Hydroxycitric acid delays intestinal glucose absorption in rats

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Hydroxycitric acid (HCA) is used in food supplements to reduce food intake and body weight. HCA reduces food intake and body weight in rodents (4, 6, 7, 16, 17). However, the precise mechanism by which HCA exerts this effect is unknown. One option is the ability of HCA to change lipid metabolism in rat liver cells by inhibition of de novo lipogenesis through blockade of the enzyme ATP-citrate-lyase, an enzyme that catalyzes the cleavage of citrate to acetyl-CoA and oxaloacetate (8, 15, 21). As a consequence, malonyl-CoA levels are reduced (10), which has been suggested to decrease food intake (2).

Besides a reduction in food intake and body weight, HCA might have other beneficial health effects. We recently found that intragastrically administered HCA reduced postprandial blood glucose and insulin responses after intragastric administration of a highly nutritive solution (22). Reduced postprandial glucose and insulin profiles have been suggested to be beneficial for health, in particular in relation to a reduced risk for the development of type 2 diabetes mellitus and coronary heart disease (18).

The aim of the present study is to identify the underlying mechanism(s) of the reduced postprandial glucose response after HCA administration. Two possible mechanisms: 1) a delay in gastric emptying and 2) reduced intestinal glucose absorption. To test the relative contribution of these possible mechanisms, we investigated the effect of HCA on gastric emptying and intestinal glucose absorption in rats by comparing the glucose response after an intragastric or intraduodenal glucose load. The systemic appearance of glucose from the intestine can be monitored by using an intravenous infusion with labeled glucose, an oral loading with differently labeled glucose, and the intermittent sampling of blood for the analysis of isotopic enrichment of blood glucose. To calculate the rates of appearance of glucose in this type of dual tracer study, the Steele’s one-compartment model can be employed (3, 12). In our study, using a primed continuous intravenous infusion of [U-13C]glucose and intraduodenal bolus administration of [U-13C]glucose, the effect of HCA on systemic glucose appearance after an intraduodenal glucose load was evaluated.

These experiments cannot exclude an enhanced postabsorptive glucose clearance. For that reason, we studied the effect of HCA on the presence of glucose in the enterocytes, portal and systemic blood, and liver after intragastric bolus administration of [U-13C]glucose, to discriminate between intestinal and postabsorptive effects of HCA.

MATERIALS AND METHODS

Animals and Housing

Male Wistar rats from our own breeding colony, weighing 300–330 g at the beginning of the study, were individually housed in Plexiglas cages (25 × 25 × 30 cm) on a layer of wood shavings under controlled temperature (21 ± 1°C), relative humidity of 55 ± 5%, and on a 12:12-h light-dark cycle (lights on 0700 to 1900 h). Water and standard rat chow (Hope Farms, Woerden, The Netherlands) were available ad libitum, unless otherwise stated. All experiments were approved by the Animal Experiments Ethical Committee of the University of Groningen (DEC 2477).

Surgery

Three different experiments were performed (see below). For experiment 1, rats were equipped with an intragastric infusion catheter...
and a jugular vein catheter for blood sampling. The animals in experiment 2 were equipped with an intraduodenal infusion catheter and two heart catheters, one for infusion and one for blood sampling. For experiment 3 rats were equipped with an intragastric infusion catheter and a heart catheter for blood sampling.

Surgery was performed under N2O-isoflurane anesthesia. The blood sampling heart catheter (silicon, 0.95-mm OD, 0.50-mm ID) was inserted into the heart through the right jugular vein according to techniques described elsewhere (13). The intravenous infusion catheter (silicon, 0.64-mm OD, 0.28-mm ID) was inserted and fixed into the left jugular vein. The intragastric infusion catheter (silicon, 1.40-mm OD, 0.80-mm ID) was inserted and fixed into the antrum wall of the stomach. The opening of the intragastric catheter extended 0.5 cm into the stomach lumen (14). The intraduodenal infusion catheter (silicon, 0.95-mm OD, 0.50-mm ID) was inserted and fixed into the duodenum, 2 cm distal from the pylorus. The opening extended 0.3 cm into the duodenal lumen. Tubings were drawn under the skin and externalized on the top of the skull with dental cement. After surgery, finadyne (0.1 ml/kg sc, Schering-Plough, Utrecht, The Netherlands) was injected once as analgesia. The animals were allowed to recover for at least 1 wk after surgery.

