

## How the house sparrow *Passer domesticus* absorbs glucose

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### Summary

According to the hypothesis that most glucose absorption occurs passively across intestinal tight junctions (paracellular absorption), one would predict fairly similar rates of *in vivo* absorption of L-glucose, the stereoisomer of D-glucose that is absorbed only passively and is not catabolized, and of 3-O-methyl-D-glucose (3OMD-glucose), the D-glucose analogue that is actively and passively transported and not catabolized. In house sparrows *Passer domesticus*, we applied a pharmacokinetic method to measure simultaneous *in vivo* absorption of [<sup>14</sup>C]L-glucose and [<sup>3</sup>H]3OMD-glucose in a situation in which intestinal glucose transporters were relatively saturated (gavage solution contained 200 mmol l<sup>-1</sup> 3OMD-glucose). Fractional absorptions (*F*) were not significantly different between [<sup>3</sup>H]3OMD- and [<sup>14</sup>C]L-glucose (0.80 vs 0.79), and the apparent rates of absorption did not differ significantly. When we performed the same experiment on other sparrows in a situation in which intestinal

glucose transporters were relatively unsaturated (200 mmol l<sup>-1</sup> mannitol replaced 3OMD-glucose in the gavage solution), the apparent rate of absorption was significantly reduced for [<sup>14</sup>C]L-glucose by 39% and for [<sup>3</sup>H]3OMD-glucose by 26%. A simulation model showed that a reduction is not predicted if most of the [<sup>3</sup>H]3OMD-glucose is actively absorbed, because the absorption rate of the tracer should increase when competitive inhibitor (unlabeled 3OMD-glucose) is removed. The similar extent and rates of absorption of [<sup>3</sup>H]3OMD- and [<sup>14</sup>C]L-glucose, and the acceleration of their rates of absorption in the presence of luminal 3OMD-glucose, are most consistent with Pappenheimer's hypothesis that the majority of dietary D-glucose is absorbed passively.

Key words: D-glucose, 3-O-methyl-D-glucose mediated absorption, passive absorption, house sparrow, *Passer domesticus*.

### Introduction

The mechanism(s) by which house sparrows and many other species of birds absorb the majority of their dietary sugars is somewhat of a mystery. All vertebrates that have been studied possess intestinal sugar transporters such as the Na-coupled glucose transporter SGLT1 in the apical membrane and GLUT2 in the basolateral membrane (Karasov and Hume, 1997). In mammals such as the rat, mouse and rabbit, the intestine's absorptive capacity by these mediated mechanisms, as measured *in vitro*, meets or exceeds dietary carbohydrate intake (Ferraris and Diamond, 1989). In contrast, in many avian species (Karasov and Cork, 1994; Caviades-Vidal and Karasov, 1996; Levey and Cipollini, 1996; Afik et al., 1997) the rates of mediated glucose transport measured *in vitro* were much lower than rates of glucose absorption actually achieved by freely feeding birds.

Pappenheimer and colleagues (Pappenheimer and Reiss, 1987; Pappenheimer, 1990) suggested that the intestine's capacity to absorb glucose by mediated pathways is inadequate to meet the daily intake of glucose and that most absorption occurs by solvent drag across intestinal tight junctions (paracellular absorption), secondary to active sugar

and amino acid transport. In *in vivo* experiments, a number of wild avian species achieved nearly complete absorption of ingested L-glucose, the stereoisomer of D-glucose that does not interact with the intestine's glucose transporters and can only be absorbed passively (Chang et al., 2004): in nectarivorous rainbow lorikeets, 80% (Karasov and Cork, 1994); granivorous house sparrows, 80% (Caviades-Vidal and Karasov, 1996); and omnivorous yellow-rumped warblers, 91% (Afik et al., 1997). These findings in birds with diverse diet and taxonomic associations suggest that, in birds, passive absorption is important for nutrient intake. However, Schwartz et al. (1995) pointed out that L-glucose might be absorbed at a much slower rate than D-glucose, but over the entire length of the intestine and the extended time of digesta residence in the gut, its absorption could still be fairly complete. Many birds have intestine lengths and digesta residence times that are relatively short, not long (Karasov and Levey, 1990), however, so how could birds exhibit nearly complete glucose absorption with low rates of mediated and passive uptake?

An elegant approach to resolving this issue has been to

compare the extent and/or rate of absorption of L-glucose (absorbed only passively) vs D-glucose or its analogue (absorbed actively and passively) simultaneously in intact animals. In laboratory rats, the absorption rate of the nonmetabolizable, actively transported 3-O-methyl-D-glucose apparently exceeded that of L-glucose by about 9:1, implying that most glucose was absorbed actively (Uhing and Kimura, 1995). Similar conclusions have been drawn for dogs (Lane et al., 1999) and humans (Fine et al., 1993). In this study, we report the first measurements of this kind in an avian species, and the findings are very different from those in the mammals studied so far.

Based on the earlier findings in birds, we predicted that

passive absorption must be important to glucose absorption, and we applied a pharmacokinetic method focusing on appearance of radiolabeled 3-O-methyl-D-glucose (3OMD-glucose) and L-glucose in blood to measure absorption in intact birds. Both of these compounds are metabolically inert, which removes the possibly confounding factor of post-absorptive catabolism from the analysis, but only the former is transported by mediated processes at the brush border of house sparrows (Chang et al., 2004) and at the basolateral membrane (Kimmich, 1981; Burant and Bell, 1992). If mediated absorption is relatively most important, then this will be apparent in slower absorption of L-glucose over an extended period of time, relative to 3OMD-glucose. Alternatively, if the

