The Metabolic Syndrome of ω3-Depleted Rats. IX. Food Intake

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Abstract: The present study complements recent investigations on the changes in fatty acid content and pattern of liver phospholipids and triglycerides found in ω3-depleted rats which, when exposed for 2 weeks to a flaxseed oil-enriched diet, display aggravated liver steatosis, visceral obesity and excess body weight. In the present experiments, the ω3-depleted rats exposed to the flaxseed oil-enriched diet still displayed insulin resistance, with higher plasma glucose and insulin concentrations than in control animals undergoing the same dietary manipulations. The food intake and gain in body weight after the switch in diet were also higher in the ω3-depleted rats than in control animals exposed for the same period to either soybean or flaxseed oil-enriched diets, there being a close analogy between these two variables in the 3 groups of rats. Emphasis is placed on the orexigenic effect attributable to the brain accumulation of C22:5n3 and C22:6n3, on the flaxseed dietary self-selection in the ω3-depleted rats, and on the increase in the lipid content of the diet from 5 to 10% (w/w) as possible determinants of the increase in food intake. In a clinical perspective, these findings indicate that, when considering the most suitable dietary approach to correct a deficiency in long-chain polyunsaturated ω3 fatty acids, equal attention should be paid to the lipid and ω3 fatty acid content of the diet, as well as to food intake, in order to avoid undesirable increases in adipose tissue mass and body weight.

Keywords: Long-chain polyunsaturated ω3 fatty acid-depleted rats, soya, sunflower and flaxseed oils, insulin resistance, food intake and body weight.

INTRODUCTION

Rats exposed to a diet deprived of long-chain polyunsaturated ω3 fatty acids display several features of the metabolic syndrome, including insulin resistance, hepatic steatosis, visceral obesity, hypertension and cardiac hypertrophy. The optimal modality for correction of these perturbations remains to be defined.

In the preceding article in this series [1], attention was drawn to the undesirable aggravation of liver steatosis and further increase in both adipose tissue mass and body weight observed in rats first deprived, for 7 months from the 6th week after birth onwards, of a dietary supply of long-chain polyunsaturated ω3 fatty acids and then given access for about 2 weeks to a flaxseed oil-enriched diet in order to restore a sufficient ω3 fatty acid content of tissue lipids [2, 3]. The analysis of data concerning the fatty acid content and pattern of liver phospholipids and triglycerides suggested that the situation found, under the present experimental conditions, in the ω3-depleted rats was mainly attributable to an increase in food intake [1]. The present study deals with the plasma glucose, and insulin concentrations, insulin resistance index, and the changes in body weight and food intake recorded in the ω3-depleted rats, as well as in control animals exposed to comparable dietary manipulations. The results document that, after the switch in diet, the food intake and body weight increase to a greater extent in ω3-depleted rats than in control animals exposed for the same last period to either a soybean or flaxseed-oil enriched diet.

MATERIALS AND METHODS

The three groups of rats examined in this study and the several diets offered to them were the same as those defined in the preceding report in this series [1]. Control rats first exposed for 7 months to a diet containing 5% soya oil were then exposed for about 2 weeks to the same diet enriched with 5% of either soya oil (CS rats) or flaxseed oil (CF rats). Experimental rats first exposed for 7 months to a diet containing 5% sunflower oil were then exposed for about 2 weeks to the same diet enriched with 5% flaxseed oil (DF rats).

The food intake by each group of 6 rats was measured during the week preceding the switch in diet and during the 2 weeks thereafter over periods of 24 hours or, on occasion, 3-4 days. The food intake was judged, over each successive period, by the difference in weight of the metallic container in which the food was placed and which was itself placed in the cage containing the six rats. Sixteen days after the switch in diet, the rats were eventually euthanized by carbon dioxide inhalation. Blood was collected from the heart for measurement of plasma glucose concentration [4] and insulin concentration by radioimmunoassay [5].

