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Short-term consumption of a high-sucrose diet has a pro-oxidant effect in rats

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The underlying mechanisms for the detrimental consequences of a high-fructose diet in animal models are not clear. However, the possibility exists that fructose feeding facilitates oxidative damage. Thus, the aim of the present study was to assess, in weaning rats, the effect of a high-sucrose diet *v.* starch diet for 2 weeks on oxidative stress variables. Plasma lipid levels were measured and lipid peroxidation was evaluated by urine and plasma thiobarbituric acid-reactive substances (TBARS). The susceptibilities of several tissues to peroxidation were determined in tissue homogenates after *in vitro* lipid peroxidation. Antioxidant defence variables were evaluated by measuring plasma and heart vitamin E levels, and heart superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities. Higher plasma triacylglycerol ($P<0{\cdot}01$) and TBARS ($P<0{\cdot}01$) levels were found in rats fed the sucrose diet as compared with the starch-fed group, whereas plasma α-tocopherol levels were significantly decreased in the sucrose-fed group compared with the starch-fed group ($P<0{\cdot}01$). Higher urine TBARS ($P<0{\cdot}01$) were found in the sucrose-fed group compared with the starch-fed group, suggesting increased production of these substances from lipid peroxidation *in vivo*. Higher susceptibility to peroxidation in heart, thymus and pancreas was also found in the sucrose-fed group *v.* the starch-fed group. No statistical differences were observed for liver TBARS level between the two groups. Heart SOD activity was significantly decreased ($P<0{\cdot}001$) in the sucrose-fed group compared with the starch-fed group, whereas heart vitamin E level and GPX activity were not different between the groups. However, the *in vitro* generation of superoxide radical in heart homogenate, measured by electron spin resonance detection and spin trapping, was not increased in the sucrose-fed group compared with starch-fed rats. Altogether, the results indicate that a short-term consumption of a high-sucrose diet negatively affects the balance of free radical production and antioxidant defence in rats, leading to increased lipid susceptibility to peroxidation.

High-sucrose diet: Oxidative stress: Free radicals: Rats

D-Fructose is a sugar that exists in foods as a simple sugar and as a component of the disaccharide sucrose, consisting of one molecule of glucose and one of fructose. Because of the use of high-fructose corn sweeteners and of sucrose in manufactured foods, the dietary consumption of fructose has increased several-fold from that present in natural foods (Henry *et al.* 1991). Although there is little evidence that modest amounts of fructose have detrimental effects on carbohydrate and lipid metabolism, larger doses of fructose have been associated with numerous metabolic abnormalities in human subjects and laboratory animals, suggesting that high-fructose consumption induces adverse effects on health (Hallfrisch, 1990; Henry *et al.* 1991). High-sucrose and high-fructose diets were used in animal models to induce the metabolic changes observed in syndrome X, a disorder in which insulin resistance, hypertension, dyslipidaemia and high incidence of cardiovascular diseases are described (Reaven, 1988). The underlying mechanisms for the detrimental consequences of a high-fructose diet in animal models are not clear. However, the possibility exists that fructose feeding facilitates oxidative damage (McDonald, 1995). The hypothesis is supported by previous findings showing that fructose has a deleterious effect both when antioxidant defences are

Abbreviations: DMSO, dimethylsulfoxide; GPX, glutathione peroxidase; PBN, phenyl *N-tert*-butylnitrone; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances.

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decreased or when free-radical production is increased. It is well known that inadequate antioxidant protection is a consequence of Cu deficiency due to a reduction of superoxide dismutase (SOD) activity and increased lipid peroxidation is a contributing factor in the pathophysiology of low-Cu status (Rayssiguier *et al.* 1993). In Cu-deficient rats, the consumption of sucrose or fructose as the carbohydrate source causes severe pathology and mortality. In contrast, the consumption of starch does not produce such pathology and Cu-deficient animals survive (Fields *et al.* 1984). Increased oxidative stress occurs in Mg-deficient rats due to inflammation (Malpuech-Brugère *et al.* 2000) and sucrose has been shown to be an aggravating factor as compared with starch (Rayssiguier *et al.* 1981). The role of oxidative stress has also been suggested since a free-radical scavenger such as vitamin E improves insulin sensivity in high-fructose-fed rats (Faure *et al.*1997). Although oxidative stress has been suggested as one mechanism for detrimental effects of fructose (McDonald, 1995), corroborating measures of lipid peroxidation are lacking. Altogether in these experiments, the possibility exists that a high-fructose diet has a pro-oxidant effect in rats and that the detrimental effect of a high-sucrose diet may be attributed to its content of fructose. Thus, the aim of the present study was to assess in weaning rats the effect of a high-sucrose diet *v.* starch diet on oxidative stress variables. The consequence of sucrose feeding in rats was assessed by measuring several variables related to oxidative stress in blood, urine and tissues.

