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Loads, capacities and safety factors of maltase and the glucose transporter SGLT1 in mouse intestinal brush border

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Safety factors are defined as ratios of biological capacities to prevailing natural loads. We measured the safety factor of the mouse intestinal brush-border hydrolase maltase in series with the glucose transporter SGLT1, for comparison with previous studies of sucrase and lactase. Dietary maltose loads increased 4-fold from virgin to lactating mice. As in previous studies of intestinal adaptive regulation, that increase in load without change in dietary composition resulted in an increase in maltase and SGLT1 capacities mediated non-specifically by an increase in intestinal mass, without change in maltase or SGLT1 activities per milligram of tissue. Maltase and SGLT1 capacities increased only sublinearly with load during lactation, such that safety factors decreased with load: from 6.5 to 2.4 for maltase, and from 1.1 to 0.5 for SGLT1. The apparently high safety factor for maltase may be related to the multiple natural substrates hydrolysed by the multiple sites of maltase activity. The apparently low safety factor for SGLT1 is made possible by the contribution of hindgut fermentation to carbohydrate digestion. SGLT1 activity is paradoxically higher for mice consuming sucrose than for mice consuming maltose, despite maltose hydrolysis yielding double the glucose load yielded by sucrose hydrolysis, and despite glucose constituting the load upon SGLT1.

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The emerging field of quantitative evolutionary design concerns the evolved relations between biological capacities (in the most general sense) and the natural loads upon them (Alexander, 1981; Weibel *et al.* 1987; Diamond, 1993, 2002). Examples include the relation between a bone's strength and the peak stress on that bone during operation, and the relation between an enzyme's or transporter's V_{max} value and its peak natural velocity. Do biological capacities exceed peak natural loads by some reserve capacity? If so, by how much, and why?

Pioneering studies in this area were those by Taylor and Weibel and their colleagues of the multi-step O_2 delivery pathway through the heart, lungs, mitochondria and capillaries during aerobic exercise (Taylor *et al.* 1987; Weibel *et al.* 1991, 1996). They coined the term 'symmorphosis' to describe the close match of capacity to load that they observed for several steps of this pathway. More often, however, biological capacities are found to exceed peak loads. The ratio of capacity to peak load is defined as the 'safety factor' by analogy to safety factors routinely calculated for human-engineered systems. Safety factors of both engineered and evolved biological systems fall in the range 1–13, mostly 2–7 (Alexander, 1981; Diamond, 1991, 1993, 2002). For instance, elevator cables are required by law to be built with safety factors of 4–11, and most bones are found to have safety factors of 3–6. In other words, a elevator cable can actually support a load 4–11 times the maximum weight that the elevator is certified to hold, and bones are far short of breaking during normal operation.

Safety factors of both biological and engineered systems can be understood as the outcome of compromises between costs and benefits. Larger capacities (e.g. stronger bones or elevator cables) fail less often but cost more to make and maintain and also occupy more space. The essential difference between engineered and biological safety factors is that the former are designed consciously by humans who calculate the costs and benefits, while the latter are the outcome of natural selection, which in effect calculates the costs and benefits unconsciously. Because animals have only finite energy and space in their bodies, excessively large biological capacities are selected against because they consume energy and occupy space that could have been devoted to other capacities. That is, one expects physiological capacities to be characterized by the phrase 'enough but not too much' (Diamond, 1991).

A host of unsolved questions arises within this framework, such as changes in safety factors during adaptive regulation of capacities by their loads, the relation between the capacities of steps in series in the same pathway, accounting for the different safety factors of different biological capacities and identifying the costs that cause excess capacities to incur an evolutionary penalty. Our

laboratory has been studying these questions for nutrient transporters of the small intestinal brush border, and more recently for series pairs of brush-border hydrolases and nutrient transporters. All other things being equal, one might expect elements operating in series to have evolved equal capacities, otherwise the smaller capacity would constitute a rate-limiting step, and the larger capacity would remain partly unutilized. However, numerous other considerations often invalidate this simple reasoning, such as unequal costs or variabilities of the two capacities (Alexander, 1997) or else multiple functions of one of the steps.