Experimental Design

On the basis of comparison of experiments 1 and 2, the effect of HCA on gastric emptying was evaluated. In experiment 2 the effect of HCA on the systemic total rate of glucose appearance (RaT), the rate traced in intestinal epithelium, portal blood, and liver after intragastric HCA and [U-14C]glucose. Food was removed 24 h before the experiment. The start of intragastric [U-14C]glucose infusion was defined as time point t = 0 min. A primed-continuous intravenous infusion of d-[6,6-2H2]-labeled glucose in isotonic NaCl (17.8 μmol/ml, bolus: 1.0 ml, and continuous: 1.0 ml/h) was started at t = −180 min and continued until the end of the experiment at t = 150 min. At t = −120 min, HCA (n = 7) or vehicle (n = 7) was administered intraduodenally. At t = 0 min, intraduodenal glucose infusion (2 ml; 0.123 g/ml distilled water in 5 min) enriched with 10.0% [U-13C]glucose was started. Blood samples (0.15 ml) and blood spots for analysis of isotopic enrichments were taken frequently between t = −180 and t = 150 min.

Experiment 3: effect of HCA on distribution of intragastrically administered glucose across enterocytes, portal and systemic blood, and liver. Intragastrically infused radioactive-labeled glucose was traced in intestinal epithelium, portal blood, and liver after intragastric HCA and [U-14C]glucose. Food was removed 24 h before the experiment. The start of intragastric [U-14C]glucose infusion was defined as time point t = 0 min. At t = −120 min, HCA (n = 6) or vehicle (n = 6) was infused intragastrically. At t = 0 min, intragastric glucose infusion (9 ml; 0.123 g/ml distilled water in 5 min) enriched with 31 kBq/ml [U-13C]glucose was started. At t = 15 min, the rat was anesthetized by intravenous administration of 0.35 ml pentobarbital. A portal blood sample (0.5 ml) was collected in a tube containing 10 μl EDTA and stored on ice. A liver biopsy from the ventral medial lobe was biopsied and stored at −20°C. Distal from the stomach and proximal from the cecum, the gut was tied off. The gut was flushed with 10 ml saline. Biopsies of ~1 cm of the proximal part of the gut (−2 cm below the pylorus), medial part of the gut (the middle between pylorus and cecum), and distal part of the gut (~2 cm before cecum) were taken and stored at −20°C until analysis for radioactivity.

Analysis

For determination of blood glucose levels, 50 μl blood was added to a mixture of 450 μl distilled water with heparin (10 ml/l heparin in distilled water) and stored at −20°C. Samples were analyzed by the ferricyanide method of Hoffman in a customized auto analyzer (Skalar, Breda, The Netherlands).

The isolation and derivatization of glucose out of the blood spots to glucose penta-acetate for the analysis of the isotopic enrichment of blood glucose in experiment 2 is described in detail elsewhere (20). In short, glucose was extracted by incubating a disk (6.5 mm) punched out of a bloodspot with ethanol/water (10:1 vol/vol) mixture. The extract was dried under nitrogen gas, and thereafter glucose was derivatized to its penta-acetate-ester. After being dried under nitrogen gas, the dry residue was dissolved in 100 μl ethylacetate for injection. The glucose penta-acetate was analyzed by gas chromatography quadrupole mass spectrometry (GCMS) (cat. no. SSQ7000; Thermo-Finnigan, San Jose, CA) under conditions previously described (20).

![Fig. 1](http://ajpgi.physiology.org/) Design of experiment 2. At t = −180 min an intravenous bolus of d-[6,6-2H2]-labeled glucose was injected and an infusion of d-[6,6-2H2]-glucose was started. At t = −120 min an intraduodenal (id) bolus of hydroxyoctic acid (HCA) was administered, and at t = 0 min [U-13C]-labeled glucose was given intraduodenally. Blood spots are indicated as x; blood samples combined with blood spots are indicated as •.
RESULTS

Experiment 1

Data are shown in Fig. 2. Baseline blood glucose levels did not differ between the HCA and control group (5.5 ± 0.1 and 5.6 ± 0.2 mM, respectively, P > 0.05). After intragastric infusion of 9 ml of glucose solution, blood glucose levels increased in the HCA group to 7.1 ± 0.2 mM at t = 25 min (P < 0.02) and in the control group to 8.5 ± 0.4 mM at t = 15 min (P < 0.02). ANOVA with repeated measures revealed significant interaction between treatment and time [F(7,84) = 2.897, P < 0.01]. Post hoc analysis showed a significantly lower glucose response after HCA treatment from t = 5 to t = 20 min compared with control treatment.

