereby all of the probes utilize the paracellular pathway. The differences in permeation rates are then explained by differences in rates of passage based on the tightness of junctions and the accessibility of probes to the crypts (Bjarnason et al., 1995).
**1.4.2 Mono- and disaccharide probes**

Mono- and disaccharide probes have been widely used in the measurement of intestinal permeability by assessing their recovery from either urine or blood. The most common sugar probe is lactulose but other suitable probes include rhamnose, melibiose, raffinose, stachyose and dextrans (Bjarnason et al., 1995). These sugars cover many of the “ideal” probe characteristics but consideration must be paid to analytical procedures and their accuracy, precision, sensitivity, and resolution. The dose of oligosaccharide probe administered should also not exceed five grams as poorly permeating solutes retain fluid in the intestine and decrease the transit time, leading to less contact between the probes and intestinal wall (Bjarnason et al., 1995). Some of the probe sugars may also be found in common food items, which must be taken into consideration (Bjarnason et al., 1995).

Monosaccharides commonly used for the measurement of intestinal permeability are rhamnose and mannitol. The urinary excretion of rhamnose 24 hours after intravenous administration is incomplete but mannitol excretion was found to be complete (Laker et al., 1982). However, small amounts of mannitol is naturally present in urine but should be negligible with 1g mannitol administration (Laker et al., 1982). The lactulose-to-rhamnose ratio had been used in patients with Crohn’s disease (Howden, 1991), cystic fibrosis (Leclercq-Foucart, 1987), and celiac disease (Greco et al., 1991) and it has been demonstrated to correlate with excretion of
chromium-labeled EDTA (Maxton et al., 1986). The inclusion of rhamnose improves the discriminatory power of the probe since factors that affect transit and excretion affect both lactulose and rhamnose equally. Thus, the lactulose-to-rhamnose ratio is preferred instead of a single oligosaccharide probe for permeability measurements (Meddings, 1997). Lactulose-mannitol ratio is another commonly used probe that functions based on the same principle as lactulose-rhamnose. Cellobiose and mannitol have also been used together to minimize external factors (Cobden et al., 1985).

Sucrose is sometimes used as a method of determining gastric permeability. In theory, sucrose reaching the small intestine should be digested completely and thus any sucrose collected in urine would serve as a marker of damage to gastric mucosa (Bjarnason et al., 1995).

1.4.3 Cr-EDTA and Tc-DTPA

Cr-EDTA and Tc-DTPA function very similarly to oligosaccharide probes but have the added benefit of easier detection based on radioactivity. However, the radioactivity is also a major disadvantage of the probes. The radiation dose from Cr-EDTA is <0.12 milliSieverts and even less for an equivalent dose of Tc-DTPA (Bjarnason et al., 1995). Both probes are often used for measuring glomerular filtration rates. The probes are administered in water orally after an overnight fast and urine is collected for 24 hours similarly to oligosaccharide probes, as the distinction between patients with small intestinal abnormalities and controls is greatest at that time (Bjarnason et al., 1983). However, a 5 hour urinary recovery can be used together with a monosaccharide probe to increase specificity and simply interpretation of results (Bjarnason et al., 1989) although a possible disadvantage is that Cr-EDTA is not degraded and may lead to bacterial overgrowth that can influence the urinary excretion ratio and decrease intestinal transit time without actual increase in permeability (Bjarnason et al., 1995). However, the non-degradation allows Cr-EDTA to be used as a marker of both small intestinal and large intestinal permeability. Usually the choice between the two probes comes down to ease of handling as due to differences in half-life, Cr-EDTA can be made in batches but Tc-DTPA must be made individually and be analyzed rapidly after collection (Bjarnason et al., 1995).
1.4.4 Polyethylene glycols

Polyethylene glycols (PEGs) have a general formula of H(OCH$_2$CH$_2$)$_n$OH. Most commercially used permeability probes (PEG 400, 600, 900, 1000, 3000, and 4000) are sold as mixtures of different molecular weight polymers. Although polyethylene glycol 400 is used in permeability studies, its use in permeability measurements has encountered some criticism because of the great variability in demonstrated urinary recovery rates and low recovery after intravenous administration (Bjarnason, 1994). PEGs are also commonly used as food additives, solvents, toothpaste, and as a water-soluble base for ointments (Bjarnason et al., 1995). PEG 6000 at high concentrations facilitates cell membrane fusion and is toxic to cells (Aldwinckle et al., 1982). Although the safety margin of PEGs is wide, there is a considerable amount of variation in toxicity between different polymers (Bjarnason et al., 1995). The most common PEG used in permeability studies is PEG 400 of which 5-10g is usually administered orally and urine is collected for 5-6 hours thereafter. The polymers are then isolated from urine samples and quantified by gas or high pressure liquid chromatography (Bjarnason et al., 1995). However, the lack of consensus on expression of PEG data results makes interpretation of results difficult despite common use in permeability studies (Chadwick et al., 1977; Kerckhoffs et al., 2010; Parlesak et al., 1994; van Wijck et al., 2012).

1.4.5 Iohexol

Iohexol is a water-soluble contrast agent that partially permeates through the small intestinal wall and is excreted unchanged in urine. It has been used for small bowel examinations and intestinal perforation when administered orally (Aakhus, 1983). Studies in rats have shown that iohexol can be used to differentiate between strangulation and obstruction because increased urinary excretion of iohexol occurs from an ischemic small intestine (Stordahl, 1988). Excretion of iohexol was also increased in rats with selective irradiation of the small intestine (Solheim et al., 1991). These investigations indicated that iohexol was absorbed and excreted unchanged and that the urinary recovery of iohexol reflects damage to small intestine. Halme et al. (1993) showed that iohexol can be used in Crohn’s disease patients to determine severity of intestinal inflammation. In that study, permeation of iohexol in patients was significantly higher than in
controls (Halme et al., 1993). A previous study showed iohexol permeation in intact baby and neonatal mucosa to be very low (less than 1% of enterally administered dose) (Langer et al., 1987). Halme et al. (1993) also determined that while serum iohexol does not produce a reliable result, oral iohexol urinary excretion can be used effectively and that body weight shows no correlation to excretion of iohexol. The same dose can thus be used for each test subject. The results also suggested that iohexol has a better correlation with C-reactive protein (CRP), clinical activity index, and endoscopic findings in Crohn’s patients than previous test probes and that mucosal damage or inflammation is strongly indicated with urinary excretion of over 0.5% iohexol (Halme et al., 1993). In a second study, Halme et al. (1997) investigated the use of iohexol as a marker of disease activity in inflammatory bowel disease patients. The results again showed significant increase in urinary excretion of iohexol in active disease patients compared to controls. Halme et al. (2000) also compared the use of iohexol to the use of lactulose-mannitol ratio which is a more commonly used permeability marker in inflammatory bowel disease. The correlation between urinary excretion of iohexol and lactulose-mannitol ratio in the study was positive and urinary excretion of iohexol correlated positively with endoscopic disease activity and modified Harvey-Bradshaw index which measures disease activity index in Crohn’s disease patients. In contrast, the lactulose-mannitol ratio only correlated positively with endoscopic disease activity but not the clinical index (Halme et al., 2000). Thus the authors conclude that, "the the iohexol test is a superior activity marker compared to the lactulose-mannitol ratio" (Halme et al., 2000).
2. Experimental Study

2.1 Study aims

The goal of the study was to measure running-induced changes in intestinal permeability and permeability marker LPS and investigate their association with gastrointestinal symptoms. A secondary aim was to inspect possible correlations between intake of certain nutrients and experiencing gastrointestinal symptoms.