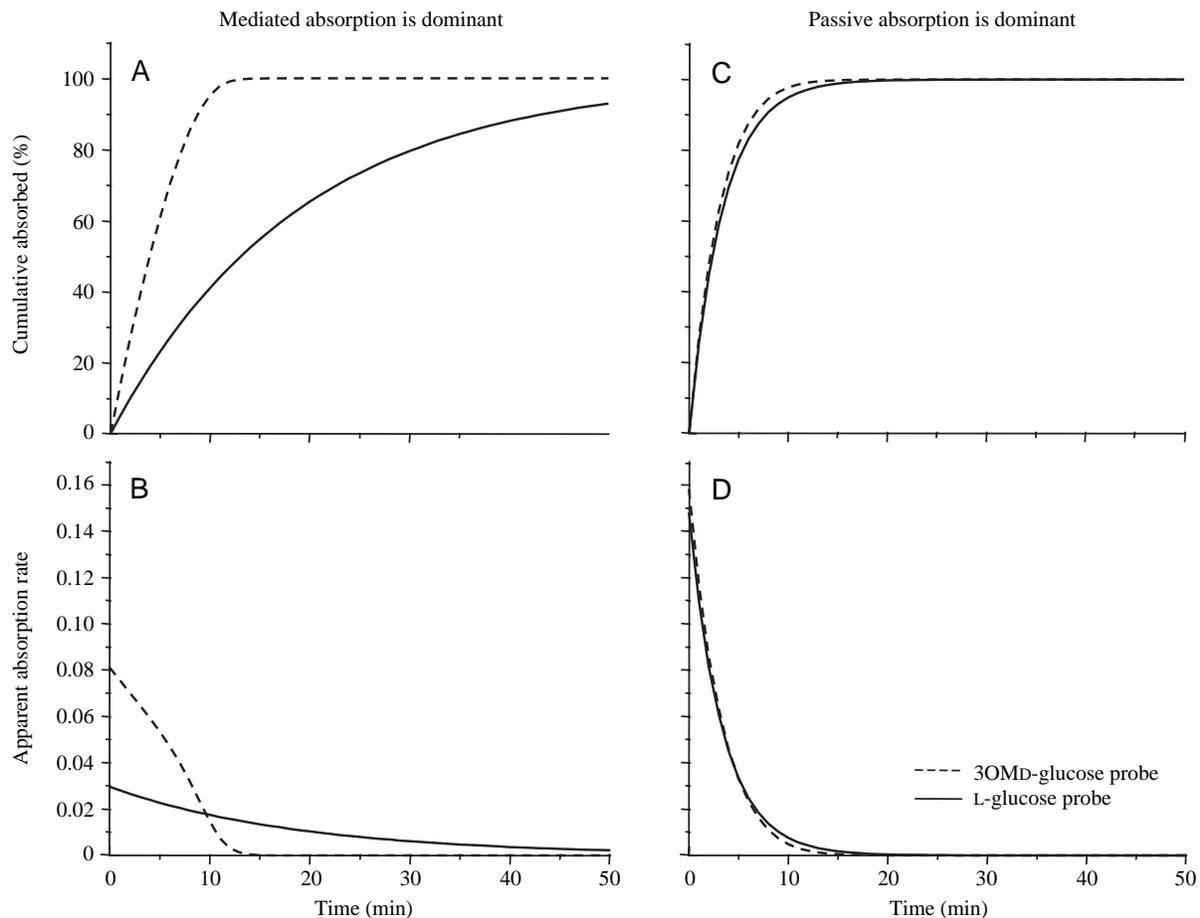


Fig. 1. The time course for absorption of radiolabeled 3OMD-glucose and L-glucose differs according to whether most glucose absorption is mediated by the  $\text{Na}^+$ -glucose cotransporter or is passive. The predicted patterns shown were generated using a chemical reactor model of intestinal absorption of radiolabeled probes, with a starting substrate concentration of  $100 \text{ mmol l}^{-1}$  (see Discussion). (A,B) Mediated uptake is assumed to dominate and so the maximal rate of 3OMD-glucose transport ( $\dot{V}_{\text{max}}$ ) was taken to be  $5\times$  that which was measured in house sparrow small intestine *in vitro* ( $2.083 \text{ nmol min}^{-1} \mu\text{l}^{-1}$ ; Caviedes-Vidal and Karasov, 1996) whereas the passive permeability coefficient ( $K_a$ ) was taken to be the measured value ( $0.05 \text{ nmol } \mu\text{l}^{-1}$ ). In this situation, percent cumulative absorption of the 3OMD-glucose (A), whose uptake is both mediated and passive (broken line), increases faster and is overall greater than the percent cumulative absorption of L-glucose, whose uptake is only passive (solid line). The apparent absorption rate (i.e. instantaneous slope; B) of 3OMD-glucose exceeds that of L-glucose. (C,D) Passive uptake is assumed to dominate and so the  $\dot{V}_{\text{max}}$  was taken to be the measured value and  $K_a$  was taken to be  $5\times$  the measured value. In this situation, the percent cumulative absorption of the 3OMD-glucose is only marginally faster and higher than L-glucose (C), and the apparent absorption rate of the 3OMD-glucose is only marginally higher than that of L-glucose (D). The Michaelis constant for mediated uptake (apparent  $K_m$ ) was fixed at  $16.5 \text{ mmol l}^{-1}$ ,  $3\times$  the measured value. These figures are shown because these types of data can be empirically generated using the pharmacokinetic methods described in Materials and methods and Results.

majority of glucose is absorbed passively, then the absorption of the radiolabeled 3OMD-glucose and L-glucose will be similar in rate and extent (Fig. 1).

Because of uncertainty about normal luminal nutrient concentrations (Ferraris et al., 1990; Pappenheimer, 1993), and how they might influence the comparisons between the radiolabeled compounds, we took measurements under substrate conditions that were both nonsaturating and relatively more saturating for SGLT1, whose half-saturation glucose concentration (apparent  $K_m$ ) is 5 mmol l<sup>-1</sup> (Caviedes-Vidal and Karasov, 1996). Also, we point out at the outset that in our comparison, 3OMD-glucose is handicapped relative to L-glucose for several reasons. First, the molecular mass of 3OMD-glucose (194.2 Da) is greater than that of L-glucose (180.2 Da), which lowers its diffusion coefficient in water and may decrease its rate of permeation, relative to L-glucose, through the paracellular space, which discriminates according to molecular size (Chediack et al., 2003). Also, the affinity of the glucose transporters for 3OMD-glucose is lower than for D-glucose (Kimmich, 1981; Ikeda et al., 1989), so the former is an imperfect substitute for the latter. To evaluate the implications of these differences, and other assumptions, on our conclusions, we utilize a simulation model in conjunction with our results.

## Materials and methods

### *Birds and their maintenance*

House sparrows *Passer domesticus* L. were captured in the vicinity of the University of Wisconsin-Madison, USA. They were housed in cages (0.80 m × 0.60 m × 0.60 m) indoors under relatively constant environmental conditions (25.0 ± 0.5°C, relative humidity 50 ± 9%, photoperiod 12 h:12 h light:dark) and received *ad libitum* water and commercial bird seed mix. Sparrows were acclimated to laboratory conditions for at least 2 weeks prior to use in experiments. The routine animal care procedures and experimental protocol used in this study were reviewed and approved by the University of Wisconsin Research Animal Resources Center.

### *Measurement of L-glucose and 3OMD-glucose absorption in vivo*

Food was removed from birds overnight prior to an experiment, when these diurnal birds would not normally eat, and measurements began 2.5–3 h after lights went on in the morning. When measuring absorption under conditions relatively nonsaturating for SGLT1, seven house sparrows were gavaged with 500 µl of a solution containing 200 mmol l<sup>-1</sup> mannitol, 10 µCi [<sup>14</sup>C]L-glucose and 20 µCi [<sup>3</sup>H]3OMD-glucose (American Radiolabeled Chemicals St Louis, MO, USA), and 80 mmol l<sup>-1</sup> NaCl. When measuring absorption under relatively saturating conditions, the mannitol was replaced with 200 mmol l<sup>-1</sup> 3OMD-glucose ( $N=6$ ). To determine elimination rate for absorption calculations, these same individuals were injected into the pectoralis on other days with [<sup>14</sup>C]L-glucose and [<sup>3</sup>H]3OMD-glucose (5 × µCi and

10 µCi, respectively, in 250 µl 200 mmol l<sup>-1</sup> mannitol and 80 mmol l<sup>-1</sup> NaCl). The sequence of trials was random, and repeated measures on individuals were performed after intervals of at least 7 days, easily enough time for residual radioactivity to return to background (normally less than 24 h; see Results). The total osmotic pressure of gavage or injection solutions was controlled at 360 mOsm with 5% variation (tested using a vapor pressure osmometer; Wescor vapor 5502, Logan, UT, USA) so that solutions were isosmotic with avian blood, and aliquot samples were saved for radioactivity analysis. Following either gavage or injection, 7–9 blood samples (20 µl each; total volume collected was <10% of blood volume in normal house sparrows; Stangel, 1986) were collected from the brachial vein with heparinized capillary tubes over the next 3–4 h. Immediately after collection, blood was centrifuged (1500 g) for 5 min, plasma was separated and weighed (10–20 ± 0.1 mg), then mixed with 2.0 ml of scintillation fluor (Hionic-Fluor, Packard, USA) and counted in a scintillation spectrometer (Tracor Analytic, MarkIII, USA) for disintegrations per minute (d.p.m.) with automatic external standardization (AES) and background correction (Caviedes-Vidal and Karasov, 1996).

In order to test for radiopurity, plasma samples from three different birds gavaged with different radioactive solutions were collected and analyzed along with three aliquot samples from each radioactive solution. Radiopurity was checked by high performance liquid chromatography (HPLC) using an NH<sub>3</sub> column (Alltech, USA) with an acetonitrile:water (85:15%) mobile phase. All <sup>3</sup>H activity was associated with 3OMD-glucose in the solution (92%) and in plasma (91%) and all <sup>14</sup>C activity was associated with L-glucose in the solution (97%) and in plasma (93%). We also checked and found in preliminary injection and gavage experiments (not shown) that we obtained similar results for absorption of L- and 3OMD-glucose regardless of whether the <sup>14</sup>C and <sup>3</sup>H labels were on L-glucose or 3OMD-glucose.

