

The metabolic syndrome of fructose-fed rats: Effects of long-chain polyunsaturated ω 3 and ω 6 fatty acids. VI. Further post-mortem investigations

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Abstract. The present study documents the increases in systolic arterial blood pressure, plasma leptin concentration and kidney proliferating cell nuclear antigen index, as well as the decreases in glutathione reductase, superoxide dismutase and catalase enzymatic activities in the liver, heart, kidney, soleus muscle and visceral adipose tissue homogenates of female rats exposed for 8 weeks to a diet containing 64% (w/w) D-fructose instead of 64% starch. In the fructose-fed rats, the partial substitution of sunflower oil by either safflower oil or salmon oil often opposed the fructose-induced changes in these variables. The present results, thus, extend to these functional, hormonal and enzymatic parameters the knowledge that the dietary supply of long-chain polyunsaturated ω 6 fatty acids, mainly C18:2 ω 6, and long-chain polyunsaturated ω 3 fatty acids opposes the undesirable features of the fructose-induced metabolic syndrome, with salmon oil demonstrating particular efficacy.

Introduction

The preceding article in this series (1) concerned post-mortem measurements of several plasma variables, percentage of glycated hemoglobin, liver glucokinase activity and hepatic content of cholesterol, triglycerides and phospholipids found in control female rats exposed from the 8th week after birth and for the ensuing 8 weeks to a diet containing 64% (w/w) starch and 5% (w/w) sunflower oil (Ssun rats) or to diets containing 64% D-fructose and 5% sunflower oil (Fsun rats) or 3.4% sunflower oil mixed with 1.6% salmon oil (Fsal rats)

or safflower oil (Fsaf rats). Generally, the differences between the Ssun and Fsun rats were attenuated or even eliminated in the Fsaf and the Fsal rats, particularly in the latter. The present report extends comparable observations to a further set of post-mortem investigations dealing with systolic arterial blood pressure, plasma leptin concentrations, the activities of glutathione reductase, superoxide dismutase and catalase in liver, heart, kidney, soleus muscle and visceral adipose tissue, and the kidney proliferating cell nuclear antigen index.

Materials and methods

The four groups of rats (Ssun, Fsun, Fsal and Fsaf) examined in the present study were the same as those defined in the first report in this series (2).

The systolic arterial blood pressure was measured by a plethysmographic procedure at the tail level, the individual results representing the mean of four measurements. The plasma leptin concentration was assessed using a SPI Bio kit (Bertin Pharma, Montigny-le Bretonneux, France). The kidney proliferating cell nuclear antigen index was determined as previously described (3). The activity of glutathione reductase was measured by the procedure recommended by Goldberg and Spooner (4), the results being expressed as μ mol NADPH consumed per min and per mg tissue wet weight. The activity of superoxide dismutase was assayed according to the method described by Elstner *et al* (5) and expressed as units per g protein. The activity of catalase was measured by the method proposed by Aebi (6), the results being indicated as mmol H₂O₂ consumed per min and per g protein.

All results are presented as mean values (\pm SEM) together with the number of individual observations (n). The statistical significance of differences between mean values was assessed using the Student's t-test.

Results

Systolic arterial blood pressure. As illustrated in Fig. 1, the systolic arterial blood pressure remained fairly stable in the Ssun rats, there being no significant correlation between the mean measurements above basal value and the length of the

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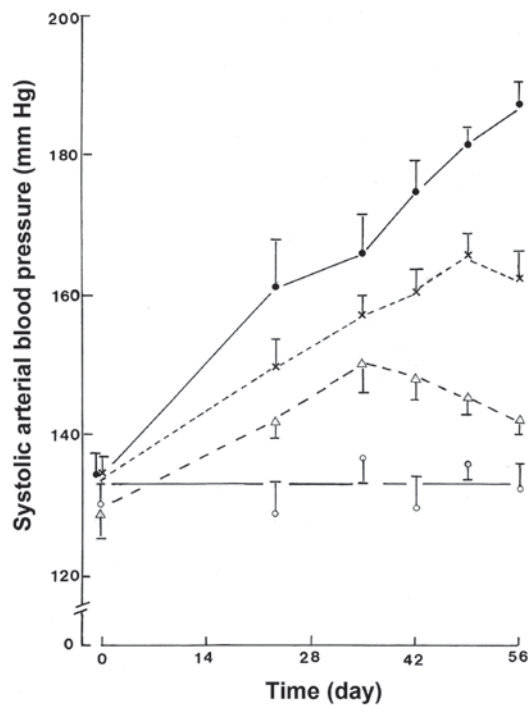


