Effect of Simmondsin Derivatives on Food Intake: Dose−Response Curves in Rats

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Simmondsin is a food intake-reducing molecule found in the seeds of the jojoba plant (*Simmondsia chinensis*). Three naturally occurring analogues of simmondsin, simmondsin 2′-*trans*-ferulate, 4-demethylsimmondsin, and 4,5-didemethylsimmondsin, were tested for anorexic properties, and dose response curves were constructed. Demethylsimmondsin and didemethylsimmondsin showed no anorexic properties, while simmondsin 2′-*trans*-ferulate showed dose-dependent food intake-reducing activity, but, on an equimolar basis, was less active than simmondsin. The food intake-reducing activity of two synthetic derivatives of simmondsin, hydroxymethoxyphenylacetonitrile and simmondsinamide, was also determined, and both were found to be inactive.

Keywords: *Simmondsin; simmondsin derivatives; jojoba meal; food intake; rats*

INTRODUCTION

Jojoba meal, the coproduct of oil production from jojoba nuts (*Simmondsia chinensis*), is not used as animal feed, since it causes food intake reduction and emaciation in various animal species (Booth et al., 1974; Ngou Ngoupayou et al., 1982, 1985; Cokelaere et al., 1993; Manos et al., 1986; Arnouts et al., 1993).

In rats and other mammals, the most important anorexigen in jojoba meal, occurring at a concentration of about 5%, is simmondsin [(2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl β-D-glycoside] (Elliger et al., 1973; Booth et al., 1974; Cokelaere et al., 1992, 1996). Jojoba meal also contains lower amounts of simmondsin analogues, namely, simmondsin 2′-*trans*-ferulate, simmondsin 3′-*trans*-ferulate, 4-demethylsimmondsin, 4,5-didemethylsimmondsin, 4-demethylsimmondsin 2′-*trans*-ferulate, and 5-demethylsimmondsin 2′-*trans*-ferulate (Elliger et al., 1974; Van Boven et al., 1994a, 1995).

In rats, simmondsin causes a dose-dependent sustained reduction of food intake (FI) (Cokelaere et al., 1992, 1996). Simmondsin 2′-*trans*-ferulate also induces FI reduction, but at a dose of 0.5%, its activity on a weight basis is only 62.5% of that of simmondsin (Cokelaere et al., 1996). As the molecular weight of simmondsin is only 68% of that of simmondsin ferulate, it has been postulated that equimolar amounts of these two molecules could have the same activity (Cokelaere et al., 1996).

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It has also been suggested that simmondsin exerts its anorexic effect only after metabolization by gut microorganisms (Booth et al., 1974; Verbiscar et al., 1980), and hydroxymethoxyphenylacetonitrile (HMPA) has been proposed as a possible active metabolite (Verbiscar et al., 1980). However, as simmondsin exerts its anorectic effect almost immediately after ingestion and is also active after intraperitoneal injection (Cokelaere et al., 1992; Flo et al., 1997), this hypothesis seems improbable. In order to compare the anorexic effect of various naturally occurring simmondsins and to test the anorexic potency of HMPA and another possible simmondsin metabolite, simmondsin amide (SA), the following experiments were performed.

MATERIALS AND METHODS

Simmondsin and Its Derivatives. Simmondsin, simmondsin 2′-*trans*-ferulate (SF), 5-demethylsimmondsin (DMS), and 4,5-didemethylsimmondsin (DDMS) were extracted from jojoba meal and purified on a silica gel column, as previously described (Van Boven et al., 1994b, 1996); the products used were pure on HPLC.

SA was prepared by hydrolysis of simmondsin with hydrogen peroxide in basic solution, as described previously by Verbiscar et al. (1980), and consisted of a single product when tested by HPLC and TLC. HMPA was prepared according to a modification of the method described by Elliger et al. (1973) as follows. After dissolving simmondsin in 1% NaOH and flushing the solution in nitrogen, it was left for 24 h at room temperature, then the pH was adjusted to 3−4 with hydrochloric acid, and the reaction mixture was stored in a refrigerator for 24 h. The crystals formed were isolated and shown to consist of a single product by HPLC and TLC.

Each component was mixed with normal rat chow at concentrations equivalent, on a molar basis, to a simmondsin concentration of 0.1%, 0.25%, or 0.5%. Table 1 shows the molecular weights of the derivatives and analogues of simmondsin and the proportional weight of the molecules corresponding to 1 g of simmondsin.