All results are presented as means (± SEM) together with either the number of individual observations (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.
**RESULTS**

**Plasma Glucose and Insulin Concentrations**

The values for plasma glucose and insulin concentrations and insulin resistance index (plasma insulin-glucose product) found at sacrifice in the three groups of rats are provided in Table 1. The mean values for these three variables were higher in the DF rats than in either the CF or CS rats. Relative to the overall mean value for each of these variables, the measurements made in DF rats indeed averaged 109.8 ± 8.1 % (n = 18), as distinct (p < 0.06) from 95.1 ± 3.4 % (n = 36) in the other two groups. Moreover, expressed in the same manner, a highly significant correlation (r = 0.8802; n = 9; p < 0.003) was found between the present data and those of our prior study [3].

**Body Weight**

At the age of 6 weeks, i.e. at the onset of the present experiments, the body weight failed to differ significantly (df = 10; p > 0.1) in the two control groups of rats (CS and CF). It was also comparable (df = 16; p > 0.4) in these control animals and those later exposed to the ω3-depleted diet (DF), with an overall mean value of 156 ± 3 g (n = 18). Over the first 31 weeks of the present experiments, the increase in body weight, whether expressed in absolute terms or relative to the initial body weight, was virtually identical in the two control groups with overall mean values of 106 ± 5 g and 69.1 ± 2.2 % (n = 12 in both cases). In the ω3-depleted rats, such an increase in body weight was slightly but significantly higher (p < 0.04 or less), averaging in absolute and relative terms respectively 134 ± 13 g and 92.7 ± 6.2 % (n = 6 in both cases). It should be underlined, however, that over the last 7 weeks preceding the switch in diet, the mean increase in body weight was no more significantly different in the control and ω3-depleted rats (df = 16; p > 0.15). It indeed averaged 0.19 ± 0.04 g/day (n = 12) in the control rats (CS and CF) as compared (p > 0.18) to 0.34 ± 0.14 g/day (n = 6) in the ω3-depleted rats (DF), with an overall mean values of 11.3 ± 2.6 g (n = 18).

Table 1: Plasma Glucose and Insulin Concentrations

<table>
<thead>
<tr>
<th>Rats</th>
<th>CF</th>
<th>CS</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mM)</td>
<td>13.88 ± 0.64*</td>
<td>12.53 ± 0.38</td>
<td>14.71 ± 1.13</td>
</tr>
<tr>
<td>Plasma insulin (pM)</td>
<td>937 ± 114</td>
<td>994 ± 91</td>
<td>1061 ± 134</td>
</tr>
<tr>
<td>Insulin resistance index (mM.pM.10⁻³)</td>
<td>13.0 ± 1.77</td>
<td>12.38 ± 1.10</td>
<td>15.90 ± 2.77</td>
</tr>
</tbody>
</table>

*Mean values (± SEM) refer to 6 individual observations in all cases

Table 2: Daily Body Weight Gain Before and After the Switch in Diet

<table>
<thead>
<tr>
<th>Rats</th>
<th>CF</th>
<th>CS</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 30 to 37 (before)*</td>
<td>0.252 ± 0.077 (6)</td>
<td>0.124 ± 0.030 (6)</td>
<td>0.344 ± 0.136 (6)</td>
</tr>
<tr>
<td>Week 38 and 39 (after)</td>
<td>0.821 ± 0.214 (6)*</td>
<td>1.893 ± 0.220 (6)*</td>
<td>2.191 ± 0.278 (6)*</td>
</tr>
<tr>
<td>Δ (after minus before)</td>
<td>0.570 ± 0.228 (10)*</td>
<td>1.769 ± 0.222 (10)*</td>
<td>1.847 ± 0.310 (10)*</td>
</tr>
</tbody>
</table>

*Mean values (± SEM) are indicated together with the number of individual observations (6) or degree of freedom (10)

* p < 0.005; ** p < 0.01; *** p < 0.003

In all 3 groups of rats, the switch from a diet containing 5 % lipids to one containing 10 % lipids provoked over 2 weeks a daily increase in body weight gain higher (p < 0.05 or less) than that recorded over the preceding 7 weeks (Table 2). As judged from the measurements made before and after the switch in diet, the increment in daily weight gain caused by the latter switch was thrice higher (p < 0.002), however, in the CS rats than in the CF rats, whilst failing to differ significantly (p > 0.8; df = 20) in CS and DF rats.