Figure 1. Time course of the changes in systolic arterial blood pressure in Ssun rats (○), Fsun rats (●), Fsal rats (x) and Fsaf rats (△). Mean values (\pm SEM) from 5-6 individual measurements. The lower horizontal line represents the mean value recorded over the entire experimental period for the Ssun rats.

experimental period ($r=+0.435$; $n=6$; $P>0.1$). In the Fsun rats, however, the blood pressure progressively increased during the experimental period ($r=+0.989$; $n=6$; $P<0.001$). This was also the case in the Fsal rats ($r=+0.971$; $n=6$; $P<0.002$). However, covariance analysis indicated that the slope of the regression line was significantly lower ($F=15.56$; $f=1, 8$; $P<0.005$) in the Fsal rats (0.537) than in the Fsun rats (0.919). The increase in blood pressure was even less pronounced in the Fsaf rats. Indeed, in the latter rats, the coefficient of correlation between the mean blood pressure above basal value and the length of the experimental period did not achieve statistical significance ($r=+0.722$; $n=6$; $P<0.11$). Moreover, the slope of the regression line was not significantly different ($F=1.54$; $f=1, 8$; $P<0.25$) in the Fsaf (0.271) and Ssun rats (0.080). Only the elevation of the regression line remained significantly higher in the Fsaf rats than in the Ssun rats ($F=16.42$; $f=1, 9$; $P<0.005$).

The incremental area under the curve, expressed as mmHg.day, provided comparable information, averaging 89.6 ± 2.7 , 1617.0 ± 41.7 , 997.2 ± 23.5 and 756.4 ± 16.2 in the Ssun, Fsun, Fsal and Fsaf rats, respectively ($n=6$ in each case), as illustrated in Fig. 2A. The four mean values were all significantly different from one another ($P<0.001$).

Plasma leptin concentration. The plasma leptin concentration in the Fsun rats (12.85 ± 0.44 ng/ml; $n=6$) was double that ($P<0.001$) in the Ssun rats (6.18 ± 0.29 ng/ml; $n=6$). It did not differ significantly ($P>0.18$) between the Fsun and Fsaf rats (12.20 ± 0.07 ng/ml; $n=6$). In the Fsal rats, however, it averaged 8.24 ± 0.20 ng/ml ($n=5$) and was much lower ($P<0.001$) than

