

# Consumption of carbohydrate solutions enhances energy intake without increased body weight and impaired insulin action in rat skeletal muscles

J Ruzzin, YC Lai, J Jensen

## SUMMARY

**Objectives:** In the present study, we investigated whether replacement of tap water by fructose or sucrose solutions affect rat body weight and insulin action in skeletal muscles.

**Methods:** Rats were fed standard rodent chow *ad libitum* with water, or water containing fructose (10.5% or 35%) or sucrose (10.5% or 35%) for 11 weeks. Body weight and energy intake from chow and drinking solutions were measured. Urinary catecholamines secretion was determined after 50-60 days. At the end of the feeding period, soleus and epitrochlearis were removed for *in vitro* measurements of glucose uptake (with tracer amount of 2-[<sup>3</sup>H]-deoxy-D-glucose) and PKB Ser<sup>473</sup> phosphorylation (assessed by Western Blot) with or without insulin.

**Results:** Fructose and sucrose solutions enhanced daily energy intake by about 15% without increasing rat body weight. Secretion of urinary noradrenaline was higher in rats drinking a 35% sucrose solution than in rats drinking water. In the other groups, urinary noradrenaline secretion was similar to rats consuming water. Urinary adrenaline secretion was similar in all groups. Insulin-stimulated glucose uptake and insulin-stimulated PKB phosphorylation were not reduced by intake of fructose or sucrose solution.

**Conclusions:** Fructose and sucrose solutions enhanced energy intake but did not increase body weight. Although noradrenaline may regulate body weight in rats drinking 35% sucrose solution, body weight seems to be regulated by other mechanisms. Intake of fructose or sucrose solution did not impair insulin-stimulated glucose uptake or signaling in skeletal muscles.

**Key-words:** Diet • Soft drinks • Beverages • Obesity • Catecholamines • Fructose • Sucrose.

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## RÉSUMÉ

**La consommation de solutions de glucides augmente l'apport énergétique sans élever le poids corporel et affecter l'action de l'insuline dans les muscles squelettiques chez le rat**

**Objectifs :** Dans la présente étude, nous avons étudié si le remplacement de la consommation d'eau par une solution de fructose ou sucrose affectait le poids corporel des rats ainsi que l'action de l'insuline dans les muscles squelettiques.

**Méthodes :** Les rats ont eu accès à une nourriture standard *ad libitum* avec de l'eau ou bien de l'eau contenant du fructose (à 10,5 % ou 35 %) ou du sucrose (à 10,5 % ou 35 %) durant 11 semaines. Le poids corporel et la consommation d'énergie ont été mesurés. La sécrétion urinaire de catécholamines a été déterminée après 50-60 jours. À la fin du protocole expérimental, les muscles soleus et épitrochléaris ont été disséqués pour permettre la mesure *in vitro* de la consommation de glucose (2-[<sup>3</sup>H]-deoxy-D-glucose) et la phosphorylation de la PKB sur Ser<sup>473</sup> (Western Blot) avec ou sans insuline.

**Résultats :** La consommation de solutions de fructose ou sucrose a augmenté l'apport énergétique journalier d'environ 15 % sans entraîner d'augmentation du poids corporel des rats. La sécrétion urinaire de noradrénaline était plus élevée chez les rats buvant une solution contenant 35 % de sucrose par rapport aux rats buvant de l'eau. Dans les autres groupes, la sécrétion urinaire de noradrénaline était comparable à celle observée chez les rats buvant de l'eau. La sécrétion urinaire d'adrénaline était similaire dans tous les groupes. La stimulation de la consommation de glucose induite par l'insuline et la phosphorylation de la PKB stimulée par l'insuline n'étaient pas réduites par la consommation des solutions de fructose ou sucrose.

**Conclusions :** La consommation de solutions de fructose ou sucrose a augmenté l'apport énergétique sans toutefois avoir augmenté le poids corporel. Bien que la noradrénaline puisse réguler le poids corporel des rats buvant la solution de 35 % de sucrose, le poids corporel semble être régulé par d'autres mécanismes. La consommation de solutions de fructose ou sucrose n'a pas affecté l'action de l'insuline et sa voie de signalisation dans les muscles squelettiques.

**Mots-clés :** Régime • Boissons sucrées • Obésité • Catécholamines • Fructose • Sucrose.

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It has been estimated that at least 171 million people worldwide were diabetics in 2000 [1]. Diet habits and reduced physical activity are critical factors for the high prevalence of obesity and type 2 diabetes [2, 3]. Abundant intake of saturated fat is a risk factor for the development of insulin resistance [4, 5], but the relative energy intake from fat decreased from 41% to 37% between 1980 and 1997 [6] when the prevalence of obesity and type 2 diabetes increased by 80% and 47%, respectively [6]. Total energy intake, however, increased by approximately 500 kcal/d since the 1980s mainly due to increased carbohydrate intake [6]. Part of the increased carbohydrate consumption comes from soft drinks [6, 7] and epidemiological studies have reported an association between soft drinks consumption and obesity [8, 9] and type 2 diabetes [10, 11].

Body weight results from a balance between energy intake and energy expenditure. Mammals have, however, the ability to avoid weight gain despite enhanced energy intake [12]. During periods of excess caloric intake, activation of the sympathetic nervous system and catecholamine release stimulate thermogenesis and stabilise body weight [13–15]. Diet-induced thermogenesis is regulated by activation of  $\beta$ -adrenergic receptors [16, 17] and impaired  $\beta$ -adrenergic stimulation is also hypothesised to participate in the development of age-associated obesity [18]. Adrenergic receptors are widely expressed in skeletal muscles [19] and this tissue, due to its large mass, may have an important role in the regulation of body weight.

Sucrose is a disaccharide composed of a fructose and glucose molecule. The fructose moiety is believed to be the primary nutrient component that causes insulin resistance induced by high-sucrose diets [20, 21]. The effects of carbohydrate diets on insulin action in skeletal muscle are unclear. Some studies have reported that rats fed with solid diet rich in fructose develop insulin resistance due to reduced activation of the early steps in the insulin signaling pathway in skeletal muscles [22, 23]. Other studies have reported that consumption of chow containing 50–70% of energy from either fructose or sucrose does not impair insulin-stimulated glucose uptake or glycogen synthesis in soleus, extensor digitorum longus, gastrocnemius, [24–26] or hemidiaphragm muscles [27–29].

