DECREASED SUCRASE AND LACTASE ACTIVITY IN IRON DEFICIENCY IS ACCOMPANIED BY REDUCED GENE EXPRESSION AND UPREGULATION OF THE TRANSCRIPTIONAL REPRESSOR PDX-1

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Short Title: Disaccharidase Activity & Expression in Iron Deficiency

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Abstract

The disaccharidases are important digestive enzymes, whose activities can be reduced by iron deficiency. We hypothesise that this is due to reduced gene expression; either by impairment to enterocyte differentiation, or by iron-sensitive mechanisms that regulate mRNA levels in enterocytes.

Iron deficient Wistar rats were generated by dietary means. The enzyme activities and kinetics of sucrase and lactase were tested, as well as the activity of alkaline phosphatase (IAP-II) since it is unrelated to carbohydrate digestion. mRNA levels of β-actin, sucrase, lactase, and the associated transcription factors PDX-1, CDX-2, GATA-4 and HNF-1 were measured by real-time PCR. Spatial patterns of protein and gene expression were assessed by immunofluorescence and in situ hybridisation respectively.

It was found that iron deficient rats had significantly lower sucrase (19.5% lower) and lactase (56.8% lower), but not IAP-II activity than control rats. Kinetic properties of both enzymes remained unchanged from controls, suggesting a decrease in the quantity of enzyme present. Sucrease and lactase mRNA levels were reduced by 44.5% and 67.9% respectively by iron deficiency, suggesting enzyme activity is controlled primarily by gene expression. Iron deficiency did not affect the pattern of protein and gene expression along the crypt to villus axis. Expression of PDX-1, a repressor of sucrase and lactase promoters, was 4.5 fold higher in iron deficiency while CDX-2, GATA-4 and HNF-1 levels were not significantly different.

The data suggest that decreases in sucrase and lactase activities result from reduction in gene expression, following from increased levels of the transcriptional repressor PDX-1.
Keywords
Anemia, Sucrase, Lactase, Transcription, Nutrition

Introduction
Carbohydrates are a vitally important dietary component, and the disaccharidases are an essential subset of digestive enzymes required for the terminal step of carbohydrate digestion (13). The enzymatic activity of each individual disaccharidase is influenced primarily by the dietary level of their corresponding substrate (2, 12), and under normal conditions this level of activity is controlled by the abundance of the mRNA encoding the proteins (12, 34). However, it has been shown on many occasions that dietary iron deficiency can cause a significant decrease in disaccharidase activity (2, 4, 5, 9, 16, 20, 31, 38), and that this decrease is reversible by iron supplementation (9, 20, 38).

It is unlikely that iron is directly required for disaccharidase activity (16), or that iron deficiency alters the kinetic properties of sucrase (5). In addition, it has been shown that many other functions of the intestinal mucosa are adversely affected by iron deficiency, including a significant decrease in the secretory component (4, 5), a decrease in the specific activity of diamine oxidase (4, 5), and an apparent inability to manufacture membrane-bound high molecular weight glycoproteins (20). As such, most hypotheses explaining the mechanisms of the relationship between iron status and disaccharidase activity focus on an impaired ability to manufacture all brush border glycoproteins, either at the level of initial synthesis, post-translational modification, or trafficking to the brush border membrane (4, 5, 9, 20, 38).

However, many other important characteristics of the small intestine do not change during iron deficiency suggesting that enterocyte function is not impaired to such a large degree. Most importantly, cell proliferation requires iron (21) yet intestinal morphology is rarely different from
normal, even under severe iron deficiency (4, 9, 16, 30). More specifically, the DNA synthesis rate of intestinal enterocytes is not affected by iron deficiency (4, 27), nor is the proportion of mitotic cells in the mucosa (9). Finally, while the activities of some enzymes in the mucosa are reduced, the activities of folate conjugase (16) and lysosomal β-galactosidase (4) remain unchanged.

