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RESEARCH ARTICLE

Cold exposure increases intestinal paracellular permeability to nutrients in the mouse

Edwin R. Price*, Lisa J. Ruff, Alberto Guerra and William H. Karasov

Department of Forest and Wildlife Ecology, University of Wisconsin-Madison, Madison, WI 53706, USA

*Author for correspondence (eprice2@wisc.edu)

SUMMARY

In situations of increased energy demand and food intake, animals can often acclimate within several days. The intestine generally responds to elevated digestive demand by increasing in size. However, there is likely a limit to how quickly the intestine can grow to meet the new demand. We investigated the immediate and longer-term changes to intestinal properties of the mouse when suddenly exposed to 4°C. We hypothesized that paracellular permeability to nutrients would increase as part of an immediate response to elevated absorptive demand. We measured absorption of L-arabinose, intestinal size and gene expression of several tight junction proteins (claudin-2, claudin-4, claudin-15 and ZO-1) at three time points: pre-exposure, and after 1 day and 2 weeks of cold exposure. Cold exposure increased food intake by 62% after 2 weeks but intake was not significantly increased after 1 day. Intestinal wet mass was elevated after 1 day and throughout the experiment. Absorption of arabinose rose by 20% after 1 day in the cold and was 33% higher after 2 weeks. Expression of claudin-2 increased after 1 day of cold exposure, but there were no changes in expression of any claudin genes when normalized to ZO-1 expression. Our results indicate that intestinal mass can respond rapidly to increased energy demand and that increased paracellular permeability is also part of that response. Increased paracellular permeability may be a consequence of enterocyte hyperplasia, resulting in more tight junctions across which molecules can absorb.

Key words: claudin, paracellular absorption, cold, arabinose, intestinal acclimation, tight junction.

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INTRODUCTION

Ingested hydrosoluble nutrients, such as carbohydrates and amino acids, are absorbed at the intestine *via* two pathways: transcellular absorption, in which nutrients pass through enterocytes *via* protein-mediated transport; and paracellular absorption, in which nutrients move unmediated through the tight junctions between enterocytes to reach the circulation. Paracellular absorption is size and charge selective, and may be driven by solvent drag (as water passes through the tight junctions) and/or *via* simple diffusion of the molecules (Pappenheimer and Reiss, 1987; Fine et al., 1994; Chediack et al., 2003; Chediack et al., 2006). Absorption *via* the paracellular route often represents a low proportion of total nutrient absorption in humans, mice and other non-flying mammals (Delahunty and Hollander, 1987; Fasulo et al., 2013a), and this prevents other non-target molecules, e.g. small water-soluble toxins, from gaining access to the circulation (Diamond, 1991). In fact, increased intestinal permeability to hydrosoluble molecules is sometimes associated with human disease (Berkes et al., 2003; Shen et al., 2009). Nonetheless, paracellular absorption of nutrient-sized molecules (in the size range of amino acids and glucose) can be quite high in small birds and bats, and even accounts for the majority of nutrient absorption in these species (Levey and Cipollini, 1996; Chang and Karasov, 2004; Caviedes-Vidal et al., 2007; Caviedes-Vidal et al., 2008; McWhorter et al., 2010), suggesting that high intestinal permeability to small molecules can be beneficial in certain cases by augmenting transporter-mediated absorption. In mammals, paracellular permeability may be regulated acutely in healthy tissues, although there have been few studies that document this (Rodgers and Fanning, 2011).

There is a growing body of evidence that intestinal paracellular permeability increases during times of elevated demand for energy and digestive processing. Absorption of nutrient-sized paracellular probes is higher when glucose is simultaneously delivered to the gut in mammals (Pappenheimer and Reiss, 1987; See and Bass, 1993; Turner et al., 2000) and birds (Chediack et al., 2003; Chang et al., 2004; Napier et al., 2008), indicating that permeability increases immediately after meal ingestion because of the presence of luminal nutrients. Changes in paracellular permeability can also be associated with particular aspects of the life history of a species. Birds that arrive at a migratory stopover often have decreased intestine mass, but are under selective pressure to gain energy reserves quickly. At a migratory stopover, carrier-mediated absorption of a glucose analog tended to be lower in blackcaps, but non-mediated absorption of nutrient-sized probes was elevated in birds that had recently arrived compared with those that had been refueling for several days (Tracy et al., 2010). Another example is pythons, sit-and-wait predators with infrequent meals, in which the percentage of glucose that is absorbed passively increases to ~50% on the day after meal ingestion, and then returns to low levels by the third day (Secor and Diamond, 1997).