Epidemiological studies underline that soft drinks may play important role for the development of obesity and insulin resistance. However, cross sectional studies do not show causal relationships. By using experimental animals, it is possible to control diets and assess its effects on different parameters. Very few studies have investigated the effects of carbohydrate drinking solutions on body weight and insulin action in rats. However, it has surprisingly been reported that intake of Coca-Cola for 9 weeks did not cause weight gain despite that energy intake was increased by about 50% [30]. In the same study, drinking a 32% sucrose solution did not enhance rat body weight despite increased energy intake.

The present study was designed to evaluate whether intake of a fructose or a sucrose solution for 11 weeks affects rat body weight. Furthermore, we have for the first time investigated the effects of intake of carbohydrate solutions on insulin-stimulated glucose uptake in skeletal muscles.

## Materials and methods

### Animal housing

Male Wistar rats (Bk1:Wist; B & K Universal AS, Nittedal, Norway) weighing 90–100 g were housed individually under a 12:12-h light-dark cycle (light from 07.00 to 19.00 h) at 21 °C. The rats were provided a standard rat chow [RM1 (Metabolisable energy: 10.74 kJ/g) Special Diet Services, Essex, UK] and tap water *ad libitum* for one week. After this acclimatization period, tap water was replaced by a 35% fructose solution (F35%;  $n = 8$ ), 35% sucrose solution (S35%;  $n = 8$ ), 10.5% fructose solution (F10.5%;  $n = 4$ ) or 10.5% sucrose solution (S10.5%;  $n = 4$ ) for 11 weeks. Control rats continued to drink tap water (W;  $n = 11$ ). The experimental and feeding protocols were approved and in accordance with the laws and regulations controlling experiments on live animals in Norway and the European Convention for the Protection of Vertebrate Animals used in Experimental and Other Scientific Purposes.

### Drinking solutions preparation

Solutions were made to provide a concentration of 35% and 10.5% (wt/ml). Commercial soft drinks like Coca-Cola, contain approximately 10.5 g of sucrose/100 ml. Solutions were prepared by dissolving commercial fructose (Dr. Lindbergs, Haugen-Gruppen AS, Vestby, Norway) or sucrose (Danisco Sugar, Oslo, Norway) in tap water. Given that 1 g of fructose and sucrose equal to 17.2 kJ [31], F35% and S35% contain 6.02 kJ/ml, whereas F10.5% and S10.5% contain 1.81 kJ/ml. Solutions were prepared and changed every 2–4 days to avoid aggregation of fructose and sucrose on the bottle walls. Body weight, food and drinking solution intakes were measured at 10.00 h each time the solutions were changed.

### Tissue harvest

At the end of the experimental diet period, rats were fasted for 5 hours (05.00 to 10.00 h) and anaesthetized with an intraperitoneal injection of ~0.8 ml pentobarbital (50 mg/ml). Soleus (slow-twitch fiber) and epitrochlearis (fast-twitch fiber) muscles were dissected out and mounted on apparatus at their approximate resting length. Other soleus muscles were mounted on contraction apparatus. Liver and heart muscle were rapidly removed, frozen in liquid nitrogen and stored at -80 °C until analysis. Finally, epididymal fat pad were removed and weighed.

## Skeletal muscle incubation

Soleus and epitrochlearis were pre-incubated for ~30 min in 3.5 ml Krebs Henseleit buffer containing 5.5 mM glucose, 2 mM pyruvate, 5 mM HEPES and 0.1% bovine serum albumin, pH 7.4. After pre-incubation, rested or contracted muscles were incubated for 30 min in Krebs-Henseleit buffer containing 0.25  $\mu\text{Ci/ml}$  2-[1,2- $^3\text{H}$ (N)]-deoxy-D-glucose (25.5 Ci/mmol; DuPont-New England Nuclear) and 0.1  $\mu\text{Ci/ml}$  [ $^{14}\text{C}$ ]-mannitol (51.5 Ci/mmol; DuPont-New England Nuclear) with 0, 500 or 10,000  $\mu\text{U/ml}$  insulin (Actrapid, Novo Nordisk, Denmark). Other soleus muscles were incubated with 2-[ $^3\text{H}$ ]-deoxy-D-glucose and electrically stimulated with impulse trains of 200 ms delivered every 0.5 s (100 Hz; square wave pulses of 0.2 ms duration and 10 V amplitude) for 30 min as described previously [32]. Incubations buffer were continuously gassed at 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  and maintained at 30 °C. Immediately after incubation, muscles were removed from the apparatuses, blotted on filter paper, frozen in liquid nitrogen and stored at -80 °C before analysis.

## Glucose uptake

For analysis of glucose uptake after incubation with 2-[ $^3\text{H}$ ]-deoxy-D-glucose, muscles were freeze-dried, weighed, and dissolved in 600  $\mu\text{l}$  1M KOH for 20 min at ~70 °C. Then, 400  $\mu\text{l}$  of dissolved muscles were added to 3 ml scintillation solution (Hionic Fluor, Packard Bioscience B.V., Groningen, The Netherlands) mixed and counted for radioactivity (TRI-CARB 1900TR, Packard, USA).

## Glycogen concentration

For glycogen analysis in skeletal muscles, 100  $\mu\text{l}$  of the KOH digest was neutralized with 25  $\mu\text{l}$  7 M acetic acid and 500  $\mu\text{l}$  0.3 M acetate buffer (pH 4.8) containing 0.2 U/ml of amyloglucosidase (Boehringer-Mannheim Corp., Indianapolis, IN, USA) was added. The glycogen was hydrolyzed at 37 °C for 3 hours and the glucose formed was determined to Lowry and Passonneau [33].