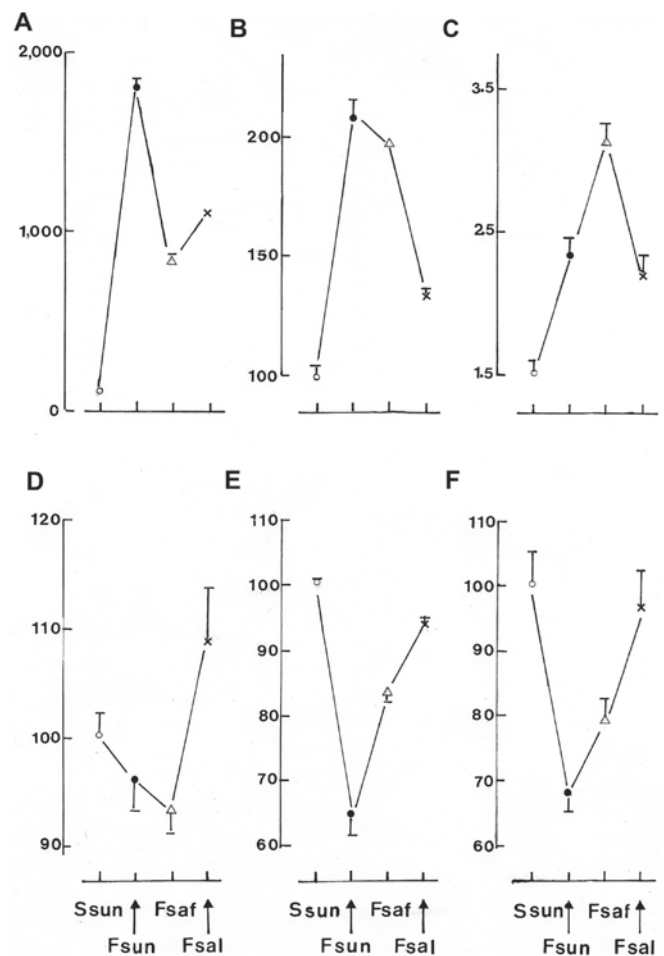


Figure 2. Comparison between Ssun rats (○), Fsun rats (●), Fsaf rats (△) and Fsal rats (x), in terms of systolic arterial blood pressure, plasma leptin concentration, kidney proliferating cell nuclear antigen index, glutathione reductase activity, superoxide dismutase activity and catalase activity. (A) The incremental area in blood pressure over the experimental period expressed as mmHg.day; mean values (\pm SEM) of 5-6 individual observations. (B) The plasma leptin concentration expressed as a percentage of the mean value in Ssun rats; mean value (\pm SEM) of 5-6 individual measurements. (C) The mean values (\pm SEM) for the kidney proliferating cell nuclear antigen index, expressed as percentages, of 100-120 separate determinations. (D) The activity of glutathione reductase in liver, heart, kidney and soleus muscle expressed as percentages of the mean corresponding values in Ssun rats; mean values (\pm SEM) of 20-24 individual measurements. The activity of superoxide dismutase (E) and catalase (F) in liver, heart, kidney, soleus muscle and visceral adipose tissue expressed as percentages of the mean corresponding values in Ssun rats; mean values (\pm SEM) of 25-30 individual measurements.

that in the Fsun rats and somewhat higher ($P<0.001$) than that in the Ssun rats (Fig. 2B).

Enzymatic activities. The results of the two separate assays of glutathione reductase activity performed in this study yielded comparable results. In the heart, which yielded one of the lowest mean values, the coefficient of correlation between the individual measurements made in these two separate assays was 0.880 ($n=23$; $P<0.001$). In general, only minor differences of glutathione reductase activity in the liver, heart, kidney, soleus muscle and visceral adipose tissue homogenates were observed among the four groups of rats (Table I). Nevertheless, the trend was, in most cases, towards lower values in the Fsun

Table I. Glutathione reductase activity.

	Ssun	Fsun	Fsal	Fsaf
Liver	8.39±0.16 ^a	8.01±0.14 ^a	9.68±0.60 ^b	8.42±0.20 ^a
Heart	1.96±0.10 ^a	2.00±0.09 ^a	2.26±0.36 ^b	1.60±0.03 ^a
Kidney	13.44±0.48 ^a	12.25±0.22 ^a	14.16±0.69 ^b	13.32±0.32 ^a
Soleus muscle	1.81±0.14 ^a	1.73±0.27 ^c	1.78±0.09 ^b	1.65±0.10 ^b
Visceral adipose tissue	0.75±0.03 ^a	0.83±0.12 ^a	0.70±0.06 ^b	0.89±0.14 ^a

Mean values (± SEM) are expressed as μ mol of NADPH consumed per min and per mg wet weight. ^an=6; ^bn=5; ^cn=4.

Table II. Superoxide dismutase activity.

	Ssun (n=6)	Fsun (n=6)	Fsal (n=5)	Fsaf (n=6)
Liver	264±1	131±2	281±5	225±6
Heart	84±1	75±1	79±1	77±1
Kidney	146±6	112±3	135±2	133±2
Soleus muscle	245±1	146±1	216±1	203±1
Visceral adipose tissue	265±2	124±1	230±2	179±1

Mean values (± SEM) are expressed as unit per g protein.

Table III. Catalase activity.

	Ssun (n=6)	Fsun (n=6)	Fsal (n=5)	Fsaf (n=6)
Liver	113±15	82±9	116±13	88±2
Heart	118±14	82±5	115±12	104±8
Kidney	194±23	130±10	217±40	162±23
Soleus muscle	115±15	80±8	98±16	83±10
Visceral adipose tissue	63±9	39±4	54±6	46±5

Mean values (± SEM) are expressed as mmol of H₂O₂ consumed per min and per g protein.

and Fsaf rats than in the Ssun or Fsal rats. Indeed, when ignoring the measurements made in adipose tissue, which yielded the mean lowest values among the five sampled organs in each group of rats, the activity of glutathione reductase found in the liver, heart, kidney and soleus muscle, expressed relative to the mean reference value found in the same organ in Fsun rats, averaged in Fsun and Fsal rats 104.0±2.7% (n=44), as distinct (P<0.005) from only 94.6±1.8% (n=45) in the Fsun and Fsaf rats.