2.2 Materials and methods

2.2.1 Selection of study participants

Study participants were recruited by a recruitment notice (Suppl 1) posted in online running groups. Voluntary participants received an informational letter about the study (Suppl 2), a consent form (Suppl 3), and a symptom history questionnaire (Suppl 4) used to determine the participant’s suitability for the study. Consenting volunteers suitable to the study were selected as participants. Persons with a diagnosed gastrointestinal illness or asthma, heart, or cardiovascular diseases were excluded from participation. Pregnant or breastfeeding women were also excluded as well as persons with iodine allergy because of the iohexol used in determining permeability.

The participants were 24- to 44-year-old active male and female runners. The average age of the participants was 33 years. A total of 24 participants were recruited based on the sample size of 20 used by Nieuwenhoven et al. (2003) to detect a significant difference in intestinal permeability between symptomatic and asymptomatic athletes. The final sample size of our study was 17 participants after 7 participants dropped out due to injury or illness. Of the 17 participants completing the study, 9 were male and 8 were female.

The study was conducted in adherence to the ethical regulations outlined in the Declaration of Helsinki. Ethical approval was obtained from the HUS Coordinating ethical committee.
2.2.2 Study design

The study protocol is outlined in Table 1 (p.35). Study participants were allocated into two groups, symptomatic (gastrointestinal symptoms >50% of runs, n=8) and asymptomatic (gastrointestinal symptoms <10% of runs, n=9), based on their completion of a symptom history questionnaire. The symptom history questionnaire probed the severity of the symptoms as well as possible gastrointestinal symptom associations with certain food items. Baseline samples at rest were taken from participants approximately three weeks before the running test. At this visit, a blood sample was taken and intestinal permeability measurement (Measurement of intestinal permeability, pp. 37) was started. The subjects returned the urine sample as well as a fecal sample the next day. Subjects were also instructed to keep a food diary for three days before the baseline measurements. The food diary was repeated for three days before the running test day. During the test day, intestinal permeability measurement was started before the running test where subjects ran for 90 minutes at 80% of their best 10 km race speed. After the run, a blood sample was taken. The subjects also received a symptom questionnaire (Suppl 5) where they were asked to score on a visual analog scale (VAS) how much stomach pain they experienced during the run. Gastrointestinal symptom occurrence during the run was also reported as well as gastrointestinal symptoms experienced later that day. The next day, participants returned the urine sample, fecal sample and completed symptom questionnaire. The study duration for subjects was approximately a month with four test-site visits.
Table 1. Timeline and study protocol

<table>
<thead>
<tr>
<th>DAY</th>
<th>Procedure, collected samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>-23</td>
<td><em>Food diary 1:day 1</em></td>
</tr>
<tr>
<td>-22</td>
<td><em>Food diary 1:day 2</em></td>
</tr>
<tr>
<td>-21</td>
<td>1. Meeting</td>
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<tr>
<td></td>
<td><em>Food diary 1:day 3</em></td>
</tr>
<tr>
<td></td>
<td>Intestinal permeability measurement (iohexol, 24h urine collection)</td>
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<tr>
<td></td>
<td>Blood sample</td>
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<tr>
<td>-20</td>
<td>2. Meeting</td>
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<tr>
<td></td>
<td>24h urine sample collection</td>
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<tr>
<td></td>
<td>Fecal sample collection</td>
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<tr>
<td>-2</td>
<td><em>Food diary 2:day 1</em></td>
</tr>
<tr>
<td>-1</td>
<td><em>Food diary 2:day 2</em></td>
</tr>
<tr>
<td>Test day</td>
<td>3. Meeting</td>
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<tr>
<td></td>
<td><em>Food diary 2:day 3</em></td>
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<tr>
<td></td>
<td>90 min run at approximately 80% of 10k PR speed</td>
</tr>
<tr>
<td></td>
<td>Intestinal permeability measurement (iohexol, 24h urine collection)</td>
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<tr>
<td></td>
<td>Blood sample</td>
</tr>
<tr>
<td>1</td>
<td>4. Meeting</td>
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<tr>
<td></td>
<td>24h urine sample collection</td>
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<td></td>
<td>Fecal sample collection</td>
</tr>
<tr>
<td></td>
<td>Symptom questionnaire collection</td>
</tr>
</tbody>
</table>
2.2.3 Serum sample

A blood sample was taken from the subjects approximately three weeks before the running test day at rest and then immediately after the running test. The blood sample volume was at most 5 ml. The blood samples were collected in serum separation tubes (VenoSafe™ Clot Act. (Z), Terumo Europe, Leuven, Belgium) by a licensed physician. Serum was separated by centrifugation (1500 g, 10 min), collected and stored in -80°C for later analysis.

2.2.4 Fecal sample

The study subjects were asked to provide a fecal sample at rest approximately three weeks before the running test and then after the running test. The subjects were given written instructions and they collected the first fecal sample after iohexol ingestion independently at home in a provided specimen container. The samples were returned the next day and stored in -20°C for later analysis. Fecal samples were collected for Calprotectin measurement but were not analyzed in this thesis.

2.2.5 Food diary

The subjects were asked to keep a food diary for three days before the baseline measurements and for three days before the running tests. All food and drink was recorded in the food diary. The participants were provided with detailed instructions for completion of the food diary which was returned by the participants either electronically or in person during sample collection. Food diaries were analyzed using Fineli Ruokakori (Foodbasket) database (http://www.fineli.fi/foodbasket.php) for average intake of macronutrients and selected micronutrients over each three-day food diary period. Daily intake of nutrients and group averages were calculated in Excel from the obtained data.
2.2.6 Running test day

During the running test day, the subjects ran for 90 minutes at a challenging pace which was suggested as approximately 80% of the speed of their best 10 km race time. The effort should have been challenging, but able to be maintained for the full 90 minutes. The running pace was determined individually by the athlete according to their perceived exertion. After the running test, subjects received a symptom questionnaire to fill out possible gastrointestinal symptoms. The rest of the form concerning the appearance of gastrointestinal symptoms later during the day was completed at home and returned the next day.