### *Pharmacokinetic calculations*

The radioactivity in each plasma sample at time  $t$  was normalized to the mass of each sample ( $C_t$ , dpm mg<sup>-1</sup> plasma) and plotted against sampling time. The integration of the area under this curve ( $AUC_t$ ) represents the amount of radiolabeled probe that has been absorbed from time 0 up to time  $t$ , whereas  $AUC_{total}$  denotes the total amount of probe absorbed from 0 up to infinity time ( $\infty$ ). Following typical procedures in pharmacokinetics (Gibaldi and Perrier, 1982), the area from  $t=0$  to  $t=x$  min (when the final blood sample was taken) was calculated using the trapezoidal rule. The area from  $t=x$  min to  $t=\infty$  was calculated as  $AUC^{x \rightarrow \infty} = C_t$  (at  $t=x$ )/ $K_{el}$ , where  $K_{el}$  is the elimination rate constant, which can be determined for each bird in each experiment based on the terminal portion of its absorption curve. The total  $AUC^{0 \rightarrow \infty}$  was obtained by summing the two areas. Fractional absorption ( $F$ ), or bioavailability, for each probe was estimated based on the ratio between the area under the probe plasma concentration *versus* time curve for oral gavage experiments ( $AUC_{oral}$ , in units of

d.p.m. min g<sup>-1</sup> plasma) and injection experiments (AUC<sub>inj</sub>) normalized to the respective dosage given to the animal:

$$F = (\text{AUC}_{\text{oral}}/\text{dose}_{\text{oral}}) / (\text{AUC}_{\text{inj}}/\text{dose}_{\text{inj}}) . \quad (1)$$

This method of calculating  $F$  is favored because it makes no major assumptions about compartments or kinetics. Fractional absorption estimates how much of the gavaged probe was absorbed into the animal's system.

Additional analyses relating to the time course and apparent rate of absorption were made assuming an open two-compartment model and first order elimination. A two-compartment model was selected following inspection of the curves of  $C_t$  vs sampling time post-injection (see Results). In 20 out of 26 cases, a bi-exponential elimination model fit the data significantly better ( $P < 0.05$ ) than a mono-exponential model, using an  $F$ -test (Motulsky and Ransnas, 1987). For each individual, parameters for the biexponential model were derived by the curve-stripping method (Gibaldi and Perrier, 1982):

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} . \quad (2)$$

In the two-compartment model, rate constants and distribution spaces are derived from the constants  $A$ ,  $B$ ,  $\alpha$  and  $\beta$ . For example, the elimination rate constant of the probe from the apparent central compartment, to which we will refer later, is estimated as  $(A+B)/[(A/\alpha)+(B/\beta)]$ . The rate constants and spaces are used in conjunction with the data from gavage experiments to calculate the cumulative proportion of  $F$  that was absorbed at each blood sampling time point ( $P_t$ ), according to the Loo-Riegelman method (Wagner, 1975; Gibaldi and Perrier, 1982). The true cumulative absorption at any sampling time point ( $t$ ) is the product of  $F$  and  $P_t$ , and the apparent absorption rate during two adjacent sampling time points is  $F\Delta P/\Delta t$ .

In the few cases where a mono-exponential model fit elimination best, or if elimination data were not available because an injection experiment was not performed (4 of 13 birds), we used the mean distribution space measured in other birds and applied the one-compartment model (Wagner, 1975) for calculating  $F$ ,  $P$  and apparent rates. As discussed by Wagner (1975), the two models can yield very similar results under many circumstances, which we also found to be the case ( $N=9$ ). Also, fractional absorptions did not differ significantly when estimated using a compartmental model *versus* the compartment-independent method [e.g. for L-glucose respectively,  $0.73 \pm 0.03$  (mean  $\pm$  S.E.M.) vs  $0.72 \pm 0.04$ ;  $P > 0.6$ ,  $N=9$ ]. Although the two-compartment model is more complex, we retained it when appropriate because, theoretically, the two-compartment model would be better able to detect experimental differences (Loo and Riegelman, 1968; Wagner, 1975; Gibaldi and Perrier, 1982). If there are some organs and tissues in an organism where blood circulation is not as high as the rest of body, this may lead to a higher concentration of probe in these peripheral compartment(s) compared to the central compartment. Thus these poorly perfused peripheral compartments can serve as another source for probes entering

the central compartment, secondary to an absorption site like the small intestine. In comparison to the one-compartment model, a two-compartment model is more conservative in that it provides corrections for the redistribution of probe from these peripheral compartments (Gibaldi and Perrier, 1982; Riviere, 1999).

### Statistical analysis

Numerical data are presented as means  $\pm$  S.E.M. ( $N$ =number of animals). Although data shown in the figures are mean values, statistical analyses were performed based on data for individuals. Fractional absorption ( $F$ ) was arcsine square-root transformed before statistical analysis. Results were analyzed by analysis of variance (ANOVA), repeated-measures ANOVA, and Student's  $t$ -test (SAS, SAS Institute Inc, Cary, NC, USA). The  $T$ - and  $F$ -values of these and other ANOVAs are presented in the text with the relevant degrees of freedom as subscripts. Linear regression was by the method of least squares. Nonlinear curve fitting (Gauss-Newton algorithm, SYSTAT; Wilkinson, 1992) was used to fit kinetic data, and kinetic models were compared according to Motulsky and Ransnas (1987). Statistical significance was accepted for  $P < 0.05$ . One-tailed tests were used for *a priori* predictions.

### Results

When the birds were gavaged with [<sup>14</sup>C]L-glucose and [<sup>3</sup>H]3OMD-glucose simultaneously, the average concentration of both radiolabeled carbohydrates in the plasma peaked within 30 min and then declined exponentially (Fig. 2). At least three patterns were apparent from simple inspection of these plots. First, the time course for absorption of both probes seemed rather similar. Second, within each treatment group, the plasma values of 3OMD-glucose slightly exceeded those of L-glucose. Areas under the curve (AUCs) for 3OMD-glucose were significantly higher than for L-glucose in both treatment groups (Table 1). Third, simply comparing peak concentrations, there seemed to be more of both probes appearing in blood when the measurement was made under more saturating conditions (i.e. 200 mmol l<sup>-1</sup> 3OMD-glucose in the gavage solution) than when under relatively nonsaturating conditions (200 mmol l<sup>-1</sup> mannitol replaced 3OMD-glucose). Indeed, the AUCs for both probes were significantly higher when measured under relatively saturating compared with nonsaturating conditions (Table 1). Observations two and three suggest, respectively, that the fractional absorption of 3OMD-glucose exceeded that of L-glucose and that absorption of the probes was enhanced when measured under conditions that are relatively more saturating for SGLT1. Although there is merit in this visual inspection of the patterns in these plots, because it affords the reader a simple and direct way to evaluate the data, less intuitive but more exact calculations of fractional absorption require additional data from the injection/elimination experiments.

In the injection/elimination trials the probes equilibrated rapidly, because the first blood samples at 7 or 10 min had the

