Influence of Pure Simmondsin on the Food Intake in Rats

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The jojoba plant (*Simmondsia chinensis*) and more specifically jojoba meal contain a series of molecules considered to be toxic, with simmondsin [2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl β-D-glucoside] as the most important. Indeed, the extracted and purified simmondsin from jojoba meal caused a food intake reduction in our experiments in adult rats. Taste is apparently not involved because the same response was seen with intragastric intubation as with oral administration. The food intake reduction is probably due to an inhibition of hunger, rather than to an enhancement of satiation. Simmondsin provokes weight reduction probably by an inhibition of food intake because the same weight reduction is seen with simmondsin administration as in pairfed animals. The action of simmondsin is observed within the first hour after oral administration and lasts for several hours. Simmondsin treated with β-glucosidase and taken into the gastrointestinal tract seems to be more active than simmondsin itself with respect to inhibition of food intake.

INTRODUCTION

Since 1974, it has been known that the food intake inhibition seen with jojoba meal, obtained by deoiling the nuts of the jojoba shrub (*Simmondsia chinensis*), is caused by a group of glycosides, with simmondsin [2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl β-D-glucoside] as the most important toxic factor (Booth et al., 1974). Simmondsin is more active than its analogues (Verbiscar et al., 1981). It is possible to obtain this molecule in a pure form (Elliger et al., 1973; 1974a,b; Verbiscar et al., 1978). According to Verbiscar et al. (1980), the aglycon of simmondsin could be the more active metabolite. Besides the reduced food intake caused by simmondsin and its physiological consequences, a direct toxic effect of simmondsin can as yet not be excluded according to Booth et al. (1974) and Verbiscar et al. (1980). A number of intriguing questions remain as yet unanswered such as (1) is the reduction of food intake partially or totally caused by the taste of simmondsin itself, (2) is the weight reduction during simmondsin intake related only to the reduction in food intake or has simmondsin by itself an emaciation effect, (3) is the food intake reduction due to a restraint of appetite or to an enhancement of the satiation feeling, (4) does deglycosylation of simmondsin increase its food intake reduction activity? An attempt to answer these questions was made in the following experiments.

EXPERIMENTAL PROCEDURE

Extraction and Purification of Simmondsin. Hexane-extracted jojoba meal was reextracted with acetone in a Soxhlet extractor for 8 h. After crystallization, the mixture of simmondsin and its analogues, diluted in water, was put on a Sephadex column for preparative chromatography. The simmondsin fraction, pure on TLC (silica gel TLC plates Merck no. 5553; solvent, ethyl-acetate/ethanol 70:30; detection with 10% H_2SO_4) was controlled by IR spectrophotometry. This product had a melting point of 96 °C and an identical R_f value when compared on TLC with an authentic sample provided by Dr. Anthony J. Verbiscar, Anver Bioscience Design. Inc., Sierra Madre, CA. It was dried and lyophilized.

Animals. We used adult, 18-week-old male Wistar rats. They were housed in groups of 3 or 2 in plastic cages, depending on the experimental design. The room temperature was 20–22 °C, the relative humidity 40–60%. Water was offered ad libitum. The normal laboratory mash for rats was presented as meal, in special feeders to avoid spilling of the food.

Experiments. In a first experiment the effect of simmondsin given in the food, or with gastral intubation, on food intake and body weight change was followed and compared with control rats fed ad libitum or pairfed. Twelve rats were divided in four groups of three animals each receiving, respectively, (a) normal mash food ad libitum (group C); (b) 50 mg of simmondsin dissolved in 2 mL of tapwater by gastral intubation once a day at 3:30 p.m. and food ad libitum (group SI); (c) normal food mixed with 50 mg of simmondsin/10 g of food, which is about the amount eaten in 24 h by the rats as tested before (group SM); (d) normal food pairfed with the amount eaten by group SI and SM as a mean; this group started 1 day after the others in order to establish the amount to be given (group PF). After 7 days all animals were returned to normal mash given ad libitum (refeeding period). Food consumption and body weight was monitored daily at 3:30 p.m. Lights were on from 8 a.m. to 8 p.m.

In a second experiment 18 rats were followed for 28 days. They were divided in three groups of six animals each and housed in groups of three. They received, respectively, (a) normal mash food ad libitum (group C); (b) normal food mixed with 50 mg of simmondsin/10 g of food (group S), (c) normal food pairfed with the amount of food daily eaten by the S group (group PF). Food consumption and body weights were followed as described above.