Materials and methods

Experimental design

Weaning male Wistar rats (IFFA-CREDO, L'Arbresle, France), 3 weeks old, weighing 61 (SEM 2) g were randomly divided into starch or sucrose groups (sixteen per group). The Institution's guidelines for the care and use of laboratory animals were followed. Rats were housed in wired-bottomed cages in a temperature-controlled room (22°C) with a 12 h light–dark cycle and they were fed the appropriate diets for 2 weeks. Diet and distilled water were provided *ad libitum*. The synthetic diets contained (g/kg): casein 200, starch or sucrose 650, corn oil 50, alphacel 50, DL-methionine 3, choline bitartrate 2, AIN-76 mineral mix 35, AIN-76 A vitamin mix 10 (ICN Biomedicals, Orsay, France).

Sample collections

Eight rats from each group were held individually in stainless-steel metabolic cages and had access to water and food *ad libitum* for 4 d before killing. The urine samples were collected (for 24 h before killing) into 50 ml graduated tubes attached to urine collection funnels with screens to prevent contamination from faeces. Urine volumes were accurately measured and the samples were centrifuged and stored at −80°C until analysis. Non-fasted rats were weighed, anaesthetised with sodium pentobarbital (40 mg/kg body weight, intraperitoneally) and killed. Blood was collected from the abdominal aorta into heparinised tubes. Plasma samples obtained after low-speed centrifugation (2000 ***g***, 15 min) were stored at −80°C for biochemical analysis. The heart, liver, pancreas and thymus were rapidly removed, washed in ice-cold saline (9 g NaCl/l), placed in liquid N_2 and stored at −80°C before performing the lipid peroxidation assay. The remaining rats (*n* 8 per group) from each group were used for the spin trapping experiment.

Plasma and urine analysis

Plasma thiobarbituric acid-reactive substances (TBARS) levels were determined by spectrofluorometry (LS 5; Perkin Elmer, Norwalk, CT, USA) as previously described (Rayssiguier *et al.* 1993). The levels of TBARS in urine samples were measured as previously described (Lee *et al.* 1992) and calculated on the basis of 24 h urine volume. Plasma vitamin E was assayed by reversed-phase HPLC (Kontron serie 400; Kontron, St Quentin en Yvelines, France) using a hexane extract. Briefly, α-tocopherol acetate (Sigma, Saint Quentin Fallavier, France) was added to samples as an internal standard, then they were extracted twice with hexane, after ethanol precipitation of the proteins. This extract was evaporated to dryness under N_2, dissolved in ethanol–methylene chloride (65:35, v/v) and injected onto a C_{18} column (Interchim, Montluçon, France) (Nucleosil; 250 mm long, i.d. 46 mm, 5 μm particles). Pure methanol, at a flow rate of 2 ml/min eluted α-tocopherol in 5·0 min and tocopherol acetate in 6·3 min. The compounds were detected by u.v. (292 nm) spectrometry then quantified by internal and external calibration using daily-controlled standard solutions. Triacylglycerol (Biotrol, Paris, France) and cholesterol (Biomerieux, Charbonnière les Bains, France) were determined in plasma by enzymatic procedures. Cu and Zn contents in plasma were determined by flame atomic absorption spectrometry (Perkin-Elmer series 800; Perkin Elmer).

Tissue susceptibility to peroxidation

The susceptibilities of heart, liver, thymus and pancreas to peroxidation were determined in tissue homogenates after lipid peroxidation was induced with 2 μM-$FeSO_4$–50 μM-ascorbate for 30 min in a water bath at 37°C, using a standard of 1,1,3,3-tetraethoxypropane, as previously described (Rayssiguier *et al.* 1993).