Animals, Housing, and Test Conditions. Sixty male adult Wistar rats (270−310 g), divided into 6 groups of 10,

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Table 1. Molecular Weights and Proportional Weight of Simmondsin (S), Simmondsin 2′-*trans*-Ferulate (SF), 4-Demethylsimmondsin (DMS), 4,5-Didemethylsimmondsin (DDMS), Hydroxymethoxyphenylacetonitrile (HMPA), or Simmondsin Amide (SA) To Correspond to 1 g of Simmondsin

	molecule					
	S	HMPA	SA	SF	DMS	DDMS
molecular weight	375	163	393	551	361	347
proportional weight corresponding to 1 g of simmondsin	1	0.43	1.1	1.4	0.96	0.92

were caged in pairs and housed under normal laboratory conditions (light from 08.00 to 20.00, temperature of 20 ± 2 °C, relative humidity of 40−60%). All groups had free access to water and food. Normal rat chow (Carfil, Oud-Turnhout, Belgium) was offered as flour and the 24 h food intake (FI) recorded by weighing the mangers, which were specially designed to avoid food spillage (Scholz, Overijse, Belgium). During the test periods, each group of rats received normal rat food supplemented with simmondsin, HMPA, SA, SF, DMS, or DDMS, at one of the above-mentioned concentrations in a Latin square design. The test periods consisted of 3 days and were separated by at least 4 control days during which normal food was offered in order to avoid any influence of the preceding treatment. FI per pair of rats was recorded daily during the test periods, as described above, and expressed as a percentage of FI on the control day preceding the test days.

Statistical Analysis. The results are expressed as the mean ± SEM and analyzed by ANOVA (Microstat, 1984) with a value of $p < 0.05$ being taken as statistically significant. Regression analysis was calculated using Origin (MicroCal software, 1991).

RESULTS

The control FI was similar in all groups and did not change during the experiment. Therefore, the data for control food intake were pooled, averaging 21.25 ± 0.25 g × rat^{-1} day^{-1}.

Figure 1 shows the dose-dependent FI-reducing effect of simmondsin; the linear regression is: food intake (g) = 19.8(±0.5) − 17.0 (±1.7)*S*%, with *r*, the linear regression coefficient, being 0.84 for an extremely significant correlation ($p < 10^{-11}$). DMS and DDMS did not show any FI-reducing activity. SF caused dose-dependent FI reduction [FI (g) = 21.5(±0.5) − 17.2(±1.5)*S*%, *S*% being the simmondsin concentration that is in molar terms equivalent to the used SF concentration; with $r = 0.91$ and $p < 10^{-11}$]; FI reduction was significant at concentrations of SF equal to, or greater than, 0.35%, equivalent in equimolar terms to a concentration of 0.25% simmondsin. At the same molar concentration, SF had a lower FI-reducing activity than simmondsin over the entire dose−response curve, the difference averaging 10 ± 4% for the different doses and being statistically significant at 0.14% and 0.7% SF, equivalent, respectively, to 0.1 and 0.5% simmondsin.

Figure 2 shows that neither SA nor HMPA caused any FI reduction at the concentrations used.

DISCUSSION

As previously described (Cokelaere et al., 1996), simmondsin produced a dose-dependent reduction in FI. SF was less active than simmondsin in reducing FI, even when used on an equimolar basis. Our earlier

