Increased hepatic de novo lipogenesis and mitochondrial efficiency in a model of obesity induced by diets rich in fructose

Raffaella Crescenzo, Francesca Bianco, Italia Falcone, Paola Coppola, Giovanna Liverini & Susanna Iossa

European Journal of Nutrition

ISSN 1436-6207
Volume 52
Number 2

Eur J Nutr (2013) 52:537-545
DOI 10.1007/s00394-012-0356-y

Springer

Eur J Nutr (2013) 52:537–545
DOI 10.1007/s00394-012-0356-y

ORIGINAL CONTRIBUTION

Increased hepatic de novo lipogenesis and mitochondrial efficiency in a model of obesity induced by diets rich in fructose

Raffaella Crescenzo · Francesca Bianco · Italia Falcone · Paola Coppola · Giovanna Liverini · Susanna Iossa

Received: 21 December 2011 / Accepted: 2 April 2012 / Published online: 28 April 2012

Abstract
Purpose To assess hepatic de novo lipogenesis and mitochondrial energetics as well as whole-body energy homeostasis in sedentary rats fed a fructose-rich diet.
Methods Male rats of 90 days of age were fed a high-fructose or control diet for 8 weeks. Body composition, energy balance, oxygen consumption, carbon dioxide production, non-protein respiratory quotient, de novo lipogenesis and insulin resistance were measured. Determination of specific activity of hepatic enzymes of de novo lipogenesis, mitochondrial mass, oxidative capacity and degree of coupling, together with parameters of oxidative stress and antioxidant defence, was also carried out.
Results Body energy and lipid content as well as plasma insulin and non-esterified fatty acids were significantly higher in fructose-fed than in control rats. Significantly higher rates of net de novo lipogenesis and activities of hepatic lipogenic enzymes fatty acid synthase and stearoyl CoA desaturase-1 were found in fructose-fed rats compared to controls. Mitochondrial protein mass and degree of coupling were significantly higher in fructose-fed rats compared to controls. Hepatic mitochondria showed oxidative damage, both in the lipid and in the protein component, together with decreased activity of antioxidant defence.
Conclusion Liver mitochondrial compartment is highly affected by fructose feeding. The increased mitochondrial efficiency allows liver cells to burn less substrates to produce ATP for de novo lipogenesis and gluconeogenesis. In addition, increased lipogenesis gives rise to whole body and ectopic lipid deposition, and higher mitochondrial coupling causes mitochondrial oxidative stress.

Keywords Mitochondrial uncoupling · Insulin resistance · Body lipids · De novo lipogenesis

R. Crescenzo · F. Bianco · I. Falcone · P. Coppola · G. Liverini (✉) · S. Iossa
Department of Structural and Functional Biology, University of Naples, Complesso Universitario di Monte Sant'Angelo, Edificio 7, Via Cinthia, 80126 Naples, Italy
e-mail: liverini@unina.it

Introduction

Obesity is a major public health problem that is obviously multifactorial, even if diet composition plays a major role. Fructose, present in small amount in many fruits, is now consumed by humans in large quantities due to the popularity of convenient, prepackaged foods and the consumption of soft drinks and juice beverages containing sucrose or high-fructose corn syrup (HFCS) [1–3]. HFCS can be produced with various fructose-to-glucose ratio, with the most commonly used being HFCS-55, containing 55 % fructose and 45 % glucose, that is, a fructose-to-glucose ratio close to the 1:1 ratio found in sucrose. Interestingly, the approximate 25 % increase in per capita fructose consumption over the past 30 years in the United States coincides closely with the increase in the prevalence of obesity [1–3]. Reports also suggest that the increasing intake of soft drinks is associated with an increase in the risk of diabetes, cardiometabolic disease and gout, as well as with lipid disturbances [4]. However, in humans, it is difficult to assess the contribution of fructose intake alone to the development of the above metabolic disorders, since in everyday life, additional factors, such as hypercaloric diet rich in saturated fat and low physical activity, are involved.

Animal models could help to shed light on the question of whether dietary fructose per se leads to excessive lipid

depots and correlated metabolic diseases. We therefore assessed the impact of long-term, high-fructose, low-fat diet on whole-body energy homeostasis in sedentary rats. To this end, we measured body composition, energy balance, oxygen consumption (VO_2), carbon dioxide production (VCO_2), non-protein respiratory quotient (NPRQ) and de novo lipogenesis in adult rats fed a high-fructose diet for 8 weeks. Since fructose is mainly metabolised by the liver, the major site of de novo lipogenesis, whole-body measurements were associated with analysis of the activity of hepatic markers of this metabolic pathway.

We have previously shown that long-term high fat feeding in adult rats is associated with ectopic fat storage and alterations in mitochondrial compartment in the liver [5], in agreement with the emerging idea that mitochondrial dysfunction can lead to the development of metabolic diseases, such as obesity, type 2 diabetes mellitus and non-alcoholic steatohepatitis [6–8]. Therefore, we decided to investigate whether high fructose feeding, in analogy with high fat feeding, is associated with hepatic mitochondrial derangement, by measuring mitochondrial mass, oxidative capacity and degree of coupling, together with parameters of oxidative stress and antioxidant defence.

Research methods and procedures

Male Sprague–Dawley rats (Charles River, Italy) of 90 days of age were caged singly in a temperature-controlled room (23 ± 1 °C) with a 12-h light/dark cycle (06.30–18.30) and divided in three groups. One group of rats was killed at the beginning of the dietary treatment and used for the determination of initial body energy and lipid content. The other two groups of rats were fed a high fructose or control diet (Mucedola 4RF21; Settimo Milanese, Milan, Italy) for 8 weeks. The composition of the two diets is shown in Table 1. Treatment, housing and killing of animals met the guidelines set by the Italian Health Ministry. All experimental procedures involving animals were approved by "Comitato Etico-Sientifico per la Sperimentazione Animale" of the University "Federico II" of Naples.