In liver and heart, glycogen was hydrolyzed in 1 M HCl for 2.5 h at 100 °C. Because of the high glycogen concentration in the liver, 100  $\mu\text{l}$  of hydrolysate were diluted (1:4) with 1 M HCl. The digests were centrifuged (2500  $g$  for 10 min at 4 °C) before glucose units were measured according to Lowry and Passonneau [33].

## Urine collection and catecholamine analysis

Between day 50 and 60, rats were housed individually in metabolic cages for two days. Urine collection was performed as described by Young *et al.* [34]. Briefly, urine was collected for 24 h in vials containing 1.5 ml of 1 M HCl under oil (Dow Corning 200/100cS fluid, BDH Silicone Products, USA). Urine was stored at -80 °C until analysis. Urine was centrifuged (3000 rpm for 3 min at 4 °C) and diluted (1:20) in deionised water. Adrenaline and noradrenaline were ana-

lysed using HPLC-ED as described by Holmes *et al.* [35]. For extraction of catecholamines, 500  $\mu\text{l}$  of diluted urine was added to ~10 mg alumina oxide (A-1772, Sigma) in 400  $\mu\text{l}$  1 M Tris/HCl buffer containing 53 mM EDTA (pH 8.6) in Supelco filtration tubes (Supelco Inc., Bellefonte, USA). The columns were rotated for 20 min at room temperature. The columns were washed with 1 ml 200 mM  $\text{NaHCO}_3$  and twice with 1 ml deionised water before catecholamines were eluted with 200  $\mu\text{l}$  0.2 N acetic acid, and 50  $\mu\text{l}$  were injected into the HPLC system. Adrenaline and noradrenaline were separated by a 100  $\times$  2 mm column packed with Chromspher 3 C18 (Chrompack, The Netherlands). The mobile phase was 80% phosphate buffer (containing 0.1 mM laurylsulphate; 1 mM KCl; 0.1 mM EDTA; pH = 3.0) and 20% methanol, and pumped at a rate of 0.25 ml/min. Adrenaline and noradrenaline were detected by electrochemical oxidation (Antec Decade electrochemical detector, The Netherlands) at +650 mV vs an Ag/AgCl reference electrode.

## Western Blot Analysis

Skeletal muscles were weighed and homogenized (1 mg: 25  $\mu\text{l}$ ) in ice-cold buffer containing 50 mM HEPES, 150 mM NaCl, 10 mM  $\text{Na}_4\text{P}_2\text{O}_7$ , 30 mM NaF, 1 mM  $\text{Na}_3\text{VO}_4$ , 10 mM EDTA, 2.5 mM benzamidine, 0.5  $\mu\text{g}/10\text{ mg}$  muscle of protease inhibitor cocktail (Sigma P-8430), and 1% Triton X-100, pH 7.4. Homogenates were rotated for 1 hour at 4 °C and centrifuged at 11,500  $g$  at 4 °C for 10 min. Protein concentration of supernatants were determined by a DC protein assay (Bio-Rad laboratories, Hercules, CA, USA) according to the instruction manual. Supernatants were diluted to a protein concentration of 3  $\mu\text{g}/\mu\text{l}$  and aliquoted.

For electrophoresis, aliquots were prepared with Laemmli buffer [36], heated at 95 °C for 5 min to completely dissociate proteins, and centrifuged at 22,000  $g$  for 15 s. Muscle proteins were separated by electrophoresis in 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Transfer of proteins from the gel onto the polyvinylidene difluoride (PVDF) membrane was performed for 1 h at 200 V in a Mini-trans-Blot cell with Bio-Ice cooling unit (Bio-Rad laboratories, Hercules, CA, USA). The transfer buffer contained 25 mM Tris, 192 mM Glycin, and 10% methanol. Membranes were washed (3  $\times$  10 min) in PBS-T (80 mM  $\text{Na}_2\text{HPO}_4$ , 20 mM  $\text{NaH}_2\text{PO}_4$ , 100 mM NaCl, 0.1% Tween 20). To avoid nonspecific binding of antibody to the PVDF, membranes were blocked in PBS-T containing 5 % nonfat milk for 2 hours at room temperature. After blocking, membranes were washed in PBS-T (2  $\times$  30 s), and incubated overnight at 4 °C with the primary antibody. After wash in PBS-T (6  $\times$  10 min), membranes were incubated with the secondary antibody conjugated to horseradish peroxidase (HRP) for 1 hour at room temperature, and washed again (6  $\times$  10 min). Antibody binding was detected by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech, Buckinghamshire, UK) as described by the manufacturer. Blots were scanned (Scan Jet

Ilcx skanner, Hewlett Packard Co., USA) and signals quantified by densitometry with Scion Image software (Scion Corporation, Maryland, USA).

### Antibodies

Anti-phospho-PKB (Ser<sup>473</sup>) was from Cell Signaling (Beverly, MA, USA). Anti-rabbit HRP-linked antibody was from New England Biolabs (Beverly, MA, USA).