Heart superoxide dismutase, glutathione peroxidase activities and vitamin E level

Heart homogenates were used for SOD and glutathione peroxidase (GPX) determination. Tissue SOD activity was determined using Ransod kit from Randox (Randox Laboratories, Crumlin, N. Ireland, UK). Tissue GPX activity was determined by the modified method of Paglia & Valentine (1967) using *tert*-butyl hydroperoxide as substrate. The results were expressed as U/mg protein for both SOD and GPX activities. Protein content was determined by bicinchoninic acid protein assay kit (Pierce, Rockford, IL, USA) using bovine serum albumin

as the standard. Tissue vitamin E level was determined with the same procedure as described for plasma vitamin E determination, after heart homogenisation in Triton 1X.

Spin trapping experiment

To investigate free radical production, heart homogenates were incubated at 37°C with phenyl *N-tert*-butylnitrone (PBN) as the spin trap in the presence of dimethylsulfoxide (DMSO). Hearts were homogenised in 10 ml 20 mM-HEPES–150 mM-KCl buffer (pH 7·2)/g, and then centrifuged at 10 000 ***g*** for 7 min. To 150 μl homogenate was added 25 μl 2 M-PBN in DMSO, leading to a final concentration of 333 mM-PBN. Buffer (25 μl) and 6·47 M-$FeSO_4$ (5 μl) were added at room temperature and used immediately as previously described (Rock *et al.* 1995). Three capillaries of 20 μl were filled with the sample and transferred into a quartz tube. The electron spin resonance spectra was recorded at room temperature using an electron spin resonance spectrometer (Brucker ECS 106, Brucker, Karlsruhe, Germany) operating at 9·79 GHz with the following conditions: microwave power 20 mW; modulation frequency 10 KHz; modulation amplitude $1{\cdot}016 \times 10^{-4}$ T; time constant 61·92 ms; receiver gain 5×10^5. The three line spectrum obtained with the samples was subtracted from a basal spectrum obtained with capillaries containing heart homogenate without $FeSO_4$.

Statistical analysis

Statistical analysis was performed using GraphPad InStat (GraphPad Inc., San Diego, CA, USA) software package. Results were expressed as mean values with their standard errors. The statistical significance of differences between means were assessed by Student's *t* test. Differences were considered statistically significant at $P<0{\cdot}05$

Results

The sucrose-enriched diet did not affect the normal growth of the rats. The mean body weight was 156 (SEM 2) g for the starch group and 154 (SEM 3) g for the sucrose group respectively. Plasma triacylglycerol levels were significantly greater in the sucrose group compared with the starch group ($P<0{\cdot}01$) whereas total plasma cholesterol levels were not significantly different between the two experimental groups. Plasma α-tocopherol levels were significantly lower in the sucrose group compared with the starch group ($P<0{\cdot}01$). The α-tocopherol:triacylglycerol ratio was significantly decreased in the sucrose group as compared with the control group ($P<0{\cdot}001$) (Table 1).

Higher plasma and urine TBARS ($P<0{\cdot}01$) were found in the sucrose group compared with the starch group (Table 2). After exposure of tissue homogenates to Fe-induced lipid peroxidation, TBARS were significantly higher in heart ($P<0{\cdot}01$), thymus ($P<0{\cdot}001$) and pancreas ($P<0{\cdot}01$) from the sucrose group compared with the starch group (Table 3). No statistical differences were observed for liver TBARS levels between the two groups.

The vitamin E levels in the heart homogenates were not significantly different between the starch and sucrose groups. Heart SOD activity was significantly decreased ($P<0{\cdot}001$) in the sucrose group compared with the starch group (Table 4), whereas heart GPX level was not different between the groups. Plasma Cu was significantly lower ($P<0{\cdot}001$) and plasma Zn higher ($P<0{\cdot}01$) in the sucrose group than in the starch group.

The characteristic electron spin resonance spectra obtained from the incubation of heart homogenates with PBN in the presence of DMSO are shown in Fig. 1. The hyperfine coupling constants for this signal ($a^N = 16{\cdot}34 \times 10^{-4}$ (SEM $0{\cdot}03 \times 10^{-4}$) T and ($a^H = 3{\cdot}61 \times 10^{-4}$ (SEM $0{\cdot}02 \times 10^{-4}$) T are close to those determined for the PBN·CH_3 adduct detected under similar conditions (Burkitt & Mason, 1991). When heart homogenates were incubated with 200 μM-$FeSO_4$, higher signal intensities were observed. We used these conditions to determine the kinetics of the reaction by following the intensity of the first line of the second doublet (results not shown). After 60 min incubation, for which a higher signal intensity was reached, we observed no difference in the amount of the spin adducts produced in homogenates from sucrose *v.* starch-fed rats.