At the end of the experimental period, the animals were killed by decapitation, the liver harvested, and the carcasses used for body composition determination.

Twenty-four-hour VO_2, VCO_2, urinary nitrogen and NPRQ

Twenty-four-hour VO_2, VCO_2, urinary nitrogen and NPRQ were measured at 0, 2, 4, 6 and 8 weeks point; 24-h VO_2 and VCO_2 of the rats were recorded with a four-chamber indirect open-circuit calorimeter (Panlab s.r.l., Cornella, Barcelona, Spain). Measurements were taken every 15 min for 3 min in each cage. Urine was collected for the whole (24-h) period, and urinary nitrogen levels were measured by an enzymatic colorimetric method (FAR S.r.l., Settimo di Pescantina, Verona, Italy); 24-h NPRQ was then calculated. Fat balance was calculated from NPRQ by using tabulated values [9], and then, rates of net whole-body de novo lipogenesis were obtained by applying the formula [10]:

$$\text{net de novo lipogenesis}\,(\text{g/day}) = \text{fat balance} - \text{fat intake}.$$

Daily energy expenditure was calculated from VO_2, VCO_2 and urinary nitrogen according to [11] using the equation:

$$\text{Energy expenditure} = 1.1\,VCO_2 + 3.91VO_2 - 1.93\text{N}.$$

Plasma glucose, insulin and non-esterified fatty acids

Rats were fasted for 6 h from 09.00. Samples were obtained from venous blood from a small tail clip and centrifuged at $1{,}400 \times g_{av}$ for 8 min at 4 °C. Plasma was removed and stored at −20 °C. Plasma glucose and non-esterified fatty acids (NEFA) concentration were measured by colorimetric enzymatic method (Pokler Italia, Genova, Italy for glucose and Randox Laboratories ltd., Crumlin, United Kingdom for NEFA). Plasma insulin concentration was measured using an ELISA kit (Mercodia AB, Uppsala,

Table 1 Composition of experimental diets

	Control diet	Fructose diet
Component (g/100 g)		
Standard chow	100.0	50.5
Sunflower oil		1.5
Casein		9.2
Alphacel		9.8
Fructose		20.4
Water		6.4
AIN-76 mineral mix		1.6
AIN-76 vitamin mix		0.4
Choline		0.1
Methionine		0.1
Gross energy density (kJ/g)	17.2	17.2
Metabolisable energy density (kJ/g)[a]	11.1	11.1
Protein (% metabolisable energy)	29.0	29.0
Lipids (% metabolisable energy)	10.6	10.6
Carbohydrates (% metabolisable energy)	60.4	60.4
Of which		
Fructose	–	30.0
Starch	45.3	22.8
Sugars	15.1	7.6

[a] Estimated by computation using values (kJ/g) for energy content as follows: protein 16.736, lipid 37.656, and carbohydrate 16.736

Sweden) in a single assay to remove inter-assay variations. HOMA index was calculated as (Glucose (mg/dL) × Insulin (mU/L))/405 [12].

Energy balance and body and liver composition

Guts were cleaned of undigested food, and the carcasses were then autoclaved. After dilution (1:2 distilled water) and subsequent homogenisation of the carcasses with a Polytron homogeniser (Kinematica, Luzern, Switzerland), duplicate samples of the homogenised carcass were analysed for energy content by bomb calorimetry. To take into account the energy content of the liver, tissue samples were dried and the energy content was then measured with the bomb calorimeter. Total body and hepatic fat content were measured by the Folch extraction method [13]. Total body water content was determined by drying carcass samples in an oven at 70 °C for 48 h. Total body protein content was determined using a formula relating total energy value of the carcass, energy derived from fat and energy derived from protein [14]; the caloric values for body fat and protein were taken as 39.2 and 23.5 kJ/g, respectively [15]. Liver glycogen content was assessed by direct enzymatic procedure [16]. Energy balance measurements were taken by the comparative carcass technique over the experimental period, as detailed previously [17]. Briefly, during the experimental period, metabolisable energy (ME) intake was determined by subtracting the energy measured in faeces and urine from the gross energy intake, determined from daily food consumption and gross energy density of the diet. Body energy and fat gain were calculated as the difference between the final and initial content of body energy and fat. Energetic efficiency was calculated as the percentage of body energy retained per ME intake, efficiency of lipid deposition was calculated as the percentage of energy gained as body lipids per ME intake, and energy expenditure was determined as the difference between ME intake and energy gain.

Stearoyl CoA desaturase and fatty acid synthase activity

Stearoyl CoA desaturase (SCD1) activity was measured polarographically in liver homogenates at 37 °C in a solution containing 0.1 M K_2HPO_4, pH 7.4, 1 μM myxothiazol, 0.12 mM NADH and 0.06 mM stearoyl CoA as cyanide (5 mM)-sensitive [18], and myxothiazol-insensitive oxygen consumption. Fatty acid synthase (FAS) activity was measured in liver homogenates according to [19].

Liver homogenate and isolated mitochondria

Liver homogenates and isolated mitochondria were prepared as previously reported [17]. Control experiments of enzymatic and electron microscopy characterisation have shown that our isolation procedure (centrifugation at $3{,}000 \times g_{av}$ for 10 min) results in a cellular fraction, which is constituted essentially by mitochondria.

Mitochondrial respiration, degree of coupling and uncoupling effect of fatty acids

Oxygen consumption was measured polarographically with a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH, USA) at 30 °C. Samples of homogenates or isolated mitochondria were incubated in a medium containing 80 mM KCl, 50 mM HEPES, 5 mM K_2HPO_4, 1 mM EGTA, 0.1 % (w/v) fatty acid-free BSA, pH 7.0. The substrate used was 10 mM succinate + 3.8 μM rotenone, 10 mM glutamate + 2.5 mM malate, 40 μM palmitoyl-coenzyme A + 2 mM carnitine + 2.5 mM malate, 40 μM palmitoyl-carnitine + 2.5 mM malate, or 10 mM pyruvate + 2.5 mM malate. State 3 oxygen consumption was measured in the presence of 0.3 mM ADP.