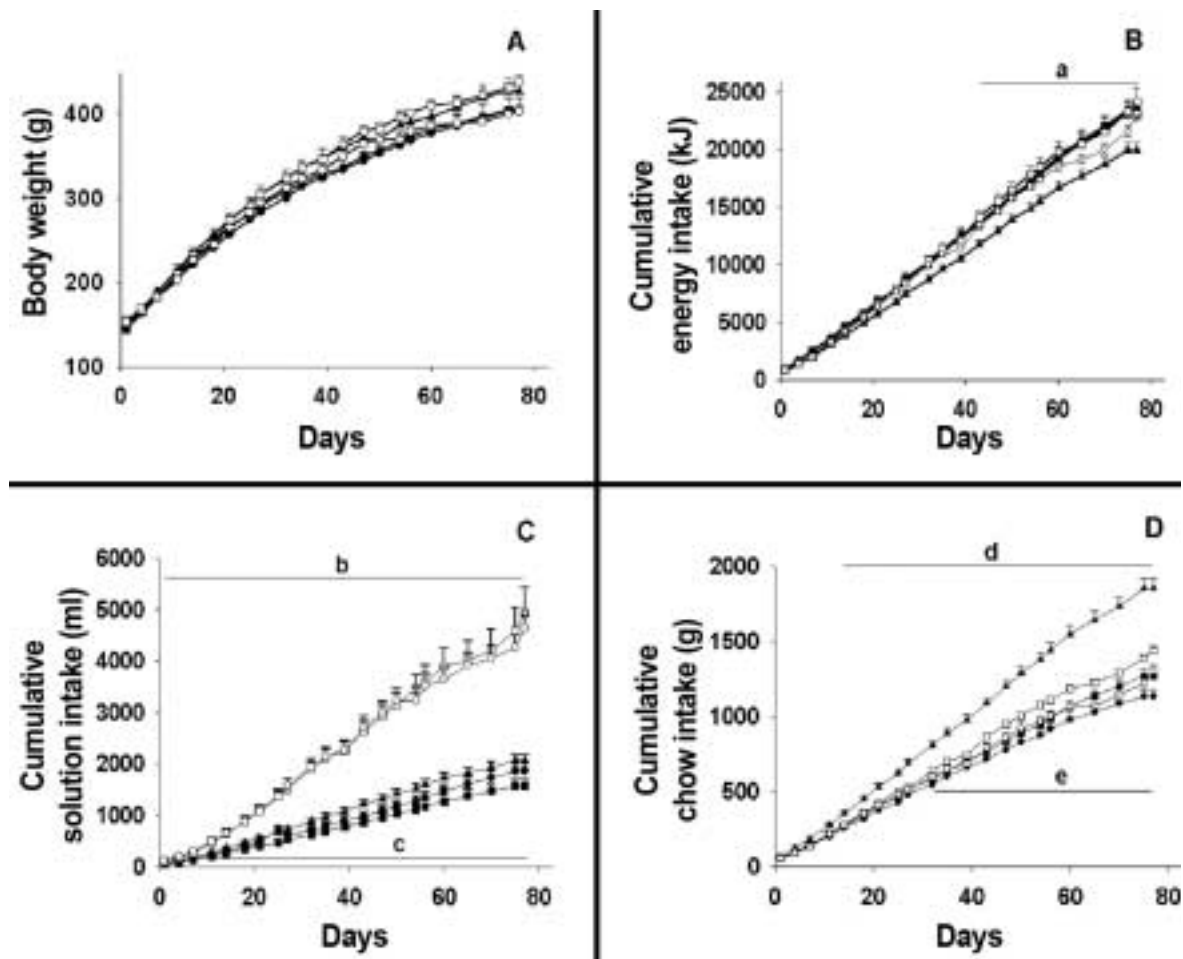
### Statistics

Data are presented as means  $\pm$  SEM. One-way analysis of variance (ANOVA) was performed to determine the differences between experimental groups. When analysis of variance revealed significant differences, further analysis was performed using LSD *post hoc* tests. P values below 0.05 were considered as statistically significant.

## Results

### Effects of carbohydrate solutions intake on body weight, energy intake and epididymal fat pad weight

Body weight increased gradually in all groups throughout the feeding protocol (Fig 1A). Body weight did not differ between the groups at any time point. Energy intake was higher in rats drinking F35% (~14%), S35% (~16%), and F10.5% (~17%) compared to rats consuming W (Tab I and Fig 1B). Rats drinking S10.5% tended to have a higher energy intake (~11%) but the increase did not reach statistical significance ( $P = 0.08$ ) (Tab I). Rats drinking fructose or sucrose solution had similar daily energy intake (Tab I). Moreover, rats drinking S35% had higher energy intake from fluid compared to rats consuming F35%, F10.5%, and



**Figure 1**

Body weight, cumulative intake of energy, solution, and chow in rats drinking water or a fructose or a sucrose solution. Rats were fed with tap water ( $\blacktriangle$ ;  $n = 11$ ), a 35% ( $\blacksquare$ ;  $n = 8$ ) or 10.5% ( $\nabla$ ;  $n = 4$ ) fructose solution or a 35% ( $\triangle$ ;  $n = 8$ ) or 10.5% ( $\circ$ ;  $n = 4$ ) sucrose solution for 77 days. A: Effect of carbohydrate solutions on body weight. Rat body weight was measured each 2–4 days throughout the feeding protocol. B: Effect of carbohydrate solutions on energy intake. Energy intake was calculated from intake of solution and chow. C: Effect of fructose or sucrose on solution intake. Solution intake was measured each 2–4 days throughout the feeding protocol. D: Effect of carbohydrate solutions on chow intake. Chow intake was measured each 2–4 days throughout the feeding protocol. Values are means  $\pm$  SEM. a:  $P < 0.04$  W and S10.5% vs F35%, S35% and F10.5%; b:  $P < 0.02$  F10.5% and S10.5% vs F35%, S35% and W; c:  $P < 0.05$  W vs F35%; d:  $P < 0.04$  W vs all other feeding groups; e:  $P < 0.04$  F10.5% vs S35%.

**Table I**

Body weight and intake of fluid, chow, and total energy in rats drinking water or a carbohydrate solution.

	Body weight	Fluid intake		Chow intake		Total energy intake	Epididymal fat pad
	gain (g)	(ml/day)	(kJ/day)	(g/day)	(kJ/day)	(kJ/day)	weight (g)
W	287.0 ± 12.9	28.2 ± 1.7	0 <sup>c</sup>	25.2 ± 0.9 <sup>c</sup>	270.4 ± 9.2 <sup>c</sup>	270.4 ± 9.2 <sup>e</sup>	12.2 ± 1.0
F35%	264.4 ± 15.2	20.9 ± 1.7 <sup>a</sup>	126.0 ± 10.4	17.0 ± 0.6	182.9 ± 6.4	308.9 ± 11.0	13.1 ± 0.9
S35%	266.6 ± 13.7	25.0 ± 1.8	150.3 ± 11.1 <sup>c</sup>	15.2 ± 0.6	163.4 ± 6.3	313.7 ± 10.6	13.9 ± 1.0
F10.5%	258.2 ± 7.6	64.2 ± 6.4 <sup>b</sup>	116.1 ± 11.7	18.7 ± 0.4 <sup>d</sup>	200.9 ± 4.4 <sup>d</sup>	317.1 ± 13.9	12.5 ± 1.4
S10.5%	283.5 ± 7.4	65.2 ± 3.7 <sup>b</sup>	117.9 ± 6.7	16.9 ± 0.5	181.9 ± 5.6	299.9 ± 8.2	14.4 ± 0.6