Experiment 2

In this experiment, the effect of HCA on the intestinal glucose absorption after intraduodenal glucose was examined. The data are shown in Figs. 3–6. Blood glucose did not change from t = −180 min at the start of the intravenous infusion of D-[6,6-2H2]glucose until the start of the intraduodenal infusion at t = 0 min [F(2,24) = 1.12, P > 0.05] (data not shown). There was also no difference in baseline blood

Fig. 2. Glucose response to 9 ml intragastric glucose solution (0.123 g/ml) during 5 min of treatment of rats with 310 mg/kg regulator HCA intragastrically (n = 7) or vehicle intragastrically (n = 7) 120 min before glucose infusion. The gray bar indicates the glucose infusion from t = 0 to t = 5 min. *P < 0.05, **P < 0.01.

Fig. 3. The glucose response to 2 ml glucose solution (0.123 g/ml id) during 5 min of rats treated with 310 mg/kg regulator HCA intragastrically (n = 7) or vehicle intragastrically (n = 7) 120 min before glucose infusion. The gray bar indicates the glucose infusion from t = 0 to t = 5 min. Inset, incremental area under the curve (iAUC) from time point t = −1 to the end of the study period was calculated, and differences were tested with two-tailed independent t-test. Significance was set at P < 0.05.
glucose at \( t = -1 \) min between the HCA and control group (4.2 ± 0.2 and 4.3 ± 0.2 mM, respectively, \( P > 0.05 \)). After intraduodenal administration of glucose, blood glucose levels significantly increased in both the HCA and control animals [to a maximum of 6.7 ± 0.4 mM at \( t = 45 \) min (\( P < 0.02 \)) and 7.9 ± 0.4 mM at \( t = 15 \) min (\( P < 0.02 \)), respectively]. ANOVA with repeated measures showed a significant interaction between treatment and time \([F_{(11,132)} = 12.14, P < 0.0001]\). Blood glucose levels of the HCA group were significantly lower from \( t = 5 \) to \( t = 20 \) min (\( P < 0.05 \)) compared with values in the control group. At \( t = 60 \) min (\( P = 0.01 \)) and \( t = 150 \) min (\( P = 0.01 \)), blood glucose levels of HCA rats were significantly higher compared with the values in the control group. \( \Delta\text{AUC} \) from \( t = -1 \) to \( t = 150 \) min did not differ significantly (\( P = 0.34 \)) between control and HCA-treated animals (224.5 ± 23.0 and 263.2 ± 32.0, respectively).

The RaT is shown in Fig. 4. Basal levels of the RaT, which equals the EGP, because no exogenous glucose is administered yet, did not differ between HCA and controls (34.9 ± 1.1 and 33.4 ± 1.5 \( \mu \text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} \), respectively, \( P > 0.05 \)). After intraduodenal infusion of glucose, the RaT of the HCA group increased to 68.4 ± 6.4 \( \mu \text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} \) at \( t = 45 \) min (\( P < 0.02 \)) and the RaT of the control group increased to 83.7 ± 3.7 \( \mu \text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} \) at \( t = 15 \) min (\( P < 0.02 \)). ANOVA with repeated measures showed a significant interaction between treatment and time \([F_{(10,110)} = 12.22, P < 0.0001]\). The RaT of the HCA group was significantly lower from \( t = 5 \) to \( t = 30 \) min (\( P < 0.05 \)) and higher from \( t = 60 \) to \( t = 150 \) min (\( P < 0.05 \)).

