Heat stress increases apical glucose transport in the chicken jejunum

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Heat stress increases apical glucose transport in the chicken jejunum. Am J Physiol Regul Integr Comp Physiol 290: R195–R201, 2006. First published September 8, 2005; doi:10.1152/ajpregu.00393.2005—In chickens, elevated environmental temperature reduces food intake. We have previously reported that, during heat stress, the intestinal mucosa has an increased capacity to take up sugars. To investigate whether the effects of warm environment on sugar uptake are an intestinal adaptation to lower energy intake or a response attributable to heat stress, we examined the glucose transport kinetics of apical and basolateral membranes of the jejunum and the mucosal morphology of broiler chickens maintained in climatic chambers for 2 wk. Experimental groups were 1) control ad libitum (CAL), fed ad libitum and in thermoneutral conditions (20°C); 2) heat stress ad libitum (HSAL), fed ad libitum and kept in a heated environment (30°C); and 3) control pair-fed (CPF), maintained in thermoneutral conditions and fed the same amount of food as that consumed by the HSAL group. Both the CPF and the HSAL groups showed reduced body weight gain, but only the HSAL chickens had lower plasma thyroid hormones and higher corticosterone than CAL and CPF groups. The fresh weight and length of the jejunum were only reduced in the HSAL group. The activity and expression of apical sodium-dependent glucose transporter 1 (SGLT-1) were increased by ~50% in the HSAL chickens, without effects in the CPF group. No changes in Kᵦ or in SGLT-1 and glucose transporter-2 Kᵦ were observed in the pair-fed and heated birds. These results support the view that increased intestinal hexose transport capacity is entirely dependent on adaptations of apical SGLT-1 expression to heat stress and is not due to reduced food intake.

chronic heat exposure; glucose transporter 2; hexose transport; intestinal absorption; sodium-dependent glucose transporter 1

The mucosal epithelium of the small intestine constitutes a highly dynamic interface with the external environment through the delivery processing and absorption of nutrients. The intestinal mucosa is capable of rapid and extensive morphological and functional adaptation in response to evolutionary, genetic, and ontogenetic demands (12), as well as to environmental and nutritional challenges (25). In the domestic fowl, intensive artificial genetic selection has resulted in commercial meat breeds (broiler chickens) whose growth rates and food conversion efficiencies greatly exceed those of their genetic predecessors (21). It may be proposed that the increased growth of “demanding” tissues, such as skeletal muscle, should be accompanied by appropriate adaptations in structure and function of “support” tissues, such as the gastrointestinal tract (38). Indeed, previous studies have demonstrated such adaptations in the small intestine of the highly selected broiler chicken in terms of crypt cell dynamics and enterocyte migration rates (40). Such birds, however, appear to have an intestinal mucosal compartment, which is relatively smaller, compared with body size, than genetically unselected lines (36). Thus it is proposed that functional adaptations in terms of nutrient absorption at the enterocyte level have supported the increased demands of elevated growth rate (36). In fact, it is now considered that nutrient absorption and adaptations therein may represent the rate-limiting step in further genetic improvements in broiler chicken growth rates (9).

It is well established that chronic heat stress reduces growth rate and the feed conversion efficiency in broiler chickens (19, 41). While these effects are, in part, attributable to the hyperthermia-induced decrease in food intake, growth depression may also be mediated directly by the associated metabolic and endocrine responses (29), as indicated by paired feeding studies. Broiler chickens are more susceptible to heat stress than slower growing domestic fowl, and both their adaptive and pathological responses to extended thermal challenge involve multiple organs and systems (1). It is, therefore, possible that both inanition and changes in metabolic and endocrine status will induce adaptive responses in intestinal absorptive function.

In the domestic fowl, the capacity to absorb nutrients depends both on the mucosal surface area of the small and large intestines and on the functional properties of the specific nutrient transporters present in the brush-border and the basolateral membranes (2). There are specific transport systems for the major dietary hexoses. Glucose is absorbed across the apical sodium-dependent glucose transporter 1 (SGLT-1) system, expressed along the small and large intestine (13, 15); fructose is taken up by the apical facilitated glucose transporter (GLUT)-5-type system (16); and both sugars are transported to the interstitial compartment through the basolateral GLUT-2 transporter (15).