Over the period of 21 days during which the food intake was monitored the daily gain in body weight was not significantly different (p > 0.15; df = 10) in the CF and CS rats (Fig. 1, right panel). It was much higher, however, in DF rats than in either the CF or CS rats (p < 0.008 or less). A comparable, but not identical, hierarchy was found for the difference in daily weight gain over the 7 weeks before and 2 weeks after the switch in diet (Table 2) or for the daily gain in body weight over the period of 14 days after the switch in food intake. For instance, in the latter case, the value recorded in DF rats (2.19 ± 0.28 g/day; n = 6) was again much higher (p < 0.004) than that recorded in CF rats (0.82 ± 0.21 g/day; n = 6). Over this period of 14 days, the daily gain in body weight was higher (p < 0.01) in CS than in CF rats, as already observed in our prior study [3]. When pooling together the results of these two studies, the daily weight gain after the switch in diet represented in the CF rats no more than 37.0 ± 12.4 % (n = 12; p < 0.002) of the mean corresponding value recorded within the same study in the CS rats (100.0 ± 12.0 %; n = 12).

**Food Intake**

Over the period of 7 days preceding the switch in diet, the mean daily food intake was, as expected, not significantly different (df = 8; p > 0.1) in the two groups of rats exposed to the soya oil-containing diet, with an overall mean value of 15.2 ± 0.3 g per rat (n = 10). It also failed to differ significantly (df = 13; p > 0.13) in these control animals and the ω3-depleted rats exposed to the sunflower oil-containing diet (14.7 ± 0.4 g/day per rat; n = 5). In the control rats even-
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Eventually exposed to a flaxseed oil-enriched diet, the switch from a diet containing 5% lipids to a diet containing 10% lipids only caused a short-lived increase in food intake (Fig. 2). Thus, such a food intake only exceeded the upper limit of the 95% confidence interval for the measurements made before the switch in diet during the first and second day after this switch. None of the 7 further measurements exceeded this upper limit. Their mean value (15.6 ± 0.4 g; n = 7) failed to differ significantly (df = 10; p > 0.19) from that recorded before the switch in diet (14.7 ± 0.5 g; n = 5). Even if including the measurements made on the first and second day after the switch in diet, the mean value after this switch (16.5 ± 0.7 g; n = 9) failed to differ significantly (df = 12; p > 0.1) from that recorded theretofore.

In the control rats eventually exposed to a soybean oil-enriched diet, 4 measurements made after the switch in diet exceeded the upper limit of the 95% confidence interval for the measurements made before such a switch. Over the period of 14 days following the switch in diet, the mean value for daily food intake (20.2 ± 0.8 g; n = 9) was significantly higher (df = 12; p < 0.004) than that recorded before such a switch (15.7 ± 0.4 g; n = 5). Nevertheless, the difference between the latter two mean values (4.4 ± 1.2 g; df = 12) failed to differ significantly (df = 24; p > 0.10) from the corresponding difference found in the control rats eventually exposed to a flaxseed oil-enriched diet (1.8 ± 1.0 g; df = 12). Such was also the case (df = 24; p > 0.13) when the increment in food intake after the switch in diet was expressed relative to the mean value recorded theretofore in the control rats eventually exposed to either the flaxseed oil-enriched diet (+12.2 ± 7.0%; df = 12) or the soybean oil-enriched diet (+28.3 ± 7.5%; df = 12). Moreover, in both cases, the increase in food intake represented a short-lived phenomenon. Thus, the slope of the regression line over the first four days after the switch averaged –10.80 ± 1.90 g/day (n = 2), as distinct (df = 3; p < 0.02) from only 0.00 ± 0.98 g/day (n = 2) between the 4th and 14th day after the switch. During the latter period, no significant further decrease in daily food intake was anymore observed whether in CF or CS rats. A further difference between CF and CS rats consisted in the finding that, over the 2-week period after the switch in diet, a significant decrease in food intake was observed in the CF rats (r = -0.7303; n = 14; p < 0.05), whilst such was not the case in the CS rats (r = -0.3029; n = 14, p > 0.1).