Based on the available data, we hypothesise that the decrease in disaccharidase activity seen during iron deficiency is not caused by reduced ability to synthesise membrane bound glycoproteins per se, but rather due to an alteration of the expression of the genes encoding the enzymes. Since sucrase and lactase mRNA expression and the presence of enzymatically active protein are considered to be markers for differentiated enterocytes (10, 36), it is possible that iron status alters the normal pattern of differentiation as the cells migrate to the villus tip, or that a specific regulatory mechanism sensitive to the effects of iron deficiency controls the abundance of the disaccharidase mRNA in each individual differentiated enterocyte.

To test our hypothesis, iron deficient and control conditions were established by dietary means in Wistar rats. The enzymatic activities of two disaccharidases, sucrase-isomaltase (sucrase) and lactase phlorizin hydrolase (lactase), were tested for both conditions, as well as the activity of intestinal alkaline phosphatase II (IAP-II) since it is an inducible brush border membrane glycoprotein unrelated to carbohydrate digestion (23, 41). The kinetic properties of both sucrase and lactase were also examined in order to confirm that any decreases result from lower quantities of enzyme present, rather than due to reduced enzyme efficiencies. The location of sucrase protein and gene expression along the crypt to villus axis was observed by immunofluorescence and in situ hybridisation respectively, so as to determine any changes in the normal pattern of enterocyte differentiation. Semi-quantitative real time PCR was performed to determine sucrase and lactase mRNA levels and assess whether decreases in enzyme
quantity are due to an inability to manufacture membrane bound glycoproteins, or attributable to mechanisms that specifically control levels of disaccharidase gene expression. Finally, mRNA levels for activators of sucrase and lactase gene expression caudal related homeobox 2 (CDX-2) protein, GATA binding protein 4 (GATA-4) and hepatocyte nuclear factor (HNF-1) (3, 26, 37) and the repressor pancreatic duodenal homeobox 1 (PDX-1) protein (15, 39) were measured to gain insight into possible mechanisms by which any alterations in gene expression may be regulated by iron deficiency.
Methods

Animals

The Animal Ethics Committee at the University of Western Australia approved all procedures concerning the use of animals in this study. Male Wistar rats were born to normal timed-pregnant mothers, and weaned onto two different synthetic diets (iron deficient and control) at 21 days of age. Both diets contained 42% starch, 22% sucrose, 20% protein, 6% fat, 3% supplementary cellulose, 5% supplementary minerals and 2% supplementary vitamins as recommended by the American Institute of Nutrition (AIN-93). The iron deficient diet had no iron added, whereas the control diet was supplemented with ferrous ammonium sulphate. Iron content of the diets was approximately 10mg/kg and 80mg/kg respectively. All rats were maintained on this diet *ad libitum* with free access to distilled water for an additional 5 weeks.

Assessment of conditions

All rats were fasted overnight before testing, but still allowed free access to distilled water. Blood was collected from animals anaesthetised with 60mg/kg sodium pentobarbital (Nembutal) by drawing directly from the left ventricle. Samples were tested for hemoglobin (Hb) content (measured by a freshly calibrated Hemocue Hemoglobin meter (Helsinborg, Sweden)) and hematocrit (Ht). Iron deficiency was considered sufficient for this study if hemoglobin and hematocrit values were less than 65% of those measured for the control group.

Mucosa Collection

Preliminary studies indicate that sucrase and lactase activities and expression in iron deficient and control animals are highest in the proximal jejunum, and that in all cases samples taken from this region closely correlate to whole intestine activity and expression (data not shown). As such, enzyme activities were obtained from the whole length of the intestine, whereas histological samples and gene expression data were obtained from the proximal jejunum.
The entire length of the small intestine was carefully removed from the pylorus to the ileo-caecal junction, and placed on paper towels soaked with isotonic saline over ice. The lumen of the intestine was washed out with 50 ml of ice-cold saline and a small section from the proximal jejunum was removed and set aside for RNA isolation. The serosal surface was then patted dry, weighed, opened along its length, and the mucosa scraped off with a glass side. Care was taken to use the same amount of force along the entire length of the intestine, and for each rat. The mucosa was then added to 50 ml of ice-cold saline and placed on ice, while the remaining serosal tissue was weighed to allow for calculation of wet mucosa mass. The mucosa was homogenised for 20 seconds at medium speed in a Heidolph Diax 600 homogenizer, then stored at –20°C.