Previous studies have shown that exposure of chickens to elevated environmental temperature for 2 wk markedly reduced food intake and that the associated lower growth rate was accompanied by increased in vivo uptake of galactose and methionine when measured on a tissue dry weight basis (33). This apparently enhanced absorption capacity was confirmed in in vitro studies in which enterocytes from chronically heat-adapted birds showed a 50% increase in galactose accumulation ratio compared with cells from control chickens (34). However, the precise mechanisms of this adaptation and the contributions of reduced food intake associated with heat stress...
and hyperthermia per se are not clear. The present study was designed to address these areas and specifically to characterize the transport properties of SGLT-1 and GLUT-2 transporters in isolated membrane vesicles from the small intestine of broiler chickens during heat stress adaptation. To distinguish between the adaptive responses attributable to prolonged heat stress per se or to the reduced food intake, our experiment employed paired feeding.

**MATERIALS AND METHODS**

**Animal model.** Male broiler chickens (*Gallus gallus domesticus*), 28 days old, were randomized into three experimental groups and maintained in climatic chambers for 2 wk, with free access to water. Birds were fed a diet formulated at the Roslin Institute containing (per kg diet) 183 g crude protein, 43 g lipid, and 398 g carbohydrate; the metabolizable energy content was 12.35 MJ/kg. The experimental groups were as follows: 1) control ad libitum (CAL), chickens fed ad libitum and exposed to thermoneutral conditions (20°C, 50% relative humidity); 2) heat stress ad libitum (HSAL), chickens fed ad libitum and exposed to heat stress conditions (30°C, 70% relative humidity); and 3) control pair-fed (CPF), chickens exposed to thermoneutral conditions and fed with the same amount of food consumed by the HSAL group. Initially, each experimental group consisted of 12 birds, but natural losses and culls reduced the final numbers in the HSAL and CPF groups. Body weight and rectal temperatures were monitored throughout the 14-day experimental period (between 0900 and 1000 on measurement days to avoid circadian variations).

At 42 days, birds were killed in the morning by cervical dislocation without previous starvation. The jejunum was removed, immediately flushed with ice-cold saline, opened lengthwise, frozen in liquid nitrogen, and then stored at −80°C. The brush-border (BBMV) and basolateral membrane vesicles (BLMV) were prepared according to Garriga et al. (15). Manipulation and experimental procedures were in accordance with Spanish regulations for the use and handling of experimental animals, and the protocol was approved by the Ethical Committees of both the Universitat de Barcelona and the Roslin Institute.

**Blood sampling.** Blood samples (2.5 ml) were obtained by simple venepuncture of the brachial vein from 9–12 birds in each group at 42 days of age. All samples were taken between 0900 and 1000 and placed in heparinized tubes in ice. Plasma samples were immediately obtained by centrifugation at 1,500 g and at 5°C for 10 min and stored at −20°C. Packed cell volume or hematocrit was determined as described by Maxwell (31).

**Plasma glucose.** Plasma concentration of glucose was measured by using a commercially available kit (Wako glucose) obtained from Alpha Laboratories (Hampshire, UK) and modified for use in an automated plate-reading spectrophotometer (Titertek 2, Autoflow Laboratories).

**Thyroxine and tri-iodothyronine.** Plasma thyroxine (T₄) concentration was measured by radio immunoassay with the use of a commercially available kit (Gamma B T₄; IDS, Tyne and Wear, UK). The assay sensitivity was 2.0 ng/ml. Plasma tri-iodothyronine (T₃) concentration was measured with the use of a commercially available ELISA assay (T₃ Microwell EIA; IDS). The assay sensitivity was 0.4 ng/ml. Both assays were adapted for use with avian plasma, and the standard concentrations were adjusted appropriately.

**Glucagon.** Plasma glucagon concentration was measured by radio immunoassay (Linco Research), as previously described (4, 18). The sensitivity of the assay was 20 pg/ml. Because the primary antibody is directed at a mammalian glucagon molecule, the values are expressed as glucagon-like immunoreactivity for avian plasma, as previously described (4, 18).

**Corticosterone.** Plasma corticosterone concentration was determined by radio immunoassay (Gamma B ¹²⁵I-labeled corticosterone; IDS) following extraction of the plasma with dichloromethane, as described by Mitchell et al. (35). The assay sensitivity was 0.2 ng/ml.

**Intestinal morphometry and light microscopy.** For the morphometric study, the entire jejunum was removed and immediately flushed with ice-cold saline. The length was determined as described by Mitjans et al. (37). For the light microscopy study, two pieces ~10 mm long were excised from the proximal and distal ends of the segment. Samples were fixed with Bouin solution, dehydrated in a graded series of ethanol, and finally embedded in paraffin wax (37). Five sections 10 μm thick were obtained and stained with hematoxylin and eosin. Villous lengths were obtained for at least 10 structures in each section, and the average value was calculated for each chicken; therefore, in results, “n” refers to the number of animals processed.

