Dietary Manipulation of the Disappearance of trans-Octadecenoates in Rat Tissues

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Summary The effects of dietary manipulations on the fate of trans-octadecenoates deposited in the tissues of rats were examined. Male rats were fed on a 15% fat diet containing trans-octadecenoic acids (46.6% of total fatty acids) for 35 days followed by various diets free of trans-fatty acids. After removal of trans-fatty acids from the diet, there were phased disappearances of trans-octadecenoates from the circulation; a rapid and broad reduction in one day and a slow and gradual reduction thereafter. The rate of the initial reduction in serum trans-octadecenoates was highest on a high fat (20%) diet in relation to low fat (1 or 5%) diets. However, the disappearance rate at the later stage was apparently the same among the various groups and trans-fatty acid contents in the serum declined to about 10% of the initial value in 2 weeks and thereafter. After 35 days, the concentration of trans-octadecenoates remaining in the adipose tissue was markedly lower in rats fed on a high protein (40%) diet. The effects of dietary fat type and cholesterol on the fate of serum trans-octadecenoates were virtually the same, but livers from rats fed on the cholesterol-free safflower oil diet contained more trans-fatty acids than those from rats fed on the corresponding olive oil diet. Thus, the amounts of trans-fatty acids stored in the tissues cannot be merely predicted from serum levels. It seems that both dietary fat and protein affect the metabolic rate of trans-octadecenoates in rats.

Key Words trans-octadecenoates, serum, liver, adipose tissue

A wealth of information indicates the active incorporation of dietary trans-fatty acids into the various lipid fractions of animal tissues (1–3). Although no conclusive study is so far available, some reports claim that the accumulation of trans-fatty acids, in particular trans-octadecadienoates, in animal tissues may alter

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the membrane fluidity and the function of organelles and hence provoke several types of disorders such as atherosclerosis, ischemic heart disease and cancer (4–6). In addition, the possibility exists that trans-fatty acids perturb the metabolism of polyunsaturated fatty acids and thus aggravate the essential fatty acid deficiency (7, 8).

As trans-fatty acids, similar to the cis-counterparts, are oxidized via the \(\beta\)-oxidation pathway and hence used as an energy source (9–11), it is anticipated that trans-fatty acids are cleared from animal tissues in a relatively short period. However, Moore et al. (12) observed in rats that trans-fatty acids once deposited in the adipose tissue remained for at least 8 weeks even when the animals were maintained on a diet free of the trans-isomers, although they were rapidly cleared from the plasma. Accordingly, when animals are once fed trans-fatty acids, their tissues may be exposed to a certain level of the trans-isomers for a relatively long period. On considering the undesirable, but undefined effects of trans-fatty acids, it would be of considerable importance to seek for the dietary components which enhance the catabolism and clearance of trans-fatty acids. In this context, the effects of dietary components on the rate of disappearance of trans-octadecenoates, the prototype of trans-fatty acids in commercial hydrogenated fats, in rat tissues were examined in the present study.

MATERIALS AND METHODS

Animals and diets. Two sets of experiments (Exps. 1 and 2) were carried out in this study. Male Sprague-Dawley rats purchased from Seiwa Experimental Animals, Fukuoka, weighing approximately 100 g were housed individually in an air-conditioned room (20–23°C, lighting on 08:00 to 20:00 h) and given a purified diet containing trans-fatty acids for 35 days to accumulate the trans-isomers in the tissues. The composition of the purified diet was in weight percent: casein 20, partially hydrogenated corn oil 13.7, safflower oil 1.3, vitamin mixture (13) 1, mineral mixture (13) 4, choline chloride 0.15, cellulose 2 and sucrose to 100. The diet contained, in total fatty acids, 46.6% of trans-octadecenoates and an adequate amount of linoleic acid (6.8% of total fatty acids or approximately 1.8% of total energy). At the last day on the trans-fat diet, animals were deprived of the diet at around 08:00 h and a small volume of blood was withdrawn from the tail vein at 13:00–14:00 h. Animals were then randomly divided into 5 and 4 groups in Exp. 1 (mean body weight 335 g) and Exp. 2 (mean body weight 350 g), respectively and then switched to various purified diets free of trans-acids. Exp. 1 was designed to determine the effects of dietary fat (corn oil 1, 5 and 20%) and protein (casein 10, 20 and 40%) levels on the metabolic rate of trans-octadecenoates. These dietary manipulations were accomplished by adjusting the content of sucrose. The effects of the type of dietary fats (10%) and cholesterol (0.5%) were examined in Exp. 2 using safflower oil (77% linoleic acid) and olive oil (78% oleic acid). In both trials a small volume of blood was withdrawn from the tail vein periodically for 28 and 14 days in J. Nutr. Sci. Vitaminol.
Exps. 1 and 2, respectively, 5 to 6 h after the removal of diets as described above (the blood specimens at days 35 in Exp. 1 were obtained when rats were killed by decapitation). At the end of the experiments, rats were killed by decapitation, blood was collected and liver and adipose tissues were excised.

