

## The metabolic syndrome of $\omega$ 3-depleted rats. II. Body weight, adipose tissue mass and glycemic homeostasis

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**Abstract.** Exposure of 7-week-old normal rats for 3-7 months to a diet deprived of long-chain polyunsaturated  $\omega$ 3 fatty acids was recently reported to induce changes in the fatty acid content and pattern of liver phospholipids and triglycerides similar to those otherwise found in second generation  $\omega$ 3-depleted rats. In the present study, the changes in body weight, parametrial adipose tissue mass, plasma glucose and insulin concentrations and insulin resistance index were investigated in the same control and  $\omega$ 3-depleted rats, which were then given access for 2 to 4-5 weeks to either a flaxseed oil-enriched diet (control and  $\omega$ 3-depleted rats) or a soybean oil-enriched diet (control rats). The body weight failed to differ between control and  $\omega$ 3-depleted rats. The latter rats, however, displayed increases in adipose tissue mass, plasma glucose and insulin concentrations, and insulin resistance index. In the control rats given access to the soybean or flaxseed oil-enriched diet, body weight and adipose tissue mass were little affected, but both the plasma glucose concentration and insulin resistance index decreased. In the  $\omega$ 3-depleted rats given access to the flaxseed oil-enriched diet, both body weight and adipose tissue mass underwent a rapid, pronounced and sustained increase, whilst the plasma glucose concentration and insulin resistance index decreased similarly to those in the control rats. The present design of  $\omega$ 3 fatty acid dietary deprivation thus reproduces the visceral obesity and insulin resistance otherwise observed in second-generation  $\omega$ 3-depleted rats. However, the supply of exogenous  $\omega$ 3 fatty acids to the  $\omega$ 3-depleted rats failed to oppose visceral obesity, possibly as a result of the orexigenic effects of these  $\omega$ 3 fatty acids.

### Introduction

In the first report in this series, it was documented that exposure of 7-week-old normal rats to a sunflower oil-containing diet for 3-7 months is sufficient to provoke changes in the fatty acid content and pattern of both liver phospholipids and triglycerides similar to those previously found in second-generation rats depleted in long-chain polyunsaturated  $\omega$ 3 fatty acids (1-3). Hence, it was proposed that such a procedure represents a simple and realistic animal model to study the consequence of  $\omega$ 3-depletion. Attention was also drawn to the changes in hepatic lipid variables occurring when the  $\omega$ 3-depleted rats were exposed after the 7 months of  $\omega$ 3 dietary deprivation, to the sunflower oil-containing diet enriched with flaxseed oil for a further period of 2 to 4-5 weeks (4).

The present report deals mainly with the changes in body weight, parametrial adipose tissue mass, and both plasma glucose and insulin concentration in the same rats. The measurement of the latter two plasma variables also allowed to assess changes in insulin sensitivity.

### Materials and methods

The 8 groups of 5-6 female rats each were the same as those indicated in our first report in this series (4). Briefly, 4 of these groups included control rats exposed for 3 or 7 months to a diet containing 5% (wt/wt) soybean oil and then given access for 4-5 weeks to the same diet enriched with either another 5% of soybean oil or 5% of flaxseed oil. The other 4 groups consisted of rats exposed for 3 or 7 months to a diet containing 5% sunflower oil and then given access for 2 or 4-5 weeks to the same diet enriched with 5% flaxseed oil. The fatty acid composition of these diets and the modalities of sacrifice, heparinized blood collection and tissue sampling were also described in our prior publication (4). The plasma glucose (5) and insulin (6) concentrations were measured by methods described in the cited references.

In the Tables, the following symbols are used: 3mC and 7mC for the control rats examined 3 and 7 months after the onset of the present experiments, 3 mD and 7 mD for the  $\omega$ 3-depleted rats ( $\omega$ 3D) also examined 3 and 7 months after the start of the experiments, 7mC/4wS and 7mC/4wF for the control rats eventually exposed for 4-5 weeks to either the

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Table I. Body weight and parametrial adipose tissue weight.