Table 1. Plasma triacylglycerol, cholesterol and vitamin E concentrations and plasma vitamin E:triacylglycerol ratios in rats consuming a starch- or sucrose-based diet†

(Mean values with their standard errors for eight rats per group)

Diet...	Starch-based		Sucrose-based	
	Mean	SEM	Mean	SEM
Triacylglycerol (mmol/l)	0·73	0·12	1·63**	0·20
Cholesterol (mmol/l)	2·04	0·07	1·84	0·08
Vitamin E (μg/ml)	9·92	0·71	7·06**	0·62
Vitamin E:triacylglycerol (μg/mol)	8·49	0·57	3·95***	0·78

† For details of diets and procedures, see pp. 338–339.
Mean values were significantly different from those of the starch-based group: **$P<0{\cdot}01$, ***$P<0{\cdot}001$.

Discussion

The level of the peroxidation marker (TBARS) significantly increased in plasma from rats fed the sucrose diet as compared with the starch group. In addition, sucrose feeding is accompanied by hypertriacylglycerolaemia and

Table 2. Plasma and urine thiobarbituric acid-reactive substance values in rats consuming a starch- or sucrose-based diet†

(Mean values with their standard errors eight rats per group)

Diet...	Starch-based		Sucrose-based	
	Mean	SEM	Mean	SEM
Plasma TBARS (nmol/ml)	1·97	0·04	2·17**	0·05
Urine TBARS (nmol/24 h)	0·57	0·10	1·29**	0·20

TBARS, thiobarbituric acid-reactive substances.
† For details of diets and procedures, see pp. 338–339.
Mean values were significantly different from those of the starch-based groups **$P<0{\cdot}01$.

Table 3. Susceptibility of tissues to peroxidation in rats consuming a starch- or sucrose-based diet†

(Mean values with their standard errors for eight rats per group)

Diet...	Starch-based		Sucrose-based	
	Mean	SEM	Mean	SEM
Heart TBARS (nmol/g wet wt)‡	94	10	152**	11
Liver TBARS (nmol/g wet wt)‡	117	11	142	20
Thymus TBARS (nmol/g wet wt)‡	40	8	104***	12
Pancreas TBARS (nmol/g wet wt)‡	28	4	53**	7

TBARS, thiobarbituric acid-reactive substances.
† For details of diets and procedures, see pp. 338–339.
‡ TBARS were measured in tissue homogenates after lipid peroxidation induced with $FeSO_4$–ascorbate for 30 min at 37°C (for details, see p. 339).
Mean values were significantly different from those of the starch-based group: $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.

Table 4. Superoxide dismutase and glutathione peroxidase activities and vitamin E levels in heart, and plasma copper and zinc levels in rats consuming a starch- or sucrose-based diet†

(Mean values with their standard errors for eight rats per group)

Diet...	Starch-based		Sucrose-based	
	Mean	SEM	Mean	SEM
Vitamin E (μg/g wet wt)	28·72	0·91	26·45	0·97
SOD (U/mg protein)	39·89	2·08	29·75***	0·91
GPX (U/mg protein)	52·10	4·02	55·43	5·60
Cu (mg/l)	0·74	0·04	0·23***	0·07
Zn (mg/l)	1·21	0·01	1·42**	0·05

SOD, superoxide dismutase; GPX, glutathione peroxidase.
† For details of diets and procedures, see pp. 338–339.
Mean values were significantly different from those of the starch-based group: $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.