The series pairs of brush-border hydrolases and transporters that we previously studied were the pair consisting of sucrase (which hydrolyses sucrose to glucose and fructose) plus the glucose transporter SGLT1 (which transports one of the two products of sucrase action) (Weiss *et al.* 1998), and the pair consisting of lactase (which hydrolyses lactose to galactose and glucose) plus again the glucose transporter SGLT1 (which transports both products of lactase action) (O'Connor & Diamond, 1999). The present paper studies capacity–load relations of a third brush-border hydrolase–transporter pair: maltase (which splits maltose into two glucose molecules) and again the glucose transporter SGLT1. This pair is of interest for several reasons. First, whereas the molar loads on both members of the sucrase–SGLT1 pair are identical (one mole of sucrose yielding one mole of glucose to be transported by SGLT1), in the maltase–SGLT1 and lactase–SGLT1 pairs the molar load on the transporter is double that on the hydrolase (because one mole of maltose or of lactose yields two moles of glucose or of aldohexose SGLT1 substrate, respectively). Second, whereas in the cases of sucrase and lactase enzymatic activity is virtually confined to one active site, maltase activity is divided among three types of active sites on two proteins (Sörensen *et al.* 1982; Nören *et al.* 1986; Gayland, 1989). (Similarly, while we refer to SGLT1 as 'the' glucose transporter of the brush border, it has recently been argued that brush-border glucose transport in rat intestine arises from GLUT2 as well as from SGLT1 (Kellett, 2001); if that were true for mice, then glucose transporter as well as maltase activity would be divided among different active sites.) Finally, maltose is not the only, and possibly not even the most important, load on those sites: they also hydrolyse longer oligosaccharides (Gray, 1975). As we shall see, these distinctive features of the maltase–SGLT1 system, which are of course shared with many other biological series pathways, may contribute to understanding the load–capacity relations that we shall describe. We report three findings: the load–capacity relation for each element separately (maltase and SGLT1); the capacity–capacity relation for those two elements in series; and the effect upon these relationships of a large increase in maltose load associated with the energetic demands of lactation.

METHODS

Animals and maintenance

Virgin Swiss-Webster mice were obtained from Charles River Laboratories (Wilmington, MA, USA) and housed individually in the UCLA Health Sciences Vivarium. Mice were maintained on a 12 h light : 12 h dark schedule at a constant temperature of 24 °C and had *ad libitum* access to water and a maltose-based diet prepared in pelleted form by ICN Biochemicals (Cleveland, OH, USA; identical to the sucrose-based diet described by Lee *et al.* (1998) except for replacement of sucrose by maltose). Female mice were separated randomly into two groups. The lactating group ($n = 8$) was mated with male mice, and the virgin group ($n = 8$) was not mated. Approximately 21 days after mating, pregnant mice in the lactating group gave birth to 6–12 pups. Three days after birth, litters of eight were achieved by either cross-fostering additional same-aged pups or by culling (by anaesthetic overdose). On the day of peak lactation (15 days after birth, just before the pups start to nibble solid food), lactating mice were killed for experiments, along with virgin mice. Mice were killed by overdose of pentobarbitone anaesthesia. All animal experiments were carried out in accordance with the guidelines of the 2000 report of the Panel on Euthanasia of the American Veterinary Medical Association. All mice were 108–138 days old on the day they were killed.

Experimental design

For each mouse we measured food intake and apparent digestive efficiency before killing, and body mass, digestive and vital organ morphology, and maltase and SGLT1 activities at the time of killing. Mice were killed and tissues were removed between 10.00 and 14.00 h in order to minimize effects of circadian variation.

From those data we calculated the dietary maltose and glucose loads and the maltase and SGLT1 capacities, in order to study the relationships between capacities and loads in maltose digestion. Determining the relationship between maltase capacity and dietary maltose load is straightforward: if a mouse consumes 1 mmol day^{-1} of maltose, then the load upon maltase is 1 mmol of its maltose substrate per day; and if the intestinal maltase capacity in this hypothetical example is 3 mmol of maltose hydrolysed per day, then the maltase safety factor equals 3 (3 divided by 1). However, the relationship between maltose load and SGLT1 capacity requires explanation. Since the disaccharide maltose is composed of two monosaccharides of glucose, hydrolysis of 1 mmol of maltose yields 2 mmol of glucose. Therefore, a dietary maltose load of 1 mmol day^{-1} represents a load of 1 mmol day^{-1} on maltase, but of 2 mmol day^{-1} upon SGLT1. If (in our hypothetical example) the SGLT1 capacity were 3 mmol day^{-1}, then the SGLT1 safety factor would equal 1.5 (3 divided by 2). Hence, in mice consuming maltose, the SGLT1 capacity would have to be double the maltase capacity in order for the two series capacities to possess equal safety factors.

Food intake and digestibility

Three days prior to killing, each mouse was placed in a separate cage with an elevated wire bottom, and its daily food intake and faecal production were measured. Dry-matter content of the food was 95–99 %. Orts (uneaten food) and faeces that dropped to the bottom of the cage were separated from each other, dried in an oven at 60 °C to constant mass, and weighed. We then calculated apparent dry-matter digestive efficiency as (food intake − faecal output)/(food intake); we refer to this efficiency as 'apparent' because it is uncorrected for possible contributions of gut bacteria and shed protein to faecal mass. A day's food intake was calculated for each mouse as the mass of food disappearing from the food dispenser that day, minus the day's mass of orts.