In this paper, we investigate paracellular absorption in another context of increased digestive demand: that caused by cold exposure in mice. Because of the extra energy expenditure required to maintain euthermic body temperature, long-term cold exposure should increase the quantity of food consumed (Gross et al., 1985). Indeed, previous experiments with mice exposed to cold demonstrated that they dramatically increase food intake within 2–7 days (Toloza et

al., 1991; Konarzewski and Diamond, 1994). The size of the gastrointestinal tract organs, particularly the small intestine, increased over the first 5–15 days of cold exposure in response to this increased demand for digestive processing (Toloza et al., 1991; Konarzewski and Diamond, 1994). The increase in small intestine mass was driven by an increase in mucosa and was accompanied by greater carrier-mediated uptake of glucose and proline (Toloza et al., 1991). However, it is plausible that an increase in carrier-mediated uptake is not enough to meet total demand and that increased intestinal permeability is also part of the response to cold. In particular, increasing intestinal permeability to nutrients might be an effective immediate response to cold exposure before the intestine can compensate with growth of the absorptive surface area. That is to say, in the face of a potential short-term limitation to absorption (before the intestine can adequately grow in size and surface area), mice might be able to partially compensate for increased absorptive demand by allowing increased paracellular nutrient absorption. Although the paracellular proportion of nutrient absorption is normally low in mice, to meet an 'emergency demand', we hypothesized that mice could transiently increase paracellular permeability *via* acute regulation of tight junction proteins.

We investigated this further using a temperature manipulation in mice. While previous studies have examined intestinal responses after several days of cold exposure, there has been very little study of 'immediate' responses on a time scale of just 1 day, and no previous measurements of how paracellular permeability to nutrients changes with cold exposure. An interesting previous observation was that although food consumption increased in the cold, it did not reach its plateau value after the first day (Toloza et al., 1991), indicating there could be a short-term digestive limitation. We hypothesized that mice would increase intestinal permeability to nutrients after 1 day of cold exposure, when the intestine may not yet have compensated for increased food intake through other means (increased mucosal mass, nominal surface area, etc.). Further, we hypothesized that intestinal permeability would return to baseline after 2 weeks, when intestinal mass and mediated transport mechanisms had compensated for the increased food intake. Two weeks was chosen conservatively as a duration long enough to observe a plateau of changes to gross morphology of the intestine (Green and Millar, 1987; Toloza et al., 1991; Konarzewski and Diamond, 1994). Additionally, at these time points we measured mRNA expression of several tight-junction genes that are hypothesized to be important in regulating permeability to nutrient-sized molecules.

MATERIALS AND METHODS

Animals and husbandry

Adult male mice (*Mus musculus* strain ICR) were purchased from Harlan (Indianapolis, IN, USA) and were housed individually in standard shoebox cages in our animal facility at 20°C for 1 week before any measurements. In their cages, mice had woodchip bedding and were provided with a small amount of nesting material (crinkle paper) for enrichment but not enough to form large insulative nests. During this acclimation period, mice were placed for ~1 h every 2 days into metabolic chambers designed for separate urine and feces collection (Harvard Apparatus, Holliston, MA, USA) to allow them to acclimate to this test apparatus. Except during the assessments of paracellular absorption of L-arabinose (see below), the mice were at all times provided *ad libitum* water and rodent chow (2018 Teklad Global 18% Protein Rodent Diet, Harlan). All experimental procedures were approved by the University of Wisconsin-Madison Animal Care and Use Committee (protocol A01441).

