Low calorie commercial sugar is a sensitive marker of glomerular filtration rate

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Background. Glomerular filtration rate (GFR) in humans and animals might be determined with precision by measuring the clearance of an ideal marker, such as inulin. However, the use of inutest, an inulin analog, is limited by its cost and accessibility. The present study tested whether low calorie commercial sugar (LC sugar) can be used to measure GFR during normal and renal dysfunction.

Methods. Two groups of 6 male Wistar rats weighing 300 to 350 g were included. One group was treated with a daily dose of cyclosporine (CsA) 30 mg/kg subcutaneously for 7 days and the other group was formed by nontreated control rats. In one half of each group, GFR was evaluated by using inutest and in the other half by using LC sugar. GFR was also evaluated by using a wide LC sugar plasma concentration range in an additional group.

Results. In nontreated rats, the mean GFR evaluated with LC sugar was 2.2 ± 0.1 mL/min. This value is equal to that obtained with inutest: 2.3 ± 0.1 mL/min. CsA administration produced a significant reduction of renal blood flow and renal function. The GFR reduction induced by CsA was similarly determined by both LC sugar and inutest to be at 1.0 ± 0.2 and 1.1 ± 0.2 mL/min (P = NS), respectively. In addition, GFR did not change when LC sugar plasma concentration gradually increased.

Conclusion. Our results show that in both normal and pathophysiologic conditions, LC sugar is a good marker of GFR similar to the gold standard inutest.

Quantifying glomerular filtration rate (GFR) with sensitive and specific techniques allows testing the extent and prognosis of renal dysfunction and contributes substantially to our understanding of a variety of renal disease mechanisms, as well as potentially increasing the accuracy of diagnosis and directing appropriate therapy. Moreover, GFR estimation is a powerful tool in experimental models of renal disease that not only evaluates renal viability or success of new therapies, but also for studying mechanisms of renal injury.

Accurate renal function is evaluated through the measurement of the renal clearance of an ideal filtration marker. Inulin, an exogenous marker, is the only compound that is neither toxic nor metabolized, the excretion of which occurs exclusively by glomerular filtration, with no tubular reabsorption or secretion. Therefore, inulin clearance provides the most accurate method to measure GFR, as well as for evaluating single nephron GFR by micropuncture techniques, and is considered as the “gold standard” of renal function evaluation [1–6]. However, inulin solubility is low and tends to form precipitates in urine and plasma, complicating samples handling and inulin concentration determination [7, 8]. The introduction of polyfructosan (inutest), a more soluble compound, avoided this problem [6, 9–13]; nevertheless, the use of inutest is limited due to its high cost and because it is only produced in some European countries. Finally, a third alternative to accurately evaluate GFR is the use of radiolabeled markers, such as [3H]inulin, [51Cr]EDTA, and p-aminohippurate [10, 14–17], but these strategies have also a number of disadvantages associated with their cost and safety issues that are inherent to the use of radioisotopes, including the fact that in the case of [3H], isotope concentration declines, resulting in underestimation of GFR [18].

In order to have an inexpensive and accessible exogenous GFR marker, we reasoned that the artificial sweetener LC sugar (http://www.metco.com.mx/azucarbe2.html); a low calorie sugar made of sucrose, that is widely available at very low cost, could be a GFR marker if possessing similar inulin properties such as being freely eliminated, nonsecreted, or tubular reabsorbed, as well as being neither toxic nor metabolized. To test this hypothesis, glomerular filtration rate in nontreated rats and in rats with certain degree of renal failure induced by acute CsA nephrotoxicity was evaluated by using LC sugar and compared with inutest as a gold standard. Our results show that LC sugar is an accessible, sensitive, and inexpensive...
marker of GFR similar to inutest or inulin markers, in both normal and pathophysiologic conditions.