The degree of thermodynamic coupling, q, was determined in liver mitochondria by applying equation 11 by Cairns et al. [20]: $q = \sqrt{1 - (J_o)_{sh}/(J_o)_{unc}}$, where $(J_o)_{sh}$ represents the oxygen consumption rate in the presence of oligomycin that inhibits ATP synthase, and $(J_o)_{unc}$ is the uncoupled rate of oxygen consumption induced by FCCP, which dissipates the transmitochondrial proton gradient. $(J_o)_{sh}$ and $(J_o)_{unc}$ were measured as above using succinate (10 mM) + rotenone (3.75 μM) in the presence of oligomycin (2 μg/mL) or FCCP (1 μM), respectively, both in the absence and in the presence of palmitate at a concentration of 45 μM.

Uncoupling effect of fatty acids was assessed by measuring mitochondrial membrane potential before and after addition of increasing concentration of the fatty acid palmitate. Mitochondrial membrane potential recordings were performed with safranin O using a JASCO dual-wavelength spectrophotometer (511–533 nm). Measurements were taken at 30 °C in a medium containing 80 mM LiCl, 50 mM HEPES, 1 mM EGTA, 50 mM Tris-PO_4, pH 7.0, 0.1 % (w/v) fatty acid-free BSA, in the presence of succinate (10 mM), rotenone (3.75 μM), oligomycin (2 μg/mL), safranin O (83.3 nmol/mg), both in the absence and after the addition of 15, 30 and 45 μM palmitate. The absorbance readings were transformed into mV membrane potential using the Nernst equation: $\Delta\psi = 61$ mV log $([K^+]_{in}/[K^+]_{out})$. Calibration curves made for each preparation were obtained from traces in which the extramitochondrial K^+ level ($[K^+]_{out}$) was altered in the 0.1–20 mM range. The change in absorbance caused by the addition of 3 μM valinomycin was plotted against $[K^+]_{out}$. Then, $[K^+]_{in}$ was estimated by extrapolation of the line to the zero uptake point.

Mitochondrial protein mass

Mitochondrial mass was evaluated by Western blot analysis of cytochrome c as described previously [21], as well as by measuring polarographically [22] the activity of a mitochondrial marker enzyme, cytochrome oxidase (COX), in liver homogenate and isolated mitochondria, and by evaluating mitochondrial protein yield as mg of isolated protein per g of starting wet tissue.

Mitochondrial lipid peroxidation, aconitase and superoxide dismutase (SOD) specific activity

Lipid peroxidation was determined according to Fernandes et al. [23]. Aconitase specific activity was measured spectrophotometrically by the method of Gardner [24], while SOD specific activity was measured according to Flohè and Otting [25].

Statistical analysis

Data are given as means ± SEM. Statistical comparisons were performed by the two-tailed unpaired Student's *t* test, two-way ANOVA for main effects and interaction followed by Bonferroni post-test or linear regression analysis. Probability values less than 0.05 were considered to indicate a significant difference. All analyses were performed using GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA).

Materials

All chemicals used were of analytical grade and were purchased from Sigma (St. Louis, MO, USA).

Results

Body composition measurements taken at the end of 8 weeks of dietary treatment show that body energy (+11 %) and lipid (+20.5 %) content were significantly higher, while body water content was significantly lower (−4 %), in fructose-fed than in control rats (Table 2). No variation was found in final body weight, as well as in body protein content. Liver lipid (+13 %) and glycogen content (+20 %), as well as plasma NEFA (+30 %) and insulin (+42 %) and HOMA index (+48 %), were significantly higher in fructose-fed rats compared to controls. As for energy balance measurements (Table 3), energy gain (+90 %), energetic efficiency (+113 %) and efficiency of lipid deposition (+110 %) were found to be significantly higher in fructose-fed than in control rats, while no significant variation was found in energy expenditure.

Table 2 Body composition and glucose homeostasis in rats fed a high-fructose or control diet for 8 weeks

	Control	Fructose
Final body weight (g)	527 ± 31	528 ± 32
Body energy (kJ)	8.9 ± 0.3	9.9 ± 0.3*
Body lipids (%)	12.2 ± 0.5	14.7 ± 0.5*
Body proteins (%)	17.4 ± 1.0	17.8 ± 0.4
Body water (%)	63.7 ± 0.6	61.4 ± 0.6*
Hepatic lipids (mg/g)	47.0 ± 1.3	53.3 ± 1.6*
Hepatic glycogen (mg/g)	25.3 ± 1.2	30.3 ± 1.1*
Plasma insulin (μg/L)	2.27 ± 0.21	3.23 ± 0.12*
Plasma glucose (mg/dL)	97.8 ± 3.2	101.7 ± 2.5
HOMA index	15.7 ± 1.1	23.2 ± 1.3*
Plasma NEFA (mM)	0.47 ± 0.02	0.61 ± 0.02*

Values are the means ± SEM of 6 different experiments

NEFA non-esterified fatty acids

* $P < 0.05$ compared to controls (two-tailed unpaired Student's *t* test)

The 24-h energy expenditure measurements, taken at 0, 2, 4, 6 and 8 weeks of dietary treatment (Fig. 1a), as well as area under the curve of 24-h energy expenditure over the whole experimental period (Fig. 1b), show no significant variation due to fructose feeding, although values of fructose-fed rats were slightly lower. Significantly higher mean 24-h NPRQ values and rates of net de novo lipogenesis were found in fructose-fed rats as from the second week of treatment (Fig. 1c). Finally, significantly higher activities of hepatic lipogenic enzymes FAS and SCD-1 were found at the end of the dietary treatment in fructose-fed rats compared to controls (Fig. 1d).