Cold manipulation and experimental design

The capacity for paracellular nutrient absorption was assessed by measuring the fractional absorption (oral bioavailability) of L-arabinose. L-arabinose (M_r=150) is a carbohydrate in the size range of water-soluble nutrients, e.g. glucose (M_r=180) and proline (M_r=115), and has no affinity for the glucose transporter and therefore its absorption is completely paracellular (Lavin et al., 2007). Absorption of L-arabinose was measured at up to three time points in each mouse: before cold exposure (N=23), following 1 day of cold exposure (N=14) and following 2 weeks of cold exposure (N=6). Before cold exposure, the temperature in the animal room was maintained at 20°C. For the cold exposure, the temperature in the room was lowered quickly (over 30 min) to 4°C and maintained at that temperature for the following 2 weeks. Immediately following each assessment, a subset of mice was euthanized for measurements of organs and tissue samples (details below). The remaining mice were returned to their normal housing (either at room temperature or 4°C, as appropriate). Food consumption was measured immediately prior to each absorption assessment by the change in the mass of food in the cage over the previous 24 h period.

Assessment of L-arabinose fractional absorption

Mice were moved from the animal facility to the laboratory and tested at 20°C. Mice were orally gavaged with [^{14}C]-L-arabinose in ~120 µl of an aqueous solution containing 50 mmol l^{-1} glucose and 125 mmol l^{-1} NaCl. Glucose was added to the solution to increase intestinal permeability, because previous studies have demonstrated that the presence of glucose in the intestine can increase the permeability of the paracellular pathway (Pappenheimer and Reiss, 1987; See and Bass, 1993). We simultaneously injected ~60 µl of [^{3}H]-L-arabinose i.p. in 150 mmol l^{-1} NaCl. Syringes were weighed before and after dosing to obtain accurate measurements of the dose for each mouse. Mice were then placed in the metabolic chambers (20 cm diameter by 10 cm height) and we collected the urine that was produced after 90, 180, 270 and 1200 min following dosing. Previous work showed that this time period is long enough to measure fractional absorption of arabinose (Fasulo et al., 2013a). Mice had access to 10% glucose in water, but no food, during this period. Radioactivity was measured using 5 ml Ecolume scintillation cocktail in 8 ml glass scintillation vials and a Wallac 1414 liquid scintillation counter (PerkinElmer, Waltham, MA, USA). We calculated the cumulative percentage of both the gavaged [^{14}C]-L-arabinose dose and the injected [^{3}H]-L-arabinose dose that were recovered in the urine. A preliminary experiment, conducted with three mice simultaneously injected i.p. with both [^{14}C]- and [^{3}H]-arabinose, showed that recovery of [^{3}H]-arabinose was only 89.9±0.9% of that of [^{14}C]-arabinose recovery. We therefore multiplied [^{3}H]-L-arabinose recoveries by 1.11 to account for this difference in probe recovery, producing an 'adjusted cumulative % injection dose recovered'. Although L-arabinose is not metabolized and theoretically can be fully recovered, recoveries of injected inert probes are often less than 100% (Karasov et al., 2012). To account for this loss, we normalized our gavage data using the i.p. injection data. Thus, we calculated percent absorption as (cumulative % gavage dose recovered)/(adjusted cumulative % injection dose recovered).

Organ mass and tissue storage

Following the measurement of paracellular absorption, mice were euthanized with CO_2 and we rapidly removed and then dissected the intestinal tract in ice-cold Hanks' balanced salt solution (HBSS; 5.4 mmol l^{-1} KCl, 0.5 mmol l^{-1} KH_2PO_4, 136 mmol l^{-1} NaCl,

4 mmol l^{-1} $NaHCO_3$, 0.3 mmol l^{-1} Na_2HPO_4, 5.5 mmol l^{-1} D-mannitol, pH 7.2), recording the wet mass of the small intestine, length of the small intestine, wet mass of the large intestine, length of the large intestine, and wet mass of the cecum. We also calculated nominal surface area (smooth bore area) of the small intestine by cutting the intestine longitudinally at three points along its length to lay the intestine flat and measure circumference (flat width) with calipers. The circumference was multiplied by length and the resultant area was integrated along the three points of the intestine to calculate nominal surface area.