plasma contains many substances that react in the thiobarbituric assay. Thus the value of malondialdehyde in plasma as an indicator for lipid peroxidation is limited (Rice-Evans *et al.* 1991). However, we also found a significant decrease in vitamin E plasma level. Since vitamin E normally acts as an antioxidant, low vitamin E level probably is the result of increased vitamin E utilisation (Esterbauer *et al.* 1992). Both sucrose and fructose given at abnormally high amount in the diet have been shown to increase plasma triacylglycerol concentration (Vrana & Fabry, 1983; Mamo *et al.* 1991; Frayn & Kingman, 1995). Unlike other fat-soluble vitamins, vitamin E has no specific transport protein, but rather is transported in plasma lipoproteins and tocopherol is secreted in the liver in VLDL and protects lipoproteins by preventing oxidation. When lipoproteins are depleted of antioxidants, unsaturated fatty acids are rapidly oxidised and the vitamin E depletion in sucrose-fed rats may predispose lipoproteins to subsequent oxidative stress (Esterbauer *et al.* 1992). Thus, an increase in triacylglycerol-rich lipoprotein and increased lipoprotein susceptibility to peroxidation are factors that

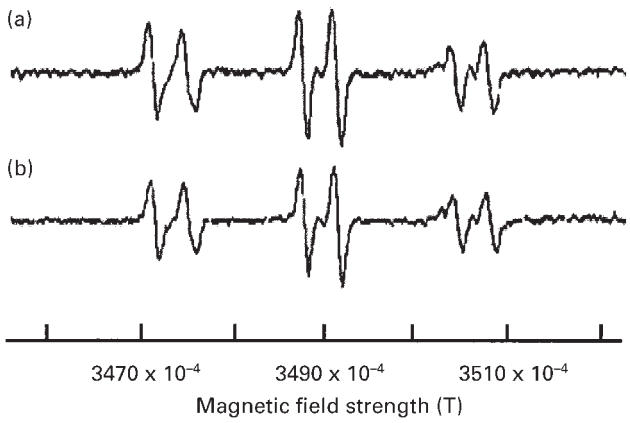

Fig. 1. Electron spin resonance spectra from (a) starch and (b) sucrose rats. Typical electron spin resonance spectra of heart homogenates with the spin-trapping agent phenyl *N-tert*-butylnitrone. The spectra for starch (a) and sucrose (b) rats were obtained at room temperature under the conditions described on p. 339. For details of diets and procedures, see pp. 338–339.

may combine to contribute to an increased risk for cardiovascular diseases (Ross, 1999). Accordingly, compared with starch, fructose feeding results in higher occurrence of aortic atherosclerotic plaque in animal models as shown previously (Kritchevsky *et al.*1980).

Sucrose feeding in rats is accompanied by a significant increase in the urinary excretion of TBARS suggesting increased production of these substances from lipid peroxidation *in vivo*. Several reports have indicated that a positive relationship exists between *in vivo* peroxidation and urinary malondialdehyde levels (Brooks & Klamert, 1968). For instance, vitamin E-deficient rats excrete higher levels of TBARS in urine compared with vitamin E-supplemented rats (Draper *et al.* 1984). This demonstrates that total urinary TBARS level corresponds to the increase *in vivo* lipid peroxidation associated with vitamin E deficiency (Lee *et al.* 1992). Moreover, the present experiment clearly indicates that heart, thymus and pancreas are more susceptible to *in vitro* peroxidation. Unlike other organs, liver susceptibility to peroxidation was not significantly increased in rats fed a high-fructose diet, suggesting a greater protection against oxidative stress in this organ. Comparing diets containing a large percentage of energy provided by sucrose results in a general decline of glucose homeostasis and an increased risk for the development of non-insulin-dependant diabetes mellitus (Reiser, 1982). In general, the development of type 2 diabetes is associated with pancreatic β-cell dysfunction occurring together with insulin resistance. The present experiments suggest that sucrose feeding can cause severe cell injury to β cells as shown by increase susceptibility of pancreas to oxidative damage. Pancreatic β cells have a much lower scavenging capacity than most other tissues and they are unable to adapt their antioxidant enzyme expression in response to chronic oxidative stress (Tiedge *et al.* 1997). Oxidative free radicals may in the first instance stimulate the growth of pancreatic β cells and then, the ongoing increased oxidative stress will progressively destroy pancreatic cells and result in insulin deficiency (Bakker *et al.* 2000). Moreover, there has been growing interest in the effects of antioxidants on insulin activity. α-Tocopherol supplementation improves insulin action in patients with non-insulin-dependant diabetes mellitus (Paolisso *et al.* 1993) and in rats fed high a dosage of

fructose (Faure *et al.* 1997). Of particular significance is the observation that hearts from fructose-fed rats are more susceptible to *in vitro* peroxidation as compared with the starch group. These results emphasise the potential detrimental effect of fructose on vascular risk (Halfrisch, 1990), as shown by hyperlipaemia, decreased lipoprotein protection and cardiovascular susceptibility to free radical-mediated injury. We also found that the high-sucrose diet may affect the balance of oxyradical production and antioxidant defence in the thymus; thus oxidative injury may occur in this tissue involved in host immune response.

