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TCDD-Induced Anorexia and Wasting Syndrome in Rats: Effects of Diet-Induced Obesity and Nutrition

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TUOMISTO, J. T., R. POHJANVIRTA, M. UNKILA AND J. TUOMISTO. *TCDD-induced anorexia and wasting syndrome in rats: Effects of diet-induced obesity and nutrition.* PHARMACOL BIOCHEM BEHAV **62**(4) 735–742, 1999.—Interactions of diet and diet-induced obesity, and the characteristic wasting syndrome caused by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were studied in TCDD-resistant Han/Wistar and TCDD-sensitive Long–Evans rats. The rats were made obese by feeding them either a high-energy diet (consisting of chocolate, cheese, and chow) or force feeding. TCDD reduced body weight in a parallel manner in lean and obese rats. The high-energy diet diminished the body weight loss and increased the survival time in L-E rats after a lethal dose of TCDD, while energy supplement with high-fat/low-protein food had an opposite effect. In conclusion, diet-induced obesity and TCDD had additive effects on body weight. Dietary manipulations were able to modify the weight loss and survival time after TCDD. Fat seems to have a negative impact, while carbohydrate or protein may have a positive impact in this respect. The results are in agreement with a view that TCDD-exposed rats have a negative fat balance favoring fat loss. © 1999 Elsevier Science Inc.

TCDD 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Wasting syndrome Body weight Lethality Dietary obesity Fat metabolism Rat

2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) is a highly toxic man-made chemical found ubiquitously in the environment. Even after huge doses of TCDD, acute lethality occurs only after 2 or 3 weeks, following a wasting syndrome with feed intake refusal and consequent body weight loss (15,25). Decrements in body weight are permanent after a sublethal dose, and the new body weight level is defended against feeding challenges such as over- and underfeeding (14,20). A palatable, high-energy diet causes a parallel weight gain in both control and TCDD-exposed rats, and TCDD-treated rats are also fully capable of increasing their feed intake in response to repeated 24-h fasts, though TCDD-treated rats remain at a lower body weight level than the respective control rats. Thus, the effects of TCDD and dietary manipulations on body weight seem to be additive (21). The physiological mechanisms behind the wasting syndrome are unknown, but they can be depicted by a model of body weight set-point that is decreased by TCDD. The set-point is the body weight level that is maintained by physiological regulation and defended against dietary challenges. Even though much research effort has been expended on TCDD and other dioxins, the mechanism of this wasting syndrome, and also the lethality, has escaped detection (15). Depletion of energy stores is an important factor in the lethality, but it is not necessarily the only reason.

Previously we have shown that lesioning of the ventromedial hypothalamic nuclei, which normally leads to hyperphagia and obesity, paradoxically aggravated the wasting syndrome when rats were treated with TCDD (24). This aggravation was not entirely due to obesity as such, because preobese lesioned rats also showed an interaction, although it was less pronounced than that of obese rats, between the effects of TCDD exposure and ventromedial hypothalamic lesion. In the present study a complementary approach was taken by utilizing dietary obesity caused by nutritious and palatable food. Animals with access to such food become hyperphagic and gain weight, but after reverting to normal feed, body weight returns to control level unless the high-energy food has been offered for a long period of time (1).

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We studied the effects of diet and also forced feeding together with TCDD exposure for two reasons; first, to confirm our previous result that obesity as such is not a major aggravator of the wasting syndrome by testing a nonhypothalamic type of obesity; and second, to determine, whether TCDD toxicity (wasting syndrome, body weight loss or lethality) could be altered by dietary manipulations or intragastric forced feeding.

METHOD

Animal Husbandry

Outbred Han/Wistar (*Kuopio*) (H/W) or inbred Long–Evans (*Turku AB*) (L-E) rats were obtained from the breeding colony of the National Public Health Institute, Kuopio, Finland (13). The H/W rats are highly resistant to TCDD, having an LD_{50} value of over 9600 μg/kg, while the L-E rats are highly sensitive, having an LD_{50} value of 10–20 μg/kg (18,26). The rats were 10–17 weeks old at the onset of the experiments, and the age range in a single experiment did not exceed 2 weeks. Female rats were usually used, because in a previous study (24) hypothalamic lesions were carried out in female rats. However, Experiments 5 and 6 had been performed earlier and male rats had been used in those experiments. In Experiment 4, both genders were used. The rats were housed singly in metabolic cages (Tecniplast, Buguggiate, Italy) or wire mesh cages with an identical feeding tunnel to measure feed intake and feed spillage. In Experiments 1 and 3, the feed intake of certain groups was not measured, and these rats were housed in groups in wire mesh cages, five or six rats in each cage. The room temperature in the animal rooms was 21 ± 1°C and relative humidity 50 ± 10%. The lighting rhythm was 12 h on, 12 h off.