Everted sleeve assays

We measured maximal activity (V_{max}) of brush-border maltase and SGLT1 by the everted sleeve preparation summarized below and described in detail by Lee *et al.* (1998) and Karasov & Diamond (1983*a*). Briefly, mice were anaesthetized with an intraperitoneal injection of 0.08–0.10 ml sodium pentobarbitone (Nembutal, 50 mg ml^{-1}), a laparotomy was performed, and the entire small intestine from pylorus to ileocaecal junction was perfused with ice-cold Ringer solution and dissected out. The small intestine was divided into thirds of equal length, each of which was lightly blotted dry and weighed. The wet mass of the small intestine was taken as the sum of these three regional wet masses. Each of the three intestinal regions (proximal, middle and distal) was then everted over a stainless-steel rod and cut into tissue sleeves of 1.5–2.0 cm in length. From the middle of each region we used five sleeves immediately adjacent to each other: the first and fifth sleeves to assay SGLT1, the second and fourth sleeves to assay maltase, and the third sleeve as a tissue blank for maltase assays (see explanation below). Sleeves mounted on rods were maintained in ice-cold, oxygenated Ringer solution for 25–60 min prior to beginning the assays. Thus, we obtained maximal activity (V_{max}) values for maltase and SGLT1 from adjacent pieces of intestine from the same mouse. As with any physiological measurement, some uncertainty remains about how closely experimental measurements of intestinal activities approach true physiological values (for discussion see Karasov & Diamond, 1983*a*; Uhing & Kimura, 1995). For example, *in vivo* infusion of glucagon-like peptide 2 (GLP-2) into rats causes an upregulation of SGLT1 that is maximal after 1 h (Cheeseman, 1997). If there were a similar effect in mice, and if downregulation had a time course similar to upregulation, then there could be some down-regulation of SGLT1 during the *in vitro* incubation of 25–60 min prior to beginning our assays.

SGLT1 assay

Everted sleeves were pre-incubated at 37 °C for 5 min in Ringer solution at pH 7.3 aerated with 95 % O_2–5 % CO_2. The sleeves were then incubated for 2 min (also at 37 °C, pH 7.3, and aerated) in 10 ml Ringer solution containing 50 mM glucose (isosmotically replacing NaCl). Also incorporated into the incubation solution were trace concentrations of D-[^{14}C]glucose and L-[^{3}H]glucose. The incubation solution was stirred at 1200 r.p.m. with a stirring bar in order to minimize the influence of unstirred layers. At the end of the 2 min incubation, the sleeve was lightly blotted, weighed and placed in a vial for liquid scintillation counting.

Carrier-mediated D-glucose uptake was calculated as the uptake of D-[^{14}C]glucose, corrected for both passive uptake and for glucose in the adherent fluid by subtracting the simultaneously measured uptake of the stereoisomer L-[^{3}H]glucose, which is not subject to carrier-mediated transport. Uptake activity was normalized to milligrams wet mass of tissue and expressed as nanomoles of glucose per minute per milligram. Regional capacities for glucose uptake were calculated as mean SGLT1 activity times regional mass and expressed as mmoles per day for each of the three intestinal regions. The capacity of the entire length of the small intestine was calculated as the sum of the three regional capacities.

Brush-border maltase assay

Like conventional homogenate assays of disaccharidase activity (Dahlqvist, 1968), the modified everted sleeve method (Lee *et al.* 1998) measures colorimetrically the rate at which glucose is produced by the hydrolysis of maltose by maltase. The principle of the assay is to determine maltase activity by incubating an everted intestinal sleeve preparation in a maltose-containing solution, and measuring the rate of glucose production within the incubation medium. Because the glucose would normally be taken up into the tissue by the action of SGLT1, we inhibited SGLT1 by pre-incubating the sleeve for 20 min at 37 °C in Ringer solution containing 0.5 mM of the SGLT1 inhibitor phlorizin, which at that concentration and at the glucose concentration produced in our experiments was found to inhibit glucose uptake by 99.5 %. In brush-border homogenates, we found inhibition of maltase activity by 0.5 mM phlorizin to be immeasurably small (less than 5 %).

Sleeves were incubated at 37 °C in Ringer solution containing 50 mM maltose and 0.5 mM phlorizin for 4 min. (We found glucose production to be linear with time for at least 16 min.) At the end of 4 min, two 250 μl aliquots of the incubation solution were collected for determination of glucose concentration. Each aliquot was added to a tube containing 1500 μl of Glucostat solution (250 mM Tris buffer, 200 units l^{-1} horseradish peroxidase, 10 mM *p*-hydrobenzoic acid, 0.2 mM aminoantipyrine, and 4442 units l^{-1} glucose oxidase; Sigma Chemical Co., St Louis, MO, USA), which halts maltose hydrolysis and detects glucose in the solution enzymatically. The amount of Glucostat used was double that used in our earlier sucrase and lactase studies (Lee *et al.* 1998; O'Connor & Diamond, 1999) because maltose hydrolysis produces twice as much glucose as does sucrose or lactose hydrolysis. Glucostat tubes were incubated at 37 °C for 30 min, whereupon absorbance at 500 nm was read using a Beckman DU-640 spectrophotometer. The resulting absorbance was translated into maltase activity by means of absorbance measured for glucose standards. Maltase activity, like glucose uptake activity, was normalized to milligrams wet mass of the intestinal sleeve and expressed as nanmoles of glucose produced per minute per milligram tissue. The apparent coefficient of variation of maltase activity by this method for six adjacent mid-intestinal sleeves from the same mouse was 14 %. We refer to this as an apparent coefficient of variation, because part of the variation represents a real proximal-to-distal gradient in maltase activity along the intestine (Fig. 2*B*).