2.2.7 Measurement of intestinal permeability

The intestinal permeability was determined through oral administration of a contrast agent, iohexol (Omnipaque 350™, 755 mg iohexol/ml, GE Healthcare, Oslo, Norway). Subjects drank a glass of water to which 50 ml iohexol had been added. Urine was collected for 24 hours after administration of the iohexol drink. The collection was performed at the baseline measurement and during the running test day. To improve the reliability of the measurement, subjects were instructed not to eat two hours before and three hours after the testing. Drinking of liquids was not restricted. The subjects were given instructions and a collection container for the urine collection which was then returned the next day. The amount of urine was recorded, the urine was stirred and two 5 ml samples were collected. These samples were stored in -20°C for later analysis. Because non-steroidal anti-inflammatory medications and alcohol are known to affect intestinal permeability, subjects were asked to abstain from use the day before and during the measurements. Iohexol was administered in the presence of a doctor because it contains iodine, which can in some instances trigger an allergic reaction. The test location was also equipped for first-aid administration. Possible contrast medium-induced gastrointestinal symptoms were viewed as a minor disadvantage as the use of iohexol in permeability studies is widely accepted and used in literature.
2.2.8 Measurement of iohexol from urine

The iohexol concentrations in the urine were measured using the Iohexol enzyme-linked immunosorbent assay (ELISA) kit (BioPAL, Worcester, MA, USA) according to the kit instructions. First, the urine samples were prepared by diluting them 1:1000 in dilution buffer (0.1% bovine serum albumin in PBS) so that they fell within the active region of the standard curve. Then 50μl iohexol standards and diluted urine samples were pipetted onto the 96-well plate. This was followed by pipetting of 50μl rabbit anti-iohexol into all wells except blanks and incubating on an orbital shaker for 1 hour. After completion of the 1-hour incubation period, the solutions were aspirated from all wells and the plate was washed with 350 μl Wash Buffer (0.05% Tween 20 in PBS) per well for a total of three times. After washing, 100μl Goat anti-rabbit IgG-HRP was pipetted into all wells except blanks and the plate was incubated on an orbital shaker for 30 minutes. Again, the solutions were aspirated from all wells and the plate was washed with 350 μl Wash Buffer per well for a total of three times. Then 100μl HRP Substrate Reagent was pipetted into all wells including blanks and the plate was tapped gently to mix contents of each well. The substrate was incubated once more for 30 minutes at room temperature with no shaking. After incubation, 100μl of HRP Stop Reagent was pipetted into all wells including blanks. The plate was read at 450nm (Wallac Victor² 1420 Pertin Elmer Multilabel counter, Waltham MA, USA) and the concentration of iohexol in the samples was determined from the standard curve. Intestinal permeability to iohexol was then assessed by calculating the percentage of excreted iohexol using the following equation:

\[
\text{Iohexol (\%)} = \frac{\text{amount of excreted iohexol in urine after 24h (mg)}}{\text{amount of administered iohexol (mg)}} \times 100
\]
2.2.9 Measurement of zonulin from serum

Zonulin was measured using the Zonulin ELISA test kit (Immundiagnostik, Bensheim, Germany) according to the kit instructions. Serum samples were prepared by adding 475 μl dilution buffer to 25 μl sample. Then 150 μl of the sample solution was added to 150 μl tracer before vortexing. Controls and standards were prepared similarly by adding 150 μl of either standard or control to 150 μl of the tracer and mixing well. To begin the ELISA procedure, 100 μl of the prepared standards, controls or samples were pipetted into each well. Then the strips were covered and the plate was incubated for 1 hour while shaking on a horizontal shaker at 550 rpm at room temperature. After incubation, the microtiter plate was washed five times with 250 μl wash buffer. Then 100 μl conjugate was added into each well. Again, the plate was incubated for 1 hour while shaking on a horizontal shaker at 550 rpm at room temperature and washed five times with 250 μl wash buffer after the incubation. Then 100 μl of substrate solution was added into each well. The plate was incubated once more for 15 minutes at room temperature. After incubation 100 μl of stop solution was added into each well. The color changed from blue to yellow with the intensity of the yellow color being inversely proportional to zonulin concentration in the sample. The absorption was determined promptly with a photometer at 450 nm (Wallac Victor2 1420 Pertin Elmer Multilabel counter, Waltham MA, USA) and the concentration of zonulin was determined from the standard curve.

2.2.10 Inflammatory markers: LPS

LPS was analyzed using a LAL (Limulus amebocyte lysate) Chromogenic Endpoint Assay (Hycult biotech, Uden, Netherlands) according to the kit instructions. Analysis was performed at the University of Helsinki Institute of Dentistry. To start the ELISA protocol, 50 μl in duplicate from both the diluted sample (1:5) and controls were transferred to the assigned wells. Then 50 μl reconstituted LAL reagent was added to each well except for sample controls which received 50 μl endotoxin free water instead of LAL reagent. The plate was then covered and incubated for 30 minutes at room temperature. Then the samples were measured at 405 nm and endotoxin concentrations were determined from the standard curve.
2.2.11 Data Analysis

Differences between symptomatic and asymptomatic groups were analyzed using independent samples t-test. Differences within groups from baseline to running test measurements were analyzed using paired samples t-test. Statistical significance (p < 0.05) for collected data was determined using PASW Statistics software version 18.0.2. (IBM, Armonk, NY, USA).

3. Results

3.1 Symptom questionnaires

3.1.1 Background questionnaire

Symptom frequency was rated on a scale of one to five ranging from never experiencing symptoms to always experiencing symptoms. The average symptom frequency for the asymptomatic participants was 1.9. The average symptom frequency for symptomatic participants was 3.9 (Table 2). The reported frequency of gas and diarrhea was also significantly higher in the symptomatic than the asymptomatic group (Table 3 and Table 4). Likewise, the frequency of symptoms from fruit, vegetables, bread, and spicy foods was significantly higher in the symptomatic group (Table 6). Stool consistency was reported as loose in eight of the seventeen runners in the background questionnaire. Two reports were from the asymptomatic group and six from the symptomatic group. The remaining seven runners in the asymptomatic group all reported normal stool consistency.