The small intestine was divided into three equally long sections (proximal, medial and distal) A 2 cm portion was isolated from the medial section to be used in qPCR. To isolate enterocytes, this portion was minced with scissors and incubated at 4°C in a chelating solution (5 mmol l^{-1} KCl, 30 mmol l^{-1} EDTA, 52 mmol l^{-1} NaCl, 10 mmol l^{-1} Hepes, 2 mmol l^{-1} D-L-dithiothreitol, in 6% 1 mol l^{-1} HCl, pH 7.1) for 75 min [isolation technique modified from Mac Donal et al. (Mac Donal et al., 2008)]. The supernatant was discarded and the sample was shaken vigorously after the addition of 1 ml HBSS. The supernatant was transferred to a new centrifuge tube. This was washed three times by centrifuging (100 ***g***, 5 min, 4°C), discarding the supernatant and adding 1 ml HBSS again each cycle. Finally, the supernatant was discarded and 250 ml RNALater (Qiagen, Valencia, CA, USA) was added. This was placed at 4°C overnight and then stored at –20°C.

qPCR of genes encoding tight junction proteins

Expression of four tight junction proteins, zonula occludens-1 (ZO-1), claudin-2 (CLDN2), claudin-4 (CLDN4) and claudin-15 (CLDN15), and a housekeeping gene (Eef1a1) were measured using real-time PCR (primers are listed in Table 1). CLDN2 and CLDN15 are associated with 'leaky' epithelia and have pore-forming properties (Amasheh et al., 2002; Van Itallie and Anderson, 2006; Krause et al., 2008; Markov et al., 2010), while CLDN4 is associated with 'tight' epithelia and low paracellular conductance (Van Itallie et al., 2001; Van Itallie and Anderson, 2006). ZO-1 is expressed more ubiquitously in tight junctions (Holmes et al., 2006). RNA was extracted from isolated enterocytes using the Purelink RNA Mini Kit (Life Technologies, Grand Island, NY, USA) and then reverse transcribed to create complementary DNA (cDNA) (iScript cDNA Synthesis Kit, Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. We prepared a negative control using water instead of RNA. Real-time PCR was run for each gene separately with samples run in duplicate with a RealPlex^2 (Eppendorf NA, Hauppauge, NY, USA). The qPCR cycling consisted of a 10 min melt at 95°C, followed by 45 cycles of 30 s at 95°C, 30 s at 56°C and 30 s at 72°C. Fluorescence was measured at the end of each cycle, and samples were run in duplicate. The cycle threshold was compared with a single calibrator on every run. The calibrator was created from a pool of several mouse cDNA samples. Using a serial dilution of the calibrator, we also calculated reaction efficiency (E) for each gene. Expression in each sample was calculated as $E^{\Delta C_t}$, where ΔC_t is the cycle threshold of the calibrator minus the cycle threshold of the sample. For each tight junction gene, we calculated the expression ratio, which was the expression of the tight junction gene divided by that of the housekeeper (Eef1a1). For each tight junction gene, we normalized the expression ratio to the pre-exposure treatment group mean for presentation in figures.

Table 1. Primers used for real-time PCR

Gene	Primer sequence (5′–3′)
Eef1a1 forward	CTGAACCATCCAGGCCAAAT
Eef1a1 reverse	GGCTGTGTGACAATCCAG
CLDN2 forward	GGCTGTTAGGCACATCCAT
CLDN2 reverse	TGGCACCAACATAGGAACTC
CLDN4 forward	CGCTACTCTTGCCATTACG
CLDN4 reverse	ACTCAGCACACCATGACTTG
CLDN15 forward	ACTCCGCTGCACCAACGTGG
CLDN15 reverse	ACGGCGTACCACGAGATAGCCA
ZO-1 forward	AGGACACCAAAGCATGTGAG
ZO-1 reverse	GGCATTCCTGCTGGTTACA

All primers except CLDN15 are from Holmes et al. (Holmes et al., 2006).