Glucose production was corrected for glucose leaking from the intestinal tissue sleeve in the absence of maltose (tissue blank), and also for impurities in the maltose solution (reagent blank). We measured a tissue blank and a reagent blank for each intestinal region. The tissue and reagent blank absorbances were subtracted from the tissue sample absorbance, and that corrected absorbance was then translated to preliminary maltase activity. It was then multiplied by 1.08 to take account of the Michaelis-Menten constant for maltase (K_m = 4.0 mM) measured in our everted sleeve preparation by converting activity measured at 50 mM maltose into the V_{max} value. (That K_m value was calculated by measuring the maltose concentration dependence of maltase activity, which yielded a close fit to the Michaelis-Menten equation.) Finally, maltase capacity of the entire small intestine was calculated by taking the product, for each small intestinal region, of maltase activity per milligram tissue wet mass times regional wet mass, and summing these products over the three regions.

Morphometrics

From each killed mouse, we measured the wet masses of the stomach, small intestine, caecum, large intestine and vital organs (heart, lungs, liver, spleen and paired kidneys) after carefully removing adherent fat. We then measured their dry masses by drying them in an oven at 60 °C for at least 48 h and re-weighing. We calculated the dry mass of the whole small intestine by multiplying the dry-to-wet ratio of the partial small intestine (remainder from everted sleeve assays) times the wet mass of the whole small intestine.

Table 1. Specific activities of maltase and SGLT1

Treatment group	Maltase (nmol maltose $min^{-1} mg^{-1}$)	SGLT1 (nmol glucose $min^{-1} mg^{-1}$)
Virgin mice	19.2 ± 3.0	7.0 ± 0.5
Lactating mice	16.2 ± 1.2	6.7 ± 0.5

Specific activities of maltase and SGLT1 per milligram of small intestine, averaged over all three small intestinal segments (proximal, medial and distal). Note that maltase activity is more than double that of SGLT1, and that activities of both do not differ between virgins and lactating mice.

Statistics

We analysed our data using Student's two-tailed *t* tests and analysis of covariance (ANCOVA) with the programs Systat 7 and NCSS 97 (SPSS Science, Chicago, IL, USA and NCSS, Kaysville, UT, USA, respectively), with body mass as the covariate in certain analyses (to be mentioned) comparing virgin and lactating mice which proved to differ slightly in body mass. The $P < 0.05$ level was taken as significant. Values are reported as means ± standard error of the mean.

RESULTS

Food intake and digestibility

As expected from their greater energy requirements, the lactating mice ate much more than did virgin mice (15.4 ± 0.5 *vs.* 3.8 ± 0.2 g day^{-1}; $P < 0.001$). Although daily food intake of the lactating mice was thus four times that of virgins, there was no difference in dry-matter digestive efficiency between the two groups (83 ± 0.3 and 81 ± 0.3 % for virgin and lactating mice, respectively, $P = 0.42$).

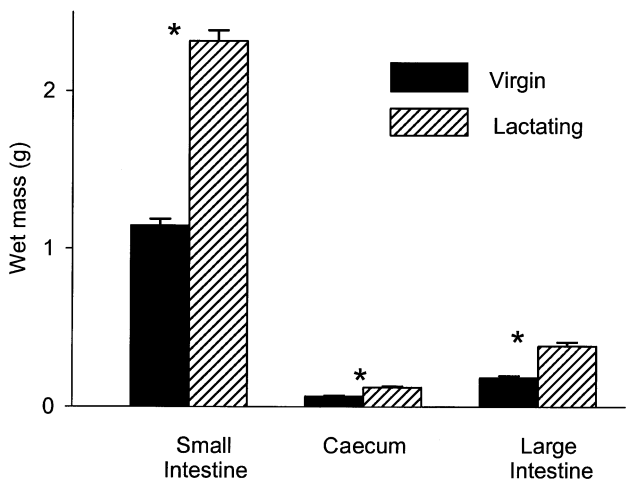

Figure 1. Morphological adaptation in the digestive organs during lactation

Wet mass of the small intestine, caecum and large intestine during two reproductive states (virgin and lactating) of Swiss-Webster mice. Here and in subsequent figures error bars denote standard error of the mean. Asterisks here and in Fig. 2 denote results differ significantly between the two reproductive states. Note the lactating mice have significantly larger ($P < 0.05$, $n = 8$) absorptive organs than virgin mice, using ANCOVA with body mass as the covariate. (The same trend is true for dry organ masses.)