3.1.2 After run questionnaire

After the run, the symptomatic group reported significantly more (2.00 cm) stomach pain than the asymptomatic group (0.30 cm) as measured by a visual analogue scale (p < 0.05). Stool consistency after the run was loose in ten of the seventeen runners, with five reports from each group (Table 5). The asymptomatic group also reported three counts of normal stool and one count of hardened stool. The symptomatic group reported two counts of hardened stools and one count of diarrhea.
Average running paces during the running test were comparable in both groups. The average pace for the control group was 5min 12sec per kilometer. The average pace for the symptomatic group was 5min 02sec per kilometer.

Table 2. Gastrointestinal symptom frequency in control (asymptomatic) vs. symptomatic runners rated on a scale from 1 (never) to 5 (always).

<table>
<thead>
<tr>
<th>Rating</th>
<th>Control (n=9)</th>
<th>Symptom (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3. Running-induced diarrhea frequency in control (asymptomatic) vs. symptomatic runners rated on a scale from 1 (never) to 5 (always).

<table>
<thead>
<tr>
<th>Rating</th>
<th>Control (n=9)</th>
<th>Symptom (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 4. Gas frequency in control (asymptomatic) vs. symptomatic runners rated on a scale from 1 (never) to 5 (always).

<table>
<thead>
<tr>
<th>Rating</th>
<th>Control (n=9)</th>
<th>Symptom (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5. Stool consistency in control (asymptomatic) and symptomatic runners after running.

<table>
<thead>
<tr>
<th>Rating</th>
<th>Control (n=9)</th>
<th>Symptom (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>loose</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>diarrhea</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>hard</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>normal/firm</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>normal but urgent</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 6. Gastrointestinal symptoms from food questionnaire. Gastrointestinal symptoms occurring during running following ingestion of various foods rated on a scale from 1 (never) to 5 (always).

<table>
<thead>
<tr>
<th>Rating</th>
<th>Fruit</th>
<th>Vegetable</th>
<th>Bread</th>
<th>Spicy food</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Symptom</td>
<td>Control</td>
<td>Symptom</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>2</td>
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<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
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<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

3.2 Intestinal permeability

Intestinal permeability increased significantly in all runners from baseline to after run measurement (p<0.001) as seen in Figure 6 (p.43). Average asymptomatic group baseline iohexol concentration was 0.21% (SD=0.17%) vs. 0.38% (SD=0.23%) after running. Average symptom group baseline iohexol concentration was 0.20% (SD=0.07%) vs. 0.36% (SD=0.12%) after running. There was no significant difference in the increase in permeability between the symptomatic and asymptomatic group.
Figure 6. A. Iohexol permeability in all subjects at baseline vs. after running test with average and standard deviation (***p < 0.001). B. Iohexol permeability in control (asymptomatic) and symptomatic groups separately at baseline (red color) and after running (green color) with average and standard deviation (*p < 0.05).

3.3 Zonulin

Serum zonulin concentrations were significantly higher in the asymptomatic group (67.5 ng/ml, SD=18.2) than in the symptomatic group (51.3 ng/ml, SD=14.6) following the run (p < 0.05). Serum zonulin concentration in the asymptomatic group was also significantly higher after the run (67.5 ng/ml, SD=18.2) compared to baseline (53.6 ng/ml, SD=24.9) (p < 0.05) as seen in Figure 7 (p.44). No significant differences were found between the groups at baseline or in the total zonulin concentration change from baseline to after the run in combined groups.
Figure 7. A. Serum zonulin concentrations in all subjects at baseline and after running with average and standard deviation. B. Serum zonulin concentrations in control (asymptomatic) and symptomatic groups separately at baseline (red color) and after running (green color) with average and standard deviation (*p < 0.05).

3.4 LPS

Serum LPS concentrations were significantly higher at baseline in the symptomatic group (0.767 EU/ml, SD=0.119) than in the asymptomatic group (0.567 EU/ml, SD=0.124) (p < 0.005) as seen in Figure 8 (p. 45). There was no significant difference in serum LPS concentrations between the groups after the run.
**Figure 8.** A. Serum LPS concentrations in all subjects at baseline and after running with average and standard deviation. B. Serum LPS concentrations in control (asymptomatic) and symptomatic groups separately at baseline (red color) and after running (green color) with average and standard deviation (**p < 0.01).

### 3.5 Food diaries

There were no statistically significant differences between asymptomatic and symptomatic groups in the average intake of various nutrients at baseline or at the running test (Table 7).
Table 7. Average nutrient intake and standard deviation in control and symptomatic groups at baseline and running test measurements (Mean ± SD).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Measurement</th>
<th>Control baseline</th>
<th>Symptom baseline</th>
<th>Control run</th>
<th>Symptom run</th>
</tr>
</thead>
<tbody>
<tr>
<td>energy</td>
<td>kcal</td>
<td>2787 ± 542</td>
<td>2580 ± 871</td>
<td>2918 ± 972</td>
<td>2581 ± 1106</td>
</tr>
<tr>
<td>carbohydrate (absorbable)</td>
<td>g</td>
<td>304 ± 122</td>
<td>293 ± 102</td>
<td>342 ± 184</td>
<td>259 ± 112</td>
</tr>
<tr>
<td>fat</td>
<td>g</td>
<td>100 ± 37</td>
<td>92 ± 53</td>
<td>100 ± 35</td>
<td>112 ± 71</td>
</tr>
<tr>
<td>protein</td>
<td>g</td>
<td>129 ± 33</td>
<td>119 ± 41</td>
<td>123 ± 36</td>
<td>113 ± 36</td>
</tr>
<tr>
<td>alcohol</td>
<td>g</td>
<td>4 ± 5</td>
<td>0 ± 0</td>
<td>2 ± 2</td>
<td>1 ± 2</td>
</tr>
<tr>
<td>fiber</td>
<td>g</td>
<td>51 ± 44</td>
<td>40 ± 25</td>
<td>57 ± 53</td>
<td>30 ± 13</td>
</tr>
<tr>
<td>fiber, insoluble</td>
<td>g</td>
<td>30 ± 26</td>
<td>24 ± 14</td>
<td>34 ± 30</td>
<td>19 ± 9</td>
</tr>
<tr>
<td>calcium</td>
<td>mg</td>
<td>1328 ± 612</td>
<td>1282 ± 366</td>
<td>1398 ± 555</td>
<td>1225 ± 498</td>
</tr>
<tr>
<td>iron</td>
<td>mg</td>
<td>24 ± 26</td>
<td>20 ± 10</td>
<td>26 ± 14</td>
<td>18 ± 8</td>
</tr>
<tr>
<td>iodine</td>
<td>µg</td>
<td>218 ± 83</td>
<td>193 ± 79</td>
<td>209 ± 85</td>
<td>218 ± 89</td>
</tr>
<tr>
<td>potassium</td>
<td>mg</td>
<td>6830 ± 4434</td>
<td>5432 ± 1897</td>
<td>7041 ± 5499</td>
<td>4536 ± 1472</td>
</tr>
<tr>
<td>magnesium</td>
<td>mg</td>
<td>709 ± 431</td>
<td>595 ± 265</td>
<td>767 ± 516</td>
<td>502 ± 161</td>
</tr>
<tr>
<td>sodium</td>
<td>mg</td>
<td>3138 ± 1003</td>
<td>2318 ± 1140</td>
<td>2993 ± 1029</td>
<td>2817 ± 1061</td>
</tr>
<tr>
<td>salt</td>
<td>mg</td>
<td>7908 ± 2554</td>
<td>5895 ± 2906</td>
<td>7458 ± 2405</td>
<td>7165 ± 2688</td>
</tr>
<tr>
<td>phosphorus</td>
<td>mg</td>
<td>2275 ± 768</td>
<td>2143 ± 558</td>
<td>2328 ± 852</td>
<td>1986 ± 636</td>
</tr>
<tr>
<td>selenium</td>
<td>µg</td>
<td>117 ± 70</td>
<td>180 ± 266</td>
<td>110 ± 28</td>
<td>89 ± 39</td>
</tr>
<tr>
<td>zinc</td>
<td>mg</td>
<td>15 ± 4</td>
<td>14 ± 4</td>
<td>16 ± 4</td>
<td>14 ± 4</td>
</tr>
</tbody>
</table>
4. Discussion and future prospects