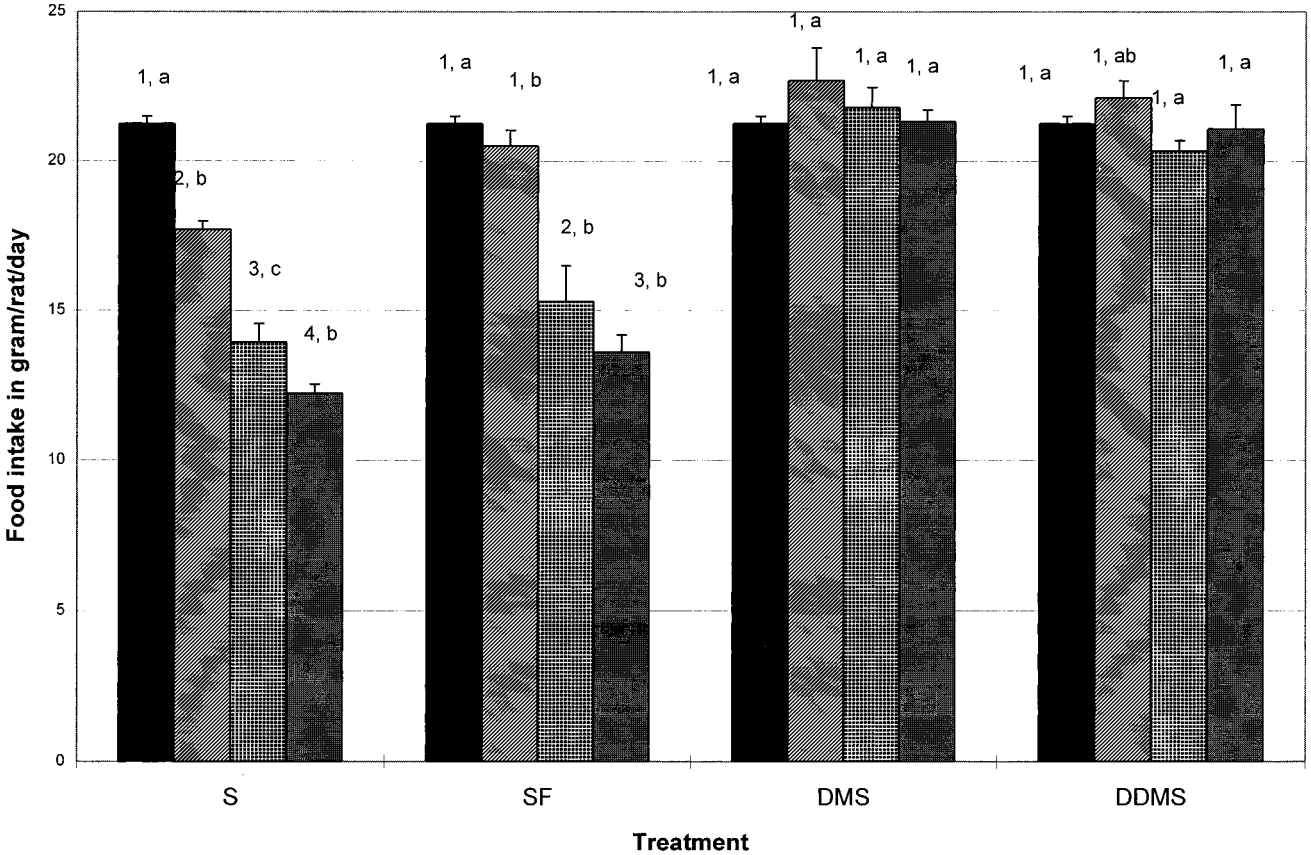

Figure 1. Food intake (FI) expressed as grams per rat per day when 0% (black bars), 0.1% (hatched bars), 0.25% (checked bars), or 0.5% (gray bars) of simmondsin (S), and equivalent doses of simmondsin 2′-*trans*-ferulate (SF); 4-demethylsimmondsin (DMS), and 4,5-didemethylsimmondsin (DDMS), were mixed in the food. The different letters denote significant differences between treatments within a given dose. The different numbers denote significant dose effects within the same treatment.