Mitochondrial state 3 respiratory capacities were assessed in liver homogenate to take into account changes in mass and capacity of mitochondria (Fig. 2a) and were found significantly higher with all the substrates in fructose-fed rats compared to controls, while no variation was found when mitochondrial capacity was measured in isolated organelles (Fig. 2b).

Table 3 Energy balance in rats fed a high-fructose or control diet for 8 weeks

	Control	Fructose
ME intake (kJ)	20,601 ± 1,144	20,394 ± 403
Energy gain (kJ)	545 ± 31	1,038 ± 123*
Energy expenditure (kJ)	20,156 ± 880	19,256 ± 517
Energetic efficiency (%)	2.3 ± 0.2	4.9 ± 0.6*
Efficiency of lipid deposition (%)	2.0 ± 0.1	4.2 ± 0.1*

Values are the means ± SEM of 6 different experiments. * $P < 0.05$ compared to controls (two-tailed unpaired Student's *t* test)

Energetic efficiency = (energy gain/ME intake) × 100; efficiency of lipid deposition = (lipid gain/ME intake) × 100

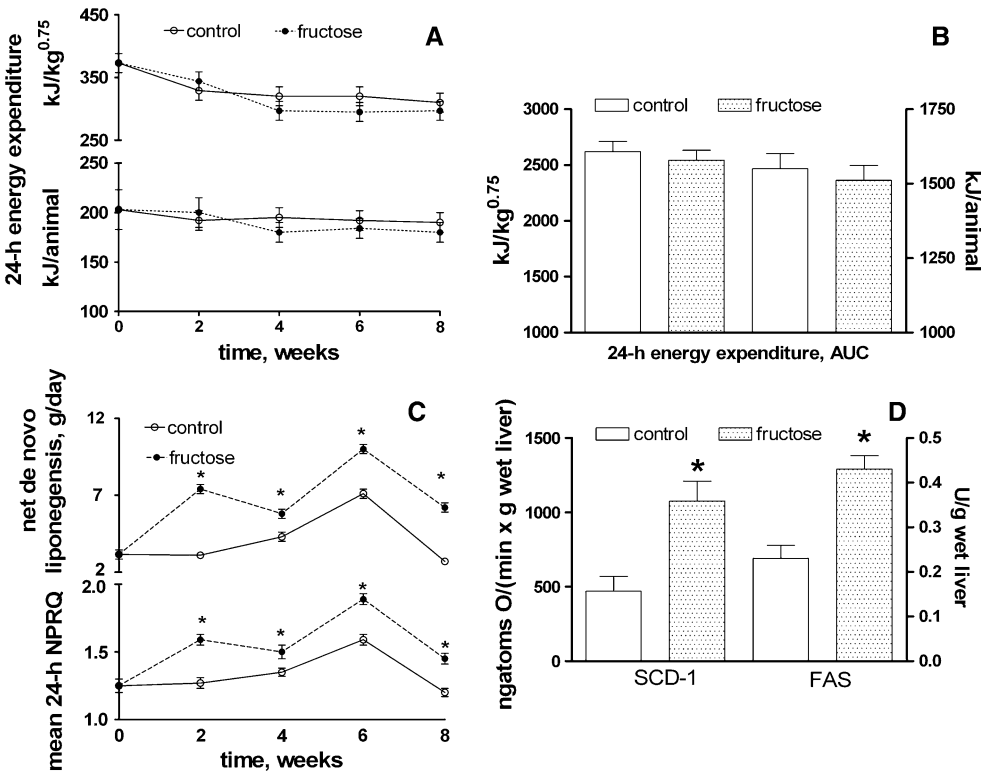

Fig. 1 Time course of 24-h energy expenditure (**a**), as well as area under the curve (AUC) of 24-h energy expenditure (**b**), time course of mean 24-h NPRQ and net de novo lipogenesis (**c**), and hepatic SCD-1 and FAS specific activity (**d**) in fructose-fed or control rats. Results are the means ± SEM of six different experiments. *$P < 0.05$ compared to controls (unpaired, two-tailed Student's t test for SCD-1 and FAS activity, or two-way ANOVA for main effects and interaction followed by Bonferroni post-test for NPRQ and de novo lipogenesis)

Mitochondrial protein mass was assessed by (1) measuring homogenate cytochrome c content, (2) measuring the activity of a mitochondrial marker enzyme COX in liver homogenates and in isolated mitochondria and (3) evaluating the mitochondrial yield. The results are reported in Fig. 2c and show that cytochrome c/actin ratio was significantly higher in fructose-fed rats compared to controls. In agreement, COX activity was significantly higher in liver homogenates from fructose-fed rats compared to controls, while no variation was found in COX specific activity measured in isolated mitochondria. As a consequence, mitochondrial protein mass, calculated from the ratio between activity in the homogenate and activity in isolated mitochondria, was significantly higher in fructose-fed rats compared to controls. Finally, mitochondrial yield was significantly higher in fructose-fed rats compared to controls.

Lipid peroxidation, aconitase and SOD specific activity were measured and taken as an index of cellular oxidative damage and antioxidant defences, respectively (Table 4). A significant decrease in active/total aconitase activity ratio and SOD specific activity was found in fructose-fed rats, compared to controls, while lipid peroxidation was found to be significantly higher.

Mitochondrial energetic efficiency was assessed by determining the degree of thermodynamic coupling, q, and the uncoupling effect of fatty acids. Significantly lower values of respiration in the presence of oligomycin, both in the absence and in the presence of palmitate, were found in fructose-fed rats (Fig. 3b), while respiration uncoupled by FCCP was not affected (Fig. 3c), so that values of thermodynamic coupling, q, were significantly higher in fructose-fed rats compared to controls, both in the absence and in the presence of palmitate (Fig. 3a). In addition, liver mitochondria from fructose-fed rats were less responsive to the uncoupling effect of fatty acids, since mitochondrial membrane potential in state 4 conditions was found to be significantly higher in fructose-fed rats compared to controls, both in the absence and in the presence of increasing concentrations of fatty acid palmitate (Fig. 3d), even after taking into account a 30 % increase in cellular fatty acid concentration.