Statistics

The sample size varied slightly among the variables we measured due to various experimental problems, e.g. spills, mice that did not urinate for most of the experiment, mice that were injected subcutaneously (detected by extremely low recovery of the injected probe), etc. Due to the strict timeline of the experiment, these samples could not be retaken. For arabinose absorption, 23, 14 and 6 animals were measured at the pre-exposure, 1 day and 2 week time points, respectively, while food consumption was measured for 14, 10 and 6 animals at these times. The sample sizes for organ measurements were 15, 6 and 6 animals for the same timepoints with the exception of large intestine length, for which the sample sizes were 12, 3 and 6. For RNA expression, sample sizes were 12, 6 and 6 for all genes except CLDN2, for which they were 11, 6 and 5. For variables without repeated measures (i.e. organ measurements and gene expression), ANOVA was used (with Tukey's honestly significant difference *post hoc* tests) to determine differences among pre-exposure, 1 day cold exposure and 2 weeks cold exposure treatment groups. For the remaining variables (i.e. percent absorption of L-arabinose, body mass and food consumption), we used a mixed model (nlme package in R statistical software) that modeled the effect of treatment group on the variable of interest while treating individual as a random effect to control for repeated measures on individuals (Pinheiro et al., 2010; R Development Core Team, 2010). Results presented are raw means ± s.e.m.

RESULTS

The proportion of the L-arabinose dose that was absorbed (fractional absorption) was altered by the cold treatment ($F_{2,23}$=3.56, P=0.045), increasing by 20% (P=0.046) after 1 day of cold exposure (Fig. 1). This proportion remained significantly elevated (33% higher than pre-exposure; P=0.047) after 2 weeks in the cold (Fig. 1).

Food consumption also varied with treatment ($F_{2,22}$=24.8087, P<0.0001). Pre-exposure mice had a mean intake of 6.5±0.3 g day^{-1}, and this was not significantly elevated after 1 day of cold exposure (7.2±0.6 g day^{-1}, P=0.15; Fig. 1). Food consumption was 62% higher (10.6±0.4 g day^{-1}, P<0.0001) in mice exposed to 2 weeks of cold compared with pre-exposure mice. Body mass did not differ significantly ($F_{2,24}$=0.381, P=0.69) between pre-exposure (39.08±1.03 g), 1 day cold exposure (37.72±1.34 g) and 2 weeks cold exposure (39.35±1.14 g). Most measures of digestive organ size did not vary among treatments (small intestine length, $F_{2,24}$=1.01, P=0.37; large intestine length, $F_{2,18}$=0.488, P=0.62; large intestine mass, $F_{2,24}$=2.050, P=0.15; cecum mass, $F_{2,24}$=1.203, P=0.32; nominal small intestinal surface area, $F_{2,24}$=3.00, P=0.069; Fig. 1). However, small intestine mass varied with treatment ($F_{2,24}$=7.526, P=0.0029) and was significantly higher in 1 day (P=0.023) and 2 week (P=0.008) cold exposure groups compared with mice that had not experienced cold (Fig. 1).

Tight junction protein expression did not vary with treatment for most of the proteins tested (Fig. 2). Expression of ZO-1 mRNA was