The decreased heart SOD activity of rats fed the sucrose diet compared with starch-fed rats is accompanied by a large decrease in blood plasma Cu concentration. The interaction of dietary fructose with Cu has received considerable attention (Fields *et al.* 1984; O'Dell, 1993). The antagonism between Zn and Cu is well known (Van Campen & Scaife, 1967). However, whether the increased plasma Zn concentration observed in rats fed the sucrose diet is the consequence of decreased Cu status is unclear. Finally, the possibility exists that Cu depletion results in reduced activity of the Cu-containing enzyme SOD. However, in previous experiments, the SOD activity of red blood cells was also found to be lower in rats fed a high-fructose diet although the plasma Cu was not different in the starch group (Faure *et al.* 1997) and vitamin E supplementation was accompanied by a normalisation of red blood cell SOD activity. Thus, the hypothesis that the protein could be damaged by oxidative stress has been advanced (Faure *et al.* 1997). Whatever the mechanisms involved, SOD depletion might participate in cardiac vulnerability to oxidative stress since this antioxidant enzyme has a key role in the cell protection against the deleterious effects of the superoxide anion (Halliwell, 1996). GPX is also important in the protection of the cell from oxygen radical toxicity. In contrast to the SOD activity, the GPX activity was not modified in heart homogenates from the sucrose group as compared with the starch group, suggesting that there is no depletion of this antioxidant enzyme or that a feed-back mechanism results in a restoration to normal enzyme level. Moreover, whereas plasma vitamin E level was lower in the sucrose group, heart vitamin E levels were not different between groups.

Heart SOD activity being decreased, the possible consequent decreased dismutation of the superoxide radical $O_2^{-\cdot}$ could result in an increased superoxide radical availability in this organ. Hence, we tested if the *in vitro* generation of the superoxide radical is affected differently in heart homogenates from sucrose- or starch-fed rats. We used electron spin resonance spectroscopy to study the production of free radicals in heart homogenates. As previously shown, in the presence of PBN and DMSO, primary free radical ($O_2^{-\cdot}$; ·OH) rather than lipid-derived secondary radicals (R·, RO·) were generated (Rock *et al.*1995). Surprisingly, electron spin resonance spectra indicate that primary free radical species ($O_2^{-\cdot}$, ·OH) were not increased in the sucrose group compared with starch-fed rats. However, these results from *in vitro* incubation of heart homogenates does not exclude a possible *in vivo* greater primary free radical production induced by a high-fructose consumption.

There are several possible pathways by which a diet rich in sucrose may alter cellular metabolism, which in turn may accelerate oxidative stress. The increased oxidative stress could be due to oxygen free radical production and/or decreased protection by non-enzymatic or enzymatic antioxidants (Halliwell, 1996). Moreover, the susceptibility of tissue to oxidative stress can be dependent on alteration in lipid composition. Another possibility is that sucrose feeding induces accumulation of advanced glycation products and that oxidative degradation of glucose or fructose adducts leads to production of free radicals (Levi & Werman, 1998). Thus, further research is needed to define the interactions among fructose, glycation and oxidative stress more clearly. Which of these possible pathways of free-radical generation occur in response to a high-fructose diet was not addressed in our present study.

In conclusion, the present study presents the evidence that short-term consumption of a high-sucrose diet negatively affects the balance of oxyradical production and antioxidant defence, suggesting that metabolic abnormalities in human subjects and laboratory animals associated with high-fructose consumption can be related to oxidative stress. Special attention should be made to the potential consequences of increased consumption of fructose combined with low dietary intake of antioxidant molecules (vitamins, minerals, phyto-micronutrients, etc.), particularly in relation to the development of cardiovascular diseases, non-insulin-dependant diabetes mellitus and the cluster of abnormalities designated as syndrome X.

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