Discussion

Increasingly worldwide fructose and sucrose consumption has drawn the attention on the metabolic effects of fructose, particularly regarding obesity development. Animal models have been considered helpful in establishing the impact of high fructose intake on obesity epidemic. Our present results give evidence that long-term high fructose intake in adult, sedentary rats induces not only metabolic derangements typical of human obesity, such as ectopic lipid deposition and altered hepatic insulin sensitivity, but also alteration in hepatic mitochondrial energetics.

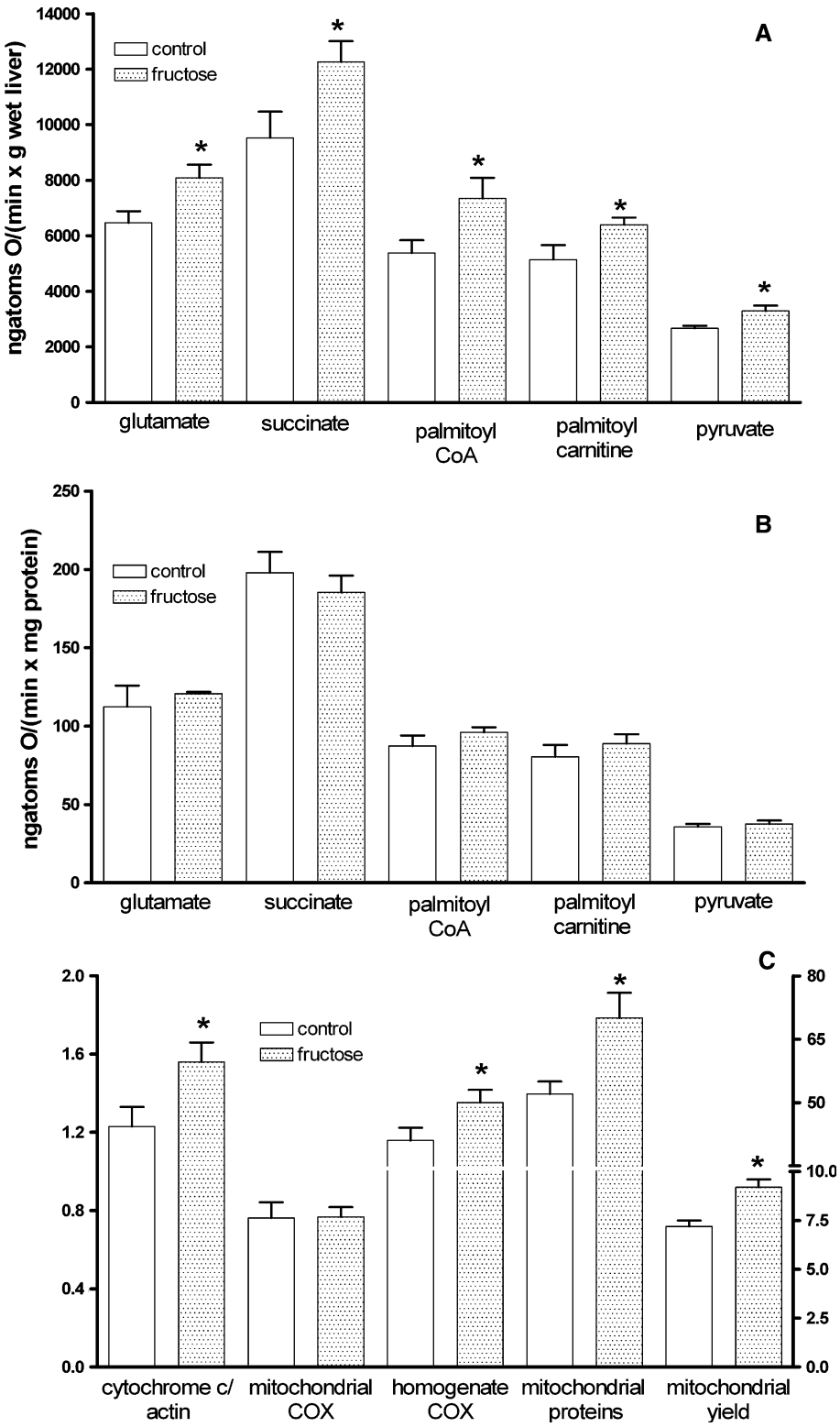

Fig. 2 Hepatic mitochondrial respiration in homogenates (**a**) and isolated mitochondria (**b**), as well as mitochondrial mass (**c**) in fructose-fed and control rats. Results are the means ± SEM of six different experiments. *COX* cytochrome oxidase. Mitochondrial COX is expressed as μg atoms O/min × mg protein, homogenate COX is expressed as μg atoms O/min × g tissue, mitochondrial proteins and yield are expressed as mg/g tissue. $^{*}P < 0.05$ compared to controls (unpaired, two-tailed Student's *t* test)

As for body composition, fructose-fed rats exhibited higher body energy and lipid content, compared to controls, despite the similar ME intake during the whole period, a result similar to our previous ones obtained in rats fed high-fat diet [5, 26]. In addition, fructose feeding did not alter body protein content, indicating that high-fat diets are more deleterious, since they are also responsible for an impairment in lean tissue maintenance [26], although the protein content of the high-fat, the low-fat and the fructose-rich diet was the same.