**Enzyme Activities**

Disaccharidase activity was assessed using the Dahlqvist method (7), with modifications. The assay system was confirmed to be linear with enzyme concentration.

Twenty five microliters of appropriately diluted mucosal homogenate was added in duplicate to 25µl of substrate solution containing 100mM maleate buffer pH 6.0, 56mM sucrose or lactose and 1% v/v toluene in a microtitre plate, sealed to prevent evaporation and incubated at 37°C for 60 minutes. Three hundred microliters of Tris glucose oxidase reagent containing 33 units/ml glucose oxidase, 500mM Tris-HCl pH 7.0, 0.01 mg/ml horseradish peroxidase, 0.1 mg/ml o-dianisidine and 0.2% v/v Triton X-100 was added to halt the reaction, and commence visualisation of produced glucose. The plate was sealed, and incubated at 37°C for a further 60 minutes for color development. Absorbance of the plate was read immediately at 415nm, and enzyme activity calculated from the quantity of glucose produced, after taking into account the dilution factor used. One unit was defined as the ability to generate 1µmol of glucose per minute,
and results expressed as units per gram of wet mucosa mass. Wet mucosa mass was used to calculate specific activity rather than protein content, as protein concentration in the mucosa does not significantly change during iron deficiency (4, 5).

A subset of the disaccharidase assays were repeated using 8 different concentrations of sucrose and lactose substrate solution diluted in 100mM maleate buffer. A Lineweaver-Burk plot was constructed, and the $K_m$ value calculated from the equation of the line.

IAP-II activity was determined by the method of Weiser (40). The assay system was confirmed to be linear with enzyme concentration. In brief, 10µl of diluted mucosal homogenate was added in duplicate to 200µl of substrate solution containing 500mM Tris-HCl pH 9.4, 10mM MgCl$_2$, 0.2mM ZnCl$_2$ and 0.23mM di-tris p-nitrophenylphosphate in a microtitre plate. The plate was then incubated at 37°C for 15 minutes, and the reaction stopped by the addition of 100µl of 500mM NaOH. Absorbance was measured at 415nm, and activity calculated from the quantity of nitrophenol produced, after the dilution factor had been taken into account. One unit was defined as the ability to generate 1µmol of nitrophenol per minute, and results expressed as units per gram of wet mucosa mass.

**Immunofluorescence**

Tissue segments approximately 0.5cm in length were removed from the proximal jejunum of a separate sub-group of iron deficient and control rats and immediately frozen in liquid nitrogen cooled isopentane and stored at –80°C. Frozen sections between 8µm to 10µm thick were cut onto positively charged slides, air dried for 20 minutes then fixed in 4% formaldehyde in PBS for 5 minutes. Fixative was washed off with 3x five minute washes with PBS.
Monoclonal antibodies against rat sucrase and lactase (BBC 1/35 and YBB 2/61 respectively) were obtained as a gift from Dr Andrea Quaroni (29) and used as primary antibodies at a 1:250 dilution in PBS with 0.2% saponin. Samples were incubated with the primary antibody for 1 hour, before unbound antibody was removed by 3x five minute washes with PBS. An anti-mouse IgG-FITC conjugate was used as the secondary antibody at a dilution of 1:250 in PBS with 0.2% saponin and incubated with the sample for 30 minutes, before removing unbound antibody with 3x five minute washes with PBS. Propidium iodide at a concentration of 0.05µg/ml was added as a nuclear stain before adding a single drop of anti-fade mounting media and cover-slipping the samples. Slides were then viewed on a Biorad MRC1000 laser scanning confocal microscope.

Laser scanning confocal microscopy was not used as a quantitative measure of immunoreactive sucrase and lactase protein. Western blots were considered for this purpose, however the antibodies used are unable to detect denatured protein.

**Generation of Radio-labelled Probes**

Rat sucrase-isomaltase partial cDNA was obtained as a gift from Dr Susan Henning (6). Riboprobes were produced from the cDNA template using the Promega Riboprobe® Combination Kit (SP6/T7), using \[^{35}S\] UTP (Amersham) as the radiolabel. The sucrase anti-sense probe was generated using T7 RNA polymerase. Probe specificity was confirmed by Northern blot analysis, with hybridisation to a single mRNA species at the correct weight range (data not shown).