Body mass and gut morphology

Lactating mice had slightly but significantly greater body mass ($P < 0.05$) than virgins of the same age (35.0 ± 0.7 *vs.* 32.6 ± 0.6 g). The wet and dry masses of the small intestine ($P < 0.001$), caecum ($P < 0.001$), and large intestine ($P < 0.001$) were also greater in lactating mice than in controls (Fig. 1). Because of the known association between body mass and these digestive structures, the latter dependent variables were compared by ANCOVA between lactating mice and virgins, using their respective body masses as the covariate. The trend remained the same after ANCOVA with significantly larger organ wet and dry masses for lactating mice ($P < 0.001$ for all variables).

For the small intestine, the morphological response to lactation involved growth in two dimensions: length, and mass per centimetre of length. Lactating mice had a 102 % increase in small intestinal wet mass over virgins. Most of that increase was due to the increase in mass per centimetre. In all three sections of the small intestine (proximal, medial and distal), lactation had a significant effect on mass per centimetre ($P < 0.001$ in all cases), which increased approximately 60 % in each of the three regions (Fig. 2*A*). In addition, the small intestine of lactating mice was 20 % longer than that of virgin mice. Both groups of mice (lactating and virgins) exhibited a positional gradient of intestinal mass along the small intestine, with mass per centimetre being highest in the proximal region and decreasing distally (Fig. 2*A*).

Specific activity of maltase and SGLT1

Regional maltase and SGLT1 activities exhibited the familiar positional gradient (Rubino & Auricchio, 1964; Levine *et al.* 1974; Toloza & Diamond, 1992), with higher activities in the proximal and medial regions than the distal region (Fig. 2*B*). Within a given intestinal region, there were no significant differences between lactating and virgin mice for either maltase activity ($P = 0.90$, 0.25 and 0.37, for proximal, medial and distal, respectively) or SGLT1 activity ($P = 0.66$, 0.45 and 0.20, for proximal, medial and distal, respectively).

Mean maltase and SGLT1 activities, averaged over the three regions, were calculated for each mouse and compared with each other. Maltase activity proved to be about 2.5 times that of SGLT1 both in lactating mice and in virgins (Table 1). Because maltase activity yields two glucose molecules for each maltose substrate molecule, the results mean that intestinal maltase can produce five times as much glucose as SGLT1 could transport.

Load and capacity of maltase and SGLT1

The loads upon maltase and SGLT1 consist of maltose and glucose, respectively. Assuming that maltose becomes

Table 2. Loads, capacities and safety factors of maltase and SGLT1

	Load (mmol day^{-1})		Capacity (mmol substrate day^{-1})		Safety factors (Capacity *vs* load)	
Treatment group	Maltose	Glucose	Maltase	SGLT1	Maltase	SGLT1
Virgin mice	5.8 ± 0.4	11.6 ± 0.7	33 ± 4	12.5 ± 1.0	6.5 ± 0.8	1.1 ± 0.1
Lactating mice	24 ± 1	47 ± 2	57 ± 5	24 ± 2	2.4 ± 0.1	0.5 ± 0.04

Note that, from virgins to lactating mice, for both maltase and SGLT1, loads increase 4-fold but capacities increase only 2-fold, so that safety factors decline 2-fold.

completely hydrolysed to glucose, the 4-fold greater daily food intake of lactating mice than of virgins means that the maltose and glucose loads upon maltase and SGLT1 were also 4-fold greater in lactating mice (Fig. 3).

Maltase and SGLT1 capacities, the integrated products of regional activities times intestinal mass over the whole length of the small intestine, both increased significantly ($P < 0.05$ for maltase, $P < 0.001$ for SGLT1) in lactating mice compared with virgins (Table 2). This was due entirely to increased small intestine mass, since neither maltase nor SGLT1 activity changed significantly with lactation (Fig. 2). However, those increases in maltase capacity (1.7-fold) and SGLT1 capacity (1.9-fold) with lactation were by smaller factors than the 4-fold increase in load, so that safety factors decreased during lactation (Table 2).

In both virgin and lactating mice the capacities of maltase and SGLT1 in series were not equal to each other. Maltase capacity exceeded SGLT1 capacity by factors of 5.2 and 4.7 in virgin and lactating mice, respectively (Table 2 and Fig. 3).