The activity of superoxide dismutase was, in all five organs, significantly lower in the Fsun rats than in the Ssun rats, averaging in the Fsun rats 64.4±3.0% (n=30) of the mean corresponding reference values in the Ssun rats (100.0±0.8%; n=30). In the fructose-fed rats, however, it increased (P<0.001) to 83.8±1.8% (n=30) of the same reference value upon partial substitution of sunflower oil by safflower oil, and further increased (P<0.001) to 93.7±1.5% (n=25) upon partial substitution of sunflower oil by salmon oil. The latter percentage was

somewhat lower (P<0.001) than the reference value recorded in the Ssun rats (Table II).

The activity of catalase in the liver, heart, kidney, soleus muscle and visceral adipose tissue was also much lower (P<0.001) in the Fsun rats than in the Ssun rats, the former averaging 68.0±2.6% (n=30) of the mean corresponding reference values found in the latter (100.0±5.4%; n=30). As indicated in Table III, in the fructose-fed rats catalase activity was increased (P<0.025) to 78.8±3.8% (n=30) upon partial substitution of sunflower oil by safflower oil and to 96.5±6.0% (n=25) of the mean corresponding reference values found in Ssun rats upon partial substitution of sunflower oil by salmon oil. The latter percentage was not significantly different (P>0.07) from that in the Ssun rats.

The correlations between the diet-induced changes and the glutathione reductase, superoxide dismutase and catalase activity in the organs under consideration are illustrated in the lower panels of Fig. 2.

Table IV. Kidney proliferating cell nuclear antigen index (%).

	Ssun	Fsun	Fsal	Fsaf
1	1.41 \pm 0.18 ^a	2.18 \pm 0.27 ^a	2.32 \pm 0.20 ^a	2.91 \pm 0.26 ^a
2	1.54 \pm 0.15 ^a	2.82 \pm 0.31 ^a	2.07 \pm 0.28 ^a	3.86 \pm 0.20 ^a
3	1.53 \pm 0.16 ^a	1.31 \pm 0.14 ^a	3.22 \pm 0.49 ^a	4.05 \pm 0.33 ^a
4	1.35 \pm 0.14 ^a	3.24 \pm 0.25 ^a	1.68 \pm 0.21 ^a	2.74 \pm 0.27 ^a
5	1.87 \pm 0.22 ^a	2.03 \pm 0.21 ^a	1.69 \pm 0.20 ^a	2.10 \pm 0.19 ^a
6	1.44 \pm 0.24 ^a	2.54 \pm 0.25 ^a		3.18 \pm 0.29 ^a
Overall mean value	1.53 \pm 0.08 ^b	2.35 \pm 0.11 ^b	2.20 \pm 0.14 ^c	3.14 \pm 0.12 ^b
Mean individual value	1.53 \pm 0.07 ^d	2.35 \pm 0.27 ^d	2.20 \pm 0.28 ^e	3.14 \pm 0.30 ^d

^an=20; ^bn=120; ^cn=100; ^dn=6; ^en=5.

Significant or close-to-significant correlations were observed between the individual values of superoxide dismutase and catalase activity in liver ($r=+0.504$; $n=23$; $P<0.02$), heart ($r=+0.493$; $n=23$; $P<0.03$), kidney ($r=+0.474$; $n=23$; $P<0.03$), soleus muscle ($r=+0.386$; $n=23$; $P<0.07$) and visceral adipose tissue ($r=+0.558$; $n=23$; $P<0.008$). Moreover, when the individual measurements of superoxide dismutase and catalase activity recorded in each group of rats (Ssun, Fsun, Fsal and Fsaf) and in each organ (liver, heart, kidney, soleus muscle and visceral adipose tissue) were expressed relative to the corresponding mean values in order to eliminate any group effect, a close-to-significant positive correlation was still observed ($r=+0.181$; $n=115$; $P<0.06$), indicating parallel changes in these two enzymatic activities at the individual level. Furthermore, such a correlation achieved statistical significance ($r=+0.236$; $n=92$; $P<0.03$) when the results recorded in visceral adipose tissue were excluded. An even higher significance ($r=+0.311$; $n=69$; $P<0.01$) was achieved when considering the data collected in the liver, heart and kidney. Statistical significance ($r=+0.416$; $n=23$; $P<0.001$) was achieved when only the measurements made in the liver were considered.