Values are means ± SEM. *n* = 11 for W, *n* = 8 for F35%, *n* = 8 for S35%, *n* = 4 for F10.5%, and *n* = 4 for S10.5%. <sup>a</sup>*P* < 0.04 vs rats drinking W; <sup>b</sup>*P* < 0.0001 vs rats drinking W, F35% and S35%; <sup>c</sup>*P* < 0.04 vs all other groups; <sup>d</sup>*P* < 0.02 vs rats drinking S35%; <sup>e</sup>*P* < 0.01 vs rats drinking F35%, S35%, and F10.5%.

S10.5% (*Tab I*). Daily energy intake from chow was higher in rats drinking W (*Tab I*). After the first day of feeding protocol, rats consuming F10.5% and S10.5% drank higher volume of solution than other groups (*Tab I and Fig 1C*). Intake of fructose or sucrose solution did not affect epididymal fat pad weight (*Tab I*).

### Effects of carbohydrate solutions intake on urinary catecholamines

*Table II* shows the daily secretions of noradrenaline and adrenaline in urine. Urinary noradrenaline secretion was higher in rats consuming S35% compared to the other groups. Urinary adrenaline secretion was similar in all groups.

### Effects of carbohydrate solutions intake on glucose uptake in skeletal muscles

In soleus muscle, basal glucose uptake was similar in the five groups (*Fig 2A*). In all experimental groups, insulin-stimulated glucose uptake was similar at both physiological and supra-physiological insulin concentrations in soleus (*Fig 2A*). In epitrochlearis, insulin-stimulated glucose uptake

was also similar in all groups at 500 and 10,000 μU/ml insulin (*Fig 2B*). In soleus muscle, contraction-stimulated glucose uptake was comparable in all groups (*Fig 2C*).

### Effects of carbohydrate solutions intake on glycogen content

Skeletal muscle, heart, and liver glycogen concentrations are shown in *Table III*. Feeding protocols did not influence glycogen content in soleus, epitrochlearis, heart or liver.

### Effects of carbohydrate solutions intake on PKB phosphorylation

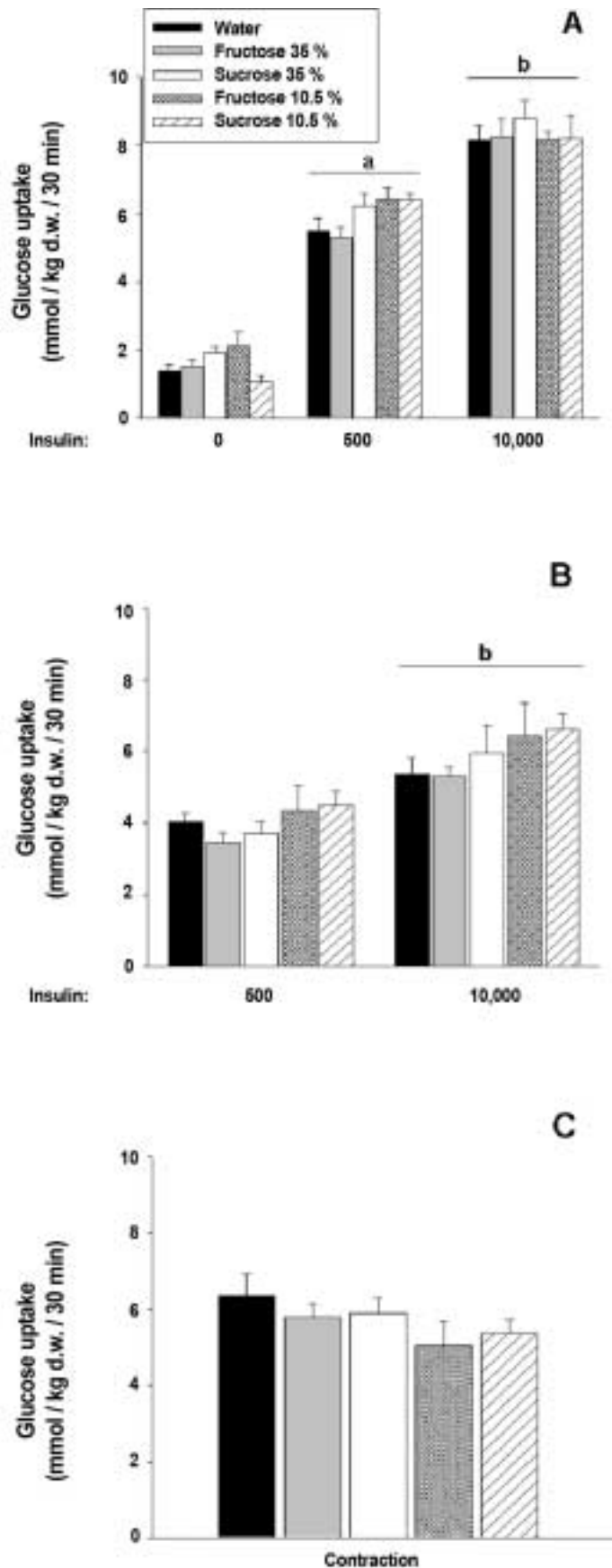
In soleus muscle, PKB phosphorylation was low in the absence of insulin in all groups (*Fig 3A*). Insulin increased PKB phosphorylation to similar extent in all five groups at both 500 and 10,000 μU/ml of insulin (*Fig 3A*). In epitrochlearis muscle, insulin increased PKB phosphorylation to the same extent in all groups at both 500 and 10,000 μU/ml insulin (*Fig 4*). Contraction did not stimulate PKB phosphorylation in soleus muscle (*Fig 3B*).