The experimental protocols were approved by the Research Animal Committee of the University of Kuopio and Provincial State Office (Institute permission 36-712-93, Experiment permissions 68Zd/6.10.1993, 33Zd/24.5.1993, 66Zd/18.9.1990). Procedures are in compliance with the Finnish Law of Animal Protection (Eläinsuojelulaki 247/96, Eläinsuojeluasetus 396/96).

The TCDD administered was >99% pure as determined by gas chromatography-mass spectrometry. It was dissolved in corn oil as described previously (16) and the rats were dosed 5 ml/kg IP.

Nutrients

The rats had free access to tap water and, if not stated otherwise, regular laboratory animal feed (R3, Ewos, Södertälje, Sweden). The feed was powdered or, if feed intake was not measured, pelleted.

Some rats were offered a palatable, high-energy diet. This comprised a self-selection of the regular feed (energy content 12.6 kJ/g; of this 13% from fat, 25% from protein and 62% from carbohydrate), chocolate (Iso vaalea kilosuklaa, Oy Panda Ab, Vaajakoski, Finland: 23.4 kJ/g; 53, 6, and 41%, respectively), and cheese (Edam 40, Ingman Foods Oy, Finland: 13 kJ/g; 66, 34, and 0%, respectively) ad lib. In addition, a 10% sucrose solution (1.7 kJ/ml) was offered in addition to tap water in Experiment 1, but only a small volume was consumed by the rats after a few days (regardless of TCDD exposure), and it was, therefore, not offered in later experiments.

Other groups were force fed with liquid food injected into the stomach through permanent intragastric cannulas. High-fat/low-protein liquid food was used in Experiments 4 and 6, and an amount of 3.3–4.7 ml was injected manually three times each day. The food consisted of a 50% glucose solution in milk, and corn oil with proportions of about 2.5:1 (energy 17.8 kJ/g; 60% from fat, 2% from protein, and 38% from carbohydrate). A balanced liquid food (Nutrison, N.V. Nutricia, Zoetelmeer, Holland; with a nontoxic amount of 5 ml of 10% formalin per liter as a preservative) was used in Experiments 2 and 5, and it was delivered to rats with a peristaltic pump or offered ad lib in bottles. It contained energy 4.2 kJ/ml; of this 35% from fat, 16% from protein, and 49% from carbohydrate.

Surgery

In forced-feeding studies, intragastric polyethene cannulas (outer diameter 1.0 mm) were placed via laparotomy under ketamine (60 mg/kg IP, Ketalar, Parke-Davis, Barcelona, Spain) and medetomidine (0.5 mg/kg SC, Domitor, Orion-Farmos, Turku, Finland) anesthesia. The cannula was guided subcutaneously between the scapulae and taken out through a small incision. After the operation, the rats were given ampicillin 25 mg/rat SC (Ampivet, Novo Industri, Copenhagen, Denmark) to prevent infections, and aroused with atipamezole (0.25 mg/rat IM, Antisedan, Orion-Farmos, Turku, Finland). The rats were allowed to recover from the operation for about a week.

If the rats were force fed with a peristaltic pump, they were harnessed and attached to the lid of a metabolic cage with a spring that had the tubing inside. The cannula was connected to the pump. The daily amount of food was delivered in several 15-min infusions (in Experiment 2, about 4 ml × 28 infusions; in Experiment 5, about 7 ml × 10 infusions). About 30% of the daily energy was given during the light hours and 70% during the dark hours.

Study Design

The effects of obesity on wasting syndrome was studied with two experiments with resistant H/W rats. In Experiment 1, rats were made obese by offering palatable high-energy food for 16 days before TCDD exposure. In some groups, the high-energy diet was started or ended on the day of exposure. In Experiment 2, obesity was induced by intragastric forced feeding with a peristaltic pump. To avoid different diets in force-fed and control groups, all rats were offered the balanced liquid food.