The changes in food intake provoked by the switch in diet differed in several respects in CF and DF rats. First, in the DF rats up to six out of nine measurements made after the switch in diet exceeded the upper limit of the 95% confidence interval for the measurements made before such a switch. Second, over the period of 14 days following the switch in diet, the mean value for daily food intake (19.6 ± 1.0 g; n = 9) remained, in the DF rats, significantly higher (df = 12; p < 0.02) than that recorded before such a switch (14.7 ± 0.4 g; n = 5). Third, over the first four days after the switch in diet, the increase in food intake above the mean value recorded before such a switch was significantly higher...
(p < 0.03) in DF rats than in CF rats. It indeed represented in the DF rats almost thrice (294 ± 47 %; n = 4) of the paired value recorded on the same day after the switch in the CF rats. Even thereafter, i.e. over the last ten days of the present experiments, the increase in food intake above the mean value recorded before such a switch remained significantly higher (p < 0.02) in DF rats (+ 2.42 ± 0.32 g/day; n = 4; p < 0.005 versus zero) than in CF rats (+ 0.23 ± 0.50 g/day; n = 4), in which case it failed to achieve statistical significance (p > 0.6). The increase in food intake provoked by the switch in diet was thus more pronounced and persisted for a longer time in DF rats, as compared to CF rats.

Several differences were also observed when comparing DF to CS rats. First, over the first eight days after the switch in diet, the daily food intake, when expressed relative to the mean value recorded before such a switch, was significantly higher (df = 4; p < 0.04) in the DF rats than in the CS rats. It indeed represented in the DF rats 115.4 ± 4.9 % (n = 5) of the paired value recorded on the same day after the switch in the CS rats. Second, when all data were expressed, in each group of rats, relative to the mean corresponding value for food intake over the 2 weeks after the switch in diet, covariance analysis revealed that the slope (b) of the regression line during these last 14 days was more than thrice steeper (F = 4.797; f = 1, 24; p < 0.05) in the DF rats (b = - 2.795 percent per day) than in the CS rats (b = - 0.813 percent per day). It failed, however, to differ significantly when comparing CF and CS rats.

As a matter of fact, there were further differences between DF rats, on one hand, and CF and/or CS, on the other hand. In the DF rats, like in the two groups of control rats, the slope of the regression line relating food intake to time was steeper over the first four days after the switch in diet (- 6.4 g/day) than between the 4th and 14th day after the switch (- 1.6 g/day). However, the difference between the latter two values (4.8 g/day) represented less than half that found in the CF and CS rats (10.8 ± 2.9 g/day; n = 2). Moreover, at variance with the situation found in CF or CS rats, a significant decrease in food intake was still observed between the 4th and 14th day after the switch in diet in the DF rats (t = - 0.6805; n = 11; p < 0.03). These findings again indicate that, in the DF rats, the increase in food intake caused by the switch in diet represented a more sustained phenomenon than in either CF or CS rats.

When all measurements of food intake after the switch in diet were expressed relative to the mean corresponding value recorded theretofore, covariance analysis confirmed that the results recorded in CF and CS rats only differed from one another by a higher elevation in the latter than former rats during the last 11 days of the present experiments (Table 3). Over the period of four days following the switch in diet, the results recorded in DF rats differed from those found in either CF or CS rats by their higher elevation in the DF animals. Between the 4th and 14th days after the switch, a significant decrease in food intake was observed in DF rats (p < 0.03) but not so in either CF or CS rats, the slope of the regression line being significantly higher in DF rats than in CS rats (Table 3). During this late period, the elevation of the regression line was also higher in both CS and DF than in CF rats.

Essentially comparable information was reached when the same analytical procedure was conducted over two successive periods of seven days each after the switch in diet. In all groups of rats, the slope of the regression line (b) was much steeper during the first week than during the second one (n = 3; p < 0.05), such a difference being more marked (p < 0.05) in the DF rats (Δb = 0.944 percent/day) than in the control animals (Δb = 3.676 ± 0.180 percent/day; n = 2). The correlation coefficient between the date (day) and food intake averaged − 0.8395 ± 0.0527 (n = 3; p < 0.005) during the first week after the switch in diet, as distinct (df = 4; p < 0.002) from + 0.0517 ± 0.1948 (n = 3; p > 0.8) during the second week after such a switch.