**Table II**

Urinary catecholamines secretion during 24 h.

	Noradrenaline	Adrenaline
	(nmol/day)	(nmol/day)
W	5.0 ± 0.4	0.8 ± 0.1
F35%	5.6 ± 0.5	0.7 ± 0.1
S35%	7.7 ± 0.7 <sup>†</sup>	0.9 ± 0.1
F10.5%	5.6 ± 0.4	0.8 ± 0.1
S10.5%	6.0 ± 0.4	1.1 ± 0.1

Catecholamine secretions are calculated as the means in urines collected during the first and second day in metabolic cages. Values are means ± SEM. *n* = 11 for W, *n* = 8 for F35%, *n* = 8 for S35%, *n* = 4 for F10.5%, and *n* = 4 for S10.5%. <sup>†</sup>*P* < 0.02 vs rats drinking W, F35%, F10.5% and S10.5%.

**Figure 2**

Glucose uptake in skeletal muscles from rats drinking water or a fructose or a sucrose solution. Glucose uptake was measured in muscles incubated with tracer amount of 2-[ $^3$ H]-deoxy-D-glucose for 30 min in the presence of 0, 500 or 10,000  $\mu$ U/ml insulin. Other soleus muscles were contracted with tracer amount of 2-[ $^3$ H]-deoxy-D-glucose without insulin for 30 min. A: Effect of insulin on glucose uptake in soleus. B: Effect of insulin on glucose uptake in epitrochlearis. C: Effect of contraction on glucose uptake in soleus. Values are means  $\pm$  SEM.  $n = 11$  for W,  $n = 8$  for F35%,  $n = 8$  for S35%,  $n = 4$  for F10.5%, and  $n = 4$  for S10.5%. a:  $P < 0.0001$  vs muscles incubated without insulin from rats exposed to similar feeding protocol; b:  $P < 0.03$  vs muscles incubated with 500  $\mu$ U/ml insulin from rats exposed to similar feeding protocol.

**Table III**

Glycogen content in skeletal muscles, heart and liver.

	Soleus	Epitrochlearis <sup>1</sup>	Heart	Liver
	mmol/kg dw			
W	159.9 ± 11.4	180.4 ± 3.6	139.1 ± 6.8	1116.0 ± 73.6
F35%	181.5 ± 7.9	195.6 ± 8.1	133.0 ± 6.5	1215.1 ± 74.6
S35%	163.9 ± 7.7	180.4 ± 8.1	129.2 ± 5.0	1185.3 ± 57.3
F10.5%	156.3 ± 15.8	190.0 ± 13.5	150.0 ± 6.4	1147.1 ± 145.7
S10.5%	169.2 ± 6.7	194.6 ± 7.2	146.1 ± 11.8	1222.2 ± 52.2

<sup>1</sup> Values from epitrochlearis incubated for 30 min with 500 µU/ml insulin. Values are means ± SEM. *n* = 11 for W, *n* = 8 for F35%, *n* = 8 for S35%, *n* = 4 for F10.5%, and *n* = 4 for S10.5%.

## Discussion

The data reported herein demonstrate that intake of fructose or sucrose solution for 11 weeks enhanced daily energy intake by 11–17% without increasing body weight or impair insulin-stimulated glucose uptake and PKB phosphorylation in skeletal muscles. By first glance these data may seem surprising. However, our results are in accordance with previous studies showing that intake of a 32% sucrose solution for 9 weeks or a 10% fructose solution for 12 weeks do not increase rat body weight despite the fact that energy intake is enhanced by 8% and 18%, respectively [30, 37]. Similarly, rats drinking tap water or a 25% fructose or sucrose solution during one year did not show different body weight [38].

Intake of Coca-Cola for 9 weeks increased energy intake by as much as 50% without any weight gain compared to control rats [30]. The reason that Coca-Cola consumption increased energy intake so much without weight gain is difficult to assess since the beverage (according to the Coca-Cola Co.) contains ingredients like caffeine, aspartame, phenylalanine, sodium, phosphoric acid, or saccharin. Interestingly, Bukowiecki *et al.* [30] reported that caffeine intake decreased body weight in rats fed chow without altering energy intake. A reason may be that caffeine stimulates catecholamine secretion, which raises metabolic rate [39–42]. However, caffeine also augments locomotive behaviour through the blockade of adenosine A<sub>2A</sub> receptors in the central nervous system [43]. To our knowledge, there is no report on the effect of Coca-Cola, fructose or sucrose solution intake on locomotor activity. This will certainly represent an exciting area for further research.