METHODS

In order to evaluate if low calorie sugar (LC sugar) is a sensitive marker of glomerular filtration rate, LC sugar GFR was determined in nontreated rats and in rats with renal dysfunction induced by cyclosporine administration. The values were compared with GFR determined by inutest. Two groups of 6 or 7 male Wistar rats weighing 300 to 350 g fed with standard chow diet were included in the study. One was treated with a daily dose of CsA 30 mg/kg subcutaneously for 7 days and the other group was formed by nontreated rats. In one half of each group, GFR was evaluated by using inutest and in the other half by using LC sugar. In addition, one group of 6 rats was included to evaluate the linearity of LC sugar excretion by using different concentrations that ranged from 5% to 12.5%. All procedures followed were in accordance with our institutional guidelines.

Functional studies

All animals were placed in metabolic cages and urine that was spontaneously voided during every 24 hours was collected. Serum and urine creatinine concentration was measured with an autoanalyzer (Technicon RA-1000; Bayer Co., Tarrytown, NY, USA).

After urine collection, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg/kg) and placed on a homeothermic table to maintain core body temperature at 37°C, by means of a rectal probe attached to a homeothermic blanket. Trachea, both jugular veins, femoral arteries, were catheterized with polyethylene tubing PE-240 and PE-50. Rats were maintained under euolemic conditions by infusing 10 mL/kg of body weight of isotonic rat plasma during surgery, followed by an infusion of 5% polyfructosan, (Inutest; Laevosan-Gesellschaft, Linz, Austria) or 5% LC sugar (METCO; Mexico City, Mexico) diluted in 0.9% saline solution at 1.6 mL/hr. Mean arterial pressure was monitored with a pressure transducer (Model p23 db; Gould, Hato Rey, Puerto Rico) and recorded on a polygraph (Grass Instruments, Quincy, MA, USA). Via a midline abdominal incision, the left renal artery was exposed. An ultrasound transit-time flow probe (1RB, Transonic, Ithaca, NY, USA) was placed around the left renal artery and filled with ultrasonic coupling gel (HR Lubricating Jelly; Carter-Wallace, New York, NY, USA) for recording renal blood flow (RBF). After an equilibrium period of 60 minutes, urine was drained from the bladder by gravity via PE-50 tube and collected for 30 to 60 minutes and blood samples were taken at the beginning and at the end of the urine collection period.

In order to determine that LC sugar is neither reabsorbed nor secreted, the LC sugar linearity of excretion over a wide plasma concentration range was studied. After the surgery, rats were maintained in euolemic conditions, as was detailed before. Four determinations of GFR were performed in the same rat by infusing increased concentrations of LC sugar that were 5%, 7.5%, 10%, and 12.5%. For each concentration, an equilibrium period of 30 to 45 minutes was allowed and thereafter urine was drained from the bladder by gravity and collected for 30 to 45 minutes and blood samples were taken at the beginning and at the end of each urine collection period.

GFR evaluation by inutest or LC sugar

Inutest and LC sugar concentrations in urine and plasma were determined by the Davidson et al [19] modified technique, which requires the use of anthrone reagent for determination of fructose. Briefly, anthrone is freshly prepared at final concentration of 0.08% in diluted sulfuric acid and stored at 4°C in a dark bottle until its use.

Standard solutions

Stock solutions containing 1 mg per mL of inutest and LC sugar were prepared. Different dilutions of these stock solutions were used as standards of inutest and LC sugar, respectively. The standard set concentrations of LC sugar were between 10 and 100 µg per milliliter.

Protein-free plasma samples were prepared by adding 250 µL of water and 125 µL of 1.0 N TCA to 20 µL of plasma. The mixture was shaken, centrifuged, and protein free supernatant isolated for subsequent analysis. Urines were diluted for maintaining LC sugar concentrations within analytical range (10 to 100 mg per mL). Anthrone reagent aliquots of 500 µL were placed in Eppendorff tubes, maintained on ice, and 100 µL of each sample (standard solution, protein-free plasma or urine) was added. All tubes were shaken vigorously and incubated on a water bath at 38°C for 50 minutes. The tubes were cooled and the absorbance at 620 nm was recorded by using a Beckman spectrophotometer. Appropriate plasma and urine blanks, collected before inutest or LC sugar infusion, were run in each determination. All samples from normal and CsA treated rats were run in duplicate and a set of standards was included in each determination.

Inutest, LC sugar, and creatinine clearances were calculated by the standard formula \( C = U \times V/P \), where \( U \) is the marker concentration in urine, \( V \) is urine flow rate, and \( P \) is the marker concentration in plasma.