The safety factor for maltase (6.5 ± 0.8 in virgins, 2.4 ± 0.1 in lactating mice) was significantly greater than 1.0 in both groups of mice ($P < 0.05$ for both; Table 2). Because of that maltase safety factor in the virgins, the 4-fold increase of maltose load during lactation did not overwhelm the maltase capacity (Fig. 3), even though that capacity increased by only 1.7-fold. In contrast, for SGLT1 the virgin group started off with no reserve capacity and a safety factor of 1.1 ± 0.1 (not significantly different from 1.0; $P = 0.38$). Therefore, even with the 1.9-fold increase in SGLT1 capacity during lactation, that capacity could not keep up

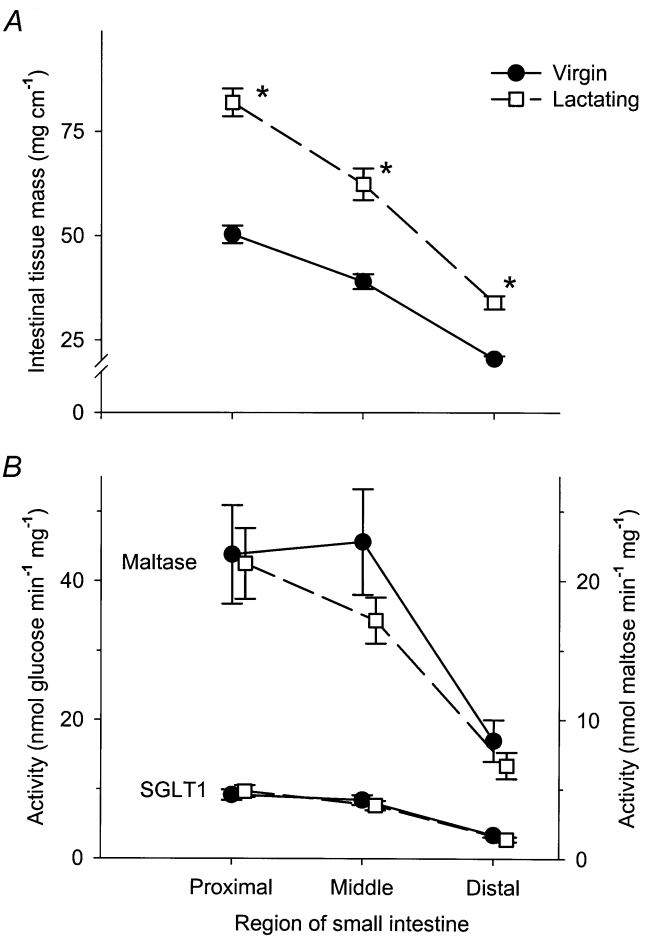

Figure 2. Morphological and functional response to lactation along the small intestine

Gradients of intestinal tissue mass (*A*) and brush-border maltase and glucose transporter (SGLT1) activities (*B*) along the small intestine of virgin and lactating mice. Ordinate units in *B* are nanomoles of glucose taken up by SGLT1 per minute per milligram of intestinal wet mass (left axis) and nanomoles of maltose hydrolysed by maltase per minute per milligram of wet intestinal mass (right axis). Data are means ($n = 8$ per treatment) ± S.E.M. Note that, in all three sections (proximal, medial and distal) of the small intestine, mass is significantly greater ($P < 0.05$) in the lactating than the virgin group (*A*), but that activities of both maltase and SGLT1 do not differ significantly ($P < 0.05$) between the two treatment groups (*B*). Because capacity = intestinal mass × protein activity, it is the increase of intestinal mass that accounts entirely for the increase of maltase and SGLT1 capacities during lactation (as seen in Fig. 3).

with the 4-fold increase in glucose load, which yielded a safety factor of 0.5 ± 0.04 (significantly less than 1.0; $P < 0.001$).

DISCUSSION

Our laboratory has now measured safety factors for three series systems of hydrolase–SGLT1 pairs, each under a range of loads: lactase in rat pups at three developmental ages, sucrase in adult mice at three dietary loads, and now maltase in adult mice at two dietary loads. The three sets of results agree in that all hydrolase safety factors fall in the range 1–13 observed for other biological and engineered systems. The maltase safety factor that we measured in virgin mice (6.5) agrees with a previous estimate of 7 for maltase in chickens (Biviano *et al.* 1993).

A synthesis of our understanding of hydrolase–SGLT1 safety factors at this stage must necessarily be tentative because of the few systems studied, only two of them in the same species. We shall discuss six issues: adaptive regulation, safety factor changes with load, values of safety factors, series matching, paradoxically low safety factors and diet-dependent changes in SGLT1 activity.