**Electron microscopy.** Pieces 2 mm long were taken from the jejunum for transmission electron microscopy. Samples were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/l phosphate buffer (pH 7.4, 4°C) for 2 h, as described by Mitjans et al. (37), and then washed in 0.2 mol/l phosphate buffer. The tissues were then processed for routine transverse electron microscopy at the Servei de Microscopì a Electrònica de Serveis Cientificotècnics of the Universitat de Barcelona. Quantitative analysis was performed on high-magnification photomicrographs. The lengths of longitudinal sections of microvilli were measured in 20–26 micrographs per chicken group.

**Membrane vesicle preparation.** BBMV were prepared by MgCl₂ precipitation (15). The vesicle suspension was obtained in a medium containing 300 mM mannitol, 0.1 mM MgSO₄, 0.41 μM LiN₃, and 20 mM HEPES-Tris (pH 7.4), with a protein concentration of 15–25 mg/ml.

BLMV were prepared according to the study by Garriga et al. (14). The vesicles were suspended in a medium containing 300 mM mannitol, 20 mM HEPES-Tris (pH 7.5), 0.1 mM MgSO₄, and 0.41 μM LiN₃ with a protein concentration of 15–20 mg/ml.

**Enzyme and protein determinations.** The activity of the ouabain-sensitive Na⁺/K⁺-activated ATPase (Na⁺/K⁺-ATPase, EC 3.6.1.3) was routinely assayed as a marker of the basolateral membrane following the study by Colas and Maroux (8). Sucrase (α-d-glucopyranosidase, EC 3.2.1.48), the marker of the brush-border membrane, was assayed according to the study by Messer and Dahlqvist (32). The protein content was determined by using the Coomassie brilliant blue method with bovine serum albumin as standard (5).

**Transport assays.** The uptake of o-methyl-d-glucoside (αGlC1Me) and d-glucose (D-Glc) was measured at 37°C by a rapid filtration technique (15). In the BBMV studies, αGlC1Me is used because it is specific for the apical SGLT-1 isoform, thus avoiding the influence of any basolateral membrane contamination. For the kinetic studies of the basolateral GLUT-2 isoform, we used D-Glc in the absence of Na⁺ to avoid any influence of apical contamination. The assays of αGlC1Me transport were performed in short-circuit conditions by loading the vesicles (30 min, 37°C) in a medium containing 200 mM mannitol, 50 mM KCl, 20 mM HEPES-Tris (pH 7.4), 0.1 mM MgSO₄, and 0.41 μM LiN₃. Valinomycin was added to the incubation medium at a final concentration of 45 μM to render the vesicles permeable to K⁺. The substrate concentrations used for the kinetic analysis of αGlC1Me uptake by BBMV were 0.01, 0.05, 0.075, 0.1, 0.25, 50, and 75 mM. For the kinetic analysis of D-Glc uptake by the basolateral membrane, vesicles were incubated with 0.01, 0.05, 0.1, 0.5, 1, 5, 15, 50, 100, 150, and 200 mM D-Glc. The osmolality of intra- and extravesicular media was kept constant at 320 osmol/kg by adjusting the total sugar concentration with mannitol.

**Binding measurements.** Steady-state phlorizin binding was assayed at 37°C, as described in a previous work (15). The density of specific phlorizin binding sites is expressed in picomoles of phlorizin bound per milligram of protein at 50 μM phlorizin (Bₐ₀).

Steady-state cytochalasin B binding was assayed at 37°C by the method described by Cheeseman and Maenz (7) with some modifi-
Animal model. Table 1 summarizes the main characteristics of the animal model. Body weight gain throughout the experimental period was higher (13% increase; \( P < 0.05 \)) in CAL than in CPF or HSAL chickens. There were no significant differences in final body weight between the CPF and HSAL groups. Food intake was reduced 21% \( (P < 0.05) \) in the raised-temperature condition. High environmental temperature significantly increased the rectal temperature in HSAL chickens (1.5–1.8°C higher than the groups kept in thermoneutral conditions) already from the second day in the climatic chamber and remained stable thereafter.