The aim of our study was to measure running-induced changes in intestinal permeability and permeability marker LPS and investigate their association with gastrointestinal symptoms. A secondary objective was to inspect possible correlations between gastrointestinal symptom occurrence and intake of certain nutrients. Participant allocation to the symptomatic and asymptomatic groups was based on the differences in reported symptom frequency and provided a good starting point for the detection of differences among the two groups. Both groups experienced a significant increase in intestinal permeability from baseline to after run measurement. However, there was no significant difference between the intestinal permeability in the two groups after the run. This result was in contrast to the findings of Nieuwenhoven et. al (2003) who reported higher intestinal permeability in symptomatic than asymptomatic athletes. To our best knowledge, this is the only other study comparing intestinal permeability in symptomatic and asymptomatic athletes. The study by Nieuwenhoven et al (2003) measured intestinal permeability via the use of sugar probes whereas our study measured intestinal permeability using iohexol, which was deemed a more reliable measure of intestinal permeability by Halme et al. (2000). The discrepancy in results indicates that more studies are needed to understand the relationship between gastrointestinal symptoms and intestinal permeability. The sample size in our study was relatively small, but comparable to other permeability studies. The significant increase in intestinal permeability in the symptomatic and asymptomatic groups in our study may explain the high incidence of stool consistency changes that were observed in both groups. The increase in intestinal permeability in both the symptomatic and asymptomatic groups suggests that the damage to the epithelia and subsequent increase in permeability is a normal response to running. However, perhaps the differences in symptom occurrence are a result of individual differences in ability to repair that damage after exercise. Research into intestinal epithelium recovery rate after exercise could help determine the variability in individual repair ability and whether this repair ability is tied to the development of gastrointestinal symptoms. Whether the difference in intestinal recovery is caused by genetic factors or by some unrelated pathology is also unclear and a source of further study.
In the symptom history questionnaires, most symptomatic athletes reported experiencing loose stools after running while only two asymptomatic runners reported loose stools. However, after the running test loose stools were reported equally by both groups with over half of runners reporting the symptom. Despite both groups experiencing changes in stool consistency, the change was accompanied by significantly more stomach pain in the symptomatic group which may indicate that the severity of the symptoms was not as high in the asymptomatic group. The asymptomatic group also included four runners with no stool changes at all while all runners in the symptomatic group reported stool changes. Clearly, the symptomatic group experienced more pain and changes in stool consistency but the cause is still unknown due to the lack of difference in intestinal permeability change between the two groups. Serum zonulin concentration increased significantly in the asymptomatic group after running compared to both the baseline measurements within the group as well as the measurements after running in the symptomatic group. Further studies are warranted to confirm and interpret the cause of the observed zonulin increase as zonulin has been previously shown to contribute to the regulation of intestinal barrier function and is up-regulated in various autoimmune conditions such as Crohn's Disease and Type I Diabetes (Fasano, 2011; Sapone et al., 2006). Additionally, exposure to bacteria in the intestine is a powerful trigger for zonulin release (Fasano, 2011).

The largest difference in reported symptom frequency between the groups occurred with gas and diarrhea. Most symptoms were reported from fruit, vegetables, bread, and spicy foods which indicates that symptomatic runners may benefit from avoidance of these foods before running to decrease symptom occurrence. However, as response to various foods varies greatly between people, no universal recommendation can be made and athletes should instead be encouraged to experiment on their own body's reaction to these foods. Complete avoidance of these foods would be unadvised as many of them exert positive effects on health and should be included in the diet regularly. Therefore it is important to focus on avoiding these food types only before runs and eating them at other times throughout the day. While research is still in its infancy, it has been suggested that nitric oxide, which improves intestinal perfusion, could be used to reduce gastrointestinal symptoms in athletes (Oliveira et al., 2014). However, a problem with this strategy is that current vegetable sources of dietary nitrate are sometimes associated with gastrointestinal symptoms (Oliveira et al., 2014). There is also indication that ingesting multiple carbohydrates reduces gastrointestinal symptoms compared to a single carbohydrate source.
Analysis of the food diaries indicated that there were no significant differences in intake of various nutrients between symptomatic and asymptomatic runners. Some of the factors examined included macronutrients, dietary fiber, and minerals. The similarity in the two groups' nutrient intake indicates that there is some sort of individual predisposition to develop symptoms and that symptoms are not caused by diet alone. Rather, it seems that certain foods aggravate symptoms and are poorly tolerated in those individuals with a predisposition for gastrointestinal problems.