Table 4 Lipid peroxidation, aconitase and superoxide dismutase specific activity in liver mitochondria from rats fed a high-fructose or control diet for 8 weeks

	Control	Fructose
Lipid peroxidation (nmol of TBARS/mg protein)	0.79 ± 0.05	0.98 ± 0.05*
Active aconitase (mU/mg protein)	14.3 ± 0.6	12.1 ± 0.7*
Total aconitase (mU/mg protein)	33.0 ± 1.8	34.4 ± 1.8
Active aconitase/total aconitase	0.44 ± 0.01	0.37 ± 0.02*
SOD (U/mg protein)	29.6 ± 0.9	24.3 ± 1.4*

Values are the means ± SEM of 6 different experiments

TBARS thiobarbituric acid reactive substances, *SOD* superoxide dismutase

* $P < 0.05$ compared to controls (two-tailed unpaired Student's *t* test)

Fructose-fed rats also exhibited a significant increase in net de novo lipogenesis, which is induced early by fructose feeding, being evident since from the second week, and is maintained throughout the experimental period. Therefore, it appears that the substitution of complex carbohydrates with the simple sugar fructose makes the low-fat diet more lipogenic. In addition, the stimulation of net de novo lipogenesis appears to be one of the main causes leading to excess lipid accumulation in fructose-fed rats. An increased de novo lipogenesis has also been found in rats and humans after long-term fructose feeding [27–29], and therefore, our animal model appears to be useful in studying the metabolic effects of fructose. In addition, the increased de novo lipogenesis, that is a very costly process, could partly explain the high thermic effect of fructose [30].

Another important result of this study is that fructose-fed rats exhibited significantly higher plasma NEFA and hepatic lipid content. Therefore, a low-fat, fructose-rich diet is able to induce metabolic effects similar to those found following high fat feeding [5, 26]. However, in high-fat-fed rats, the increase in hepatic lipid content (+77 %) was higher than that found in body lipid content (+45 %) [5], while in fructose-fed rats, the increase in hepatic lipid content (+13 %) is similar to that found in body lipids (+20 %). Our present data are similar to those previously obtained in rats and humans [31–33]. Hepatic tissue from fructose-fed rats also exhibited higher glycogen content, which could be due to increased gluconeogenesis. Accordingly, the higher HOMA index here found in fructose-fed rats may be indicative of resistance to insulin, already found by others [27, 31], with a following inability to suppress gluconeogenesis. Similar pattern of altered glycogen storage has been found already after 2 weeks of fructose feeding [29].

Liver is the main tissue involved in fructose handling and de novo lipogenesis [34], and therefore, we were also interested in investigating its role in the metabolic response to a fructose-rich diet. We firstly assessed the activity of

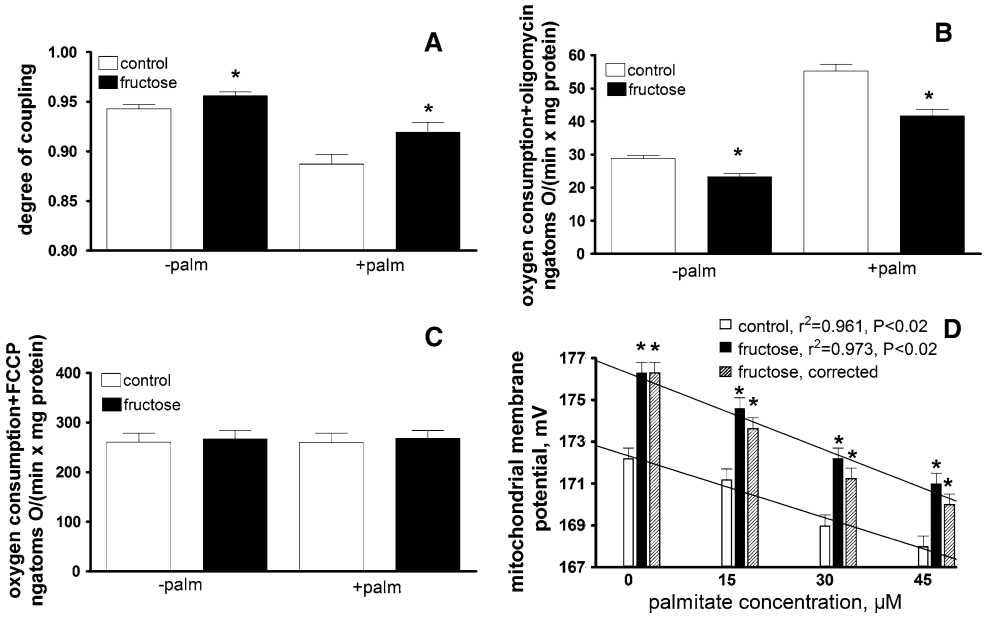

Fig. 3 Degree of coupling values calculated from oxygen consumption in the presence of oligomycin and uncoupled by FCCP (**a**), oxygen consumption in the presence of oligomycin (**b**) or uncoupled by FCCP (**c**), and membrane potential in state 4 conditions (**d**) in the absence and in the presence of palmitate in hepatic mitochondria from fructose-fed and control rats. In **d**, fructose corrected *bars* represent mitochondrial membrane potential values, which were calculated by interpolation of linear regression line of fructose-fed rats considering a 30 % increase in cellular fatty acid concentrations. Results are the means ± SEM of six different experiments. *$P < 0.05$ compared to controls (two-way ANOVA for main effects and interaction followed by Bonferroni post-test). Linear regression *lines* in **d** have significantly different intercept values ($P < 0.0001$)

two rate-limiting enzymes in the hepatic pathway of de novo lipogenesis, FAS and SCD-1, and we found that both activities were significantly increased by fructose feeding. This increase could be due to the hyperinsulinaemic condition of fructose-fed rats, since insulin is able to stimulate the pathway of de novo lipogenesis, even in conditions of hepatic insulin resistance [35, 36]. The increased hepatic de novo lipogenesis could contribute to higher body lipid deposition found in response to increased fructose intake, through secretion of neosynthesised lipids into the bloodstream and their deposition in the adipose tissue, and it could also explain ectopic lipid deposition in the liver.