In a third experiment the effect of simmondsin on the hourly food intake was followed with the aim to unravel the question whether simmondsin is causing a restraint of appetite or rather an enhancement of the satiation feeling. Rats had been trained to eat their daily rations between 11 a.m. and 3 p.m. Lights were on from 8 p.m. to 8 a.m. Twenty-two rats were divided into two

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0021-8561/92/1440-1839$03.00/0

Table I. Food Intake during 7 Days of Experiment and 11 Days of Refeeding[a]

	C	SM	SI	PF
mean body weight at start, g	386 ± 29	407 ± 5	460 ± 3	475 ± 22
weight change in 7 days, grams	+7.7 ± 1.2	−37.3 ± 1.4[1]	−49.7 ± 2.9[1]	−47.3 ± 3.7[1]
%	+1.98	−9.17[1]	−11.18[1]	−9.85[1]
food intake, g/day per rat	20.3 ± 0.7	8.9 ± 0.9[1]	8.5 ± 0.6[1]	9[1]
food intake, at refeeding, g/day per rat				
day 1	20.26 ± 0.5	25.7 ± 0.7[1,2]	20 ± 0.3	30.3 ± 0.7[1,2]
mean days 2–4	20.32 ± 0.6	21.9 ± 0.9	21.2 ± 0.7	24.4 ± 0.7
weight gain after refeeding period, g	+6.8 ± 1.1	+38.3 ± 4.5[1]	+48 ± 3.2[1]	+51 ± 1[1]
mean body weight at the end of the experiment, g	400.5 ± 31	408.3 ± 10.7	458.4 ± 4.9	478 ± 19

[a] Controls (C); simmondsin mixed in the food (SM); gastral intubation of simmondsin (SI); pairfeeding (9 g of food/day)(PF); three animals per group; (±SEM. ANOVA: 1, $p < 0.001$ compared to C; 2, $p < 0.001$ compared with SI).

groups. (a) The first group of 12 rats, housed in groups of three, received normal laboratory mash for a period of 3 days to obtain control values of food intake during the feeding time; afterward they received normal mash mixed with 50 mg of simmondsin/10 g of meal for 4 days. (b) After a control period of 3 days, 10 rats, housed in two groups of three and one group of four, received normal mash with 50 mg of simmondsin/10 g of meal during 7 days and then were returned to normal mash for another 4 days to see the effect of refeeding after treatment.

In a fourth experiment the effect of a partial or total deglycosylation of simmondsin before administration to the experimental animals was tested in order to evaluate the effect of deglycosylation on its biological activity. We monitored the daily food intake and body weight of four groups of rats (two rats per group), which obtained normal laboratory mash (meal) ad libitum for 4 days. They were gastrally intubated every day at 3 p.m. with 1.5 mL of acetate buffer solution (pH 5), containing (a) 4.5 mg of β-glucosidase (Boehringer, E.C. 3.2.1.21) (group C); (b) 50 mg of simmondsin (group S); (c) 50 mg simmondsin with 4.5 mg of β-glucosidase (group AS-50); or (d) 25 mg of simmondsin with 4.5 mg of β-glucosidase (group AS-25). The acetate buffer was incubated with the simmondsin during 45 min at 38 °C before intubation. The hydrolysate was put on TLC (as described above) to be compared with a solution of glucose and with pure simmondsin. The amount of glucose generated was measured with a glucose test kit (Boehringer, catalog no. 716.251).

RESULTS AND DISCUSSION

Experiment 1. The results are summarized in Table I. Simmondsin clearly reduced the food intake both when fed orally or given with gastral intubation, without any difference between the two treatments on food intake or body weight depression. Food intake was reduced to respectively 43% and 41% of intake in control animals in the SM and SI group. During refeeding, a higher food intake was observed on the first day in the SM and PF group compared to the C group. Food intake during refeeding of the simmondsin groups and the pairfed group was followed until the animals reached their starting body weight and tended to be slightly higher in the PF group. When expressed per body weight, differences disappeared however (Table I). From the results of this experiment it can be deduced that the effect of simmondsin in the food is not related to a taste effect since intubation of simmondsin or mixing it with feed did not alter the result of food intake depression. During this experiment, no effect of simmondsin per se is observed on body weight changes since changes in body weight were similar for the simmondsin treated and pairfed groups.