The RaE is shown in Fig. 5. ANOVA with repeated measures showed a significant interaction between time and treatment for RaE \([F_{(10,110)} = 19.91, P < 0.0001]\). RaE of the HCA group was lower from \( t = 5 \) to \( t = 30 \) min (\( P < 0.05 \)) and higher from \( t = 60 \) to \( t = 150 \) min (\( P < 0.05 \)) compared with RaE of the control group. The cumulative systemic appearance of exogenous glucose at \( t = 150 \) min was slightly higher in the HCA group relative to control (\( P < 0.01 \)).

On the basis of the RaT and the RaE the EGP in the postprandial state can be calculated. The EGP is shown in Fig. 6. No significant interaction between time and treatment was found for EGP \([F_{(10,110)} = 0.72, P > 0.05]\).

**Experiment 3**

The results of experiment 2 cannot exclude an effect of HCA on postprandial glucose clearance. This is investigated in experiment 3 by measuring radioactivity of liver and portal blood after infusion of intragastric [U-\( ^{14} \text{C} \)] glucose. Furthermore, the distribution across the enterocytes is assessed.

Figure 7 shows the data of the radioactivity levels of the different samples. All the samples from the HCA group, except those taken in the proximal intestine, contained significantly less radioactivity than the corresponding samples from the control group. At \( t = 15 \) min, the radioactivity of systemic plasma was significantly lower in HCA-treated rats compared with controls (64 ± 6 and 228 ± 28 Bq/ml, respectively, \( P < 0.001 \)) (Fig. 7A). Portal vein blood samples were significantly reduced in HCA-treated rats compared with control rats (184 ± 20 and 370 ± 36 Bq/ml, respectively, \( P = 0.001 \)) (Fig. 7B). Radioactivity of the liver was significantly lower in the HCA-treated rats compared with control rats (222 ± 21 and 522 ± 34 Bq/g, respectively, \( P < 0.001 \)) (Fig. 7C). Most of the radioactivity was found in the cells of the proximal gut, with no significant difference between HCA and control (1.49 ± 0.20 and 2.01 ± 0.24 kBq/g, respectively) (Fig. 7D). Radioactivity in the distal gut was significantly lower in HCA-treated rats compared with control rats (0.92 ± 0.21 and 1.69 ± 0.17 kBq/g, respectively, \( P < 0.02 \)) (Fig. 7E). Radioactivity in the medial gut was significantly lower in HCA-treated rats compared with control rats (70 ± 9 and 354 ± 82 Bq/g, respectively, \( P < 0.01 \)) (Fig. 7F).

**DISCUSSION**

HCA treatment reduced postprandial glucose profiles in rats. Data from the present study show that the reduced glucose response after HCA treatment are caused by a delayed intestinal absorption of glucose rather than by delayed gastric emptying.

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**Fig. 4.** Rate of appearance of total glucose (RaT) after 2 ml glucose solution (0.123 g/ml id) during 5 min of rats treated with 310 mg/kg regulator HCA intragastrically (\( n = 7 \)) or vehicle intragastrically (\( n = 7 \)) 120 min before glucose infusion. The gray bar indicates the glucose infusion from \( t = 0 \) to \( t = 5 \) min. Inset, \( \Delta\text{AUC} \) from \( t = 0 \) to \( t = 150 \) min. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \).

**Fig. 5.** Rate of appearance of exogenous glucose (RaE) after 2 ml glucose solution (0.123 g/ml id) during 5 min of rats treated with 310 mg/kg regulator HCA intragastrically (\( n = 7 \)) or vehicle intragastrically (\( n = 7 \)) 120 min before glucose infusion. The gray bar indicates the glucose infusion from \( t = 0 \) to \( t = 5 \) min. Inset, \( \Delta\text{AUC} \) from \( t = 0 \) to \( t = 150 \) min. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \).
Glucose was administered intraduodenally. On the basis of measurements, we determined the rate of appearance of exogenous glucose (RaE) before and 150 min after the administration of glucose. To determine the total rate of glucose appearance (RaT), the RaE, reflecting the rate of intestinal glucose absorption, can be calculated (19). It was found that the total RaT was lower in HCA-treated rats compared with control rats. For that reason, we may conclude that the difference in total RaT was completely due to a reduction in the entrance of exogenous glucose and not to changes in EGP.