Rats	Body weight (g)	Adipose tissue weight (g)	Adipose tissue/body weight (%)
3mC	253±6 (6)	4.50±0.63 (6)	17.6±2.1 (6)
7mC	284±11 (5)	6.62±1.32 (5)	22.8±4.0 (5)
7mC/4wS	296±18 (6)	7.86±1.56 (6)	25.7±3.7 (6)
7mC/4wF	287±11 (6)	6.68±0.89 (6)	22.9±2.3 (6)
3mD	265±12 (6)	5.60±0.75 (6)	21.2±2.6 (6)
7mD	303±15 (6)	10.61±1.09 (6)	34.6±2.3 (6)
7mD/2wF	306±12 (6)	12.93±1.41 (6)	42.0±3.6 (6)
7mD/4wF	344±13 (6)	16.42±1.65 (6)	47.2±3.3 (6)

Table II. Plasma glucose and insulin concentrations.

Rats	Plasma glucose (mM)	Plasma insulin (pM)	Insulin resistance index (mM.pM)
3mC	13.45±0.69 (6)	625±100 (6)	7,863±1,460 (6)
7mC	14.61±1.43 (5)	564±61 (5)	7,862±672 (5)
7mC/4wS	9.68±0.94 (6)	620±57 (4)	5,738±796 (4)
7mC/4wF	8.80±1.30 (6)	545±31 (6)	4,497±841 (6)
3mD	16.09±0.65 (6)	679±44 (6)	10,766±496 (6)
7mD	17.52±2.37 (6)	760±96 (6)	12,316±2,437 (6)
7mD/2wF	14.61±1.68 (6)	769±104 (6)	10,306±2,558 (6)
7mD/4wF	12.92±1.34 (6)	761±61 (6)	9,389±950 (6)

soybean (S) or flaxseed (F) oil-enriched diets, and 7mD/2wF and 7mD/4wF for the  $\omega$ 3D rats eventually exposed for 2 or 4-5 weeks to the flaxseed oil-enriched diet.

All results are presented as means  $\pm$  SE together with either the number of individual determinations (n) or degree of freedom (df). The statistical significance of differences between mean values was assessed using Student's t-test and confirmed by variance analysis with Bonferroni post-test. Geometric means were used for the insulin resistance index, the SE mentioned in text and table referring to the mean of the upper and lower deviation from such a geometric mean (7).

## Results

**Body weight.** At the age of 6 weeks, the initial body weight averaged 123.7±0.7 and 124.8±1.1 g (n=24 in both cases) in the control and experimental group. After either 3 or 7 months of  $\omega$ 3-deprivation, the mean body weight of  $\omega$ 3D rats was slightly but not significantly higher (p>0.3 or more) than that of the control animals of the same age (Table I). Over the 3 or 7 months of exposure to the  $\omega$ 3-deficient diet, the body weight gain failed indeed to be significantly higher than that recorded in the control rats. It averaged, in the control and  $\omega$ 3D rats, respectively, 130.4±3.4 and 139.9±4.0 g (n=24 in both cases) after 3 months, and 156.4±7.2 and 164.4±5.7 g (n=17-18) after 7 months.

Over the 4-5 weeks of exposure to the soya diet, i.e. from about the 39th to 43rd week after birth, the mean value for

the body weight gain in the control rats averaged 10.8±2.4 g (n=6) and, as such, appeared somewhat higher (p<0.06) than that recorded in the control rats exposed for the same period to the flaxseed oil-enriched diet (3.3±2.5 g; n=6).

In the  $\omega$ 3D rats, the body weight gain averaged 16.0±4.6 g and 25.7±2.1 g (n=6 in both cases) after, respectively, 2 and 4-5 weeks exposure to the  $\omega$ 3-enriched diet. Such a body weight gain was thus much higher (p<0.001) in the  $\omega$ 3D rats than in the control animals, all exposed for 4-5 weeks before sacrifice to the  $\omega$ 3-enriched diet (Fig. 1).

