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Obesogenic diets have deleterious effects on fat deposits irrespective of the nature of dietary carbohydrates in a Yucatan minipig model

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ABSTRACT

The effects of digestible carbohydrates, fructose in particular, on the development of metabolic disturbances remain controversial. We explored the effects of prolonged consumption of high-fat diets differing in their carbohydrate source on fat deposits in the adult Yucatan minipig. Eighteen minipigs underwent computed tomographic imaging and blood sampling before and after 8 weeks of three isocaloric high-fat diets with different carbohydrate sources (20% by weight for starch in the control diet, glucose or fructose, n = 6 per diet). Body adiposity, liver volume, and fat content were estimated from computed tomographic images (n = 18). Liver volume and lipid content were also measured post mortem (n = 12). We hypothesized that the quantity and the spatial distribution of fat deposits in the adipose tissue or in the liver would be altered by the nature of the carbohydrate present in the obesogenic diet. After 8 weeks of dietary exposure, body weight (from 26 ± 4 to 58 ± 3 kg), total body adiposity (from 38 ± 1 to 47 ± 1%; $P < .0001$), liver volume (from 1156 ± 31 to 1486 ± 66 mL; $P < .0001$), plasma insulin (from 10 ± 1 to 14 ± 2 mIU/L; $P = .001$), triacylglycerol (from 318 ± 37 to 466 ± 33 mg/L; $P = .005$), and free-fatty acids (from 196 ± 60 to 396 ± 59 μmol/L; $P = .0001$) increased irrespective of the carbohydrate type. Similarly, the carbohydrate type did not induce changes in the spatial repartition of the adipose tissue. Divergent results were obtained for fat deposits in the liver depending on the investigation method. In conclusion, obesogenic diets alter adipose tissue fat deposits and the metabolic profile independently of the nature of dietary carbohydrates.

1. Introduction

Dietary sugar intake may strongly influence the development of metabolic diseases, such as non-alcoholic fatty liver disease, dyslipidemia, and insulin resistance [1,2]. Consistent evidence exists concerning the implications of fructose or high-fructose corn syrup as potential risk factors for weight gain, abdominal adiposity, and liver steatosis as well as

Abbreviations: CT, computed tomography; BW, body weight; SD, starch-containing diet; GD, glucose-containing diet; FD, fructose-containing diet; TAG, triacylglycerol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free-fatty acids; H, Hounsfield units; ΔH, attenuation change; ROI, region of interest.

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several features of metabolic syndrome in rodents and cats [3-8]. However, in large animal models, such as pigs, or in humans, this concept remains controversial. Data obtained from humans [9] and Ossabaw minipigs [10] suggests that the association between high fructose intake with other components in the diet, such as glucose, sucrose, fat, and cholesterol, was the main factor responsible for the development of metabolic syndrome and liver steatosis. Conversely, high-fructose intake did not appear as a critical element. Nevertheless, in humans, this conclusion must be handled with care, since the experiments were performed in patients with previously established hepatic steatosis [11-13]. This exemplifies the need for a suitable animal model that resembles humans physiologically and metabolically, such as pigs [14-16]. Ideally, this animal model must be of equal size with humans so as to be able to use non-invasive imaging methods in a translational manner.

Various imaging methodologies are available for the estimation of liver volume [17-19] and fat content [17,20,21] in pigs and humans. Estimation of liver volume is mainly achieved using segmentation of 3D volumes of computed tomography (CT) abdominal scans [22]. Dual-energy CT scans appear to be a useful method for discriminating between non-fatty hypodense liver masses (eg, iron, copper, glycogen) in humans [21] that might affect hepatic attenuation [23].

In the present study, we aimed at examining the effect of prolonged consumption of three high-fat isocaloric diets differing in a fraction (20% by weight) of their digestible carbohydrates on fat accumulation and distribution in adult Yucatan minipigs. We hypothesized that prolonged intake of isocaloric high-energy diets with fructose, and, to a lesser extent glucose, but not starch (control diet), would modify fat deposits either in the adipose tissue or/and in the liver together with an alteration of the metabolic profile.