Statistical analysis

Linear regression and correlation were calculated by the least-squares method and the values represent mean
value ± standard deviation. Results of the quantitative traits are expressed as mean ± standard error. Statistical comparisons among experimental groups were performed by using analysis of variance (ANOVA), and for comparing different experimental periods in the same group, repeated measures ANOVA was used. When ANOVA showed a statistically significant difference, Student–Newman–Keuls test was performed. Statistical significance for all tests was judged at P < 0.05.

RESULTS

Figure 1 depicts the LC sugar standard concentration curve. Ten different concentrations varying from 10 to 100 µg/mL were analyzed. Each point represents the mean of absorbance ± standard deviation of 6 different determinations and shows minimal fluctuations among the performed analysis. LC sugar determined by Davidson et al [19] technique by using anthrone gives a linear and highly reproducible standard curve. The correlation coefficient was 0.9939 and r squared 0.9879. Increased LC sugar concentrations showed a linear pattern with respect to absorbance values. Thus, LC sugar concentration could be determined by Davidson’s technique by using anthrone reagent.

LC sugar infusion modified neither MAP nor RBF when they were compared with their respective controls infused with inutest. Although CsA treated rats infused with inutest or LC sugar tended to present minor values of MAP than nontreated rats (96.4 ± 3 and 92 ± 3.4 mm Hg, vs. 112.6 ± 2.8 and 109.0 ± 2.4, respectively), there were no significant differences among the studied groups. These results can be also seen in Figure 2.

The fall of RBF evidenced that both groups that received CsA presented renal dysfunction. In inutest infused rats, RBF for CsA-treated rats was 2.9 ± 0.5 versus 5.9 ± 0.6 mL/min, respectively. Thus, similar reduction was recorded in CsA treated rats infused with inutest or LC sugar. These results showed that LC sugar infusion did not alter these physiologic parameters and evidenced that both groups of CsA presented nephrotoxicity.

Renal creatinine clearance is shown in Figure 3. Nontreated and CsA treated rats that were used for inutest infusion had similar renal function that those used for LC sugar infusion assessed by creatinine clearance. The values of creatinine clearance in nontreated and CsA treated rats that were used for inutest infusion were 1.8 ± 0.2 and 0.6 ± 0.1 mL/min, respectively, and for LC sugar infusion were 1.7 ± 0.3 and 0.7 ± 0.2 mL/min, respectively. These results also show that CsA-treated rats present renal dysfunction.

Figure 4 shows the estimation of glomerular filtration rate by using LC sugar as a marker of GFR compared with inutest. In nontreated rats, the mean GFR evaluated with LC sugar was 2.2 ± 0.1. This value was similar to that obtained with inutest: 2.3 ± 0.1 mL/min. The GFR values normalized by body weight (BW) were 0.72 ± 0.04 and 0.73 ± 0.05 mL/min/100 BW, respectively.

CsA administration produced a significant reduction of GFR that was equally determined by both LC sugar and inutest. GFR in LC sugar infused rats was 1.1 ± 0.2 and in inutest infused rats was 1.0 ± 0.2 mL/min (P = NS). The GFR normalized by body weight was 0.36 ± 0.06 and 0.35 ± 0.05 mL/min/100 BW, respectively. In addition, as we detailed before, the GFR assessed by creatinine clearance was lower than inutest and LC sugar. All these results indicate that LC sugar is a GFR marker comparable to the “gold standard” inulin.

Figure 5 depicts the assessment of GFR by using different LC sugar concentrations, ranging from 5% to 12.5%. At the beginning and at the end of each urine recollection, blood samples were taken to evaluate LC sugar plasma concentration, and the mean value was used to estimate GFR in each urine recollection. As expected, LC sugar plasma concentration gradually raised with increased concentration of LC sugar infusion, as shown Figure 5A. Each point represents the mean value of the LC sugar plasma concentrations at the beginning and at the end of each urine recollection. For LC sugar infusion at 5% the concentration was 0.4 ± 0.05 mg/mL, for 7.5% it was 0.7 ± 0.07 mg/mL, for 10% the value was 0.9 ± 0.12 mg/mL, and for 12.5% it was 1.2 ± 0.05 mg/mL. All these differences were statistically different.