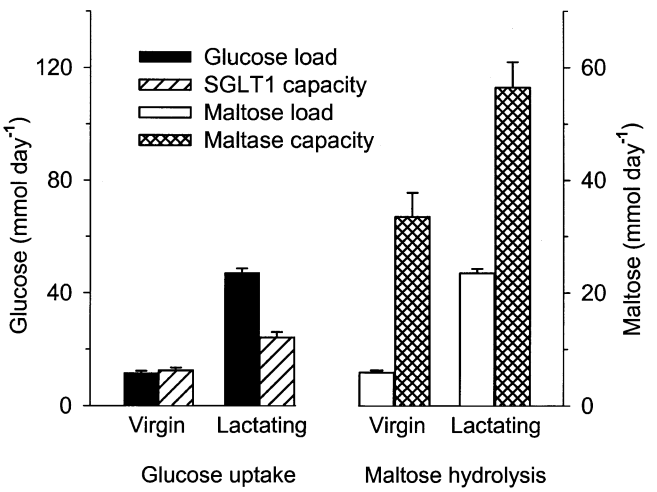

Figure 3. Load and capacity of maltase and SGLT1 at different caloric requirements

Comparisons of maltase capacity, SGLT1 capacity and their respective functional loads of maltose (in the direct form of dietary maltose) and glucose (after hydrolysis of dietary maltose by maltase). Dietary maltose load (mmol day^{-1}) equals the product of dietary intake (g day^{-1}) times dietary maltose concentration (mmol g^{-1}); glucose load upon SGLT1 = 2 times the dietary maltose load; capacity = intestinal mass (mg) times protein activity (mmol day^{-1} mg^{-1}). Glucose load on SGLT1 (left axis) is double the disaccharide load (right axis) on maltase because each maltose potentially yields two glucose molecules for transport by SGLT1. Note that, in each reproductive state (virgin and lactating) the maltase capacity considerably exceeds the SGLT1 capacity. Note also that maltase capacity increases by a smaller factor from virgin to lactating mice than does maltose load, and that SGLT1 capacity increases by a smaller factor than does glucose load, so that safety factors of both proteins decrease during lactation.

Adaptive regulation

All three pairs prove to be adaptively regulated: capacities of both hydrolase and SGLT1 increase with increasing carbohydrate load, no matter by what means that load increase is produced. In the one case where substrate load was increased by changing dietary substrate content (shifting from a no-carbohydrate to a sucrose-containing diet), the mechanism of the capacity increase proved to be an increase in activity per milligram of intestinal tissue for both the hydrolase and the transporter, without a change in intestinal mass (Weiss *et al.* 1998). In the remaining cases, where substrate load was increased by an increase in the animal's caloric requirements without any change in diet, the mechanism of the capacity increase proved instead to be an increase in intestinal mass without a change in hydrolase or transporter activity, regardless of whether the increase in caloric requirements came about as a result of pregnancy, lactation, cold ambient temperature, or growth; that conclusion applies to all three hydrolases. These findings fit with many previous studies demonstrating two types of adaptive regulation for intestinal membrane proteins: an increase in specific substrate load without a change in caloric requirements causes up- and down-regulation of specific transporters and hydrolases, whereas a change in caloric requirements causes a trophic response by the intestine (Karasov & Diamond, 1983*b*; Ferraris & Diamond, 1989).

Safety factor changes with load

For the adaptive regulation of all three hydrolases by caloric requirements, and for all four modes of change in caloric requirements, the increase of capacity of both the hydrolase and SGLT1 with load is sublinear, such that safety factors decrease towards 1.0 with increasing load (see also Fig 3. of Diamond, 2002). On reflection, of course, this finding was to be expected: the potential scope for upregulating biological systems cannot be infinite, and capacities must eventually become limiting.

Values of safety factors

Why should an animal go to the biosynthetic expense of maintaining reserve capacities likely to languish unutilized? For both biological and engineered systems, the reason is unpredictability of both loads and capacities. Biological safety factors are observed to be higher, and engineered safety factors are consciously designed to be higher, for components with more unpredictable loads or capacities, higher penalties for failure and lower costs of synthesis or maintenance (Alexander, 1981; Buddington & Diamond, 1989; Diamond, 1993; Alexander, 1997). Unpredictability of load is obvious for our hydrolase–SGLT1 systems: a wild mouse cannot 'know' what will be the carbohydrate content of tomorrow's meal, how much food will be available to it tomorrow, or how cold it will be tomorrow and hence how

much food the mouse will have to eat in order to stay warm. The severe malabsorption and osmotic diarrhoea observed in humans with congenital lack of sucrase–isomaltase illustrate the penalties that result from digestive loads exceeding capacities.

Among our three hydrolases, the ones with the two highest safety factors are lactase and maltase, whose loads are especially unpredictable because these two hydrolases actually act on many substrates. In addition, in the case of lactase, among its multiple substrates (lactose and glycoproteins and glycolipids) the last-named inhibit lactose hydrolysis (Büller *et al.* 1989), such that lactase's capacity for hydrolysing lactose will be unpredictably depressed depending on the glycolipid content of tomorrow's milk. Furthermore, a pup cannot 'know' how much milk its mother will be able to deliver tomorrow. These considerations mandate what at first would appear to be a wastefully high reserve capacity for lactase.