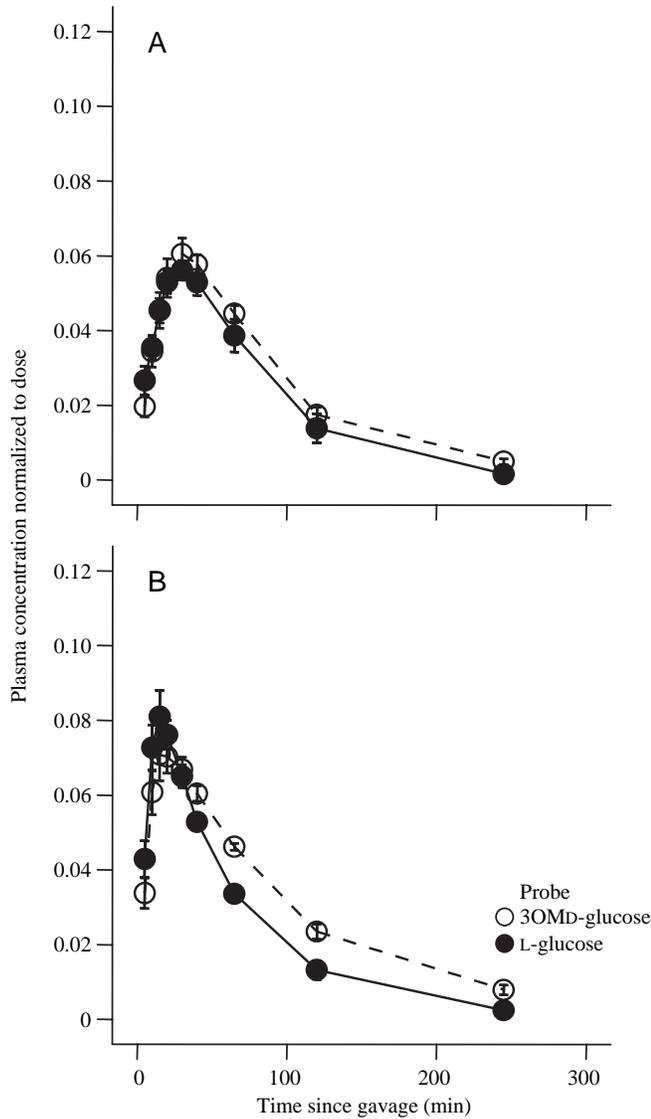


Fig. 2. Plots of plasma  $[^3\text{H}]3\text{OMD}$ -glucose and  $[^{14}\text{C}]\text{L}$ -glucose concentration (mean  $\pm$  S.E.M.) as a function of time since feeding the probes (gavage). The units for concentration are proportion per gram plasma, because values of d.p.m.  $\text{g}^{-1}$  plasma were normalized to d.p.m. dose for each individual. (A) Measurements made under relatively nonsaturating conditions (200  $\text{mmol l}^{-1}$  mannitol in the gavage solution,  $N=7$  birds); (B) measurements made under more saturating conditions (i.e. 200  $\text{mmol l}^{-1}$  3OMD-glucose replaced mannitol in the gavage solution,  $N=6$ ). Filled symbols and solid lines,  $[^{14}\text{C}]\text{L}$ -glucose; unfilled symbols and broken lines,  $[^3\text{H}]3\text{OMD}$ -glucose.

highest probe concentrations (i.e. no extended lag for distribution from pectoralis to blood; Fig. 3). Birds in the two treatment groups did not differ significantly from each other in AUCs (Table 1) or parameters fitted to the bi-exponential elimination model (Table 2) (all  $P>0.2$ ). However, 3OMD-glucose was eliminated more slowly from the apparent central compartment than was L-glucose (respectively,  $0.016\pm 0.001$  vs  $0.028\pm 0.002$   $\text{min}^{-1}$ , paired  $T_8=14.5$ ,  $P<0.001$ ), with the result

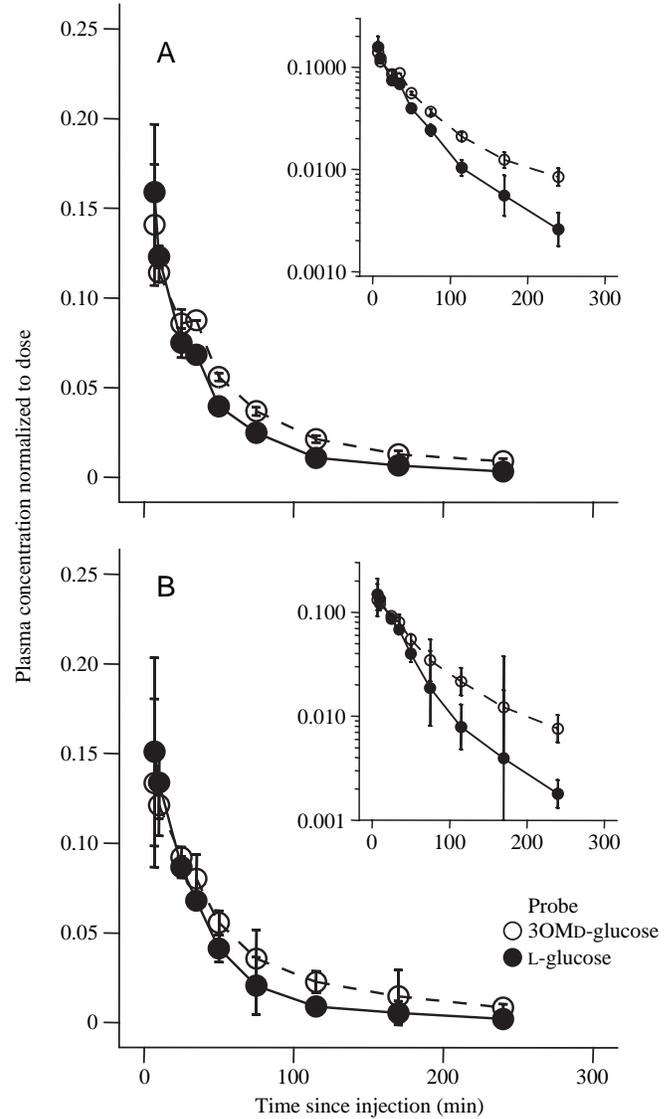


Fig. 3. Plots, and semi-log inset plots, of plasma  $[^3\text{H}]3\text{OMD}$ -glucose and  $[^{14}\text{C}]\text{L}$ -glucose concentration as a function of time since injecting the probes. The units for concentration are proportion per gram plasma, because values of d.p.m.  $\text{g}^{-1}$  plasma were normalized to d.p.m. dose for each individual. (A) Measurements made under relatively nonsaturating conditions (200  $\text{mmol l}^{-1}$  mannitol in the gavage solution,  $N=6$  birds); (B) measurements made under more saturating conditions (i.e. 200  $\text{mmol l}^{-1}$  3OMD-glucose replaced mannitol in the gavage solution,  $N=4$ ). Filled symbols and solid lines,  $[^{14}\text{C}]\text{L}$ -glucose; unfilled symbols and broken lines,  $[^3\text{H}]3\text{OMD}$ -glucose.

that the post-injection AUC was larger for the former than for the latter (Table 1). This difference, which may relate to kidney reabsorption and hence longer retention of  $[^3\text{H}]3\text{OMD}$ -glucose relative to  $[^{14}\text{C}]\text{L}$ -glucose, underscores why the extent and rate of absorption of the two probes cannot be compared simply from comparison of plasma probe concentrations and their AUCs after gavage (i.e. Fig. 2). But, the qualitative conclusions drawn from inspection of Fig. 2 were borne out by

Table 1. Values for AUC (units per gram plasma, normalized to dose) for the data shown in Figs 2 and 3

Treatment	Labeled probe administered	Administration method		P-value for gavage vs injected*
		Gavage	Injected	
100 mmol l <sup>-1</sup> mannitol	L-[ <sup>14</sup> C]glucose	4.861±0.321 (7)	7.260±0.521 (5)	0.001
	3-O-[ <sup>3</sup> H]methyl-D-glucose	6.883±0.448 (7)	9.606±0.735 (5)	<0.001
P-value for comparison of labeled probes*		P<0.001	P=0.001	
100 mmol l <sup>-1</sup> 3-O-methyl-D-glucose	L-[ <sup>14</sup> C]glucose	6.434±0.71 (6)	7.926±1.17 (4)	0.093
	3-O-[ <sup>3</sup> H]methyl D-glucose	8.376±0.476 (6)	9.534±0.86 (4)	0.164
P-value for comparison of labeled probes*		P=0.005	P=0.065	
Treatment effect on L-[ <sup>14</sup> C]glucose		P=0.014	P>0.6	
Treatment effect on 3-O-[ <sup>3</sup> H]methyl-D-glucose		P=0.031	P>0.9	

AUC, area under curve.  
 Values are means ± S.E.M. (N=number of birds).  
 \*P value obtained by repeated measures.

the more exact calculations of  $F$  that take into account the probes' respective elimination kinetics. Fractional absorptions were significantly higher for 3OMD-glucose than for L-glucose when measured simultaneously under relatively nonsaturating conditions (respectively,  $0.71 \pm 0.02$  vs  $0.66 \pm 0.03$ ; paired  $T_6=2.53$ ,  $P=0.045$ ), although under more saturating conditions the two did not differ significantly (respectively,  $0.8 \pm 0.05$  vs  $0.79 \pm 0.04$ ; paired  $T_5=0.8$ ,  $P>0.4$ ). Statistical comparison of these values across the treatments showed that fractional absorption was significantly enhanced for [<sup>14</sup>C]L-glucose ( $T_8=2.42$ ,  $P=0.042$ ), but not for the [<sup>3</sup>H]3OMD-glucose ( $T_5=1.5$ ,  $P>0.3$ ), when measured under conditions that were relatively more saturating for SGLT1.