Experiment 2. The results of this experiment are summarized in Table II. During the first week, a similar food intake reduction was observed as in the first experiment. The following weeks, the animals adapted somewhat to the product but there remained a food intake reduction of about 32% (Table II). There was no accumulation of the effect of simmondsin. The treated animals have been observed for outward signs of toxicity,

Table II. Daily Food Intake and Weight Changes during 28 Days of Administration of 0.5% Simmondsin Mixed in the Food[a]

	C	S	PF
mean body weight at start, g	325 ± 5	335 ± 8	326 ± 7
weight change in % of body weight	+7.6 ± 0.3	−13.7 ± 1.2[1]	−10.1 ± 1.2[1]
food intake first week, g	19.8 ± 0.9	8.8 ± 0.9[1]	8.8[1]
food intake fourth week, g	20.9 ± 0.7	13.7 ± 0.4[1]	13.7[1]
mean food intake during the experiment, g	20.5 ± 0.6	12.1 ± 0.5[1]	12.1[1]
mean body weight at the end of the experiment, g	350 ± 6	289 ± 20[1]	293 ± 7[1]

[a] Control animals (C); animals offered simmondsin containing food (S); pair-fed animals (PF); six rats per group; (±SEM; ANOVA: 1, $p < 0.001$ compared with C).

but all behaved normally. From the results of this and the first experiment, it can be concluded that there is no effect of simmondsin per se on the body weight changes but that the emaciation can be reduced to the food intake reduction caused by simmondsin. With respect to this point, our findings are in disagreement with the observations of Booth et al. (1974) who found a faster emaciation in the rats receiving a 10% non-detoxified jojoba meal mixed in their food compared to the pairfed animals receiving a 10% soybean meal mixed in their food as a substitution for the jojoba meal. These authors then concluded that simmondsin also had a supplementary effect by itself. We used however a testing process in which the food composition was identical in both the groups to be compared. Jojoba meal by itself is less digestible and so less biologically available than soybean meal (Ngoupayou et al., 1982). Therefore, we think that the enhanced emaciation by jojoba meal, as demonstrated by Booth et al. (1974), is probably due to a lower digestibility of jojoba meal than of soybean meal. However, the results of our experiment can not exclude a toxic effect of simmondsin because no histopathological or haematological investigations have been executed.

Experiment 3. The results of the hourly food intake in both trials are shown in Tables III and IV and in Figures 1 and 2. The food intake of the first day has been indicated separately. In Table IV, the food intake of the first day of refeeding also has been indicated separately. In the control animals, we see a fast food intake the first hour and a remarkable slowing down of the intake in the following hours. With simmondsin administration, we see a practically rectilinear, slowly rising curve during the whole feeding period (Figure 1). This can be seen also in the percentage comparison of the intake between controls and simmondsin-administered animals, where the percentage intake of the simmondsin-eating animals is especially low during the first hour (Table III). The food intake during the first hour is limited to ±23–24% of the normal intake. Subsequently, the reduction is smaller.

Table III. Hourly Food Intake (in Grams) and Percent of the Control Value in 12 Rats during a Control Period of 3 Days (C) and a Period of Simmondsin Intake of 4 Days (S)(50 mg of Simmondsin Mixed/10 g of Meal)[a]

		S			
		day 1		days 2–4	
hour	C	intake, g	%	intake, g	%
1	7.9 ± 0.5	1.8 ± 0.2[1]	23	1.9 ± 0.1[1]	24
2	3.2 ± 0.1	1.9 ± 0.3[1]	61	1.4 ± 0.4[1]	44
3	4.3 ± 0.4	1.8 ± 0.2[1]	42	1.7 ± 0.3[1]	40
4	4.1 ± 0.2	2.1 ± 0.3[1]	50	0.5 ± 0.2[1]	11

[a] Mean body weight at start, 393 ± 11 g (±SEM; ANOVA: 1, $p < 0.001$ compared with C).

Table IV. Hourly Food Intake (in Grams) and Percent of the Control Values (C) in 10 Rats Taking 50 mg of Simmondsin/10 g of Meal for 7 Days (S) and Being Refed with Normal Meal for 4 Days (RF)[a]

				RF	
	C	S		day 1	days 2–4
hour	intake, g	intake, g	%	intake, g	intake, g
1	8 ± 0.1	1.3 ± 0.1[1]	16	10.4 ± 0.6	10.5 ± 0.3
2	2.5 ● 0.2	0.9 ± 0.1[1]	35	2.9 ± 0.6	3.1 ± 0.4
3	4.4 ± 0.3	1.1 ± 0.1[1]	24	2.6 ± 0.6	2.8 ± 0.2
4	3.3 ± 0.4	1.4 ± 0.2[1]	41	3.3 ± 0.4	3.4 ± 0.3

[a] Control values obtained during 3 days before experiment (mean body weight at start, 312 ± 6 gram) (±SEM; Anova: 1, $p < 0.001$ compared with C).