**In Situ Hybridisation**

*In situ* hybridisation was performed as described previously (27). In brief, tissue from the proximal jejunum of the same rats used for immunofluorescence experiments was fixed in 4% paraformaldehyde and wax sections cut onto silanated slides. One million dpm of sucrase anti-
sense riboprobe was applied to each slide, and hybridised for 24 hours at 55°C. Slides were then washed in 50% formamide buffer for 4 hours, before washing for 30 minutes in 2x SSC, and then 30 minutes in 0.5x SSC at 65°C. The slides were then exposed to photographic emulsion (Amersham) for 60 hours before development of the signal. Sections were counter-stained with hematoxylin and eosin before being photographed on an Olympus BH-2 microscope fitted with a Sony DFW-SX900 digital video camera.

**RNA Isolation and Generation of cDNA**

RNA was obtained from mucosa in the proximal jejunum of the same rats used for enzyme activities. Approximately 100mg of tissue was homogenised in 1 ml of RNA-Wiz™ (Ambion) and isolated as per the manufacturer’s instructions. Concentration and purity of the RNA was assessed by spectrophotometry. Ten micrograms of RNA was then treated with the Ambion DNA-Free™ kit to remove genomic DNA. Aliquots of DNA-free RNA were reverse transcribed with AMV-RT and oligo(dT)$_{15}$ primers according to the manufacturers instructions (Promega). The cDNA products were then purified with the UltraClean PCR Cleanup Kit (MoBio) and stored at –20°C for real-time PCR.

**Real-Time PCR**

mRNA levels were determined by semi-quantitative real-time PCR on a Lightcycler instrument (Roche). The PCR reaction mix contained cDNA from an equivalent of 66ng RNA, 0.55µM of appropriate forward and reverse primers, 500µg/mL BSA, 1x Platinum SYBR Green qPCR SuperMix UDG (Invitrogen) and additional MgCl$_2$ (final concentration 3.5mM Mg$^{2+}$) in a volume of 20µL. The PCR programme involved two minutes at 50°C for UDG PCR carryover decontamination, followed by initial denaturation at 94°C for 2.5 minutes then 50 amplification cycles of denaturing at 94°C for 30 seconds, annealing for 30 seconds, and extension for 60 seconds at 72°C. A touchdown method was used for annealing temperatures, decreasing from
70°C to 56°C over 15 cycles. Primers were selected using Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/prime3/prime3_www.cgi) and the primer sequences used can be seen in table 1. Primer specificity was confirmed initially by PCR in a standard thermocycler and sequencing the products using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) at Royal Perth Hospital, Perth, Western Australia. The consistency of target sequence produced by each sample in the Lightcycler was assessed by melting curve analysis and agarose gel electrophoresis where required.

Table 1. Primer sequences used for real-time PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Accession Number</th>
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<tbody>
<tr>
<td>β-actin</td>
<td>TGTACCAAACCTGGGACGATA</td>
<td>ACCCTCATAGATGGCAGACAG</td>
<td>NM_031144 (306-585)</td>
</tr>
<tr>
<td>Sucrase</td>
<td>ATCATCCCTACCCAGGAACC</td>
<td>GCTGGTCATTTTCACCCACT</td>
<td>NM_013061 (2445-2751)</td>
</tr>
<tr>
<td>Lactase</td>
<td>AGGGATATTTCCCCCAAGTG</td>
<td>CCACAGTCCATTTTCATGACG</td>
<td>X56747 (3217-3506)</td>
</tr>
<tr>
<td>PDX-1</td>
<td>GGCTTAACCTAAACGGCCACA</td>
<td>GGGACGCTCAAGTCTTGAA</td>
<td>NM_022852 (1042-1288)</td>
</tr>
<tr>
<td>CDX-2</td>
<td>GAGCAACGGACTTGAGAAA</td>
<td>GAAAGCTTGGTGTGCTGTCAG</td>
<td>NM_023963 (1098-1344)</td>
</tr>
<tr>
<td>HNF-1</td>
<td>CTGTACCAACCAACCGGCTG</td>
<td>GAGGACCTGCTGGGACTGTT</td>
<td>X54423 (1501-1769)</td>
</tr>
<tr>
<td>GATA-4</td>
<td>TCTCAGCTATGGGCACAG</td>
<td>CGAGCAGGATTTTGAGGAGG</td>
<td>NM_144730 (1675-1918)</td>
</tr>
</tbody>
</table>