No effect was observed on blood hematocrit (Table 1). The serum glucose concentration was not affected by food restriction but showed a small but significant increase in the HSAL group compared with CAL animals. Hormone determination in plasma showed that chronic heat stress induces a significant increase in plasma corticosterone (90% increase compared with birds kept on thermoneutral conditions) and a reduction in circulating \( T_3 \) and \( T_4 \) concentration (32 and 37%, respectively). The concentration of glucagon was higher in CPF than in CAL chickens, with values in the HSAL group that were in between the controls, without statistical differences. The activity of mucosal enzymes

Table 2. Morphometry and histology

<table>
<thead>
<tr>
<th>CAL</th>
<th>CPF</th>
<th>HSAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh weight, g</td>
<td>30.9±1.3 (20)*</td>
<td>27.5±1.1 (12)*</td>
</tr>
<tr>
<td>Fresh weight/body weight, %</td>
<td>1.24±0.03 (12)*</td>
<td>1.24±0.03 (11)*</td>
</tr>
<tr>
<td>Length, cm</td>
<td>76.9±0.4 (20)*</td>
<td>78.2±0.6 (12)*</td>
</tr>
<tr>
<td>Villous length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal jejunum, ( \mu m )</td>
<td>1,318±17 (20)</td>
<td>1,285±11 (12)</td>
</tr>
<tr>
<td>Distal jejunum, ( \mu m )</td>
<td>1,053±7 (20)*</td>
<td>955±9 (12)†</td>
</tr>
<tr>
<td>Microvilli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length, ( \mu m )</td>
<td>1.57±0.26 (3)*</td>
<td>2.25±0.07 (3)†</td>
</tr>
</tbody>
</table>

Values are means ± SE; no. of experiments are in parentheses. *†‡ Values with different superscript symbols were statistically different \( (P < 0.05) \).
sucrase and Na\(^+\)-K\(^+\)-ATPase was the same in all experimental groups.

**Morphometric and ultrastructural studies.** The jejunal of animals exposed to heat stress showed a 22% reduction in fresh weight and a 5% reduction in length (see Table 2). These results should be attributed to an effect of high environmental temperature because the pair-fed animals showed values similar to those of controls (CAL group).

The microscopic study of the villous length was performed at the two ends of the jejunal to obtain information from both proximal and distal regions. In the proximal region, there were no differences between villous lengths from all groups. In contrast, in the distal jejulum, the villous length was different in each experimental group: maximum in CAL chickens, medium in CPF birds, and minimum for the HSAL group (Table 2), compatible with the changes observed in jejunal fresh weight. Results from the electron microscopy study showed that microvillous length was higher in CPF and HSAL groups than in CAL chickens (Fig. 1), indicating that food restriction can stimulate microvillous growth.

**Characterization of the membrane vesicle.** The membrane purity, vesicular orientation, and intravesicular volume of both BBMV and BLMV were determined. In the final BBMV preparation, sucrase activity was 11-fold, and the overall recovery was > 81% (n = 15), without significant differences between experimental groups. The activity of the basolateral marker Na\(^+\)-K\(^+\)-ATPase was 0.8-fold, indicating that the basolateral contamination was negligible. The intravesicular volume, calculated under equilibrium conditions using 0.1 mM αGlc1Me, was 0.80 ± 0.09 µl/mg protein, similar to previous results (14).

In BLMV, the enrichment factor for the Na\(^+\)-K\(^+\)-ATPase activity was also high (10-fold), and the overall recovery was 84% (n = 15), without significant differences between the three experimental groups. The activity of apical sucrase was 0.9-fold. The intravesicular volume, calculated under equilibrium conditions using 1 mM d-Glc, was 2.10 ± 0.33 µl/mg protein.

**Transport of αGlc1Me across BBMV.** The calculated kinetic constants are shown in Table 3. There are no significant differences between the diffusion and Michaelis constants of any experimental group. However, the ω-Glc maximal transport rate of the HSAL animals was increased by 50% compared with the control groups (CAL and CPF). Table 3 also shows that the specific binding of phlorizin to SGLT-1 is also increased by ~60% in the heat-stressed birds. The calculated kinetic constants are shown in Table 3. There are no significant differences between the diffusion and Michaelis constants of any experimental group.

**Discussion.** Chickens maintained on a high environmental temperature (HSAL group) for 2 wk showed increased rectal temperature...
and decreased body weight. The reduction in body mass was probably due to decreased food intake, because similar final weight values were observed in the CPF group. On the day of death, the fresh weight of the small intestine in the HSAL chickens was lower (in absolute value, as well as relative to body weight), and the jejunum was shorter than in the CPF animals. This indicates that, in addition to the effects of food restriction (i.e., lower intestinal growth proportional to body weight reduction), high temperature further reduces intestinal fresh weight, consistent with the observations of Mitchell and Carlisle (33) and with the hypothesis that thermal loads depress enterocyte proliferation and growth (27).