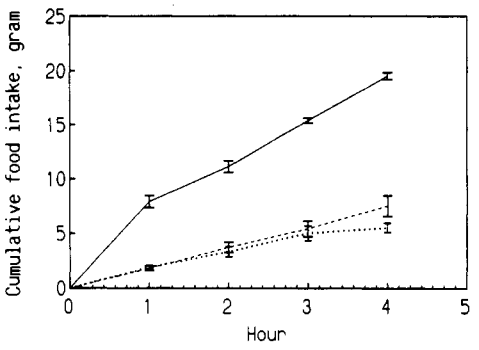

Figure 1. Cumulative food intake (4-h regimen) in control rats (—) (mean of 3 days) and in rats taking 50 mg of simmondsin/10 g of meal; results of the first day (- - -) and mean of the results of days 2–4 (···).

The total intake of simmondsin during the first day was about 78 mg/kg body weight, of which 20 mg/kg body weight was consumed in the first hour, and the following days about 60 mg/kg body weight was eaten, of which 20 mg/kg body weight was ingested in the first hour. When simmondsin is given for a longer period, the pattern of hourly food intake remains similar to that in the first trial (Table IV and Figure 2) but is somewhat lower over the 7 days compared to the rats fed simmondsin for 4 days. Total intake of simmondsin, taking into account the reduction in body weight, was 75 mg/kg body weight and hence was similar to the figure obtained in the first trial. During this experiment, in which the animals ate their daily ration in 4 h, the food intake during the control period seems to be very pronounced during the first hour and to slow down during the following hours (Figures 1 and 2). This seems logical because the animals are hungry at the start of a new feeding period 20 h after their last meals, but from the second hour on their full stomach will cause a satiation feeling. From the results of this experiment (two trials) it can be hypothesized that simmondsin primarily decreased appetite or the sensation of hunger, since the reduction in food intake was greatest during the first hour after feeding (76–84%), while differences in

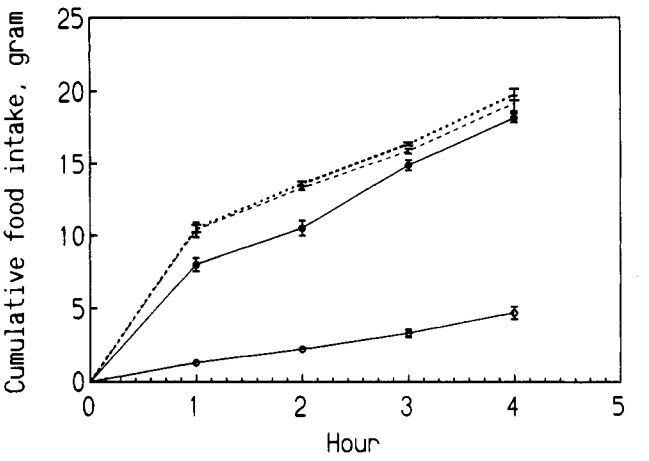

Figure 2. Cumulative food intake in control rats during 3 days (—●—) and in rats taking 50 mg of simmondsin/10 g of meal during 7 days (—●—) or being refed; results of the first day of refeeding (- - -) and mean of the results of days 2–4 (···).

absolute intake between experimental and control groups decreased during the following hours (Tables III and IV). If simmondsin would act solely on the satiety sensation, one should expect a quick increase during the first hour of feeding followed by an early slowdown and a more pronounced decrease in the cumulative food intake curve. However, if simmondsin acts immediately from the first minutes on, the measurement of the food intake after 1 h should not give enough information about a possible effect on satiation. Therefore, the results obtained do not exclude that simmondsin acts by both mechanisms and this may be compatible with the idea of an increased transit time of food through the gastrointestinal tract caused by simmondsin. The somewhat higher reduction in food intake caused by a similar concentration of simmondsin in the second trial could be explained by the differences in mean body weight of the rats in both trials, being 393 and 312 g, respectively, for trials 1 and 2. This resulted in a calculated intake of simmondsin of 60 mg/kg of body weight for the first group and 75 mg/kg of body weight for the second. The difference in food intake reduction between the first and second experiment (only ±56% reduction of food intake for ±120 mg of simmondsin/kg of body weight in the first experiment and ±72% or 75% reduction for, respectively, 60 and 78 mg of simmondsin/kg of body weight in the third experiment) can possibly be explained by the differences in procedure. In the first experiment, the animals had access to food for a period of 24 h but in the third experiment only for 4 h.