Information on the apparent rates of probe absorption was derived from plots of fractional absorption as a function of time (Fig. 4A,B). Apparent absorption rates declined with increasing time since gavage, and the rates were compared within the trials using a repeated-measures ANOVA. Under conditions relatively nonsaturating for SGLT1 (Fig. 4A), the

Table 2. Summary statistics on parameters for bi-exponential fit of plasma concentrations from injection/elimination experiments shown in Fig. 3

	[ <sup>14</sup> C]L-glucose	[ <sup>3</sup> H]3-O-methyl-D-glucose
Dose (d.p.m.)	9286671±319039	60337912±1078716
A (d.p.m. mg <sup>-1</sup> plasma)	649±55	3006±222
B (d.p.m. mg <sup>-1</sup> plasma)	214±42	1617±192
$\alpha$ (min <sup>-1</sup> )	0.0426±0.0022	0.03305±0.00132
$\beta$ (min <sup>-1</sup> )	0.0124±0.00125	0.007614±0.00069

The model was  $C_t = Ae^{-\alpha t} + B e^{-\beta t}$ .

Values are means ± S.E.M. (N=number of birds). All birds are pooled because those in the two treatment groups did not differ significantly from each other for any parameter (all  $P>0.2$ ).

least-squares adjusted mean absorption rate for 3OMD-glucose ( $1.91 \pm 0.15\%$  absorbed min<sup>-1</sup>) significantly exceeded that for L-glucose absorption ( $1.63 \pm 0.14\%$  absorbed min<sup>-1</sup>) by 17% ( $F_{1,62}=4.01$ ,  $P=0.049$ ). Under relatively more saturating conditions (Fig. 4B), the apparent absorption rates of the two probes did not differ significantly (respectively,  $2.59 \pm 0.38$  vs  $2.67 \pm 0.42\%$  absorbed min<sup>-1</sup>;  $F_{1,45}=0.1$ ,  $P>0.7$ ). Statistical comparison of these values across the treatments showed that apparent rate of absorption was significantly enhanced for the L-glucose probe by 57% ( $F_{1,52}=24$ ,  $P<0.01$ ) and for the 3OMD-glucose probe by 36% ( $F_{1,55}=8.28$ ,  $P<0.001$ ), when measured under conditions that were relatively more saturating for SGLT1.

Assuming that absorption of L-glucose is a proxy for passive absorption of 3OMD-glucose, whereas the absorption of 3OMD-glucose represents the sum of passive + mediated absorption, the ratio of the apparent absorption rates (L/D) indicates the proportion of 3OMD-glucose absorption that occurs *via* the passive pathway. Accordingly, we plotted this ratio over the course of the trials (Fig. 5). The ratio always exceeded 0.7, with no significant difference between treatments. This suggests that more than 70% of 3OMD-glucose absorption probably occurred *via* the passive pathway, regardless of whether the measures were made under conditions relatively nonsaturating or relatively saturating for SGLT1. However, the following discussion revisits a number of assumptions upon which this conclusion is based.

## Discussion

In the following sections we discuss our two main empirical results. First, when [<sup>14</sup>C]L-glucose and [<sup>3</sup>H]3OMD-glucose were gavaged simultaneously their extent and rate of absorption were very similar, never differing by even 20%. Second, the apparent rates of absorption increased when the measurements were made under conditions relatively more

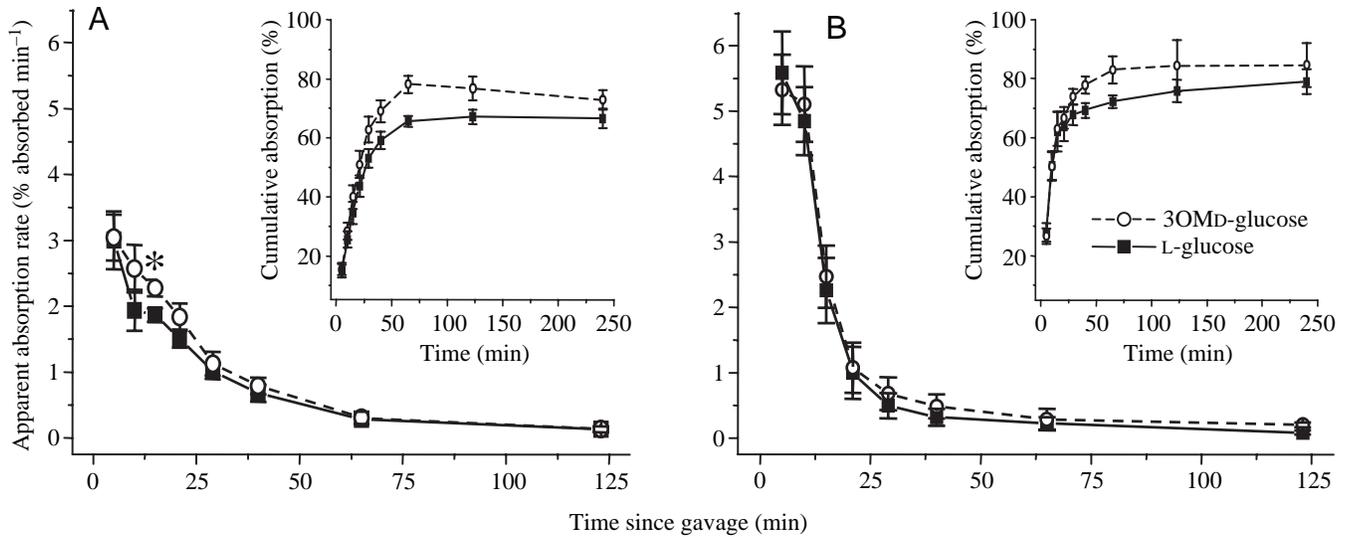


Fig. 4. The cumulative absorption (inset plots) and apparent rates of absorption of [<sup>3</sup>H]3OMD-glucose and [<sup>14</sup>C]L-glucose as a function of time since gavage of the probes to house sparrows. (A) Measurements made under relatively nonsaturating conditions (200 mmol l<sup>-1</sup> mannitol in the gavage solution, *N*=7 birds); (B) measurements made under more saturating conditions (i.e. 200 mmol l<sup>-1</sup> 3OMD-glucose replaced mannitol in the gavage solution, *N*=6). Filled symbols and solid lines, [<sup>14</sup>C]L-glucose; unfilled symbols and dashed lines, [<sup>3</sup>H]3OMD-glucose. The absorption of 3OMD-glucose was slightly but significantly higher than that of L-glucose. The asterisk in A designates the significantly higher apparent absorption rate of [<sup>3</sup>H]3OMD-glucose (*P*=0.015) than of [<sup>14</sup>C]L-glucose at the 15 min sampling point.

saturating for the brush border glucose transporter SGLT1. Part of our discussion includes a simulation that tests major assumptions important for interpreting the physiological significance of the first major result, and the simulation also aids in interpreting the second major result from a mechanistic perspective.

*The majority of 3OMD-glucose absorption is via a passive route in house sparrows*

If 3OMD-glucose is mainly actively absorbed by the intestine, then a much higher rate of absorption of [<sup>3</sup>H]3OMD-glucose than of [<sup>14</sup>C]L-glucose is predicted. For example, if 95% of the instantaneous glucose absorption is active, as suggested in some studies (UHING and Kimura, 1995; FINE et al., 1993; Lane et al., 1999), and if 3OMD-glucose is a good proxy for D-glucose, then the ratio of the absorption rate for L-glucose to 3OMD-glucose should be about 0.05. However, we found similar fractional absorption and apparent rates of absorption for L- and 3OMD-glucose in house sparrows (Fig. 4) and the ratio never fell below 0.7 (Fig. 5), indicating >70% passive absorption of glucose.

Schwartz et al. (1995) made the point that simply comparing *F* for 3OMD- and L-glucose might be misleading. Suppose that 3OMD-glucose is absorbed at a high rate in the proximal portion of the intestine, whereas L-glucose is absorbed at a very slow rate. The fractional absorption of L-glucose could still be fairly complete if its slow absorption occurred over the entire length of the intestine and over the entire time of digesta residence. We do not think that this explanation applies to the house sparrows. Absorption of L-glucose did not seem prolonged compared with that for D-glucose (insets in Fig. 4).