**Parametrial adipose tissue mass.** After only 3 months of  $\omega$ 3-deprivation, the mass of the parametrial adipose tissue, whether expressed in absolute terms or relative to body weight, was not significantly higher (p>0.2 or more) in the  $\omega$ 3D rats than in the control animals (Table I). However, after 7 months of  $\omega$ 3-deprivation, such a difference became statistically significant (p<0.05 or less).

In the control rats, there was, if anything, a trend for the further gain in parametrial adipose tissue mass to be more pronounced in the rats exposed for the last 4 weeks before sacrifice to the soybean oil-enriched diet, as distinct from flaxseed oil-enriched diet. Such a difference failed, however, to achieve statistical significance.

The exposure of the rats first deprived for 7 months of dietary  $\omega$ 3 fatty acids to the  $\omega$ 3-enriched diet for 2 or 4-5 further weeks failed to prevent a further gain in parametrial adipose tissue mass (Fig. 1). The variables relevant to the

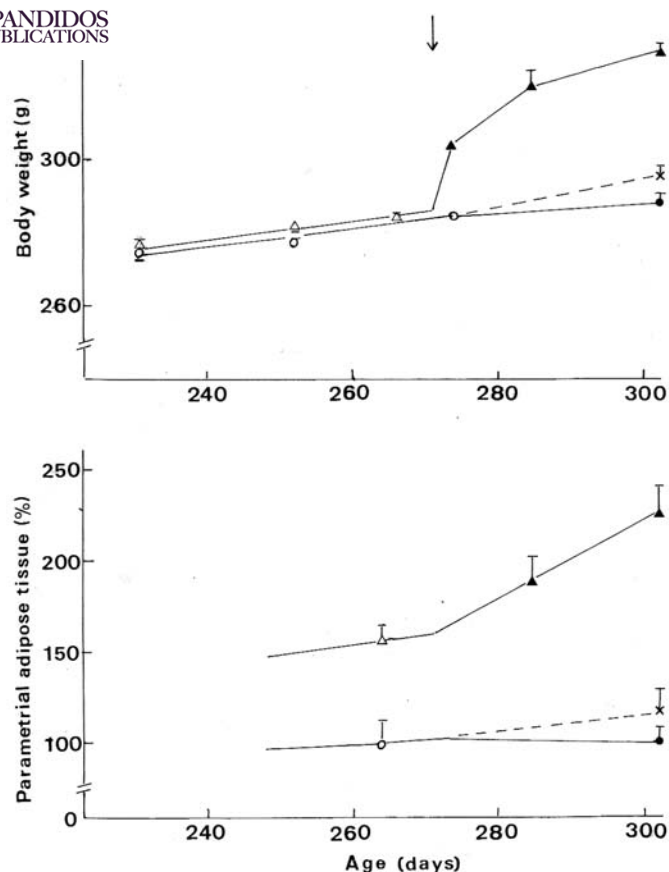


Figure 1. Time course for the changes in body weight and parametrial adipose tissue mass in control animals (circles and the cross) and  $\omega$ 3D rats (triangles) exposed from the 43rd to 271st day (vertical arrow) after birth to their respective diets. Thereafter, the control animals were exposed up to the 302nd day after birth to either a soybean oil-enriched diet (cross) or a flaxseed oil-enriched diet (closed circle), whilst the  $\omega$ 3D rats were given access over the same period to a flaxseed oil-enriched diet (closed triangles). Upper panel: mean values ( $\pm$  SE) for the changes in body weight were calculated from the differences relative to the paired value recorded at the 274th day after birth, and refer to 12 individual values up to day 274 and 6 individual values thereafter. Lower panel: The values for parametrial adipose tissue mass are expressed in percent of the mean value recorded in the control animals at day 264; before day 271, the lines were drawn by linear extrapolation with the mean values recorded at day 139. Mean values ( $\pm$  SE) refer to 5-6 animals and are derived from both the absolute values for adipose tissue weight and the corresponding values calculated relative to body weight.

latter mass indeed progressively increased from a reference value of  $100.0 \pm 5.8\%$  after 7 months of  $\omega$ 3-deprivation to  $121.6 \pm 8.0\%$  and  $145.5 \pm 9.1\%$  after, respectively, 2 and 4-5 further weeks of exposure to the  $\omega$ 3-enriched diet. The latter percentage was much higher ( $p < 0.005$ ) than that recorded in the control rats also exposed for the last 4-5 weeks before sacrifice to the flaxseed oil-enriched diet ( $100.7 \pm 8.0\%$ ).