Experiment 4. The results of the fourth experiment are summarized in Table V and demonstrate a higher food intake reduction after treatment of simmondsin with β-glucosidase. Food intake reduction was equal for 25 mg of treated simmondsin compared with 50 mg of untreated product, while 50 mg of treated simmondsin had a significant increase in activity over 50 mg of untreated. The TLC of the hydrolysate shows three spots: one with the same R_f (0.21) as glucose, one with the same R_f (0.35) as simmondsin, and one spot of a faster migrating unidentified substance. The amount of glucose formed was measured several times in different hydrolysates. The results were inconstant but suggested a deglycosylation between 0.8% and 2.1% of the total amount of simmondsin.

The results of this experiment could be explained in several ways. Simmondsin is a hydrophilic molecule. Therefore, we can not exclude the possibility that it crosses the intestinal wall only with great difficulties. Several

Table V. Food Intake (in Grams) and Percent of the Normal Intake over 24 h in Rats[a]

	C	S	AS-50	AS-25
mean BW at start, g	423 ± 7	416 ± 11	449 ± 12	404 ± 6
mean BW after 4 days of experiment, g	430 ± 6	403 ± 9	398 ± 10	388 ± 6
% weight change	+1.6	−3.1[1]	−11.3[1,2]	−3.9[1]
food intake, g/day per rat	22.5 ± 0.4	12.7 ± 1.9[1]	3.2 ± 1[1,2]	12.2 ± 0.6[1]
% of normal intake	100	57[1]	14[1,2]	54[1]

[a] (Two rats per group), gastrally intubated for 4 days with 1.5 mL of acetate buffer (C), 50 mg of simmondsin in 1.5 mL of acetate buffer (S), 50 mg (AS-50) or 25 mg (AS-25) of simmondsin in 1.5 mL of acetate buffer preincubated with 4.5 mg of β-glucosidase for 45 min at 38 °C. BW: body weight (±SEM; ANOVA: 1, $p < 0.001$ compared with C; 2, $p < 0.001$ compared with S and AS-25).

authors (i.e. Booth et al., 1974; Verbiscar et al., 1980) accept that simmondsin is broken down to its aglycon in the intestinal tract by the intestinal bacteria and that the aglycon (or a derivative of it) is responsible for the food intake reduction. This aglycon is less hydrophilic and could possibly cross the intestinal wall more easily. It has been demonstrated that glucose can be split off by β-glucosidase by Elliger et al. (1973), but they were unable to separate the aglycon of simmondsin, because their hydrolysate yielded only mixtures of extensively degraded material. However, they incubated the simmondsin for 110 h. After 45 min, we could demonstrate the presence of glucose in the hydrolysate, together with another substance. Therefore, it is supposed but not proved that the aglycon of simmondsin is generated in our experiments and that this aglycon was responsible for the increased effect on feed intake reduction of the hydrolysate. Furthermore, since the small degree of deglycosylation we measured was variable, we cannot calculate how much more potent the aglycon form of simmondsin was than the glycoside form. On the other hand, it cannot be excluded that the aglycon of simmondsin is decomposed further, in the intestines, to one or several derivatives which are responsible for the observed effect. These results strengthen the hypothesis that the aglycon of simmondsin or derivatives of it are responsible for the feed intake reduction seen after simmondsin administration. Future experiments should be aimed at measuring transit time in different parts of the gastrointestinal tract to localize the possible effect of simmondsin or its derivatives on gastrointestinal motility. Moreover, we have to work out a microanalysis of the feeding pattern to discriminate more clearly between the effect of simmondsin on the hunger sensation and/or the satiation feeling. Further physiological evidence is also needed as well as overall body metabolism measurements and toxicity studies to strengthen the hypothesis that the simmondsin effect can be attributed totally to its food intake inhibition.

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Received for review January 9, 1992. Revised manuscript received May 22, 1992. Accepted June 15, 1992.