Another factor besides individual predisposition that may contribute to the increased rate of symptoms in the symptomatic group is the elevation of serum LPS concentrations at rest. While there was no difference in LPS concentrations between the groups after running, the plasma LPS concentrations at rest were significantly higher in the symptomatic group. The observed increase in serum LPS concentrations following strenuous exercise is consistent with previous studies (Bosenberg et al., 1988; Brock-Utne, 1988; Jeukendrup et al., 2000). Serum LPS concentrations rise as exercise-induced damage to the intestinal epithelia prevents leakage of LPS into the bloodstream. The bacterial LPS toxins then trigger a host inflammatory response and have been hypothesized to cause many of the symptoms experienced by endurance athletes such as dizziness, nausea, stomach ache, intestinal cramps and diarrhea (Jeukendrup et al., 2000). Thus, it is plausible that the increase in symptoms reported by the symptomatic group was at least partially caused by chronically higher concentrations of serum LPS and subsequent inflammation. This hypothesis is supported by Brock-Utne's (1988) work, where 80.6% of runners with high LPS levels (0.329 ± 0.026 ng/ml) experienced gastrointestinal symptoms while only 17.7% of those with low LPS levels (0.075 ± 0.005 ng/ml) experienced symptoms. The lack of difference between the LPS concentrations in the two groups after the run may be explained by the fact that the symptomatic group’s LPS concentrations were already high at rest. Thus, they did not increase further above the high baseline level whereas the low baseline LPS concentrations in the asymptomatic group increased to the same higher level in response to the trauma caused by strenuous long-duration running. It is still unclear whether the high baseline LPS concentrations were caused by the mechanical damage created by training and an inability of the intestinal mucosa to repair that damage or if those runners with elevated LPS concentrations have some other underlying pathology which leads to gastrointestinal problems during running. If some other pathology leads to gastrointestinal symptoms during running, then
identifying and addressing that problem may be of greatest benefit to athletes struggling with gastrointestinal symptoms.

No difference was found in the average running pace between the two groups during the running test while both groups experienced an increase in intestinal permeability, indicating that the running effort was quite uniform between the groups and was not likely to influence the permeability results. Overall, our study suggests that running produces similar changes in the intestinal mucosa in all runners but that those runners experiencing symptoms have an individual predisposition for symptom occurrence which may be caused by impaired epithelial membrane recovery after exercise-induced damage or by some other underlying pathology. A genetic factor in gastrointestinal symptom occurrence has also been suggested previously due to a strong correlation of gastrointestinal complaints and gastrointestinal symptom history (Oliveira et al., 2014). Developing strategies to minimize symptom occurrence is important due to the high symptom incidence among runners and the negative effects of gastrointestinal symptoms on sport performance (Jeukendrup et al., 2000; Stuempfle and Hoffman, 2015). Based on the symptom history questionnaires, it may be advisable for some runners to avoid certain foods which aggravate their symptoms. These foods must be identified on an individual basis but it appears that fruit, vegetables, bread, and spicy foods are commonly identified as a cause of gastrointestinal distress. At this time avoiding symptom-aggravating foods remains the most effective treatment for prevention of running-induced gastrointestinal distress. Adding probiotics to the diet may also improve symptoms by strengthening the integrity of intestinal mucosa (Kainulainen et al., 2015; Karczewski et al., 2010; Lamprecht et al., 2012).

5. Conclusion

No significant difference was found in intestinal permeability between symptomatic and asymptomatic runners either at rest or following strenuous exercise. However, both groups experienced a significant increase in intestinal permeability from baseline to after running. Higher symptom occurrence in the symptomatic group may be caused by higher resting serum LPS concentrations. LPS in the bloodstream triggers a host inflammatory response which has been proposed to lead to gastrointestinal symptom occurrence in athletes (Jeukendrup et al., 2000). Comparison of average intake of various nutrients between the two groups showed no
significant differences, indicating an individual predisposition as the cause of symptoms rather than diet alone. The lack of difference in intestinal permeability between the groups combined with the difference in symptom occurrence indicates that intestinal permeability changes alone do not account for symptom development. A possible factor may be individual differences in intestinal mucosa repair ability or some underlying pathology.
6. Acknowledgements

This project was carried out under the supervision of the Medical Nutrition Physiology group leader Professor Riitta Korpela and Doctoral candidate Richard Forsgård M.Sc. in the Department of Pharmacology in Faculty of Medicine at the University of Helsinki. First, I would like to express my sincere gratitude to Riitta Korpela for all the support I received and for being so open to my ideas for the direction of this project. Her advice and mentorship were invaluable during this research process.

Secondly, I would like to extend my gratitude to Richard Forsgård for his continuous support throughout the project. He very graciously offered his time to help guide me both in organizing the study and in the laboratory work and was always patient in answering any questions I had. I truly do not know how to thank him enough for his support.

I would like to thank Dr Lauri Alanko MD of Liikuntalääketieteen yksikkö (Sports Medicine department) in the University of Helsinki for volunteering his time to take blood samples and offering up his practice for our test location. From him I also received great advice and inspiration both personally and professionally. I would also like to thank the rest of the staff at Helsingin Urheilulääkäriasema and Dr Katri Peuhkuri for taking the time to teach me how to analyze food diaries.

Finally, I would like to thank my parents and my brother for their unwavering support of all of my endeavors and my friend Tommi Hytönen for his encouragement throughout the process.
7. References


8. Supplements

8.1 Supplement 1

OSALLISTU TUTKIMUKSEEN, JOSSA SELVITETÄÄN JUOKSIJOIDEN VATSAVAIVOJEN SYITÄ

Haemme vapaaehtoisia juoksijoita keväällä 2015 Helsingin Yliopiston Lääketieteellisen tiedekunnan Medicum-yksikössä toteuttettavaan tutkimukseen, jossa selvitetään juoksijoiden juoksun aikaisten vatsavaivojen syitä.


Mikäli olette kiinnostunut osallistumaan tutkimukseen, ota yhteyttä:

Projektitutkija Elisa Karhu
050-3180279
elisa.karhu@helsinki.fi
8.2 Supplement 2

TIEDOTE TUTKITTAVILLE

"Liikuntasuorituksen aiheuttamat muutokset suoliston läpäisevyydessä vatsaoireilevalla ja ei-oireilevalla juoksijoilla"

TUTKIMUKSEN KUVAUS

Tutkimuksen tarkoituksena on selvittää juoksijoiden yleisesti kokemien vatsavaivojen syitä. Tutkimuksen päätavoitteena on mitata juoksusuorituksen aiheuttamia muutoksia suoliston seinämän läpäisevyydessä ja määrittää niiden mahdollinen vaikutus vatsaireiden ilmaantumiseen juoksusuorituksen aikana. Läpäisevyyttä mitataan juotavan varjoaineen avulla, joka imeytyy suolistosta verenkiertoon ja poistuu elimistöstä virtsan mukana. Lisääntynyt varjoaineen pitoisuus virtsassa merkitsee suoliston läpäisevyyden lisääntymistä. Tutkimuksessa käytettävää varjoa on yleisesti käytössä sairaaloihin kuvantamisessa ja sen todettu olevan turvallinen ja luotettava suoliston läpäisevyyden mittaamiseen.