**Lipid analyses.** Serum lipids extracted by the mixture of chloroform–methanol (2:1, v/v) (14) were saponified and fatty acids were analyzed as methyl esters by gas-liquid chromatography using OV-275 and DEGS columns as described previously (15). *trans*-Octadecenoic acids were virtually the sole *trans*-fatty acids detected (more than 98% of total *trans*-fatty acids) in serum and tissue lipids as well as partially hydrogenated corn oil used as a source of *trans*-fatty acids. In Exp. 1 the amount of serum *trans*-octadecenoates was calculated by the product of the total amount of fatty acid in serum determined by the method of Noma et al. (16) and the percentage value of *trans*-octadecenoates measured by gas-liquid chromatography. In Exp. 2 the amount of *trans*-fatty acids in serum was determined by gas-chromatography using pentadecanoic acid as an internal calibration standard. Fatty acid compositions of liver and adipose tissue lipids were also determined similarly. Serum and liver lipids were analyzed for cholesterol and triglyceride (17).

**Statistical analysis.** To evaluate the statistical significance with a probability level of 0.05, one- and two-way analysis of variance was adopted for Exps. 1 and 2, respectively (18).

**RESULTS**

**Effects of dietary fat and protein levels (Exp. 1)**

The growth of the animals for 35 days on diets free of *trans*-fatty acids was highest in the 40% protein group (157 ± 8 g) and lowest in the 10% protein group (118 ± 1 g), intermediate in the other groups (mean values 129–142 g). However, the difference among the various groups was statistically insignificant. Average daily food intake, but not energy intake, in the high fat group (18.3 ± 9 g/day) was significantly less than that in the other groups (mean values 22.9–24.3 g/day). Relative liver weight was essentially comparable in all groups (mean values 3.4–3.8 g/100 g body weight).

Sera harvested at the end of the *trans*-fat diet period contained 45.5 ± 2.3 mg/dl (or 12.3 ± 0.4% of total fatty acids) of *trans*-octadecenoates. The time course of the change in the content of *trans*-octadecenoates expressed as the percentage of the initial value is shown in Fig. 1. After removal of *trans*-fatty acids from the diet, they disappeared by stages from the circulation. One day after the replacement of the diet, the most profound reduction was noted in rats fed on the high-fat (20% o) diet while the rate of disappearance was the same in the other groups (20.7 ± 3.1 vs. 44.3 ± 11.8–46.3 ± 4.0%, of the initial value, respectively, *p < 0.05*). Concentrations of *trans*-fatty acids decreased gradually thereafter in the various groups and reached approximately 10% of the initial value at 28 days. A moderate rise of the concentration of serum *trans*-octadecenoates at 35 days when rats were killed may
Fig. 1. Time course of the disappearance of trans-octadecenoate in serum of rats fed different levels of fat and protein (Exp. 1). Values are means of 5 to 7 rats per group and expressed as percentages of the starting value, 45.5 ± 2.3 mg/dl.