### Table 3. Covariance Analysis of Food Intake (Expressed as a Percentage Relative to Mean Corresponding Values Before the Switch in Diet)

<table>
<thead>
<tr>
<th>Rats</th>
<th>CF</th>
<th>CS</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1 to day 4 after the switch in diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (± SD)</td>
<td>- 10.09 ± 3.45</td>
<td>- 13.47 ± 2.08</td>
<td>- 7.27 ± 3.73</td>
</tr>
<tr>
<td>Mean (± SEM)</td>
<td>122.7 ± 7.2 cd</td>
<td>131.8 ± 8.9 d</td>
<td>153.9 ± 5.8 e</td>
</tr>
<tr>
<td><strong>Day 4 to day 14 after the switch in diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (± SD)</td>
<td>- 1.12 ± 0.53</td>
<td>+ 1.04 ± 1.13 c</td>
<td>- 1.77 ± 0.64 c</td>
</tr>
<tr>
<td>Mean (± SEM)</td>
<td>107.6 ± 1.9 a</td>
<td>119.8 ± 3.5 b</td>
<td>120.5 ± 2.6 a</td>
</tr>
</tbody>
</table>

a: p < 0.005; b: p < 0.01; c: p < 0.05
those of our prior study [3].

In this respect, there was a close analogy between the results of the present study and assessment) for insulin resistance. In this respect, there was a close analogy between the results of the present study and assessment) for insulin resistance. In this respect, there was a close analogy between the results of the present study and assessment) for insulin resistance. In this respect, there was a close analogy between the results of the present study and assessment) for insulin resistance. In this respect, there was a close analogy between the results of the present study and assessment) for insulin resistance. In this respect, there was a close analogy between the results of the present study and assessment) for insulin resistance.

**DISCUSSION**

**Insulin Resistance**

The access of the DF rats to the flaxseed oil-enriched diet failed to fully restore to normal values for plasma glucose and insulin concentrations and HOMA (homeostatic model assessment) for insulin resistance. In this respect, there was a close analogy between the results of the present study and those of our prior study [3].

**Body Weight**

The present results reveal that exposure of 6-weeks old rats to an ω3-depleted diet provokes a greater gain in body weight than that recorded in control animals. Such a difference, however, fades out during prolonged exposure, up to 7 months, to the ω3-depleted diet. In second generation ω3-depleted rats, the excess of body weight relative to control animals also progressively fades out at increasing age [6].

The switch from a diet containing 5 % lipids to one containing 10 % lipids resulted in an increased daily body weight gain. As judged from the gain in body weight during the last 2 or 3 weeks of the present experiments or the difference in daily weight gain before and after the switch in diet, the hierarchy of results was always identical with CF data ≤ CS data ≤ DF data (Fig. 1 and Table 2). In our prior study, it was duly documented that, in the DF rats, such an increase in body weight is most pronounced immediately after the switch in diet and progressively fades out thereafter.

**Food Intake**

In all three groups of rats, the daily food intake increased when the diet containing only 5 % lipids was replaced by a diet containing 10 % lipids. Such a phenomenon was least pronounced in the CF rats. In these animals, the daily food intake only exceeded the upper limit of the 95 % confidence interval for the measurements made before the switch in diet in the first and second day after such a switch. Between the third and twelfth day after the switch in diet, the mean daily food intake was no more significantly different from that recorded over the period of seven days preceding the switch in diet. Over the total 2-week-period of exposure to the flaxseed oil-enriched diet, the daily food intake in the CF rats only represented 82.4 ± 3.4 % (n = 9; p < 0.001) of the paired value recorded on the same day in the CS rats.

In addition to the latter finding, the CS rats also differed from the CF rats by the fact that the daily food intake remained significantly higher (p > 0.08) between the third and last day after the switch in diet (19.4 ± 0.9 g/day; n = 7) than before such a switch (15.7 ± 0.4 g/day; n = 5), whilst such was not the case in the CF rats.