We also assessed whether mitochondrial alterations could be evident in rats fed a fructose-rich diet, since they are the main cellular site involved in metabolic energy conversion. Firstly, we measured mitochondrial respiratory capacity in homogenates and isolated mitochondria. Mitochondrial respiratory capacity measured in the homogenate and expressed per g wet liver reflects the product of mitochondrial protein mass and specific activity of the respiratory enzymes, while measurements made in isolated mitochondria and expressed per mg of mitochondrial protein only reflect enzyme specific activity. Therefore, changes in enzyme activity will be detectable both in homogenates and in isolated organelles, while changes in mitochondrial mass will only be detectable in homogenates measurements. As a consequence, results obtained in fructose-fed rats, showing increased respiratory capacity in homogenates but not in isolated organelles, are indicative of an increased whole-tissue respiratory capacity only due to enhanced mitochondrial mass here found. Our present result is in agreement with Nagai et al. [37], who found a significant increase in the expression of the mitochondrial-encoded gene ATPase-6 and in mtDNA copy number in livers from rats fed a high-fructose diet. It can be hypothesised that the increased hepatic mitochondrial mass support increased ATP needs for de novo lipogenesis and gluconeogenesis. In addition, increased mitochondrial mass implies increased flux through pyruvate carboxylase and pyruvate dehydrogenase (PDH), which generate substrates for gluconeogenesis and de novo lipogenesis, respectively. Accordingly, it has been previously found a significant increase in whole-tissue hepatic PDH in rats fed a high-fructose diet [38].

The efficiency of oxidative phosphorylation depends on the degree of coupling between oxygen consumption and ATP synthesis. The degree of coupling can vary according to the metabolic needs of the cell and is regulated by the amount of cellular unbound fatty acids that can act as natural uncouplers of oxidative phosphorylation [39]. Our present results indicate that the increased ATP needed for biosynthetic pathways is obtained at a lower cost, since hepatic mitochondria display increased degree of coupling and are less responsive to the uncoupling effect of fatty acids, also after taking into account a possible increase in cellular fatty acid concentrations, dictated by higher plasma NEFA here found. Higher coupling efficiency implies lower fuel burning, which could partly explain the higher body lipids found in fructose-fed rats. Another unwanted consequence of the increased degree of coupling is higher free radical production, and in fact, hepatic mitochondria showed signs of oxidative damage, both in the lipid and in the protein component, together with decreased activity of SOD, one of the enzymatic components of the antioxidant defence system. The increased oxidative stress of hepatic mitochondria from fructose-fed rats could also be one of the causes leading to the onset of insulin resistance in this tissue [40]. Accordingly, the flavonoid naringenin has been found to prevent oxidative stress and reduce insulin resistance in the liver of fructose-fed rats [41]. It should be noted that higher hepatic mitochondrial efficiency and oxidative damage have also been found in rats fed high-fat diet [5], again indicating similar effects of fructose-rich or high-fat diet.

In conclusion, liver mitochondrial compartment appears to be highly affected by fructose feeding. Increased mitochondrial efficiency allows liver cells to burn less substrates to produce ATP for de novo lipogenesis, gluconogenesis and other energy needs. In addition, increased lipogenesis gives rise to whole body and ectopic lipid deposition, and higher mitochondrial coupling causes oxidative stress. The two harmful consequences could be at the basis of the onset of non-alcoholic steatoheaptitis frequently found associated with obesity induced not only by high-fat but also by high-fructose diets [42, 43].

Acknowledgments This work was supported by a grant from University "Federico II" of Naples. The authors thank Dr. Emilia De Santis for skilful management of animal house.

Conflict of interest The authors declare no conflict of interest.

References

1. Bray GA (2010) Soft drink consumption and obesity: it is all about fructose. Curr Opin Lipidol 21:51–57
2. Tappy L, Lê KA (2010) Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev 90:23–46
3. Samuel VT (2011) Fructose induced lipogenesis: from sugar to fat to insulin resistance. Trends Endocrinol Metab 22:60–65
4. Brown CM, Dulloo AG, Montani JP (2008) Sugary drinks in the pathogenesis of obesity and cardiovascular diseases. Int J Obes 32:S28–S34
5. Crescenzo R, Bianco F, Falcone I, Prisco M, Liverini G, Iossa S (2008) Alterations in hepatic mitochondrial compartment in a model of obesity and insulin resistance. Obesity 16(5):958–964
6. Serviddio G, Bellanti F, Vendemiale G, Altomare E (2011) Mitochondrial dysfunction in nonalcoholic steatohepatitis. Expert Rev Gastroenterol Hepatol 5(2):233–244