It should be noted that the RaE in HCA rats was only lower than the control values in the first 30 min after infusion. Thereafter it changed, and the RaE in the HCA rats was higher than in the controls, a difference that persisted until the end of the experiment at t = 150 min. This means that HCA delays intestinal glucose absorption without an effect on total glucose absorption. Therefore, this experiment demonstrates that the decreased response in blood glucose levels after HCA treatment is due to delayed systemic appearance of the administered glucose.

On the basis of experiment 2, it cannot be excluded that HCA may increase the postabsorptive clearance of glucose with the concomitant reduction in blood glucose levels, because the RaE was calculated from posthepatic blood samples. Therefore, experiment 3 was performed in which [U-14C]glucose was administered intragastrically, and at t = 15 min, rats were killed after which radioactivity was measured. Increased clearance would lead to increased radioactivity in portal blood and in liver tissue samples. In contrast, we found a clear reduction in radioactivity in portal blood and in liver tissue samples after HCA treatment, which excludes that HCA increases portal clearance of glucose by the liver. This latter experiment also provided some information on the delayed absorption. By counting the radioactivity of the intestinal biopsies, it could be investigated whether the absorption of glucose from the lumen side into the mucosal cells was delayed or that glucose was retained in the mucosal cells and released at a slower rate into the bloodstream. It was found that the

Experiment 1 confirmed our previous finding (22) that prior intragastric administration of HCA reduced the increase in blood glucose levels after an intragastric load of glucose. In the following experiments, we investigated two possible underlying mechanisms: 1) an effect of HCA on gastric emptying and 2) decreased or delayed intestinal glucose absorption.

Theoretically, HCA treatment could cause a delay in the entrance of glucose in the gut by delaying gastric emptying, which would then lead to a delayed appearance of glucose into the blood. If reduced gastric emptying would contribute importantly to the observed effects of HCA, one would have expected that HCA would attenuate glucose response almost exclusively after intragastric, but not after intraduodenal administration of glucose. Our data clearly indicate, however, that the effect of HCA on blood glucose profiles were similar after intragastric or intraduodenal administration of glucose. This observation does not support an important role of gastric emptying.

To test whether HCA inhibits or delays intestinal glucose absorption, a dual isotope approach was applied. Using Steele’s one-compartment model, we measured the rate of appearance of glucose from an intraduodenal carbohydrate load and from endogenous sources in vivo (12). A primed-continuous infusion of D-[6,6-2H2]glucose was used for the determination of the total rate of glucose appearance (RaT) before and 150 min after the administration of glucose. To determine the rate of appearance of exogenous glucose (RaE), [U-13C]glucose was administered intraduodenally. On the basis of the measurement of [U-13C]glucose in blood samples and the RaT, the RaE, reflecting the rate of intestinal glucose absorption, can be calculated (19). It was found that the total RaT was lower in HCA rats from t = 5 to t = 30 min and higher from t = 60 to t = 150 min compared with control. The postprandial RaT is determined by the EGP and the rate of appearance of exogenous glucose (12, 19). The EGP did not differ between the groups, but the increase of the systemic appearance of exogenous glucose was delayed in HCA-treated rats.
counts in all tissue samples, except those taken in the proximal small intestine, were lower in HCA-treated rats, suggesting that the transport of glucose across the apical membrane is more likely to be affected than the transport across the basolateral membranes.

The mechanism underlying the delay in intestinal glucose absorption remains unknown. Glucose is predominantly actively absorbed in cotransport with Na\(^+\) by the sodium-dependent glucose transporter-1 (SGLT-1) on the apical membrane. An effect of HCA on the availability of Na\(^+\), either direct or indirect via an effect on Na\(^+\)-K\(^+\)-ATPase, might explain this delayed glucose absorption, but this is highly speculative. Another possibility might be an effect of HCA on the expression of SGLT-1.

In summary, rats treated with HCA 2 h before an intragastric or intraduodenal glucose infusion showed a reduced response of blood glucose due to delayed intestinal glucose absorption. Delayed gastric emptying or increased hepatic glucose clearance seems to be of less importance. Because reduced postprandial glucose profiles have been suggested to be beneficial for health, in particular in relationship to a reduced risk for the development of type 2 diabetes mellitus and coronary heart disease (18), one may speculate that these data open a possible therapeutic role of HCA as a food supplement.

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