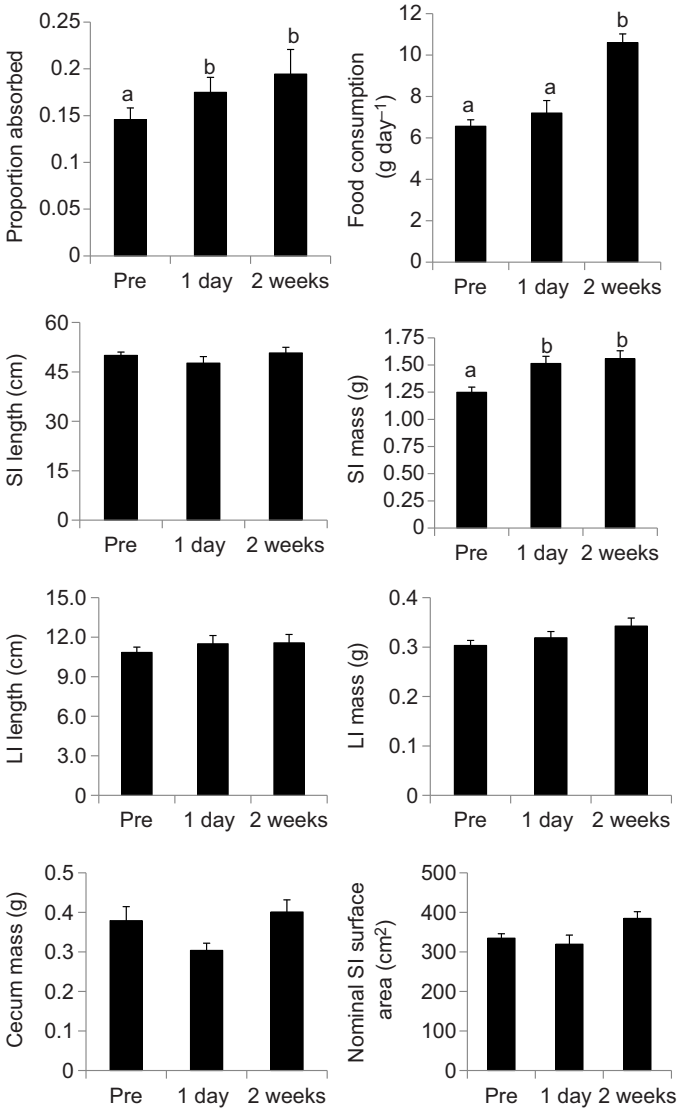

Fig. 1. Fractional absorption of L-arabinose, food consumption and intestinal morphometrics of mice exposed to 4°C for 1 day or 2 weeks, or pre-exposure. SI, small intestine; LI, large intestine. Bars that share the same letters or lack letters do not differ significantly (P>0.05). Data are means ± s.e.m.

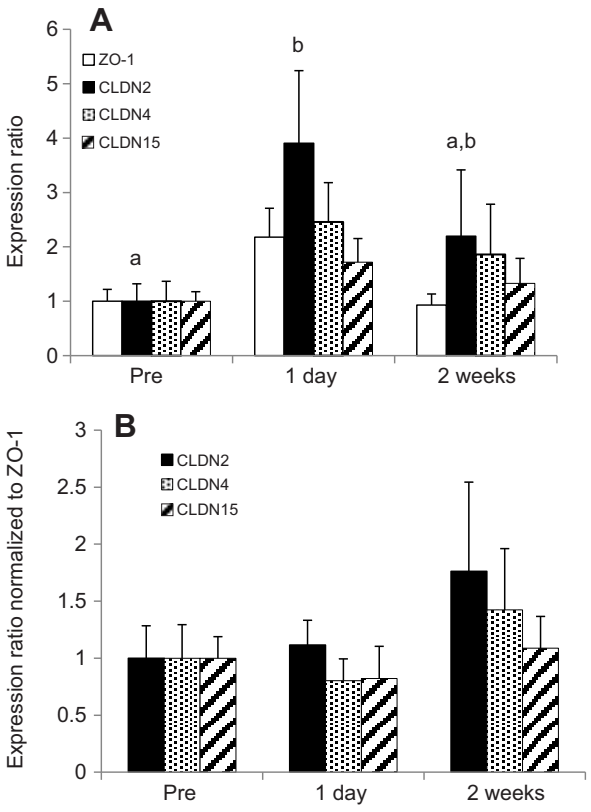

Fig. 2. (A) mRNA expression of several tight junction proteins in mice exposed to cold for up to 2 weeks. CLDN2 expression was significantly elevated after 1 day of cold exposure compared with pre-exposure. (B) Expression of claudins normalized to the expression of ZO-1. CLDN2, claudin-2; CLDN4, claudin-4; CLDN15, claudin-15; ZO-1, zonula occludens-1.