3OMD- and L-glucose had apparent absorption rates similar to each other throughout all the sampling time points (Fig. 4). When measurements were done under conditions relatively nonsaturating for SGLT1, even for the largest difference at 10 min after gavage the apparent absorption rate of 3OMD-

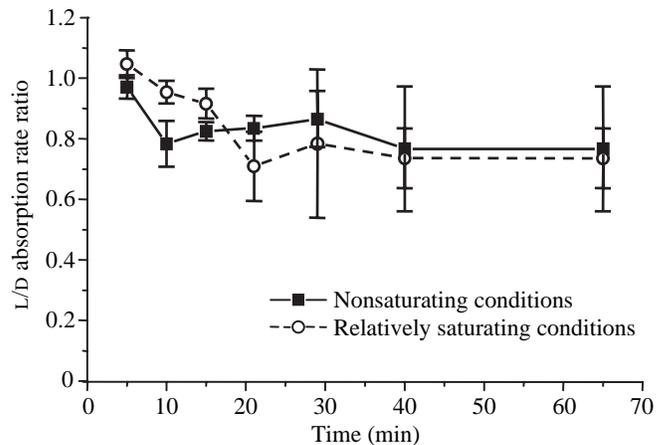


Fig. 5. The majority of 3OMD-glucose absorption is passive, as indicated by the high ratio of apparent absorption rates for L-glucose and 3OMD-glucose. Assuming that absorption of L-glucose is passive whereas the absorption of 3OMD-glucose represents the sum of passive + mediated absorption, the ratio of the apparent absorption rates (L/D) indicates the proportion of 3OMD-glucose absorption that occurs *via* the passive pathway. The ratios were calculated from the apparent rates shown in Fig. 4, for measurements made under relatively nonsaturating conditions (filled squares, solid line), and for measurements made under relatively more saturating conditions (unfilled circles, broken line).

glucose was only 1.32-times higher than that of L-glucose. When measurements were done under more saturating conditions, the fractional absorption of L-glucose was the same as that for 3OMD-glucose, the apparent absorption rate of 3OMD- and L-glucose followed each other closely throughout the course of experiment, and the mean apparent absorption rates were not statistically different from each other either.

In the Introduction we suggested that in the comparison of 3OMD- and L-glucose, the former was handicapped relative to the latter for several reasons. First, we previously found that the fractional absorption of carbohydrates absorbed passively by the house sparrow declines with increasing molecular mass (MW) of the probe, by an average of  $-0.002 \text{ Da}^{-1}$  (Chediack et al., 2003). The reasons for this probably include the fact that diffusion coefficients decline with increasing  $\text{MW}^{1/2}$ , and the paracellular space discriminates according to molecular size, much like a sieve (Chang et al., 1975; Friedman, 1987). Considering the 14 Da difference in the molecular mass of L-glucose and 3OMD-glucose, the direct comparison of their fractional absorptions (averaging, respectively, 0.73 and 0.76) might be adjusted by increasing the value for 3OMD-glucose by 0.03 (i.e. the product of 14 Da and  $0.002 \text{ Da}^{-1}$ ). This would not change the overall conclusion that their extent and rate of absorption are very similar when measured simultaneously.

The affinities of both the brush border and basolateral glucose transporter(s) are lower for 3OMD-glucose than for D-glucose (Kimmich, 1981; Ikeda et al., 1989), so the former is an imperfect substitute for the latter. To evaluate the implications of this difference for our conclusions, we utilized a simulation model of absorption by mediated and passive pathways that we discuss in the next section. Thinking about how the  $[^3\text{H}]3\text{OMD}$ -glucose interacted with a glucose transporter also helped to highlight what was notable about our second major empirical finding – that the rates of absorption increased, even for  $[^3\text{H}]3\text{OMD}$ -glucose, when the measurements were done under conditions relatively more saturating for the glucose transporter.

#### *Simulation of sugar absorption supports the interpretation of empirical findings*

In order to characterize absorption of D-, L- and 3OMD-glucose in intact animals, we constructed a model for absorption at the apical membrane using idealized simple reactor theory, following the approach of Penry and Jumars (1987), who proposed that tubular organs such as the intestine are analogous to plug-flow reactors in chemical engineering. In a plug-flow reactor, material is mixed instantaneously and continuously in the radial direction without appreciable axial mixing, and items leave the tube in the same order that they entered. At low and high substrate concentrations, we simulated several possible patterns of 3OMD-glucose absorption, including expected patterns if mediated absorption were dominant, but with different apparent affinities between the transporter and its substrate, and an expected pattern in the event that passive absorption were dominant. We used the results as a check on our conclusions based on empirical results.

#### *Nonsaturable absorption*

The simplest case when modeling the kinetics of nutrient absorption is for an animal absorbing food entirely through passive, nonsaturable process(es). In the plug-flow reactor-like intestine of the animal eating hexoses (requiring no hydrolysis), the only reaction is absorption, and its rate of removal of substrate from the reactor is  $-r_L$  in  $\text{nmol } \mu\text{l}^{-1} \text{ min}^{-1}$  (Jumars and Martinez del Rio, 1999). Note that here, and elsewhere, rates are normalized to the reactor volume, which has units of  $\mu\text{l}$ . The luminal or gut volume was estimated assuming a tube with an averaged small intestinal (SI) length equal to 18.3 cm and SI luminal radius of 0.1 cm (Caviedes-Vidal and Karasov, 1996). The passive absorption is determined by the absorption rate constant,  $K_a$  ( $\text{min}^{-1}$ ), and substrate concentration  $C_t$  ( $\text{nmol } \mu\text{l}^{-1}$ ) at the reaction or absorption site at time  $t$ :

$$-r_L = K_a C_t. \quad (3)$$

Paracellular absorption of hydrosoluble molecules across small intestinal mucosal epithelium is best described in the context of the Kedem–Katchalsky equation (Kedem and Katchalsky, 1958), which includes the contribution of both diffusion and solvent drag to the flux of solutes through porous epithelia.  $K_a$  is thus a lumped first-order coefficient of both diffusion and solvent drag flux, which both occur in linear proportion to substrate concentration  $C_t$ .

Fig. 6. The simulated pattern of D- and L-glucose radiolabeled probe absorption at the apical membrane in the presence of an initial low substrate concentration ( $C_{C0}=1 \text{ mmol l}^{-1}$ ) varies according to whether absorption is primarily mediated or primarily passive. The initial probe concentration used in the modeling was  $0.001 \text{ nmol } \mu\text{l}^{-1}$  (i.e. tracer level). (A) Cumulative absorption (%); (B) probe absorption rate. Four different situations are shown. (Ai,Bi) Absorption described by kinetic values ( $V_{\text{max}}$ ,  $K_m$  and  $K_a$ ) that have been measured *in vitro* using isolated sparrow intestine (Caviedes-Vidal and Karasov, 1996). We used the  $V_{\text{max}}$  measured in that study because *in vitro* rates of uptake of 3OMD-glucose and D-glucose under saturating conditions were comparable (Chang et al., 2004). (Aii,Bii) Analogous to Ai,Bi but for the D-glucose analogue 3-O-methyl-D-glucose, which has lower affinity (approximately  $3\times$  higher  $K_m$ , determined in rats, rabbits, guinea pigs and hamsters; Jorgensen et al., 1961; Syme and Levin, 1980; Thomson et al., 1982). (Aiii,Biii) Absorption based on the assumption that the  $V_{\text{max}}$  was underestimated *in vitro* due to handling effects on tissue viability (Starck et al., 2000), uses a  $5\times$  higher  $V_{\text{max}}$ , and thus represents absorption dominated by the mediated pathway. (Aiv,Biv) Absorption based on the assumption that the  $K_a$  was underestimated *in vitro* due to absence of solvent drag (Pappenheimer, 1993), uses a  $5\times$  higher  $K_a$ , and thus represents absorption dominated by the passive pathway. Notice that when passive absorption dominates (Aiv,Biv) the predicted cumulative absorption of D- and L-glucose probes as a function of time (Aiv) are much more similar than when mediated absorption dominates (Aiii). Analogously, when passive absorption dominates, the predicted absorption rates of D- and L-glucose probes as a function of time (Biv) are much closer to each other than when mediated absorption dominates (Biii).