Relative levels of transcript were assessed by the method of Gentle et al (11) from the crossing point, with fold-changes calculated relative to the first sample collected. Crossing point data was obtained using the second derivative maximum method from the Lightcycler 5.32 analysis software. An amplification efficiency value of 2 was used for all genes. Data is expressed as the fold-change per unit β-actin in order to account for any variations in reverse transcriptase efficiency or RNA loading, and normalised such that the condition with the highest expression for a particular gene is assigned a mean value of 1.

**Data and Statistics**

All data are expressed as mean ± standard error. The Analyse-It™ General v1.71 software package was used for all computational statistics. One-way ANOVA was used to compare groups, with p < 0.05 considered significant.
Results

Assessment of Conditions

Body mass, mucosa mass and blood properties can be seen in table 2. The iron deficient condition in Wistar rats was confirmed by a large significant decrease in hemoglobin and hematocrit compared with control rats.

<table>
<thead>
<tr>
<th>Table 2. Measured properties used for the confirmation of anemia in iron deficient rats. * Denotes a significant difference between the iron deficient and control conditions.</th>
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</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>----</td>
</tr>
<tr>
<td>Body mass (g)</td>
</tr>
<tr>
<td>Mucosa mass (g)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
</tr>
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Enzyme Activities

Table 3 shows the measured enzymatic activities of sucrase, lactase and IAP-II and the associated $K_m$ value for sucrase and lactase. Iron deficient rats exhibited significantly lower sucrase and lactase activity than control rats (19.5% and 56.8% lower respectively), whereas there was no observable difference in IAP-II activity. The $K_m$ value was not significantly different between conditions for either enzyme.

<table>
<thead>
<tr>
<th>Table 3. Enzyme Activities and $K_m$ values recorded for both conditions. N values for $K_m$ values are present in brackets. * Denotes a significant difference between the iron deficient and control conditions.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>----</td>
</tr>
<tr>
<td>Sucrese Activity (U/g)</td>
</tr>
<tr>
<td>Lactase Activity (U/g)</td>
</tr>
<tr>
<td>IAP-II activity (U/g)</td>
</tr>
<tr>
<td>Sucrese $K_m$ (mM)</td>
</tr>
<tr>
<td>Lactase $K_m$ (mM)</td>
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</table>

Sucrase and lactase activity were significantly decreased in the iron deficient condition. IAP-II activity and $K_m$ values for sucrase and lactase were unaffected.
**Immunofluorescence**

There were no apparent differences in the pattern of distribution of immunoreactive sucrase protein between either of the conditions. Sucrase expression was confined exclusively to the microvillus membrane, becoming apparent at the crypt-villus junction. There was strong expression along the entire length of the villus, with diminishing levels present in enterocytes at the villus tip. Lactase followed a similar pattern, but typically still had stronger staining present on the villus tip than for sucrase. Figure 1 shows images obtained for sucrase and lactase for both conditions.

**In Situ Hybridisation**

There were no apparent differences in the pattern of expression of sucrase mRNA between iron deficient and control conditions (data not shown). In both groups, sucrase mRNA was primarily present in the apical cytoplasm of enterocytes, commencing at the crypt-villus junction and continuing along most of the length of the villus. Expression was typically absent at the villus tip.