TUTKIMUKSEN OSALLISTUMINEN

Pyydämme Teitä osallistumaan tämän tutkimukseen, jos olette iältänne 18-45 -vuotias terve aktiivijuoksija. Sovellutte tutkimukseen, jos kärsitte juoksusuorituksen aikana usein vatsaireista (kipu, ripuli, oksentelu) tai jos koette vatsaireita juoksun aikana vain harvoin tai ette ollenkaan. Tutkimukseen osallistuminen edellyttää, että Teillä ei ole diagnosoitua suolistosairautta, astmaa
ja/tai sydän- ja verisuonisairauksia. Tutkimus ei sovellu raskaana oleville tai imettäville. Tutkimuksessa käytettävän varjoaineen takia Teillä ei myöskään saa olla yliherkkyyttä jodille tai jodiallergiaa.

**TUTKIMUksen KULKU**


**OSALLISTUMISEN VAPAHEETTOISUUS JA OSALLISTUMISEN KESKEYTTÄMINEN**

TIETOJEN LUOTTAMUKSELLISUUSS

Kaikkei tutkimuksessa kerätty tieto on luottamukSELLISTa eikä tutkimustuloksista voi tunnistaa yksittäistä henkilöä. Ainoa tutkimusrekisteriin jäävä tieto on tässä tutkimuksessa syntyvää, tautitietoja ei kerätä sairaalan tai terveyskeskuksen potilasrekisteristä eikä mistään muustakaan tiedostosta. Tutkimuksessa kerättäviä tietoja voidaan käsitellä myös muualla kuin tiedot keränneen tutkijan, Helsingin Yliopiston Lääketieteellisen tiedekunnan Medicum-yksikössä. Tällöin tiedot ovat koodattussa muodossa eikä yksittäisen henkilön tunnistaminen ole mahdollista. Tutkimuksen tuloksia käytetään tieteelliseen raportointiin (esim. tiedejulkaisut) vain sellaisessa muodossa, jossa yksittäistä tutkittavaa ei voi tunnistaa.

MAHDOLLINEN HYÖTY JA RISKIT

Tämän tutkimuksen tarkoituksena on saada tietoa liikunnan aikaisten vatsaoireiden ilmaantumisesta ja siten edesauttaa ennaltaehkäisevien hoitojen kehitystä näihin vaivoihin. Tutkimuksen aikana otetut verikokeet saattavat aiheuttaa epämukavuutta, mutta ne eivät vaikuta terveydentilaanne. Suoliston läpäisevyyden mitaamisen yhteydessä joudutte olemaan noin 5 h syömättä, mutta mittaus pyritään suorittamaan siten, että tämä lyhyt paasto ajoittuu luonnollisesti aterioiden välisiin.

TUTKIMUKSEN EETTISYYS

Eettinen toimikunta on antanut tutkimuksesta puoltavan lausunnon.

LISÄTIEETOJA

Mahdollisiin kysymyksiin tutkimuksesta vastaa

Projektitutkija: Elisa Karhu
Puhelinnumero: 050-3180279

Tutkimuksesta vastaava henkilö: Professori Riitta Korpela
Puhelinnumero: 029 4125354
SUOSTUMUS TUTKIMUKSEN OSALLISTUMISESTA

Olen osallistumassa tutkimukseen ”Liikuntasuorituksen aiheuttamat muutokset suoliston läpäisevyydessä vatsaoireilevällä ja ei-oireilevällä juoksijoilla”, jossa tutkitaan muutoksia vatsaoireilevien ja ei-oireilevien juoksijoiden suoliston seinämän läpäisevyydessa ja tulehdusta kuvaavien aineiden pitoisuksissa juoksusuorituksen aikana. Tutkimuksessa selvitetään myös mahdollisia vatsaoireisiin johtavia ruokaaineita ruokapäiväkirjan avulla.

Olen saanut, perehtynyt ja ymmärtänyt tutkimuksen kirjallisen tiedotteen (versio 07012015). Tiedotteesta minulle on selvinnyt tutkimuksen tarkoitus ja sen aikana suoritettavat toimenpiteet. Olen saanut myös riittävästi suullista informaatiota ja minulla on ollut mahdollisuus esittää kysymyksiä tutkimuksesta.

Osallistun tutkimukseen vapaaehtoisesti. Olen tietoinen siitä, että voin keskeyttää tutkimukseen osallistumisen missä tahansa tutkimuksen vaiheessa ennen sen päättymistä ilman, että siitä koituu minulle mitään haittaa. Voin myös peruttaa tämän suostumukseni, jolloin minulla on ollut mahdollisuus esittää kysymyksiä tutkimuksesta.

Allekirjoituksellani vahvistan osallistumisen tähän tutkimukseen ja suostun vapaaehtoisesti tutkimushenkilöksi.

Tutkimushenkilön nimi:_________________________    Syntymäaika:________

Kotiosoite:________________________________________________________

____________________________________

Tutkimushenkilön allekirjoitus    Päiväys

Suostumus vastaanotettu.

____________________________________

Suostumuksen vastaanottajan allekirjoitus    Päiväys

Nimenselvennys

*Tästä suostumuslomakkeesta täytetään kaksi samansisältöistä kappaletta, joista toinen jää tutkimushenkilölle ja toinen tutkimusryhmälle.*
8.4 Supplement 4

TAUSTAKYSELY GUTRUN-TUTKIMUKSEEN

"Liikuntasuorituksen aiheuttamat muutokset suoliston läpäisevyydessä vatsaoireilevilla ja ei-oireilevilla juoksijoilla"

HENKILÖTIEDOT

Nimi:______________________________________________
Syntymäaika:_______________

Katuosoite:_____________________________________________________________________

Postinumero:___________________
Postitoimipaikka:_______________________________

Sähköpostiosoite:_______________________________________________________________

Puhelinnumero(t):___________________________________

TUTKIMUSNUMERO:_________________________________  (Projektitutkija täyttää)
TUTKIMUKSEN OSALLISTUMINEN

Onko Teillä diagnosoitu suolistosairaus?  

Kyllä  Ei

Jos vastasit edelliseen Kyllä, niin mikä:_______________________________________________

Onko Teillä diagnosoitu astma?  

Kyllä  Ei

Onko Teillä diagnosoitu sydän- tai verisuonisairauksia?  

Kyllä  Ei

Oletteko raskaana tai imetättekö?  

Kyllä  Ei

Oletteko allerginen tai yliherkkä jodille?  

Kyllä  Ei

Käytättekö säännöllisesti tulehduskipulääkkeitä?  