Table 1. Content of trans-octadecenoate of liver and adipose tissue of rats fed on diets containing different levels of fat and protein (Exp. 1).

| Diets             | Liver                  | Adipose tissue              |
|-------------------|------------------------|-----------------------------|
|                   | Percentage of total fatty acids | Concentration (μg/g tissue) | Percentage of total fatty acids |
| 20% fat–20% protein | 1.4 ± 0.1              | 482 ± 71                    | 8.5 ± 0.6⁺ |
| 5% fat–20% protein  | 1.5 ± 0.4              | 464 ± 80                    | 8.6 ± 0.8⁺ |
| 1% fat–20% protein  | 1.1 ± 0.3              | 418 ± 163                   | 8.6 ± 0.4⁺ |
| 5% fat–40% protein  | 1.4 ± 0.1              | 636 ± 70                    | 1.4 ± 0.2ᵇ |
| 5% fat–10% protein  | 1.8 ± 0.7              | 502 ± 187                   | 9.4 ± 0.7⁺ |

Values represent means ± SE of 5 to 7 rats per group. ⁺,ᵇ Values not sharing common superscript letters are significantly different at p < 0.05.

be ascribed to the difference in the route of blood collection, either from the tail vein or by decapitation.

The contents of trans-octadecenoates in the liver and adipose tissue 35 days after feeding experimental diets are shown in Table 1. There were no statistically
Table 2. Serum and liver lipids of rats fed on diets containing different levels of fat and protein (Exp. 1).

| Diets               | Serum lipids (mg/dl) | Liver lipids (mg/g) |
|---------------------|----------------------|---------------------|
|                     | Cholesterol | Triglyceride | Cholesterol | Triglyceride |
| 20% fat–20% protein| 82.7±9.3<sup>ab</sup> | 167±29 | 3.2±0.2 | 15.3±4.4<sup>a</sup> |
| 5% fat–20% protein | 106±7<sup>ec</sup>  | 182±28 | 3.0±0.2 | 35.1±6.8<sup>ab</sup> |
| 1% fat–20% protein | 121±8<sup>e</sup>   | 212±27 | 3.5±0.3 | 33.0±3.9<sup>ab</sup> |
| 5% fat–40% protein | 88.0±8.1<sup>ab</sup> | 187±27 | 3.3±0.4 | 38.1±4.4<sup>b</sup> |
| 5% fat–10% protein | 64.1±4.6<sup>b</sup> | 169±40 | 2.5±0.1 | 22.2±2.3<sup>ab</sup> |

Values represent means±SE of 5 to 7 rats per group. <sup>a,b,c</sup> Values in the same column not sharing common superscript letters are significantly different at p<0.05.

There were no differences in the growth (mean values 45–54 g/14 days) and food intake (mean values 21.9–23.0 g/day) during the 14 days of the experimental period. Cholesterol feeding slightly increased the liver weight (mean values 3.7 and 4.0 vs. 4.1 and 4.2 g/100 g body weight).

The amount of trans-octadecenoates detected in the serum at the end of trans-fat feeding was 31.1±3.6 mg/dl or 13.7±0.6% of total fatty acids. Consistent with the die-away curve of the preceding experiment, the serum levels of trans-octadecenoates decreased markedly in one day (approximately 30 to 50% of the initial value, Fig. 2). Although there was no significant difference in the initial reduction rate among the groups, it tended to be higher in rats fed on a cholesterol-enriched diet containing olive oil (mean value 28.3%) than in those fed on other diets (mean values 43.9–46.5%). The levels reached after 14 days, 11 to 17% of the initial value, were comparable to those observed at the same period in the preceding

Effect of dietary fat type and cholesterol (Exp. 2)

There were no differences in the growth (mean values 45–54 g/14 days) and food intake (mean values 21.9–23.0 g/day) during the 14 days of the experimental period. Cholesterol feeding slightly increased the liver weight (mean values 3.7 and 4.0 vs. 4.1 and 4.2 g/100 g body weight).

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Fig. 2. Time course of the disappearance of trans-octadecenoate in serum of rats fed different fats with or without cholesterol (Exp. 2). Values are means of 6 to 7 rats per group and expressed as percentages of the starting value, 31.1 ± 3.6 mg/dl.