Mediated plus nonsaturable absorption

Carrier-mediated process(es) can be assumed to have Michaelis–Menten kinetics with a Michaelis constant ( $K_m$ , in

$\text{nmol } \mu\text{l}^{-1}$ ) reciprocally related to the affinity of the carrier system for the substrate and a rate of removal of substrate from the reactor ( $\dot{V}_{\text{max}}$ ,  $\text{nmol } \mu\text{l}^{-1} \text{ min}^{-1}$ ) at saturating substrate

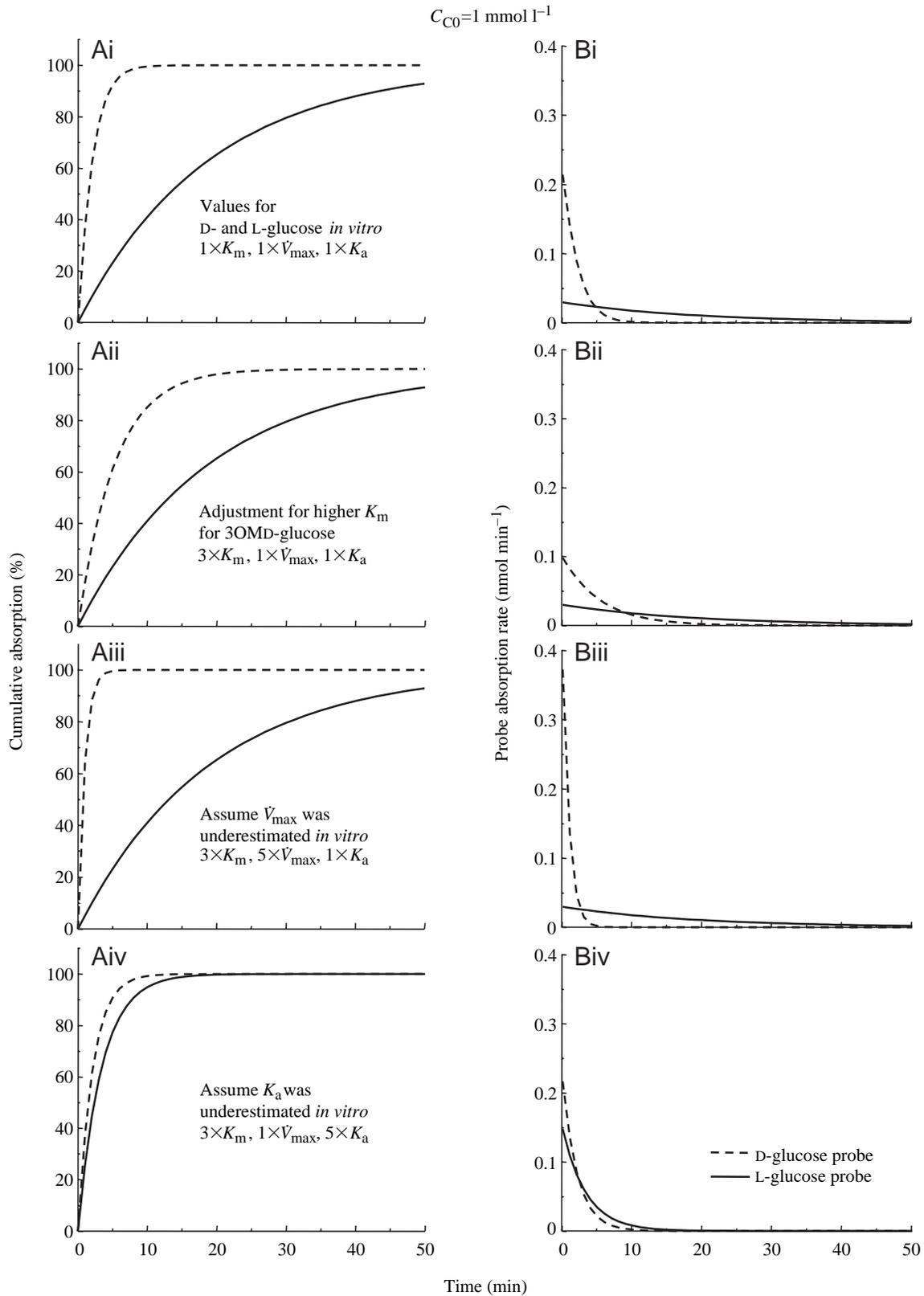


Fig. 6

concentration. The rate of intestinal absorption of a single hexose, such as 3OMD-glucose, by both mediated and nonsaturable processes ( $-r_D$ ), can thus be calculated following the approach of Dade et al. (1990):

$$-r_D = \left( \frac{V_{\max} C_t}{K_m + C_t} \right) + K_a C_t. \quad (4)$$

#### Modeling for probe absorption

The kinetics of probe absorption is the final target for this modeling exercise because radiolabeled probe was the measurable parameter in our studies.  $^{14}\text{C}$ -labeled L-glucose probe presumably behaves like a nonsaturable substrate, thus its absorption is described by Equation 3. However, the absorption of  $[^3\text{H}]3\text{OMD}$ -glucose probe, which includes mediated plus nonsaturable absorption, is more complicated.  $[^3\text{H}]3\text{OMD}$ -glucose probe will compete with labeled and unlabeled substrate for the reaction sites or transporters in the small intestine (Malo and Berteloot, 1991). Because 3OMD-glucose can be absorbed by both passive and mediated routes, the absorption rate of  $[^3\text{H}]3\text{OMD}$ -glucose probe ( $-r_{DP}$ ) at the absorption site can be estimated as follows:

$$-r_{DP} = \left( \frac{V_{\max} C_{DPt}}{K_m + C_{DPt} + C_{Ct}} \right) + K_a C_{DPt}. \quad (5)$$

Here,  $C_{DPt}$  is the 3OMD-glucose probe concentration ( $\text{nmol } \mu\text{l}^{-1}$ ) at time  $t$  at the absorption site. Luminal unlabeled 3OMD-glucose concentration at time  $t$  ( $C_{Ct}$ ) can be described by Equation 4 with any given initial concentration at time zero.

In the simulation, the reaction or absorption rate at time  $t$  is calculated by formulae as described above with any given initial substrate concentrations. Assuming that the volume of gut contents does not change with time and that the absorption rates stay unchanged from time  $t$  to time  $t+1$ , the absorption rate times the intestine luminal volume approximates how much substrate is absorbed during the time interval. By subtracting this value from the initial total substrate content at time  $t$ , the initial substrate concentration at time  $t+1$  can be calculated from the remainder. With this initial substrate concentration at time  $t+1$ , the subsequent absorption rate and the amount of absorption during the next time interval can also be calculated following the same rule. Plotting the values gathered from a simulation run from  $t=0$  to  $t=50$  min, when most absorption had occurred in theory and in fact (Fig. 4A,B), we estimated the relative rate and extent of  $[^{14}\text{C}]$ -L-glucose and  $[^3\text{H}]3\text{OMD}$ -glucose absorption. The selection of initial parameter values is described in the legend of Fig. 6.

#### Results of simulations

The simulated cumulative absorption (Figs 6A, 7A) and apparent rate of absorption (Figs 6B, 7B) of radiolabeled 3OMD-glucose are considerably greater than that of radiolabeled L-glucose in all the situations modeled (i.e. Figs 6Ai–iii, Bi–iii, 7Ai–iii, Bi–iii), except when the apparent passive permeability coefficient was increased (compare

Figs 6Aiv, Biv, 7Aiv, Biv with all other panels). Adjusting upward the apparent  $K_m$  for mediated 3OMD-glucose absorption (Figs 6Aii, 7Bii) had little effect on this comparison. The predicted rates of probe absorption were in most cases depressed in the presence of high luminal D-glucose concentration (c.f. Figs 6B, 7B), as might be expected because the labeled probe competes with non-labeled substrate for uptake from the lumen. The one situation in which high luminal substrate concentration had little effect on predicted probe absorption rate was when the apparent passive permeability coefficient was increased, and this makes sense because most of the predicted probe absorption is nonmediated and thus not competitively inhibited.

The simulation results support our interpretation of the empirical results, and they add one new insight. First, simulation results are consistent with the empirical findings that absorption by intact house sparrows was not dominated by the mediated pathway. The simulations indicate that when passive absorption dominates, then absorption of 3OMD- and L-glucose will be similar in rate and extent (see especially Figs 6Aiv, Biv, 7Aiv, Biv), which is what we observed empirically (Fig. 4). In contrast, the simulations indicate that if mediated absorption had dominated, then the absorption of 3OMD-glucose should have been more rapid and extensive than that of the L-glucose, even despite the fact that the 3OMD-glucose has a lower affinity than D-glucose for the brush border glucose transporter (see especially Figs 6Aiii, Biii, 7Aiii, Biii), but this was not observed empirically.