Kidney proliferating cell nuclear antigen index. A total of 20 kidney fields were examined in each rat. The total number of nuclei examined in each of these fields ranged between 123 in a Fsal rat and 389 in a Ssun rat. Even so, the mean number of nuclei under consideration in the twenty fields did not differ significantly between these two animals ($P>0.36$), averaging 283 ± 13 ($n=20$) in the Fsal rat and 300 ± 13 ($n=20$) in the Ssun rat.

In the Ssun rats, the kidney proliferating cell nuclear antigen index (expressed as a percentage) ranged between 1.35 ± 0.14 ($n=20$) and 1.87 ± 0.22 ($n=20$); the difference between the two mean values failing to achieve statistical significance ($P>0.05$). Pooling together all available measurements, such an index averaged 1.53 ± 0.08 ($n=120$) in the Ssun rats. A similar SEM was reached when considering only the mean values, each derived from 20 determinations; in this case the results from the Ssun rats averaged 1.53 ± 0.07 ($n=6$).

In the Fsun rats, only one animal yielded a mean index within the range of individual values recorded in the Ssun rats

(1.31 ± 0.14 ; $n=20$). All other Fsun rats yielded an index >2.00 . The difference between the Ssun and Fsun rats achieved statistical significance when judged from the mean individual values recorded in each rat ($t=2.90$; $df=10$; $P<0.02$) or from all 120 measurements ($t=6.06$; $df=238$; $P<0.001$).

The situation found in the Fsal rats was comparable to that in the Fsun rats (Table IV). Thus, two Fsal rats yielded mean indices of 1.68 ± 0.21 and 1.69 ± 0.20 which were within the range of values found in the Ssun rats, whilst the highest individual values in Fsal (3.22 ± 0.49 ; $n=20$) and Fsun rats (3.24 ± 0.25 ; $n=20$) were almost identical. The overall mean value derived from the 20 measurements made in each animal also failed to differ significantly ($P>0.38$) in Fsal (2.20 ± 0.14 ; $n=100$) and Fsun rats (2.35 ± 0.11 ; $n=120$). Moreover, the values in the Fsal rats exceeded those in the Ssun rats ($P<0.04$), even when the difference was judged from the mean individual values obtained in the Fsal (2.20 ± 0.28 ; $n=5$) and Ssun (1.53 ± 0.07 ; $n=6$) rats.

Finally, in the Fsaf rats, the index was even higher than in the Fsun rats, such a difference achieving statistical significance ($P<0.001$) when judged from the 120 measurements made in each of these two groups of rats. Furthermore, in the Fsaf rats, all mean individual values were >2.00 and the highest individual value was 4.05 ± 0.33 ($n=20$).

Discussion

The present results reveal that the fructose-induced metabolic syndrome involves increases in the systolic arterial blood pressure, plasma leptin concentration and kidney proliferating cell nuclear antigen index, and decreases in the activities of glutathione reductase, superoxide dismutase and catalase in liver, heart, kidney, soleus muscle and visceral adipose tissue homogenates. In most of these cases, the partial substitution of sunflower oil in the diet of the fructose-fed rats by either safflower or salmon oil provoked a partial or complete restoration of these functional, hormonal and enzymatic variables towards the reference values found in control rats exposed to a diet containing starch instead of D-fructose as carbohydrate. As recently indicated in an extensive review of the relevant literature (7) presented as the background information to the present study, the present findings reinforce the view that the

dietary supply of long-chain polyunsaturated ω 6 fatty acids, especially C18:2 ω 6, and long-chain polyunsaturated ω 3 fatty acids may exert favourable effects in terms of correcting the undesirable features otherwise prevailing in the fructose-induced and possibly other models of metabolic syndrome, with long-chain polyunsaturated ω 3 fatty acids appearing to be particularly effective.

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