**Plasma glucose and insulin concentrations.** Already after 3 months of exposure to the  $\omega$ 3-deficient diet, the plasma glucose concentration was higher ( $p < 0.02$ ) in  $\omega$ 3D rats than in the control animals of the same age (Table II). A comparable increase in plasma glucose concentration was observed after 7 months of exposure to the  $\omega$ 3-deficient diet. Pooling together all available data, the plasma glucose concentration in the

$\omega$ 3D rats was  $19.8 \pm 9.6\%$  higher ( $df=21$ ;  $p < 0.06$ ) than in control animals of the same age. No significant difference ( $df=10$ ;  $p > 0.5$ ) in plasma glucose concentration was observed when the control animals were exposed for the last 4-5 weeks before sacrifice to either the soybean or flaxseed oil-enriched diet. In both cases, however, the plasma glucose concentration was lower ( $p < 0.02$ ) than that recorded in the control animals 7 months after the start of the present experiments. In the  $\omega$ 3D rats, the mean plasma glucose concentration progressively decreased ( $p < 0.08$ ) from  $17.52 \pm 2.37$  mM to  $14.61 \pm 1.68$  mM and  $12.92 \pm 1.34$  mM ( $n=6$  in all cases) when these rats were given access to the flaxseed oil-enriched diet for 2 and 4-5 weeks, respectively. The latter value remained somewhat higher ( $p < 0.06$ ), however, than that recorded at the same age in the control rats also exposed to a flaxseed oil-enriched diet for the last 4-5 weeks before sacrifice.

As a rule, no significant difference in plasma insulin concentration was found in the 8 groups of rats considered in this study. Nevertheless, the plasma insulin concentration recorded in the  $\omega$ 3D rats exposed for 3 and 7 months to the  $\omega$ 3-depleted diet and in the  $\omega$ 3D rats then given access for 4-5 weeks to the flaxseed oil-enriched diet averaged  $127.7 \pm 7.5\%$  ( $n=18$ ;  $p < 0.01$ ) of the mean corresponding values found in the control animals exposed for 3 and 7 months to the control diet or then also given access for 4-5 weeks to the flaxseed oil-enriched diet ( $100.0 \pm 6.4\%$ ;  $n=17$ ).

The insulin resistance index (HOMA), taken as the product of plasma glucose times plasma insulin concentration and expressed as mM.pM, averaged in the  $\omega$ 3D rats fed for 3 to 7 months the  $\omega$ 3-deficient diet  $11,448 \pm 1,135$  ( $n=12$ ), as distinct ( $p < 0.02$ ) from only  $7,862 \pm 812$  ( $n=11$ ) in the control rats also examined during the first 7 months of the present experiments. No significant difference ( $p > 0.3$ ) was found between the control animals eventually exposed to either the soybean or flaxseed oil-enriched diet, with an overall mean value of  $4,958 \pm 622$  mM.pM ( $n=10$ ). The latter value was lower ( $p < 0.01$ ) than that recorded in the control rats during the first 7 months of the present experiments. In the  $\omega$ 3D rats, the mean values for the same index progressively decreased from  $12,316 \pm 2,437$  to  $10,306 \pm 2,558$  and  $9,389 \pm 950$  mM.pM ( $n=6$  in all cases) after 2 and 4-5 weeks of exposure to the flaxseed oil-enriched diet (Table II). Such a decrease failed, however, to achieve statistical significance ( $p > 0.1$ ). Moreover, the last of these 3 mean values remained significantly higher ( $p < 0.01$ ) than that recorded at the same age in the control rats also exposed to the flaxseed oil-enriched diet for the last 4-5 weeks before sacrifice.