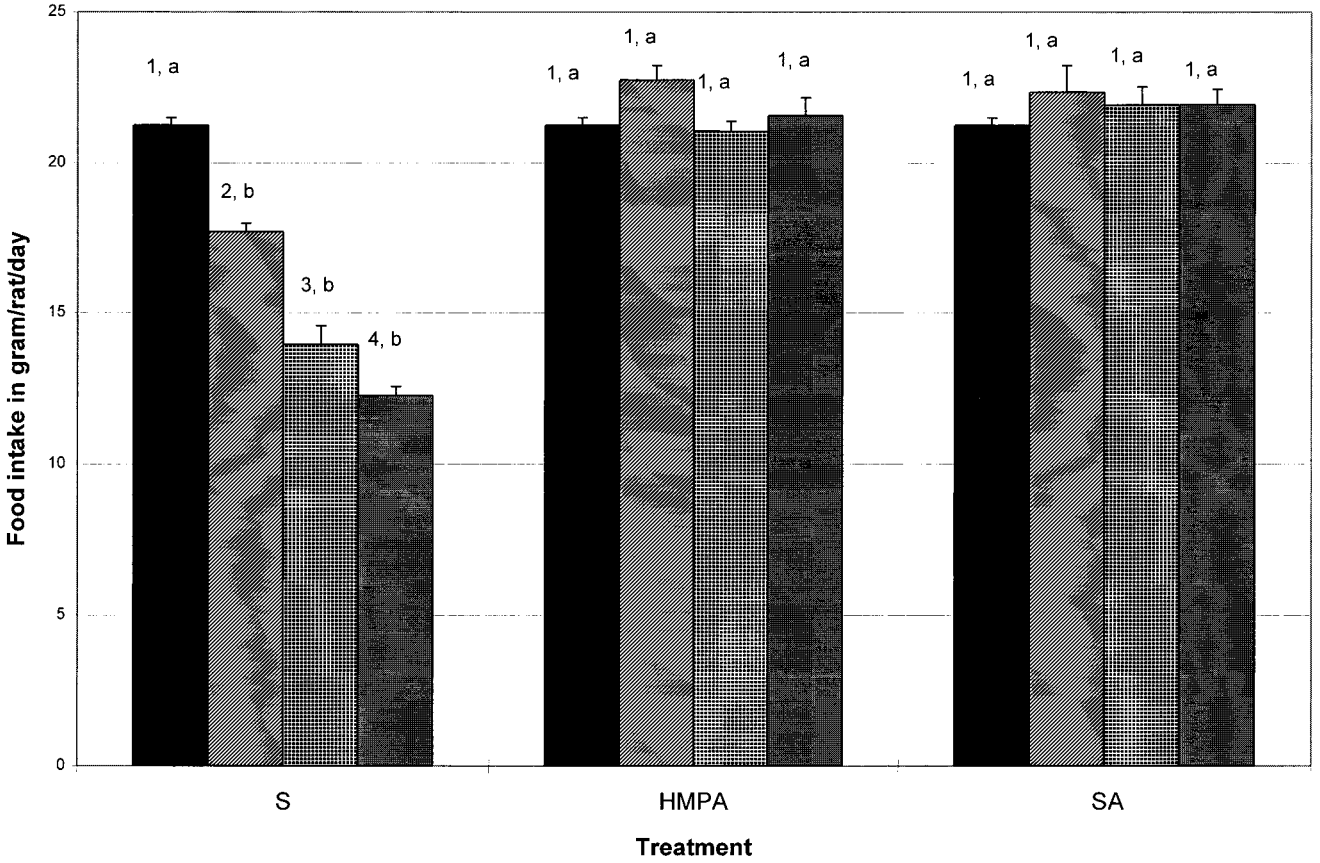

Figure 2. Food intake (FI) expressed as gram per rat per day when different doses (see Figure 1 for symbols) of simmondsin (S), and equivalent doses of hydroxymethoxyphenylacetonitrile (HMPA) and simmondsin amide (SA), were mixed in the food. The different letters denote significant differences between treatments within a given dose. The different numbers denote significant dose effects within the same treatment.

studies showed that, at equal percentage concentrations, SF is about 62% as effective as simmondsin in reducing FI (Cokelaere et al., 1996), and it was thought that the difference in activity resulted from the difference in molecular weight of the two molecules. From the present results, this is obviously not the case, and the FI-reducing activity of SF is somewhat less than the activity of simmondsin, multiplied by 0.68 as a correction factor for the MW.

Two possible explanations can be proposed. First, since it is a different molecule, SF itself has a lower anorexic effect. Second, it is possible that the activity of SF is due to the simmondsin formed after rapid hydrolysis of SF in the acidic environment of the stomach, the somewhat lower activity of SF compared to simmondsin being explained by incomplete hydrolysis.

Since DMS, DDMS, and SA did not have any anorexic effect, it seems that both methoxy groups and the cyanide group are required for the anorexic effect of simmondsin. In addition, the results show that DMS and DDMS do not contribute to the total FI-reducing effect of jojoba meal.

At the concentrations tested, SA had no effect on FI, confirming earlier results (Verbiscar et al., 1980). HMPA also had no effect on FI, even at the highest doses (equivalent to about 165 mg/kg BW), and we can therefore conclude that HMPA has no anorexic activity. This does not exclude that HMPA or SA are formed in the gut or blood.

Intraperitoneal administration of simmondsin or HMPA for several consecutive days to mice had no effect on body weight (Verbiscar et al., 1980, 1982), but the same authors reported that the oral administration of HMPA to growing mice for 14 days, at a daily dose of 120 mg/kg BW, resulted in growth retardation. Unfortunately, the FI was not measured, and the differences in BW were not analyzed statistically. From their observations, the authors concluded that simmondsin must be metabolized in the gastro-intestinal tract before it affects BW gain. However, the in vivo formation of HMPA from simmondsin in the gut remains to be proven, and our previous (Flo et al., 1997) and present results indicate that it is improbable that HMPA is responsible for the anorexic activity of simmondsin.

It is concluded that, of the tested simmondsins occurring in jojoba meal, only S and SF are active as anorexigens.

On an equimolar basis, simmondsin 2′-*trans*-ferulate is about 90% as active as simmondsin in reducing FI.

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