At the end of the experimental period, HSAL chickens had reduced plasma T3 and increased plasma corticosterone, consistent with the hormonal profile described for the model of heat stress adaptation (19). A similar pattern of responses was described for heat-acclimated desert rodents, that is, reduced intestinal weight and a large decrease in plasma T3 concentration (46). Our results also show that the plasma concentration of T3 in HSAL chickens was lower than in CPF and CAL groups, supporting the view that caloric restriction does not alter thyroid hormone metabolism (19). Acute heat exposure also induces a transient decrease in plasma T3 and a reduction of food intake in young chicks (44). Since thyroid hormones have trophic effects stimulating the growth of the intestinal mucosa (28), we suggest that functional hypothyroidism mediates the reduction of jejunal mass and villous height in the HSAL chickens.

Acclimation to a warm environment did not affect either the passive hexose permeability or the affinity constant in mucosal enzyme markers, indicating that the metabolic changes associated with heat stress do not affect basic functions of the membrane. The study of the effects of heat stress adaptation on the hexose transport properties in the chicken intestine showed that the capacity to take up hexose across the apical SGLT-1-type transporter was increased, consistent with previous results (33) and with observations from rodents (46), while the kinetic properties of the basolateral GLUT-2-type transporter remained unchanged. These observations were strengthened by the results of binding and Western blot analysis, both confirming that heat stress increases the number of hexose transporters in the apical membrane only. In rodents, diets rich in free simple sugars (glucose, fructose) stimulated the transient recruitment of GLUT-2 by the brush-border membrane (20, 26). The possibility that GLUT-2 was expressed in the chicken apical membrane of the jejunum was not addressed in the present study, but this is considered unlikely because our chickens were fed a diet with low concentrations of free sugars. However, apical SGLT-1 kinetics would not be altered by the presence of GLUT-2, because all transport experiments in BBMV were done using αGlc1Me, a glucose analog that is selective for SGLT-1 (14).

Previous studies have shown that elevated environmental temperature increases galactose and methionine uptake in perfused intestine in vivo (33). One signal mediating these effects could be glucagon, as chronic glucagon administration enhances glucose and galactose transport in the rat jejunum in vitro (42), as also happens in our HSAL condition. However, the CPF chickens, albeit with increased plasma glucagon concentration, have an intestinal SGLT-1 expression similar to that of the CAL group, indicating that other signals may be involved in the heat stress response. The effects on nutrient uptake do not seem to be related to changes in circulating T3, either, because this hormone has the opposite effects as it stimulates brush-border D-Glc Vmax by increasing SGLT-1 activity (10). Therefore, we can conclude that upregulation of SGLT-1 by heat stress is apparently mediated by signals other than thyroid hormones and glucagon. A plausible candidate to mediate these effects is corticosterone, especially considering that its plasma concentration in the HSAL group is twofold that of the controls. Glucocorticoids play a key role in glucose metabolism, including the trafficking of GLUT-4 to the plasma membrane of hepatocytes (39). They can also regulate glucose transport in intestinal epithelial cells; for example, dexamethasone induces acute expression of SGLT-1 in the rabbit small intestine (23), and Dieter et al. (11) have demonstrated that glucocorticoids induce the expression of serum and glucocorticoid-regulated kinase 1, which enhances glucose transport by increasing SGLT-1 abundance in the cell membrane.

The higher cumulative capacity due to increased number of SGLT-1 transporters will result in stimulation of net transepithelial transport, because the low-affinity/high-capacity kinetic properties of GLUT-2 enable efficient transfer of D-Glc, even at high cytosolic glucose concentrations (2). The HSAL group shows a significant hyperglycemia, an effect repeatedly observed in chickens (33, 43) and rats (6) under chronic heat stress. This effect of heat stress can be attributed to shorter intestinal transit times (43), which will extend the time that nutrients are exposed to the mucosal absorptive epithelium; to increased apical SGLT-1 activity (33; and the present study),
which will result in higher rates of absorption; or to increased gluconeogenesis due to enhanced plasma corticosterone, mainly from muscle tissue proteins (3).

At high ambient temperatures, there is a decrease in protein synthesis (19), probably due to reduced plasma amino acid concentration and to lower energy supply (41), as observed in broiler chicken muscle tissue. In addition, heat stress decreases plasma T3 concentration and increased plasma corticosterone, both changes known to reduce protein deposition through alterations in protein turnover in birds and other species (47). These effects reduce the total muscle mass, as reflected by the alterations in protein turnover in birds and other species (47).

Interestingly, the two groups with lower food intake (CPF and H9252) showed increased microvillous length, restricted to the distal region. These effects reduce the total muscle mass, as reflected by the alterations in protein turnover in birds and other species (47). Generalitat de Catalunya (Spain) is also acknowledged.

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