2. Methods and materials

2.1. Animals and diets

The present study was conducted in accordance with the current ethical standards of the European Legislation after validation by a regional Ethics Committee (R-2011-MO-01). Trained staff members provided animal care and management under the supervision of a veterinarian. Eighteen male adult Yucatan minipigs (INRA, Saint-Gilles, France) were housed in individual cages (150 × 60 × 60 cm) under controlled temperature (24°C) and relative humidity (4%-55%), and were maintained on a 12-hour light-dark cycle with free access to water.

Animals were randomly divided into three groups (initial weight 26 ± 4 kg). During 8 weeks, each group (n = 6 per group) received one of three isocaloric high-fat diets (Table 1), varying only in a fraction of their digestible carbohydrates: starch- (SD) as control, glucose- (GD), and fructose-containing (FD) diets (0.41 MJ/kg BW/day). Animals were weighed before the beginning of the procedure, then every 2 weeks, and also before the beginning of the CT scan and before euthanasia.

The number of animals per group (N = 6) comes from an a priori statistical power analysis performed using available data obtained by our group using CT scan in a minipig model of human obesity [25]. Briefly, we selected VAT values as the data source since it is the one with the smallest difference between lean and obese animals (see Table 2 of [25]). Using the means difference and SEM plus $\alpha = .05$ and the statistical power set to 0.9, we can calculate n = 6 (JMP 12 software).

Table 1 – Ingredient composition of the experimental diets

Ingredient (g/kg)	Experimental diets		
	Starch	Glucose	Fructose
Wheat	60.0	60.0	60.0
Barley	120.0	120.0	120.0
Wheat bran	140.0	140.0	140.0
Soybean meal	90.0	90.0	90.0
Sunflower meal	80.0	80.0	80.0
Soybean hulls	110.0	110.0	110.0
Corn starch	250.0	51.0	65.0
Glucose source [a]		199.0	
Fructose source [a]			185.0
Lard	120.0	120.0	120.0
Dicalcium phosphate	6.0	6.0	6.0
Calcium carbonate	13.0	13.0	13.0
Salt	6.0	6.0	6.0
Oligoelements and vitamins [c]	5.0	5.0	5.0
Nutritional value (calculated [b])			
Metabolizable energy (MJ/kg)	14.06	14.06	14.06
Net energy (MJ/kg)	10.89	10.89	10.89
Dry matter (g/kg)	896.4	896.4	896.4
Crude protein (g/kg)	121.8	121.8	121.8
Crude fat (g/kg)	134.5	134.5	134.5
Cellulose (g/kg)	80.0	80.0	80.0
Minerals (g/kg)	55.6	55.6	55.6

[a] Pure glucose and fructose sources with water content of 9.5% and 0.5%, respectively, so they both provided exactly 20.05% of the diets' dry matter.
[b] Nutritional values were calculated upon the nutritional tables from Sauvant et al (2004).
[c] Contains choline chloride 50 mg for 1 kg of diet irrespective of the type of diet.

2.2. Experimental paradigms

Anesthetized animals underwent 2 CT scan sessions and blood sampling before and after 8 weeks of dietary exposure. CT scan images were used for the estimation of body adiposity, liver fat content, and liver volume. The CT scan (Hispeed NX/I; General Electric Medical systems, Milwaukee, WI, USA) was calibrated daily against air density 30 to 60 minutes before scanning, and weekly with a water phantom. Animals were fasted for 15 to 18 hours before each imaging session. Pre-anesthesia was induced before CT scanning with an intramuscular injection of ketamine (5.0 mg/kg; Rhône-Mérieux, Lyon, France). Anesthesia and conditions for CT scan procedure were the same as previously described [24].

2.3. Blood collection and biochemical analyses

Before the CT scan sessions, venous blood samples were collected in fasted anesthetized animals in 5-mL coated tubes containing 5 μL of EDTA (0.8 mol/L, Sigma Aldrich, Saint-