**Gene Expression**

Semi-quantitative real-time PCR results can be seen in Figure 2. β-actin expression was not significantly different between the two conditions, validating the use of this gene as a measure of baseline cell function with respect to mRNA content in iron deficiency. Control rats exhibited significantly higher levels of both sucrase (1.8 fold) and lactase (3.1 fold) mRNA than iron deficient rats, whereas PDX-1 expression was 4.5 times higher in iron deficient rats. No significant change was observed for CDX-2, GATA-4 or HNF-1.
Figure 1. Immunofluorescence images showing sucrase and lactase protein expression. Green fluorescence indicates immunoreactive protein. Red fluorescence is the nuclear stain. A: iron deficient sucrase. B: control sucrase. C: iron deficient lactase. D: control lactase. In both control and iron deficient rats sucrase and lactase protein was present primarily on the apical membrane of enterocytes from the crypt-villus junction and along the length of the villus. Sucrase protein was typically absent on the villus tip.

Figure 2. Relative gene expression data for both conditions. * Denotes significance at p < 0.05, ** denotes significance at p < 0.01. Sucrase and lactase mRNA levels were significantly decreased in the iron deficient condition. Conversely, PDX-1 mRNA was significantly elevated by iron deficiency.
Discussion

In agreement with previous studies, it was observed that iron deficient Wistar rats have significantly lower sucrase and lactase activities when compared with their control littermates (2, 4, 5, 9, 16, 20, 31, 38). This decreased activity cannot be attributable to changes in the kinetic properties of the enzymes as evidenced by no significant differences in the $K_m$ values, inferring that it is primarily due to reduced quantities of enzyme present in the mucosa. In order to determine whether this change was specific for the disaccharidases, IAP-II activity was also measured since it is an inducible membrane bound glycoprotein with a function unrelated to carbohydrate metabolism (23, 41). Since IAP-II activity did not differ between the two conditions, this suggests that the decrease in sucrase and lactase activity is not due to an impaired ability of the iron deficient enterocyte to manufacture brush border membrane glycoproteins as previously thought (4, 5, 9, 20, 38).

Based on the decreased body mass of iron deficient animals, alterations in sucrase and lactase activity may be attributable to decreased voluntary food intake in iron deficient rats compared with control animals. Therefore, the use of a pair-fed control group should be considered. However, previous studies have reported significant reductions in disaccharidase activities at times when food intake of iron deficient rats was identical to controls, and alterations in body mass were not yet apparent (4, 5). In addition, it has been reported that protein deficient diets increase disaccharidase activity, irrespective of iron status (31). As such, it seems that marginal macronutrient depletion play little part in determining disaccharidase activity when compared to the effects of iron deficiency, thus pair-fed control experiments were not pursued.

To further examine the nature of the decrease in sucrase and lactase activities, the levels of jejunal gene expression were measured by performing semi-quantitative real-time PCR for
sucrase and lactase mRNA. Since results from these experiments show that iron deficient rats also express the lowest levels of sucrase and lactase mRNA, it seems unlikely that alterations in disaccharidase activity in Wistar rats are caused by an inability to synthesise the enzymes, but rather due to changes in gene expression. The lack of alteration in β-actin expression suggests that iron deficiency induced this change through a specific and selective mechanism (see below).

To investigate potential mechanisms for the observed reductions in gene expression, we explored the possibility that sucrase and lactase protein expression may be restricted to a select region of the villus due to an alteration in enterocyte differentiation caused by iron deficiency. We investigated this possibility by performing confocal immunofluorescence microscopy for immunoreactive sucrase and lactase protein. The spatial pattern of sucrase and lactase protein expression observed along the crypt to villus axis is in full agreement with previous studies (6, 12, 33), but more importantly there does not appear to be any change resultant from iron status. Specifically, in both conditions protein expression becomes apparent in enterocytes at the crypt-villus junction and along the length of the villus, with sucrase levels diminishing at the villus tip. Furthermore, the presence of sucrase and lactase protein exclusively on the microvillus membrane suggests that iron deficiency does not impair the post-translational modification or targeting of the proteins.

The pattern of sucrase gene expression was also assessed by in situ hybridisation. Consistent with immunofluorescence experiments, there was no discernable difference between the iron deficient and control conditions. Performing in situ hybridisations for lactase was considered, but considering the similarities between overall gene expression and the pattern of protein expression the experiment was not pursued. Thus it seems unlikely that reductions in sucrase and lactase gene expression and, in turn, activity, are due to any alteration in the state of
proliferation and differentiation of the mucosa cells as they migrate from the crypt to the villus tip. It is more likely that a specific regulatory mechanism controls the abundance of the sucrase and lactase mRNA in mature enterocytes, and that this is sensitive to the effects of iron deficiency.