An array of findings, such as those illustrated in Table 3, indicated that in the DF rats, the increase in food intake caused by the switch in diet was even more pronounced and more sustained than that recorded in CF or CS rats.

Tight correlations were found between the increase in daily food intake after the switch in diet, expressed relative to the mean value recorded before such a switch, and the daily gain weight over the same total period of 3 weeks in CF rats (open circles), CS rats (closed circles) and DF rats (open triangles); mean values (± SEM) refer to a degree of freedom equal to twelve (food intake) and six individual observations (body weight). In all three groups of rats, the energetic efficiency of each diet.

Fig. (3). **Upper panel:** correlation between the increase in daily food intake after the switch in diet, expressed relative to the mean value recorded before such a switch, and the daily gain weight over the same total period of 3 weeks in CF rats (open circles), CS rats (closed circles) and DF rats (open triangles); mean values (± SEM) refer to a degree of freedom equal to twelve (food intake) and six individual observations (body weight). **Lower panel:** correlation between the absolute values for the increase in food intake and the daily body weight gain over the two weeks following the switch in diet in CF, CS and DF rats (same symbols as in the upper panel); mean values (± SEM) refer to a degree of freedom equal to twelve (food intake) and six individual observations (body weight). The oblique lines were established by regression analysis.

The switch from a diet containing 5 % lipids to one containing 10 % lipids resulted in an increased daily body weight gain. As judged from the gain in body weight during the last 2 or 3 weeks of the present experiments or the difference in daily weight gain before and after the switch in diet, the hierarchy of results was always identical with CF data ≤ CS data ≤ DF data (Fig. 1 and Table 2). In our prior study, it was duly documented that, in the DF rats, such an increase in body weight is most pronounced immediately after the switch in diet and progressively fades out thereafter.
fatty acids [7]. This is not meant to deny that such an orexigenic effect, already documented by a number of prior studies [8-11], may participate to the increase in food intake during the late period of exposure to the flaxseed oil-enriched diet. This view is supported by the fact that, within 2 weeks exposure to the flaxseed oil-enriched diet, the C22:5o3 and C22:6o3 relative weight content of brain phospholipids increases in the DF rats, from a zero value to 3.37 ± 0.22 % in the case of C22:5o3 and from 16.8 ± 0.5 % to 19.2 ± 0.4 % in the case of C22:6o3 [7]. In the same respect, it should be underlined that, 4-5 weeks after the switch in diet, the C22:5o3 (but not C22:6o3) relative weight content of brain phospholipids is significantly higher (p < 0.001) in CF rats (3.70 ± 0.16 %; n = 6) than in CS rats (2.35 ± 0.12 %; n = 6) [7]. A second mechanism may well consist in a flaxseed dietary self-selection in o3-depleted rats, which indeed slowly develop a robust preference for consumption of an o3-rich diet [12]. Last, the increase in the total lipid content of the diet from 5 % before to 10 % after the switch in diet may also account, in part at least, for the increase in food intake. The latter mechanism is consistent with a prior observation [13], and may also participate to the increase in food intake observed in the control animals first exposed for 7 months to a diet containing only 5 % of lipids (soya oil) and then exposed for the last 2 weeks to the same diet enriched with either another 5 % of soya oil or 5 % of flaxseed oil.

It cannot be ignored that the increase in food intake caused by the switch in diet was more pronounced and more sustained in CS rats than in CF rats. The question comes inevitably in mind whether such a situation represents a mirror image of the induction of flaxseed dietary self-selection in o3-depleted rats [12].

In conclusion, the present study clearly documents the key role of an increase in food intake in the otherwise unexpected increase in visceral obesity and body weight recorded when o3-depleted rats are exposed to flaxseed oil-enriched diet. The clinical implications of these findings should not be overlooked. They indeed indicate that, when considering the most suitable dietary approach to correct a deficiency in long-chain polyunsaturated o3 fatty acids, as often prevailing today in Western populations, equal attention should be paid to the content of the diet in such o3 fatty acids and total food intake, in order to avoid both an undesirable gain in body weight and undesirable increase in adipose tissue mass.

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