In the case of maltase, whereas sucrase and lactase activities each mainly reside at one enzyme active site, what we call maltase activity actually resides at three active sites on two heterodimer proteins (Sörensen *et al.* 1982; Nören *et al.* 1986; Gayland, 1989). Of the total activity, 80 % comes from the sucrase–isomaltase (SI) complex, one of whose two subunits (termed 'sucrase') splits sucrose plus maltose, while the other subunit (termed 'isomaltase') splits maltose plus 1,6-glycopyranose bonds of various oligosaccharides. The remaining 20 % of maltase activity comes from the glucoamylase complex, both of whose subunits have similar affinities and split maltose and starch. Thus, maltose hydrolysis is not the sole, and possibly not even the principal, function of the sites responsible for maltase activity. 'Maltase capacity' may have actually evolved to respond mainly to an animal's requirements for splitting starch, not maltose. What may appear to be unnecessarily high capacity for maltose hydrolysis may be a by-product of starch splitting needs. We predict that this phenomenon of apparent overcapacity for one biological function will prove widespread for biological components with multiple functions: it is impossible to maintain 'ideal' safety factors for all of those functions simultaneously.

Series matching

For the two reasons explained in the introduction (avoiding rate-limitation by low-capacity steps, and avoiding unutilized waste capacities of high-capacity steps), one's simplest expectation is for natural selection to have led to approximately equal capacities of steps operating in series. This simple expectation is confirmed for sucrase and SGLT1 in virgin mice consuming high-sucrose diets: hydrolase capacity and transporter capacity differ from each other by less than 10 % (see safety factors in Table 1 of ref. Weiss *et al.* 1998). However, capacities are not matched for sucrase and SGLT1 in lactating mother mice, nor for maltase and SGLT1 in maltose-consuming mice, nor for lactase and SGLT1 in rat pups under any condition studied. The main reason in the latter two cases is presumably the conflicting demands imposed by multiple substrates, as discussed in the preceding paragraph.

Paradoxically low safety factors

The safety factor for mice consuming a maltose diet is 1.1 in virgins but only 0.5 during lactation. That is, the small intestine of lactating mice is capable of absorbing by carrier-mediated transport only half of the dietary glucose ingested in the form of maltose. Yet apparent dry-matter digestive efficiency is as high (81 %) during lactation as in virgins. How can that be? Why does digestive efficiency not drop to 50 % during lactation?

Probably the main reason is that glucose digestion can involve not only glucose transport in the small intestine but also glucose fermentation to volatile fatty acids in the hindgut. The hindguts of rats and rabbits are known to ferment any simple carbohydrates remaining unabsorbed after the small intestine, as well as dietary fibre (Carter *et al.* 1981; Buddington & Diamond, 1990; O'Connor & Diamond, 1999). The doubling of mass that we observed for both mouse caecum and mouse large intestine during lactation suggests that that same finding about hindgut fermentation may apply to mice. SGLT1 safety factors below 1.0 were not observed in our laboratory's previous studies of mice consuming a sucrose diet because that diet yields higher SGLT1 activity and lower glucose loads than does the maltose diet used in the present study (see next paragraph). In principle, passive glucose uptake by the small intestine could also contribute to the 'missing' glucose digestive capacity not explained by SGLT1, but our measurements of passive glucose uptake by means of L-[^{3}H]glucose show its contribution to be quantitatively minor.

Diet-dependent changes in SGLT1 activity

For carbohydrate diets presenting equal disaccharide loads, the glucose load for a maltose-based diet is double that for a sucrose-based diet, because maltose is split to two glucose units but sucrose is split to one glucose plus one fructose. We therefore expected SGLT1 activity to be higher for mice consuming a maltose-based diet than for mice consuming a sucrose-based diet, but the reverse proved to be true, both in virgin mice and during lactation: SGLT1 activity is 43 % higher on the sucrose-based diet (Weiss *et al.* 1998).

We do not have an explanation for this puzzle. A possible suggestion involves the many paradoxes noted previously among signals for upregulation: an enzyme's or transporter's preferred substrate is not necessarily the most potent inducing signal for its upregulation. Many examples have been noted for SGLT1 and for amino acid transporters (Solberg & Diamond, 1987; Stein *et al.* 1987). For example, in mouse intestine the acidic amino acid aspartate is the best inducer of the basic amino acid transporter, conversely

the basic amino acid arginine is a good inducer of the acidic amino acid transporter, and the ketohexose fructose is more potent than aldohexoses at inducing the aldohexose transporter SGLT1. Our results similarly suggest that sucrose is paradoxically a more potent inducer of SGLT1 than is maltose. This paradox is one of the many puzzles about load–capacity relations requiring further study.

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