not significantly affected by treatment ($F_{2,21}$=3.275, P=0.0578). Cldn2 did vary with treatment ($F_{2,19}$=4.041, P=0.0345); expression increased after 1 day in the cold (P=0.0474), but after 2 weeks was not significantly elevated over that of pre-exposed mice (P=0.43). Neither Cldn4 ($F_{2,21}$=1.383, P=0.27) nor Cldn15 ($F_{2,21}$=1.359, P=0.28) mRNA expression varied significantly with cold treatment. When the expression ratios for the claudins were normalized to the expression of ZO-1, none varied significantly with cold treatment (Cldn2: $F_{2,19}$=0.434, P=0.654; Cldn4: $F_{2,21}$=0.607, P=0.554; Cldn15: $F_{2,21}$=0.250, P=0.781; Fig. 2).

DISCUSSION

Intestinal adjustments to a cold environment

Animals can be expected to have some excess intestinal absorptive capacity, but not too much (Diamond, 1991; Toloza et al., 1991). Nutrient transporters, and other parts of the absorptive machinery in the intestinal mucosa, are energetically expensive to produce and maintain (Martin and Fuhrman, 1955; Cant et al., 1996). Thus, while an animal may maintain some capacity in excess of ordinary demands (in order to respond to short-term fluctuations in food intake), large increases in absorptive demand are unlikely to be accommodated without upregulation of absorptive capacity. Cold environmental conditions cause an increase in energetic demand on endotherms as they maintain thermal homeostasis; this should cause an increase in food intake. Indeed, our results show that mice do increase food intake, at least after 2 weeks, in accordance with other studies of chronic cold exposure in mice and other species (Gross et al., 1985; Toloza et al., 1991; Dykstra and Karasov, 1992; Konarzewski and Diamond, 1994; Naya et al., 2005). The primary response of the gut to cold exposure is to augment intestinal size, including increased mucosal mass (Gross et al., 1985; Green and Millar, 1987; Bozinovic et al., 1988; Hammond and Wunder, 1991; Toloza et al., 1991; Dykstra and Karasov, 1992; Konarzewski and Diamond, 1994; Naya et al., 2005; present study). Additionally, there was a non-significant (P=0.069) rise in nominal surface area (after 2 weeks cold exposure) that we may not have had the power to detect with statistical confidence.

Although animals can respond to increased demand for absorptive capacity by increasing the mucosal mass and surface area, this response has some drawbacks. First, maintenance of the increased mucosal mass incurs an energetic cost (Martin and Fuhrman, 1955; Konarzewski and Diamond, 1995; Cant et al., 1996). Second, there are likely limitations to how rapidly an animal can build up the

intestinal mucosa. Previous studies showed higher intestinal mass as early as 4 days in the cold (Toloza et al., 1991). Here we show that intestinal mass was significantly higher as early as 2 days (1 day in the cold plus 1 day of arabinose absorption assessment) following cold exposure. This increase, along with the observation that there was no growth (actually a slight, non-significant decline) in nominal surface area, suggests an increase in villous area per nominal surface area caused by, for example, growth in villus length.

Despite the rapid increase in intestinal mass, this immediate response still may not have been enough to meet digestive/absorptive demand, as food intake was not significantly higher after 1 day. Toloza et al. similarly showed that mice exposed to cold increased food intake substantially over baseline by the second day, but in the first day of exposure food intake was intermediate (Toloza et al., 1991). In the face of a potential short-term limitation to absorptive capacity, we hypothesized that a partial solution could be to increase paracellular absorption of nutrients. Normally, the proportion of nutrient absorption that is paracellular is maintained low in mice, presumably to prevent absorption of toxins (Diamond, 1991). However, to meet an 'emergency demand', this mechanism could augment nutrient absorption *via* acute regulation of the permeability of the tight junctions to nutrients, even before mucosal mass increases with concomitant increase in transcellular absorptive capacity. Such a mechanism might only partially offset the limitation of gut size; indeed, this is suggested by the fact that food intake did not reach its plateau by the first day (Toloza et al., 1991; present study).