7. Patti ME, Corvera S (2010) The role of mitochondria in the pathogenesis of type 2 diabetes. Endocr Rev 31(3):364–395
8. Johannsen DL, Ravussin E (2009) The role of mitochondria in health and disease. Curr Opin Pharmacol 9(6):780–786
9. Elia M, Livesey G (1988) Theory and validity of indirect calorimetry during net lipid synthesis. Am J Clin Nutr 47:591–607
10. Kovacs EMR, Westerterp-Plantenga MS (2006) Effect of (-)-hydroxycitrate on net fat synthesis as de novo lipogenesis. Physiol Behav 88:371–381
11. Even PC, Mokhtarian A, Pele A (1994) Practical aspects of indirect calorimetry in laboratory animals. Neurosci Biobehav Rev 18:435–447
12. Cacho J, Sevillano J, de Castro J, Herrera E, Ramos MP (2008) Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. Am J Physiol 295:E1269–E1276
13. Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–510
14. Dulloo AG, Girardier L (1992) Influence of dietary composition on energy expenditure during recovery of body weight in the rat: implications for catch-up growth and obesity relapse. Metab 41:1336–1342
15. Armsby HP (1917) The nutrition of farm animals. The Macmillan Company, New York
16. Roehrig KL, Allred JB (1974) Direct enzymatic procedure for the determination of liver glycogen. Anal Biochem 58:414–421
17. Iossa S, Lionetti L, Mollica MP, Crescenzo R, Botta M, Barletta A, Liverini G (2003) Effect of high-fat feeding on metabolic efficiency and mitochondrial oxidative capacity in adult rats. Br J Nutr 90:953–960
18. Strittmatter P, Spatz L, Corcoran D, Rogers MJ, Setlow B, Redline R (1974) Purification and properties of rat liver microsomal stearyl coenzyme A desaturase. Proc Natl Acad Sci 71:4565–4569
19. Penicaud L, Ferre P, Assimacopoulos-Jeannet F, Perdereau D, Leturque A, Jeanrenaud B, Picon L, Girard J (1991) Increased gene expression of lipogenic enzymes and glucose transporter in white adipose tissue of suckling and weaned obese Zucker rats. Biochem J 279:303–308
20. Cairns CB, Walther J, Harken AH, Banerjee A (1998) Mitochondrial oxidative phosphorylation efficiencies reflect physiological organ roles. Am J Physiol 274:R1376–R1383
21. Crescenzo R, Bianco F, Falcone I, Prisco M, Dulloo AG, Liverini G, Iossa S (2010) Hepatic mitochondrial energetics during catch-up fat after caloric restriction. Metab 59:1221–1230
22. Barré H, Bailly L, Rouanet JL (1997) Increased oxidative capacity in skeletal muscle from cold-acclimated ducklings: a comparison with rats. Comput Biochem Physiol 88B:5119–5522
23. Fernandes MA, Custódio JB, Santos MS, Moreno AJ, Vicente JA (2006) Tetrandrine concentrations not affecting oxidative phosphorylation protect rat liver mitochondria from oxidative stress. Mitochondrion 6:176–185
24. Gardner PR (2002) Aconitase: sensitive target and measure of superoxide. Meth Enzymol 349:9–16
25. Flohè L, Otting F (1974) Superoxide dismutase assay. Meth Enzymol 105:93–104
26. Lionetti L, Mollica MP, Crescenzo R, D'Andrea E, Ferraro M, Bianco F, Liverini G, Iossa S (2007) Skeletal muscle subsarcolemmal mitochondrial dysfunction in high-fat fed rats exhibiting impaired glucose homeostasis. Int J Obes 31:1596–1604
27. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, Hatcher B, Cox CL, Dyachenko A, Zhang W, McGahan JP, Seibert A, Krauss RM, Chiu S, Schaefer EJ, Ai M, Otokozawa S, Nakajima K, Nakano T, Beysen C, Hellerstein MK, Berglund L, Havel PJ (2009) Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 119(5):1322–1334
28. Cox CL, Stanhope KL, Schwarz JM, Graham JL, Hatcher B, Griffen SC, Bremer AA, Berglund L, McGahan JP, Havel PJ, Keim NL (2011) Consumption of fructose-sweetened beverages for 10 weeks reduces net fat oxidation and energy expenditure in overweight/obese men and women. Eur J Clin Nutr. doi:10.1038/ejcn.2011.159
29. Koo HY, Wallig MA, Chung BH, Nara TY, Simon Cho BH, Nakamura MT (2008) Dietary fructose induces a wide range of genes with distinct shift in carbohydrate and lipid metabolism in fed and fasted rat liver. Biochim Biophys Acta 1782:341–348
30. Tappy L, Randin JP, Felber JP, Chiolero R, Simonson DC, Jequier E, DeFronzo RA (1986) Comparison of thermogenic effect of fructose and glucose in normal humans. Am J Physiol 250(6 Pt 1):E718–E724
31. Pagliassotti MJ, Prach PA, Koppenhafer TA et al (1996) Changes in insulin action, triglycerides, and lipid composition during sucrose feeding in rats. Am J Physiol Regul Integr Comp Physiol 271:R1319–R1326
32. Sobrecases H, Lê KA, Bortolotti M, Schneiter P, Ith M, Kreis R, Boesch C, Tappy L (2010) Effects of short-term overfeeding with fructose, fat and fructose plus fat on plasma and hepatic lipids in healthy men. Diabetes Metab 36(3):244–246
33. Ngo Sock ET, Lê KA, Ith M, Kreis R, Boesch C, Tappy L (2010) Effects of a short-term overfeeding with fructose or glucose in healthy young males. Br J Nutr 103(7):939–943 (Epub 2009 Nov 24)
34. Hellerstein MK, Schwarz JM, Neese RA (1996) Regulation of hepatic de novo lipogenesis in humans. Annu Rev Nutr 16:523–557
35. Brown MS, Goldsein JL (2008) Selective versus total insulin resistance. Cell Metab 7:95–96
36. Choi SH, Ginsberg HN (2011) Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. Trends Endocrinol Metab 22:353–363
37. Nagai Y, Nishio Y, Nakamura T, Maegawa H, Kikkawa R, Kashiwagi A (2002) Amelioration of high fructose-induced metabolic derangements by activation of PPARα. Am J Physiol 282:E1180–E1190
38. Park OJ, Cesar D, Faix D, Wu K, Shackleton CH, Hellerstein MK (1992) Mechanisms of fructose-induced hypertriglyceridaemia in the rat. Activation of hepatic pyruvate dehydrogenase through inhibition of pyruvate dehydrogenase kinase. Biochem J 282:753–757
39. Harper ME, Green K, Brand MD (2008) The efficiency of cellular energy transduction and its implications for obesity. Ann Rev Nutr 28:13–33
40. Rains JL, Jain SK (2011) Oxidative stress, insulin signaling, and diabetes. Free Radic Biol Med 50(5):567–575
41. Kannappan S, Palanisamy N, Anuradha CV (2010) Suppression of hepatic oxidative events and regulation of eNOS expression in the liver by naringenin in fructose-administered rats. Eur J Pharmacol 645(1–3):177–184
42. Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH (2010) The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. Nat Rev Gastroenterol Hepatol 7(5):251–264
43. Yki-Järvinen H (2010) Nutritional modulation of nonalcoholic fatty liver disease and insulin resistance: human data. Curr Opin Clin Nutr Metab Care 13(6):709–714