Since the mean values for the insulin resistance index were virtually identical ( $p > 0.999$ ) in the control rats examined either 3 or 7 months after the onset of the present experiments, it could be assumed that the initial value for such an index 42 days after birth was close to the mean value recorded in the control animals indeed examined at 139 and 264 days after birth, i.e.  $7,862 \pm 812$  mM.pM ( $n=11$ ). The progressive rise in such an index, as observed over the same period of 7 months in the  $\omega$ 3D rats then yielded, in semi-logarithmic coordinates and by regression analysis, a positive slope of  $2.072 \pm 0.778 \cdot 10^{-3}$  ( $df=21$ ;  $p < 0.02$ ). Upon exposure to the flaxseed oil-enriched diet, the further change in the insulin resistance index, which failed to differ significantly ( $p > 0.25$ ; covariance analysis) in

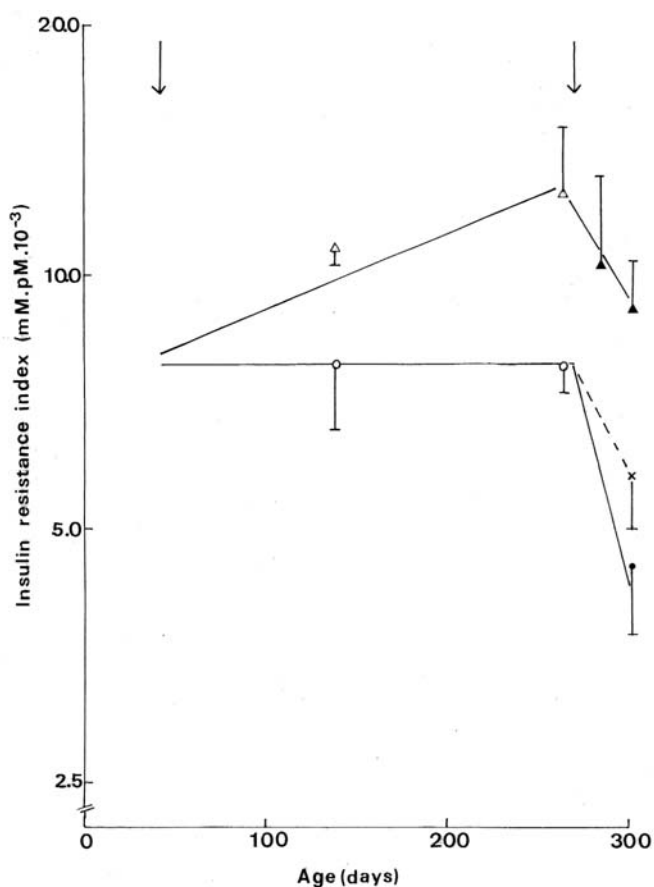


Figure 2. Time course for the changes in insulin resistance index in control animals (circles and the cross) and  $\omega$ 3D rats (triangles) exposed from the 43rd to 271st day after birth (vertical arrows) to their respective diet, and then given access to either a soybean oil-enriched diet (cross) or a flaxseed oil-enriched diet (closed circle and closed triangles). All lines were calculated by linear regression. Mean values ( $\pm$  SE) refer to 5-6 animals and are ranged on a logarithmic scale.

control and  $\omega$ 3D rats, yielded, in semi-logarithmic coordinates, an overall negative slope of  $-11.294 \pm 4.555 \cdot 10^{-3}$  ( $df=27$ ;  $p<0.02$ ). Covariance analysis indicated that these two slopes differed vastly ( $p<0.005$ ) from one another (Fig. 2).