In support of this hypothesis, the fractional absorption of arabinose (a paracellular probe that is intermediate in size between glucose and amino acids) did increase as early as 1 day after exposure to the cold, and elevated fractional absorption (which is consistent with elevated intestinal permeability) was maintained after 2 weeks of cold exposure. This increase was modest, and the proportion of our probe that was absorbed did not approach that achieved by some small birds and bats [greater than 50% bioavailability in birds and bats (Caviedes-Vidal et al., 2007; Fasulo et al., 2013b)]. An increase in paracellular permeability to nutrient-sized probes has also been observed in other cases of elevated absorptive demand in pythons 1 day post-feeding (Secor and Diamond, 1997) and in migratory birds during refueling after a long flight (Tracy et al., 2010). Nonetheless, the increase in permeability we observed need not be adaptive in nature, but could simply reflect other processes. For example, higher fractional absorption of arabinose could result from greater retention time. Although we did not measure it, this possibility seems unlikely because previous studies of animals with increased energy demand often show either no change (Toloza et al., 1991) or quicker throughput time (Hammond and Wunder, 1991; Derting and Bogue, 1993). Similarly, increased villous amplification could result in not only more enterocytes but also more tight junctions, and not necessarily higher permeability per tight junction length. In support of this explanation, intestinal mass was higher after 1 day of cold exposure in our mice. However, increased expression of certain tight junction proteins may alternatively indicate an adaptive regulation of permeability of the tight junction itself (discussed below). The observation that permeability remained high after 2 weeks could reflect the increased number of tight junctions and/or greater permeability of each tight junction. Regardless of whether increased paracellular arabinose absorption reflects an adaptive phenotypic flexibility or is simply a passive consequence of other tissue responses to the cold, an increase in paracellular permeability implies both a greater capacity to absorb hydrosoluble nutrients as well as an increased exposure to dietary hydrosoluble toxins.

Changes in gene expression of tight junction proteins

Cold exposure was associated with a transient increase in the mRNA expression of CLDN2 that was indistinguishable from baseline levels after 2 weeks exposure. CLDN2 and CLDN15 are associated with leaky epithelia and increased cation permeability (Amasheh et al., 2002; Van Itallie and Anderson, 2006; Krause et al., 2008; Markov et al., 2010). Permeability to polyethelene glycol is also associated with increased protein expression of CLDN2 and decreased expression of CLDN4 (Van Itallie et al., 2008). The increase in CLDN2 mRNA expression we observed could therefore represent a remodeling of the tight junction that increases paracellular permeability to nutrient-sized molecules. However, an increased expression of CLDN2 could also simply be associated with increased villous growth and quantity of tight junctions. Notably, when CLDN2 expression was normalized to the expression of ZO-1 to control for tight junction length (Holmes et al., 2006; Van Itallie et al., 2008), its expression was invariant with cold treatment. Overall, our results do not suggest remodeling of the tight junction but rather growth of the mucosa and associated growth of the quantity of tight junctions across which nutrients may absorb.

Conclusions

Cold exposure caused an increase in food consumption and an associated increase of the mass of the small intestine. This was accompanied by an increase in absorption of arabinose. This increase in paracellular permeability may have resulted from the increased number of tight junctions associated with enterocyte hyperplasia and not from remodeling of the tight junctions. The response of paracellular permeability to cold exposure results in a modest augmentation of absorptive capacity, as well as a modest increase in exposure to water-soluble toxins.

LIST OF ABBREVIATIONS

CLDN2	claudin-2
CLDN4	claudin-4
CLDN15	claudin-15
ZO-1	zonula occludens-1

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AUTHOR CONTRIBUTIONS

E.R.P. and W.H.K. designed the study. E.R.P., L.J.R. and A.G. executed the study. E.R.P., L.J.R., A.G. and W.H.K. interpreted the findings. E.R.P., L.J.R., A.G. and W.H.K. drafted and revised the manuscript.

COMPETING INTERESTS

No competing interests declared.

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