Kyllä  Ei

Jos vastasit edelliseen Kyllä, niin mitä ja kuinka usein:___________________________________

Tutkimuksessa Teidän tulee juosta juoksumatolla 90 minuuttia huomattavalla rasituksella,

Salliiko terveytenne tutkimukseen osallistumisen?  

Kyllä  Ei
VATSAIOREKYSELY

Vastaa vatsaoirekyselyyn ympyröimällä oikea vaihtoehto.

1 = En koskaan
2 = Harvoin (alle 10% kerroista)
3 = Joskus (alle 50% kerroista)
4 = Usein (yli 50% kerroista)
5 = Aina (yli 90% kerroista)

Kuinka usein kärsitte vatsavaivoista juostessanne tai heti juoksun jälkeen?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(En koskaan)</td>
<td>(Aina)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Oman juoksu-urasi aikana, oletteko kärsinyt näistä oireista juoksusuorituksen aikana tai heti sen jälkeen?

Vatsakipu

<table>
<thead>
<tr>
<th></th>
<th>1</th>
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<tbody>
<tr>
<td></td>
<td>(En koskaan)</td>
<td>(Aina)</td>
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</tbody>
</table>

Pahoinvointi

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</tr>
</thead>
<tbody>
<tr>
<td>koskaan)</td>
<td>(En</td>
<td>(Aina)</td>
<td></td>
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</tbody>
</table>

Oksentelu

<table>
<thead>
<tr>
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<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Aina)</td>
<td>(En</td>
<td>koskaan)</td>
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<td></td>
</tr>
</tbody>
</table>

Turvotus

<table>
<thead>
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</thead>
<tbody>
<tr>
<td>koskaan)</td>
<td>(En</td>
<td>(Aina)</td>
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<td></td>
</tr>
</tbody>
</table>

Röyhtäily

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>koskaan)</td>
<td>(En</td>
<td>(Aina)</td>
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</tbody>
</table>

Ilmavaivat

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>koskaan)</td>
<td>(En</td>
<td>(Aina)</td>
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</table>

Närästys

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>koskaan)</td>
<td>(En</td>
<td>(Aina)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hölskyminen</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<tr>
<td>-------------</td>
<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Ripuli</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Ummetus</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Ulosteenne koostumus on juoksusuorituksen jälkeen yleensä (ympyröi oikea vaihtoehto):

1. Löysä
2. Ripuli
3. Kova
4. Normaali/kiinteä
5. Normaali rakenne, mutta pakottava hätä

Stressi aiheuttaa Teille vatsaoireita juoksusuorituksen aikana

Kyllä  Ei
**VATSAOIREET JA RAVINTOAINET**

Vastaa vatsaireita ja ravintoaineita koskeviin kysymyksiin ympyröimällä oikea vaihtoehto.

1 = Ei koskaan  
2 = Harvoin (alle 10% kerroista)  
3 = Joskus (alle 50% kerroista)  
4 = Usein (yli 50% kerroista)  
5 = Aina (yli 90% kerroista)

Onko Teillä laktoosi-intoleranssi?  

Kyllä  
Ei

Aihettavatko seuraavat ravintoaineet Teille vatsaireita juoksusuorituksen aikana tai sen jälkeen?

<table>
<thead>
<tr>
<th>ravintoaine</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>(Ei koskaan)</th>
<th>(Aina)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kahvi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkoholi</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>(Ei koskaan)</td>
<td>(Aina)</td>
</tr>
<tr>
<td>Maitotuotteet</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>(Ei koskaan)</td>
<td>(Aina)</td>
</tr>
<tr>
<td>Hedelmät</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>(Ei koskaan)</td>
<td>(Aina)</td>
</tr>
<tr>
<td>Vihannekset</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>(Ei koskaan)</td>
<td>(Aina)</td>
</tr>
<tr>
<td>Leipä</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>(Ei koskaan)</td>
<td>(Aina)</td>
</tr>
<tr>
<td>Rasvaiset ruuat</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>(Ei koskaan)</td>
<td>(Aina)</td>
</tr>
<tr>
<td>Mausteiset / tuliset ruuat</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>(Ei koskaan)</td>
<td>(Aina)</td>
</tr>
</tbody>
</table>
8.5 Supplement 5

VATSAOIREKYSELY

TUTKIIVAN TIEDOT:

Nimi: ________________________________

Tutkimusnumero:________________________ (projektitutkija täyttää)

JUOKSUSUORITUKSEN JÄLKEEN TÄYTETTÄVÄKSI

Merkitää VAS-janalle juoksusuorituksen aikana kokemanne vatsakivun kovuus.

Ei kipua                                Pahin mahdollinen kipu
Kärsittekö juoksun aikana seuraavista vatsaoireista? Ympyröi oikea vaihtoehto.

<table>
<thead>
<tr>
<th>Vatsa-aine</th>
<th>Kyllä</th>
<th>Ei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pahoinvointi</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
<tr>
<td>Oksentelu</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
<tr>
<td>Turvotus</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
<tr>
<td>Röyhtäily</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
<tr>
<td>Ilmavaivat</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
<tr>
<td>Näärästys</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
<tr>
<td>Hölskyminen</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
</tbody>
</table>
**MYÖHEMIN TÄYTETTÄVÄKSI**

Kärsittekö juoksun jälkeen tai myöhemmän juoksusuorituspäivänä seuraavista vatsaoireista? Ympyröi oikea vaihtoehto.

<table>
<thead>
<tr>
<th>Vatsakipu</th>
<th>Kyllä</th>
<th>Ei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pahoinvointi</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
<tr>
<td>Oksentelu</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
<tr>
<td>Turvotus</td>
<td>Kyllä</td>
<td>Ei</td>
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<td>Röytäily</td>
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<tr>
<td>Ilmavaivat</td>
<td>Kyllä</td>
<td>Ei</td>
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<tr>
<td>Närästys</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
<tr>
<td>Hölskyminen</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
<tr>
<td>Ripuli</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
<tr>
<td>Ummetus</td>
<td>Kyllä</td>
<td>Ei</td>
</tr>
</tbody>
</table>
Ulosteen koostumus juoksusuorituksen jälkeen oli (ympyröi oikea vaihtoehto):

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>1</td>
<td>Löysä</td>
</tr>
<tr>
<td>2</td>
<td>Ripuli</td>
</tr>
<tr>
<td>3</td>
<td>Kova</td>
</tr>
<tr>
<td>4</td>
<td>Normaali/kiinteä</td>
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<tr>
<td>5</td>
<td>Normaali rakenne, mutta pakottava hätä</td>
</tr>
</tbody>
</table>