Figure 5B shows that in spite of increased LC sugar plasma concentration the GFR remained unaltered. The GFR value obtained with 5% LC sugar infusion was 2.3 ± 0.2 mL/min, with 7.5% it was 2.1 ± 0.1 mL/min, with 10% the value was 2.1 ± 0.1 mL/min, and finally, the GFR with 12.5% was 2.3 ± 0.5 mL/min. No statistical differences were observed among the different used concentrations.
DISCUSSION

Here we present an alternative marker of glomerular filtration rate by using low calorie sugar. Our results indicate that measurement of LC sugar concentration in plasma and urine is a useful and accurate marker to estimate GFR in normal conditions and during renal dysfunction. The estimation of GFR with LC sugar and inutest was virtually identical, suggesting that LC sugar is a sensitive marker of GFR with the advantages that it is easy to acquire together with its very low cost.

The assessment of glomerular filtration rate (GFR) is the most commonly used test of renal function. Endogenous markers that are widely used for the estimation of GFR, such as serum creatinine, are suitable to estimate renal function in the clinical setting and in subjects with normal renal function, but are not ideal to determine GFR in patients with reduced renal function or when an accurate GFR is required (i.e., experimental models of renal disease). In consequence, inulin clearance provides the most accurate method to measure GFR, as well as for evaluating single nephron GFR in experimental models of renal disease by using micropuncture techniques. Thus, inulin is considered as the “gold standard” of renal function evaluation [1–6], but its use is limited for the requirement of IV infusion.

Inulin concentration is detected through colorimetric assay by the action of anthrone reagent, which reacts mainly with fructose and in much lesser proportion with glucose [20]. The glucose interference was solved by the modification made by Davidson et al [19], under the condition proposed by these authors; glucose did not react with anthrone and is almost undetectable by this method. The present study was undertaken to evaluate the application of LC sugar as a new marker of GFR by using Davidson et al [19] technique.

LC sugar is a patented formula of a food industry from Mexico (METCO; http://www.metco.com.mx/azucarbc2.html) isolated from sugar cane. LC sugar contains mainly sucrose processed with sweeteners.
Because sucrose is composed by fructose, we reasoned that LC sugar could be detected by the Davidson's modified technique and that LC sugar in plasma will be freely filtrated and neither reabsorbed nor secreted. The fact that LC sugar standard curve showed to be reproducible and highly lineal, together with the observation that GFR values detected with LC sugar in rats were similar to those estimated with inutest, indicate that LC sugar could be indeed accurately analyzed with anthrone reagent. In order to know if another sweeter such as Splenda® (Splenda, McNeil Nutritional, Washington, PA, USA), which contains maltodextrin and sucralose, but not sucrose, could be also a GFR marker, some experiments were performed (data not shown). We observed that anthrone did not react with the components of Splenda.

Our results also suggest that LC sugar is not secreted or reabsorbed at tubular level, because if this were the case, GFR values might would be lesser or greater than inutest GFR. We observed that when LC sugar plasma concentration gradually rises, the GFR did not change (Fig. 5). These results suggest that LC sugar is a solely GFR-dependent excretion.

It is known that endogenous creatinine can be used to estimate GFR with reasonably degree of certain in animal or subjects with normal renal function, but reduction in renal function is associated with increased tubular secretion of creatinine. Therefore, as renal dysfunction progresses, assessment of GFR by creatinine clearance is less accurate. Thus, to determine if LC sugar could be used to estimate GFR during renal dysfunction, we used an animal model of renal failure induced by CsA administration [6, 12, 21–23]. In the present study we found a significant increased of LC sugar plasma concentrations and reduction of LC sugar renal clearance in these animals. The similitude of GFR by LC sugar and inutest in normal reduction of LC sugar renal clearance in these animals.

The data presented here demonstrate that LC sugar clearance is a convenient, accurate, and accessible marker to estimate the glomerular filtration rate.

CONCLUSION

The data presented here demonstrate that LC sugar clearance is a convenient, accurate, and accessible marker to estimate the glomerular filtration rate.

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