## Discussion

Under the present experimental conditions of dietary  $\omega$ 3 deprivation, the same rats as those examined in this study were found to display in liver phospholipids and triglycerides several perturbations of fatty acid content and pattern similar to those previously documented in second generation  $\omega$ 3-depleted rats (1-3). The present data indicated, however, that, at variance with the latter rats (8), the animals deprived of dietary  $\omega$ 3 fatty acids between the 42nd and 264th days after birth fail to display higher body weight than the control animals fed a diet containing 5% soybean oil instead of 5% sunflower oil. This difference between the present  $\omega$ 3D rats and the second generation  $\omega$ 3D rats coincides with the fact that, in the latter rats, the difference in body weight between  $\omega$ 3-depleted rats and control animals progressively fades out as a function of age (8). The two sets of findings suggest, therefore, that the occurrence of an increased body weight

may in second generation  $\omega$ 3D rats represent an early manifestation most obvious in young animals.

Despite the similarity in body weight between control animals and  $\omega$ 3D rats in the present study, the  $\omega$ 3D rats displayed both liver steatosis (4) and, as shown here, visceral obesity, in the same manner as previously observed in second generation  $\omega$ 3D rats (1-3,9). A further analogy between these two animal models of  $\omega$ 3 deprivation relates to the presence of insulin resistance and, despite higher plasma insulin concentrations, elevated plasma glucose concentration. These features were indeed observed in the second-generation  $\omega$ 3D rats, examined in the fed state or during an intragastric glucose tolerance test (10,11).

When the  $\omega$ 3D rats were given access, after 7 months of dietary  $\omega$ 3 deprivation, to a flaxseed oil-enriched diet, a progressive decrease in plasma glucose concentration and insulin resistance index were observed. However, such changes were also encountered in the control animals fed either a soybean or flaxseed oil-enriched diet, so that the values recorded in the  $\omega$ 3D rats remained significantly higher than those found in the control animals. The changes in glucose homeostasis and insulin sensitivity found in the control rats when given a diet containing 10%, instead of 5%, triglycerides are reminiscent of those previously reported in normal rats exposed to an olive oil-enriched diet (12).

In the  $\omega$ 3D rats given access to the flaxseed oil-enriched diet, however, a further unexpected event consisted in a rapid, pronounced and sustained increase in body weight and parametrial adipose tissue mass (Fig. 1). The rapidity and magnitude of such an increase argue in support of the idea that it may be attributable to an increased food intake. Although further experiments are required to assess the validity of the latter proposal, it is compatible with both preclinical (13,14) and clinical (15,16) findings dealing with the orexigenic effects of long-chain polyunsaturated  $\omega$ 3 fatty acids. Alternatively, the postulated increase of food intake may involve the induction of flaxseed dietary self-selection in  $\omega$ 3-depleted rats (17).

No obvious change in body weight and parametrial adipose tissue mass was observed in the control rats, when exposed to either the soybean or flaxseed oil-enriched diet. In this respect, the most likely explanation for the contrasting results recorded in response to the exposure to the flaxseed oil-enriched diet in control animals *versus*  $\omega$ 3D rats may well consist in the fact that the control animals, at variance with both the second generation  $\omega$ 3D rats and the present  $\omega$ 3D rats, contain sizeable amounts of  $\omega$ 3 fatty acids in brain phospholipids (9,18). Hence, a further supply of exogenous  $\omega$ 3 fatty acid, even if further increasing, within limits, the  $\omega$ 3 fatty acid content of brain phospholipids (18) may well be inefficient in augmenting appetite beyond its normal level. Once again, further experiments, such as the exposure of the  $\omega$ 3D rats to a diet supplemented with soybean oil, rather than flaxseed oil, are likely to provide suitable information to assess the role of an increased dietary supply of  $\omega$ 3 acids in their postulated orexigenic effect.

In conclusion, on one hand, the results of this study further establish the suitability of the present animal model of  $\omega$ 3-depletion to induce features of the metabolic syndrome, including liver steatosis, visceral obesity and insulin resistance.





her hand, however, the findings made in the  $\omega$ 3D n exposed to a flaxseed oil-enriched diet, whilst documenting an improvement of insulin sensitivity and glucose homeostasis, draw attention to the unfavourable effect of the dietary supply of  $\omega$ 3 fatty acids to  $\omega$ 3D rats in terms of the control of body weight and adipose tissue mass, as possibly involving an orexigenic effect of these  $\omega$ 3 fatty acids.

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