Table 3. Content of trans-octadecenoate of liver and adipose tissue of rats fed different types of fats with or without cholesterol (Exp. 2).

| Diets                | Liver                  | Adipose tissue         |
|----------------------|------------------------|------------------------|
|                      | Percentage of total fatty acids | Concentration (µg/g tissue) | Percentage of total fatty acids | Concentration (mg/g tissue) |
| Cholesterol-free     |                        |                        |                        |
| Olive oil            | 1.6 ± 0.2*             | 489 ± 43*              | 13.9 ± 0.7             | 103 ± 5               |
| Safflower oil        | 2.9 ± 0.3b             | 864 ± 135              | 14.0 ± 0.3             | 103 ± 2               |
| Cholesterol-enriched |                        |                        |                        |
| Olive oil            | 1.8 ± 0.3              | 675 ± 104              | 13.6 ± 0.6             | 99 ± 5                |
| Safflower oil        | 1.5 ± 0.2              | 758 ± 107              | 13.6 ± 1.2             | 107 ± 4               |

Values represent means ± SE of 6 to 7 rats per group. *Significantly different from the corresponding safflower oil group at p < 0.05. bSignificantly different from the corresponding cholesterol-enriched group at p < 0.05.
Table 4. Serum and liver lipids of rats fed on diets containing different fats with or without cholesterol (Exp. 2).

| Diets               | Serum lipids (mg/dl) | Liver lipids (mg/g) |
|---------------------|----------------------|---------------------|
|                     | Cholesterol | Triglyceride | Cholesterol | Triglyceride |
| Cholesterol-free    |             |             |             |             |
| Olive oil           | 69.9 ± 5.4a | 117 ± 13    | 3.4 ± 0.1a  | 30.3 ± 3.3   |
| Safflower oil       | 65.8 ± 4.8  | 80.6 ± 14.3 | 2.7 ± 0.1a  | 25.0 ± 4.1a  |
| Cholesterol-enriched|           |             |             |             |
| Olive oil           | 99.4 ± 4.0b | 146 ± 29    | 20.3 ± 1.6  | 35.5 ± 3.1b  |
| Safflower oil       | 75.0 ± 6.4  | 94.8 ± 15.4 | 23.1 ± 1.4  | 70.1 ± 4.1   |

Values represent means ± SE of 6 to 7 rats per group. a Significantly different from the corresponding cholesterol-enriched group p<0.05. b Significantly different from the corresponding safflower oil group at p<0.05.

experiment.

The content of trans-octadecenoate of the liver and adipose tissue is shown in Table 3. In rats fed on cholesterol-free diets, significantly fewer trans-octadecenoates were detected in the olive oil group than in the safflower oil group, both in terms of percentage and concentration. No such difference was, however, found in rats fed on cholesterol-containing diets due to a significant reduction of the content of the trans-isomers after feeding safflower oil with cholesterol. Concentrations of trans-isomers in the livers after 14 days were roughly comparable with those observed in the preceding experiment in which animals were killed 35 days after the replacement of the diet. The adipose tissue still contained a large amount of trans-octadecenoates compared to the liver and the percentage was approximately 1.5-fold higher than that observed in the preceding trial. However, no demonstrable effects of the fat type and cholesterol on the content of trans-octadecenoates were observed in this tissue.

As shown in Table 4, a significant hypocholesterolemic activity of safflower oil in relation to olive oil was found in rats fed on cholesterol-enriched diets, and the serum triglyceride level also tended to be low in rats fed safflower oil. The extent of the increase in hepatic cholesterol due to feeding cholesterol-enriched diets was comparable between the two fat diets. Cholesterol feeding increased the hepatic triglyceride level in rats fed safflower oil but not in those fed olive oil.

**DISCUSSION**

This study confirmed a relatively rapid clearance of trans-monoene fatty acids from the circulation. Moore et al. (12) fed a 15% fat diet containing 50% trans-octadecenoates to rats for 3 months followed by a 15% fat diet free of the trans-isomers. The percentage values of trans-octadecenoates in plasma attained at the
end of trans-fat feeding were 17% and 22% for phospholipid and triglyceride, respectively. These values were roughly comparable with those observed in the present study and the rate of disappearance of plasma trans-octadecenoates (20 to 30% of the initial value 2 weeks after discontinuation of trans-fats (12)) was very close to the results obtained currently. Ohlrogge (19) and Emken et al. (20) compared the absorption, distribution and the fate of deuterated trans- and cis-13-octadecenoic acids to those of cis-9-octadecenoic acid in plasma lipids of adult male subjects. Although slight differences existed among various fatty acids, the labeled fatty acids were readily incorporated into plasma lipids and then rapidly cleared from the circulation. The rate of disappearance differed depending on the lipid components; the labels disappeared rapidly from triglyceride, while were retained for a relatively long period in phosphatidylcholine. However, dietary factors influencing the rate of metabolism of trans-acids were not investigated in these studies.