With respect to mechanisms that may regulate sucrase and lactase gene expression in response to iron deficiency, it seems unlikely that the common IRP/IRE system (for example, divalent metal transporter 1 (DMT1), transferrin receptor and ferritin mRNAs) is responsible for our observations. These systems operate relatively rapidly in order to regulate the abundance of mRNA, and the resultant quantity of protein produced (18, 19, 32). By contrast, alterations in disaccharidase activity typically occur much more slowly, requiring weeks of iron supplementation to return to control values (9, 20). Analysis of the mRNA sequences of sucrase (NM_013061) and lactase (X56747) reveals three areas that have the C/CAGUG consensus sequence for the most common and physiologically relevant IRE bulge and loop structures (18, 19); these sequences can be seen at NM_013061 position 5013-5023, and X56747 positions 502-512 and 5908-5918. However, only X56747 position 5908-5918 is in an untranslated region, and this sequence does not appear to contain sufficient matching base pairs to form a stable stem component, supporting the hypothesis that the IRP/IRE system has little control over expression of these genes.

To further clarify potential mechanisms for the decrease in sucrase and lactase gene expression and subsequent decrease in enzyme activity, we considered the possibility that mRNA levels are primarily regulated by the level of gene transcription. For this purpose, the expression of select transcription factors in the jejunum was measured by semi-quantitative real-time PCR, including activators of the sucrase and lactase promoter regions CDX-2, GATA-4 and HNF-1 (3, 26, 37), as well as the potent repressor PDX-1 (15, 39). Strikingly, PDX-1 expression was 4.5 fold higher in the iron deficient condition. Since any potential activation of the promoter regions by CDX-2
and HNF-1 appear to be entirely negated by PDX-1 (39), this strongly suggests that the decreases in sucrase and lactase gene expression are primarily due to a reduction of gene transcription.

Our observation that PDX-1 expression is elevated in the jejunum during iron deficiency has many wide ranging implications, since there is a strong argument to suggest that PDX-1 is a major spatial regulator of proximal intestine gene transcription. PDX-1 expression is typically restricted to the pancreas and duodenum, and is widely regarded as being vitally important for pancreatic development and function (1, 17, 22, 25, 28), while duodenal expression is required for adenosine deaminase (ADA) promoter activation (8, 14). Further, it is hypothesised that the presence of PDX-1 is the primary reason sucrase and lactase expression is repressed in the duodenum (15, 39).

In this context, it is interesting to note that the expression of principal genes involved in the absorption of dietary iron, namely divalent metal transporter 1 (DMT1) and ferroportin 1 (FPN1), are spatially restricted to the duodenum under normal circumstances (24, 35). Although the promoter regions for these genes have not yet been identified, it has been demonstrated that transcription of DMT1 and FPN1 is increased by iron deficiency in the human enterocyte cell line CaCo-2 (42). From a physiological standpoint, PDX-1 appears to be an excellent candidate transcription factor for the regulation of these genes (as has been established for ADA), since increased PDX-1 expression in the jejunum may allow for reduced spatial restriction of gene expression, and an increased capacity for iron absorption. Indeed, preliminary evidence suggests that both DMT1 and FPN1 expression is significantly increased in the proximal jejunum during iron deficiency, and cannot be explained by IRE/IRP activity alone (AR West, and PS Oates, unpublished observation). Potential mechanisms for the upregulation of jejunal PDX-1
during iron deficiency, and the interaction with iron absorption proteins, should therefore be explored in further studies.

In summary, the decrease in sucrase and lactase enzyme activities observed during iron deficiency appear to be primarily attributable to a significant reduction in gene expression. This reduction in expression is most likely caused by repression of the sucrase and lactase promoter regions by PDX-1. The combined findings strongly support a role for PDX-1 in spatial regulation of numerous small-intestine-related proteins, and that this regulation is sensitive to the effects of iron deficiency.

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