Second, the simulations help us interpret a second empirical finding – that when the measurements were done under conditions relatively more saturating for the glucose transporter, the rates of absorption increased, even for  $[^3\text{H}]3\text{OMD}$ -glucose (c.f. Fig. 4A,B). In contrast to this empirical finding, the simulation indicated that if most  $[^3\text{H}]3\text{OMD}$ -glucose uptake were mediated, then its absorption would have been inhibited by unlabeled substrate in the lumen rather than accelerated. However, studies in mammals (Pappenheimer and Reiss, 1987; Madara and Pappenheimer, 1987; Pappenheimer, 1987; Pappenheimer and Volpp, 1992; Sadowski and Meddings, 1993; See and Bass, 1993; Turner and Madara, 1995; Chang et al., 2004) and in birds (Chediack et al., 2003) have shown that hydrophilic molecules cross the intestinal mucosa by diffusion and/or solvent drag *via* a

Fig. 7. The simulated pattern of D- and L-glucose radiolabeled probe absorption at the apical membrane in the presence of an initial high substrate concentration ( $100 \text{ mmol l}^{-1}$ ) varies according to whether absorption is primarily mediated or primarily passive. The same four different situations as shown in Fig. 6 are modeled, but the starting concentration of unlabeled D-glucose is 100 times higher. Notice that when passive absorption dominates (Aiv, Biv), the predicted cumulative absorption of D- and L-glucose probes as a function of time (Aiv) are much more similar than when mediated absorption dominates (Aiii). Analogously, when passive absorption dominates, the predicted absorption rates of D- and L-glucose probes as a function of time (Biv) are much closer than when mediated absorption dominates (Biii).

paracellular pathway, and that the permeability of this pathway is enhanced when  $\text{Na}^+$ -coupled nutrient transport occurs. The mechanism(s) for acceleration by luminal glucose is not known

in house sparrows, but might be increased solvent drag and/or cytoskeletal contractions (Madara and Pappenheimer, 1987; Pappenheimer, 1987; Madara et al., 1986, 1988) or protein

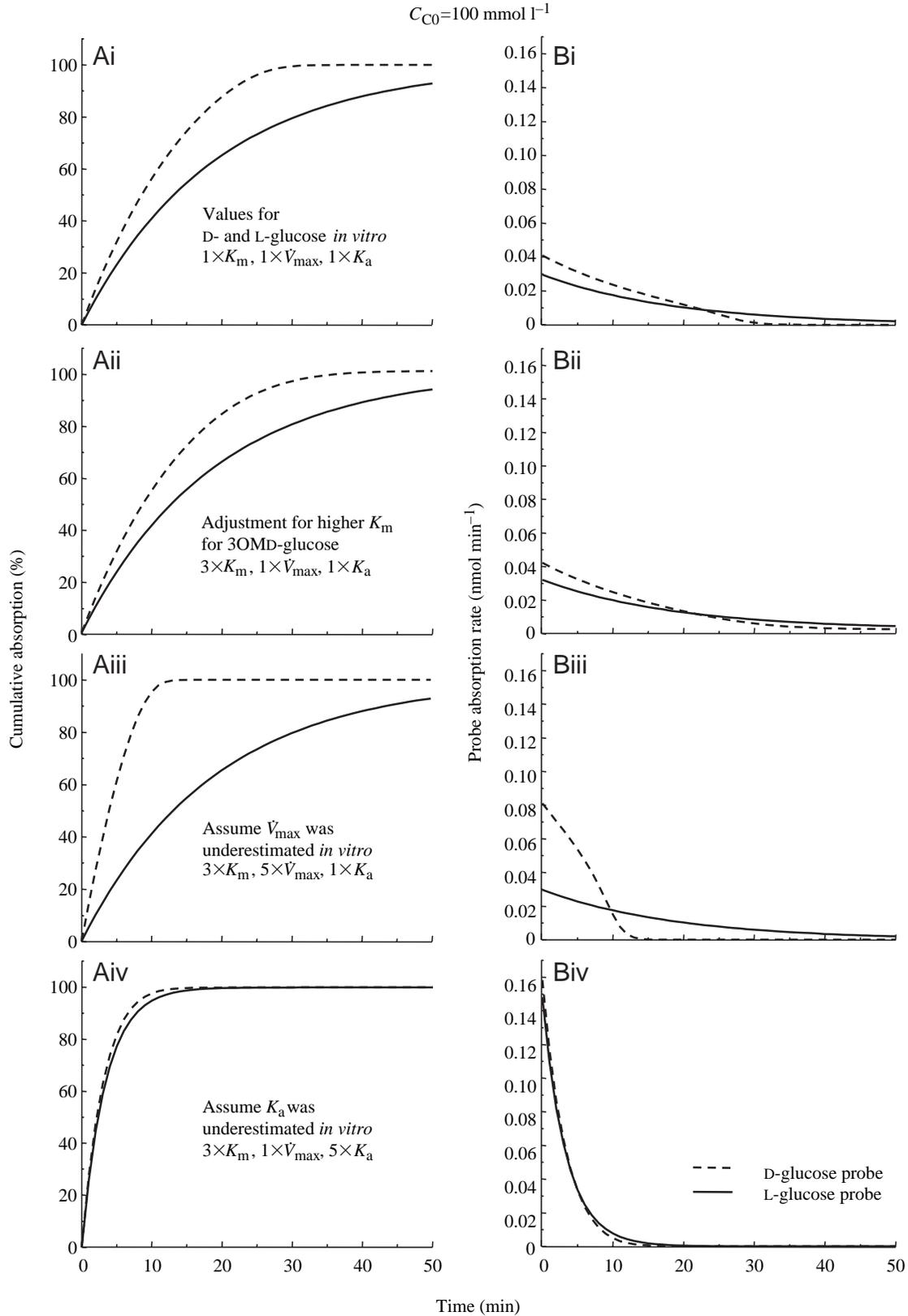


Fig. 7

strand alterations that alter the tight junction effective pore size (Pappenheimer and Reiss, 1987). This feature was not included in our simple simulation. Mediated transport by SGLT1 seems to play a role in the acceleration of passive absorption, because when the former was partially blocked by phloridzin, the specific SGLT1 inhibitor, *in vivo* passive absorption was reduced by about a third in laboratory rats (Fasulo et al., 2001).

#### *Passive absorption and its ecological and evolutionary implications*

Our results underscore how, in some animals, the glucose absorptive capacity of the small intestine cannot be accurately estimated only on the basis of *in vitro* measurements of mediated glucose uptake. The quantitative determination of physiological capacity in relation to load has been called a new research frontier (Diamond, 1993), and our research helps establish important limits on scientists' attempts to match the capacity to absorb nutrients determined on the basis of *in vitro* data with nutrient intake seen *in vivo*.

From an evolutionary perspective, one can argue that there are both costs and benefits to intestinal permeability to hydrosoluble biochemicals. A possible cost is that a high intestinal permeability that permits passive absorption might be less selective than a carrier-mediated system for nutrient absorption and might permit toxins to be absorbed from plant and animal material in the intestinal lumen (Diamond, 1991). But it has also been suggested (Pappenheimer, 1993) that passive absorption may confer a selective advantage because it requires little energy and provides a mechanism whereby rate of absorption is matched to rate of hydrolysis. Opposing costs and benefits could lead to variation among species in intestinal permeability to hydrosoluble biochemicals. This could explain why studies of some species indicate relatively low reliance on passive absorption (Fine et al., 1993; Uhing and Kimura, 1995; Schwartz et al., 1995; Lane et al., 1999) and others such as ours indicate relatively high reliance (Pappenheimer, 1990, 1998; Karasov and Cork, 1994; Karasov et al., 1996; Levey and Cipollini, 1996). For the latter type, vulnerability to hydrophilic toxins could be an important ecological driving force, constraining food exploratory behavior, limiting the breadth of the dietary niche, and selecting for compensatory behaviors such as searching for and ingesting specific substances that inhibit hydrophilic toxin absorption (Diamond et al., 1999).

To continue to advance knowledge on the nutritional, pharmacological, and ecological significance of paracellular absorption we need to determine in more species the relative importance of passive absorption with whole-animal studies like the present one and test different probes and different putative modulators of the paracellular pathway.

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