Neither the level nor the type of dietary fat apparently influenced the rate of disappearance of trans-fatty acids in the serum, although the initial rate was somewhat high when the high fat diet was fed (Figs. 1 and 2). The most rapid reduction observed in rats fed on the high fat diet was presumably due to the increased oxidation and catabolism of fatty acids (21, 22), insofar as the rate of oxidation is comparable between cis- and trans-monoene fatty acids (23). In contrast, the content of hepatic trans-fatty acids was influenced by the type, but not the level of dietary fat (Table 3). The significantly greater retention of trans-octadecenoates in rats fed safflower oil than in those fed olive oil is in line with the observation that a dietary fat-dependent increase in fatty acid oxidation activity is more prominent in rats fed dietary fat rich in oleic acid than in those fed fat rich in linoleic acid (24).

In addition, the level of protein in the diet also seemed to affect the rate of metabolism of trans-fatty acids; the percentage of trans-octadecenoates was markedly low in rats fed on a high protein diet (Table 1). Since the type and amount of dietary protein affect several aspects of lipid metabolism in experimental animals (25, 26), it is plausible to assume that the dietary protein level also influences the rate of metabolism of trans-fatty acids as well. The effect of dietary cholesterol on the percentage of hepatic trans-octadecenoates was diverse. However, the hepatic concentration was not affected by dietary cholesterol (Table 3).

There appeared to be no reciprocity between the levels of serum and liver lipids (cholesterol and triglyceride) and those of trans-fatty acids throughout (compare Tables 1 and 2 and Tables 3 and 4, respectively). Since the tissue levels of phospholipids are largely uninfluenced in these studies, the same conclusion will be drawn even when the comparison is made on the basis of total lipids, although they were not measured currently. These results at least imply a characteristic feature of the metabolism of trans-monoene fatty acids.

In conclusion, this study provided the evidence that both dietary fat and protein at least affect the rate of metabolism of trans-octadecenoates in rats.
Although the exact nature of this phenomenon and the mechanism by which different dietary components exert their effects remain to be clarified, the available information hopefully suggests that an appropriate dietary manipulation may enhance and stimulate the removal of trans-fatty acids from animal tissues. It is indeed important that the serum level does not merely reflect the amount of trans-fatty acids stored in the tissues.

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REFERENCES

1) Egwin, P. O., and Kummerow, F. A. (1972): Incorporation and distribution of dietary elaideate in the major lipid classes of rat heart and plasma lipoproteins. *J. Nutr.*, **102**, 783–792.
2) Wood, R., Chumbler, F., and Wiegand, R. (1977): Incorporation of dietary cis and *trans* isomers of octadecenoate in lipid classes of liver and hepatoma. *J. Biol. Chem.*, **252**, 1965–1970.
3) Emken, E. A. (1979): Utilization and effects of isomeric fatty acids in human, in Geometrical and Positional Fatty Acid Isomers, ed by Emken, E. A., and Dutton, H. J., American Oil Chemists' Society, Champaign, IL, pp. 99–129.
4) Kummerow, F. A. (1979): Effects of isomeric fats on animal tissue, lipid classes, and atherosclerosis, in Geometrical and Positional Fatty Acid Isomers, ed. by Emken, E. A., and Dutton, H. J., American Oil Chemists' Society, Champaign, IL, pp. 151–179.
5) Blomstrand, R., and Svensson, L. (1983): The effects of partially hydrogenated marine oils on the mitochondrial function and membrane phospholipid fatty acids in rat heart. *Lipids*, **18**, 151–170.
6) Royce, S. M., and Holmes, R. P. (1984): The saturation and isomerization of dietary fatty acids and the respiratory properties of rat heart mitochondria. *Biochim. Biophys. Acta*, **792**, 371–375.
7) Kinsella, J. E., Bruckner, G., May, J., and Shimp, S. (1981): Metabolism of *trans*-fatty acids with emphasis on the effects of *trans*, *trans*-octadecadienoate on lipid composition, essential fatty acid, and prostaglandins: an overview. *Am. J. Clin. Nutr.*, **34**, 2307–2323.
8) Emken, E. A. (1984): Nutrition and biochemistry of *trans* and positional fatty acid isomers in hydrogenated oils. *Ann. Rev. Nutr.*, **4**, 339–376.
9) Ide, T., and Sugano, M. (1984): Oxidation and esterification of *cis*- and *trans*-isomers of octadecenoic and octadecadienoic acids in isolated rat liver. *Biochim. Biophys. Acta*, **794**, 281–291.
10) Lawson, L. D., and Holman, R. T. (1981): Beta-oxidation of the geometric and positional isomers of octadecenoic acid by rat heart and liver mitochondria. *Biochim. Biophys. Acta*, **665**, 60–65.
11) Beare-Rogers, J. C. (1983): *Trans-* and positional isomers of common fatty acids. *Adv. Nutr. Res.*, **5**, 171–200.
12) Moore, C. E., Alfin-Slater, R. B., and Aftergood, L. (1980): Incorporation and disappearance of *trans* fatty acids in rat tissues. *Am. J. Clin. Nutr.*, **33**, 2318–2323.
13) Harper, A. E. (1959): Amino acid balance and imbalance. Part I. Dietary level of protein and amino acid imbalance. *J. Nutr.*, **68**, 405–424.
14) Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957): A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497-509.