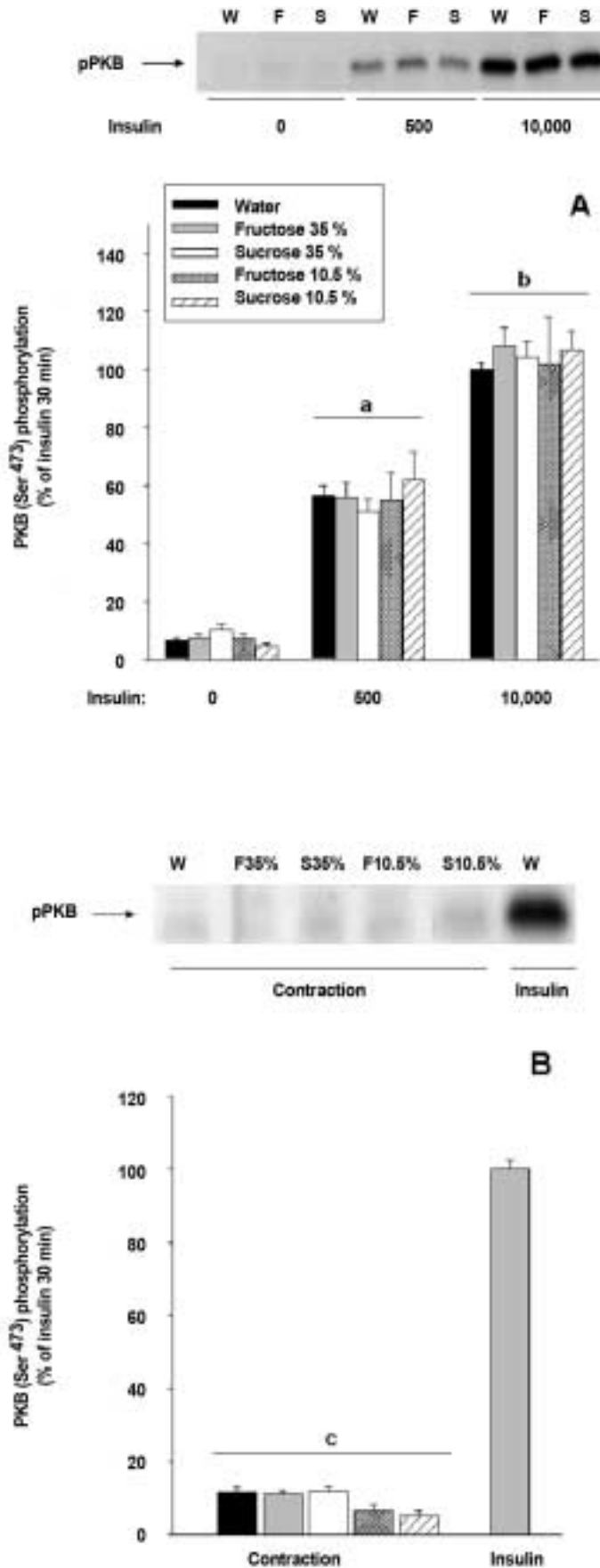
In accordance with unchanged body weight, epididymal fat pad weight was not increased by consumption of fructose or sucrose solution. Other studies feeding rats a solid diet with ~70% of total energy from sucrose for 27–30 weeks did not find increased body weight or increased weight of epididymal fat pads [26, 44].

These data agree with ours and suggest that a diet rich in carbohydrate is not sufficient to enhance epididymal fat pad weight despite increased total energy intake.

In the present study, rats drinking a 35% sucrose solution had an elevated urinary noradrenaline secretion compared to rats drinking water. Urine represents the main elimination way for catecholamines and the higher secretion suggests higher sympathetic nervous system activity [45, 46]. Sucrose consumption has been reported to activate the sympathetic nervous system in rats and to increase energy expenditure by accelerating metabolism and heat production [14, 47]. Surprisingly, urinary noradrenaline secretion was normal in rats drinking a 10.5% sucrose or in rats drinking a fructose solution. Previous studies have reported elevated noradrenaline secretion following intake of a 12% sucrose solution [48] or a solid diet rich in 50% fructose [15]. These studies, however, measured catecholamine after 3 and 10 days respectively, and it is possible that rats fed carbohydrate solutions normalize catecholamine secretion over time. Our data suggest that energy expenditure was regulated by mechanisms other than activation of the sympathetic nervous system in rats during long-term carbohydrate solution feeding.

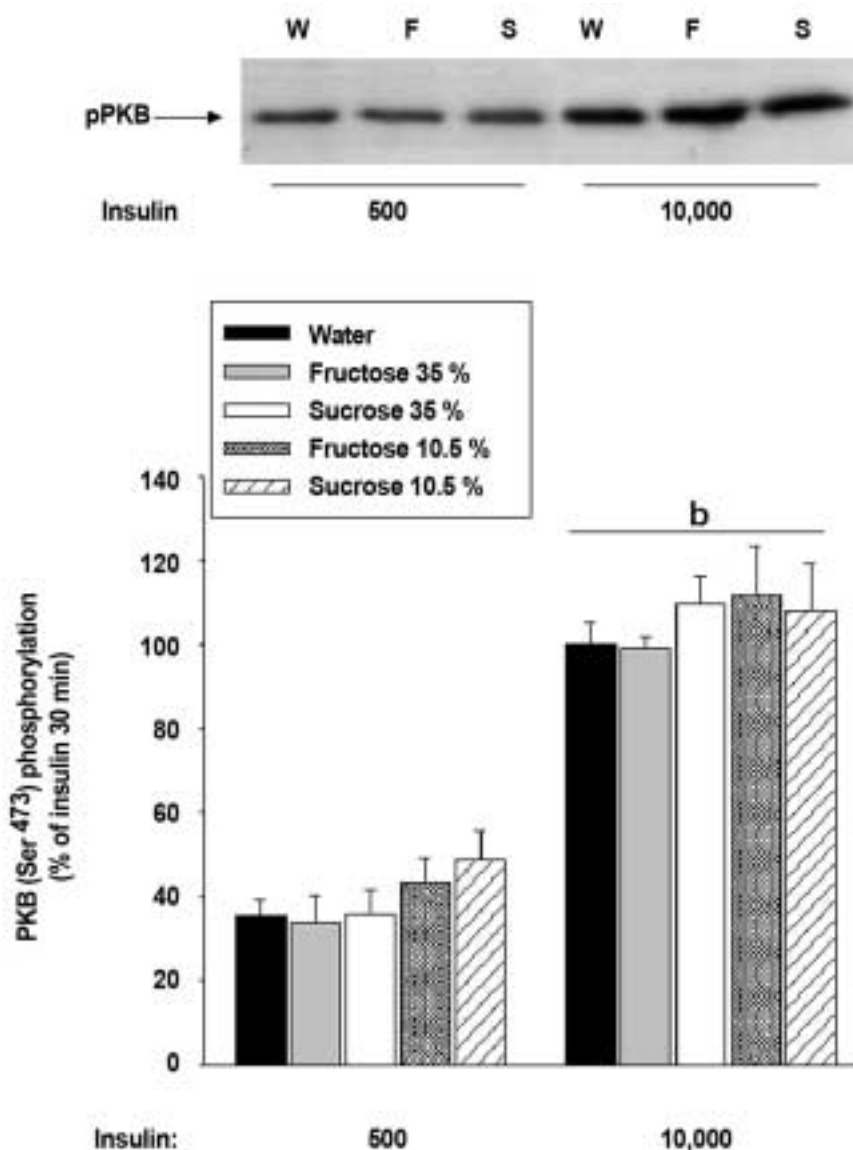
There are several mechanisms that may protect body weight in face of increased energy intake. Expression of uncoupling proteins (UCP) have been suggested to be critical for the regulation of energy expenditure [49] and mice over-expressing UCP3 in skeletal muscles are resistant to diet-induced weight gain [50]. Interestingly, it has been reported that intake of a 30% sucrose solution enhances the amount of brown adipose tissue and UCP expression [51] and Coca-Cola intake elevates UCP2 expression in skeletal muscles [52]. It is therefore possible that increased UCP expression following high-carbohydrate diet may have increased energy expenditure and regulate body weight.