15) Sugano, M., Ryu, K., and Ide, T. (1984): Cholesterol dynamics in rats fed cis- and trans-octadecenoate in the form of triglyceride. *J. Lipid. Res.*, **25**, 474-485.

16) Noma, A., Okabe, H., and Kita, M. (1973): New colorimetric microdetermination of free fatty acids in serum. *Clin. Chim. Acta*, **43**, 317-320.

17) Nagata, Y., Imaizumi, K., and Sugano, M. (1980): Effects of soya-bean protein and casein on serum cholesterol levels in rats. *Brit. J. Nutr.*, **44**, 113-121.

18) Yoneda, K., Taga, Y., and Mori, T. (1981): Toukeigaku no Ohyou to Enshuu (Application and Exercises of Statistics), Dobunshoin, Tokyo, pp. 95-99.

19) Ohlrogge, J. B. (1983): Distribution in human tissues of fatty acid isomers from hydrogenated oil. In *Dietary Fats and Health*, ed. by Perkin, E. G., and Visek, W. J., American Oil Chemists’ Society, Champaign, IL, pp. 359-374.

20) Emken, E. A., Adlof, R. O., Rohwedder, W. K., and Gulley, R. M. (1983): Incorporation of deuterium-labeled trans- and cis-13-octadecenoic acids in human plasma lipids. *J. Lipid Res.*, **24**, 34-46.

21) Kalopissis, A. D., Griglio, A., Malewiak, M. I., and Rozen, R. (1980): Effect of a high fat diet on rat very low density lipoprotein secretion. *Biochim. Biophys. Acta*, **620**, 111-119.

22) McGarry, J. D., and Foster, D. W. (1980): Regulation of hepatic fatty acid oxidation and ketone body production. *Ann. Rev. Biochem.*, **49**, 395-420.

23) Ide, T., Yamamoto, I., and Sugano, M. (1984): Effect of dietary fat on lipid secretion and ketone body production in rat liver. *J. Nutr. Sci. Vitaminol.*, **30**, 153-162.

24) Herzberg, G. R., and Rogerson, M. (1981): The role of dietary protein in hepatic lipogenesis in the young rat. *Brit. J. Nutr.*, **45**, 529-538.

25) Portman, O. W., Alexander, M., Neuringer, M., Illingworth, D. R., and Alam, S. S. (1981): Effect of long-term protein deficiency on plasma lipoprotein concentrations and metabolism in Rhesus monkeys. *J. Nutr.*, **111**, 733-745.

26) Huang, Y. S., Cunnane, S. C., and Horrobin, D. F. (1986): Effect of different dietary proteins on plasma and liver fatty acid compositions in growing rats. *Proc. Soc. Exp. Biol. Med.*, **181**, 399-403.