The dramatic increase in body weight in the Western societies has shown difficult for clinicians to prevent.

**Figure 3**

PKB phosphorylation in soleus muscles from rats drinking water or a fructose or a sucrose solution. Soleus muscles were incubated with 0, 500 or 10,000  $\mu\text{U/ml}$  insulin for 30 min. Other soleus muscles were contracted without insulin for 30 min. Soleus muscles incubated for 30 min with 10,000  $\mu\text{U/ml}$  insulin from rats drinking W were used as 100% and other values were calculated as % of this. **A:** Effect of insulin on PKB phosphorylation on Ser<sup>473</sup> in soleus. The blot shows the phosphorylation of PKB on Ser<sup>473</sup> in soleus incubated without or with 500 and 10,000  $\mu\text{U/ml}$  insulin from rats drinking tap water (W), a 10.5% fructose (F) or 10.5% sucrose (S) solution.  $n = 4-11$ . **B:** Effect of contraction on PKB phosphorylation in soleus. The blot shows the phosphorylation of PKB on Ser<sup>473</sup> in soleus electrically stimulated from different feeding groups, and in soleus incubated with 10,000  $\mu\text{U/ml}$  insulin from rats drinking W.  $n = 4-11$ . Values are means  $\pm$  SEM. a:  $P < 0.0001$  vs muscles incubated without insulin from rats exposed to similar feeding protocol; b:  $P < 0.0001$  vs muscles incubated with 500  $\mu\text{U/ml}$  insulin from rats exposed to similar feeding protocol; c:  $P < 0.0001$  vs muscles incubated with 10,000  $\mu\text{U/ml}$  insulin from rats drinking water.



**Figure 4**

PKB phosphorylation in epitrochlearis from rats drinking water or a fructose or a sucrose solution. Epitrochlearis muscles were incubated with 500 or 10,000  $\mu\text{U/ml}$  insulin for 30 min. Epitrochlearis muscles incubated for 30 min with 10,000  $\mu\text{U/ml}$  insulin from rats drinking W were used as 100% and other values were calculated as % of this. The blot shows the phosphorylation of PKB on Ser<sup>473</sup> in epitrochlearis from rats drinking drinking tap water (W), a 10.5% fructose (F) or 10.5% sucrose (S) solution.  $n = 4-11$ . Values are means  $\pm$  SEM. b:  $P < 0.0001$  vs muscles incubated with 500  $\mu\text{U/ml}$  insulin from rats exposed to similar feeding protocol.

Although studies have shown association between intake of soft drink and obesity [8, 9] not all studies find this association [53], and our data may support the idea that additional lifestyle factors co-associating with high intake of soft drink solutions have to be considered in prevention. Most interestingly, Levine *et al.* [54] showed considerable inter-individual variation in weight gain in response to overeating suggesting that humans also possess mechanisms to prevent/reduce weight gain. Clinically, these mechanisms may be of major importance for future treatment.

In the present study, we report that intake of carbohydrate as solution did not impair insulin-stimulated glucose uptake in soleus and epitrochlearis. Previously, some studies have shown that consumption of a solid diet rich in fructose develops insulin resistance in skeletal muscles [22, 23], whereas others reported that solid diet containing 65-75% of total energy from fructose or sucrose did not alter insulin-stimulated glucose incorporation into glycogen and insulin-stimulated glucose uptake in rat skeletal muscles [24-29]. This is the first study investigating the effects of a

solution containing fructose or sucrose on insulin action in skeletal muscles. We show that consumption of solutions with high or low percentage of simple carbohydrates do not influence insulin-stimulated glucose uptake in skeletal muscles from 3 months young rats.

PKB is a signaling protein situated downstream of phosphatidylinositol 3-kinase, which regulates insulin-stimulated glucose uptake [55-57]. Not surprisingly, insulin-stimulated PKB phosphorylation was not impaired by intake of carbohydrate solutions. These data support our result that insulin-stimulated glucose uptake was normal in all feeding groups. Contraction-stimulated glucose uptake was also normal in muscles from rats fed carbohydrate solutions. Therefore, regulation of glucose uptake by both insulin and contraction remained normal in skeletal muscles from rats drinking solutions with fructose or sucrose.

It is the fructose molecule that is believed to be the primary nutrient component that impairs insulin action [20, 21]. Liver is the main tissue metabolising fructose [58], and intake of high-fructose or high-sucrose diet causes hepatic insulin resistance [20, 59, 60]. In liver impaired insulin-induced suppression of hepatic glucose production [44, 61] occurs in concert with lipid accumulation [20, 62] and reduced insulin signaling [59]. The anatomical location of the liver and the abundant expression of GLUT2, which transports fructose [63], may explain why high-sucrose diet-induced insulin resistance was reported to primarily affect the liver [44, 62]. Thus, although the consumption of carbohydrate solutions did not cause insulin resistance in skeletal muscles, deteriorations of insulin action in liver or impaired physiological regulation of blood glucose cannot be excluded from the present study.

In conclusion, intake of normal and high concentration of carbohydrate solutions for 11 weeks promotes excess energy intake without enhanced body weight. Furthermore, consumption of a fructose or sucrose solution did not impair insulin- or contraction-stimulated glucose uptake in skeletal muscles. We suggest clinicians also to consider physical activity and lifestyle factors other than the diet for prevention and treatment of obesity.

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