The effects of nutrition and diet on the wasting syndrome and survival time were studied with four experiments. In Experiment 3, the effect of high-energy diet was studied with sensitive L-E rats. In Experiment 4, L-E and H/W rats were force fed with high-fat/low-protein liquid diet or offered a fixed amount of regular diet. In Experiment 5, L-E rats were force fed with the balanced liquid diet. In Experiment 6, high-fat/low-protein diet was force-fed to L-E rats that also had free access to the regular feed.

Clinical chemistry was studied 11 (L-E) or 14 (H/W) days postexposure in rats with the high-energy or regular diet. Serum free fatty acids, triglycerides, and bilirubin were measured in Experiment 7. In addition, blood glucose levels were measured in Experiment 1.

Clinical Chemistry

Blood glucose was measured with a portable refractometer (Accutrend, Boehringer–Mannheim, Mannheim, Germany). Serum free fatty acids were measured by an enzymatic colorimetric method (23) with NEFA C reagent (Wako Chemicals

GmbH, Neuss, Germany). Serum triglycerides were measured by an enzymatic VIS-photometric method (29). Serum total bilirubin was measured by an acid diazo method (30). A selective chemistry analyzer (Kone Specific, Kone Instruments, Kone Corp., Espoo, Finland) was used for measurements of free fatty acids, triglycerides, and bilirubin. Blood and serum samples were taken without fasting.

Statistics

The groups were statistically compared by the analysis of variance (ANOVA; with repeated measures when applicable), and then Duncan's multiple range test if the ANOVA showed a statistically significant difference. In the case of nonhomogenous variances, nonparametric Kruskal–Wallis H and Mann–Whitney *U*-tests were used. Two-group comparisons were performed by two-tailed Student's *t*-test. The level of significance was set at 0.05. The survival times were compared by Mann–Whitney *U*-test.

RESULTS

Effects of Obesity on the Wasting Syndrome

Experiment 1. The participation of weight regulation systems in TCDD toxicity was studied by challenging female TCDD-resistant H/W rats with palatable, high-energy food for 16 days to cause hyperphagia and obesity before they received a high but nonlethal dose of TCDD (1000 μg/kg IP). Some of the rats were switched from the high-energy diet to the regular feed or vice versa at the time of exposure.

The high-energy diet increased energy intake [2-week average: $F(1, 16) = 121$, $p < 0.001$, ANOVA]. TCDD reduced energy intake in rats both on the high energy and on the regular diets, $F(1, 16) = 33.8$, $p < 0.001$. This led to a subsequent body weight loss (Fig. 1), $F(1, 16) = 33.3$, $p < 0.001$. The body weights decreased similarly in the TCDD-exposed groups eating the different diets, showing no interaction between effects of TCDD and diet.

Effects of diet were further studied by switching the rats from high-energy diet to low-energy diet or vice versa at the time of TCDD exposure. When the high-energy diet was offered until the exposure, the elevated body weight returned to the level of the rats on the regular feed in both TCDD and control groups by day 14 (Table 1). When the high-energy diet was offered starting from the time of exposure, the control rats gained weight, whereas the TCDD-exposed rats managed to maintain their body weight.

Experiment 2. To avoid problems due to obese and lean animals having different diets, 10 H/W rats were force fed with a peristaltic pump to induce obesity, while another 10 rats had free access to the same balanced liquid food. After 18 days, the forced feeding was discontinued, the harnesses were removed, and the cannulas were closed. The rats were allowed to recover for 2 days, and then the rats were exposed to 1000 μg/kg TCDD. The weight difference was 16% between force-fed and freely eating rats at the time of TCDD exposure.

TCDD reduced the body weights of both obese and lean animals, $F(1, 14) = 11.1$, $p = 0.005$, ANOVA, to the same level within two weeks (Fig. 2). Because obese rats had more weight to lose, they ate less than lean rats over this period of time (data not shown). One TCDD-exposed obese rat died 16 days postexposure.

Effects of Nutrition on TCDD Toxicity: Weight Loss and Mortality

Experiment 3. The effects of nutrition on body weight maintenance and survival were studied in TCDD-sensitive L-E rats after a lethal dose of TCDD (50 μg/kg). Female L-E rats were offered either the high-energy diet or the regular diet ad lib after the TCDD exposure.

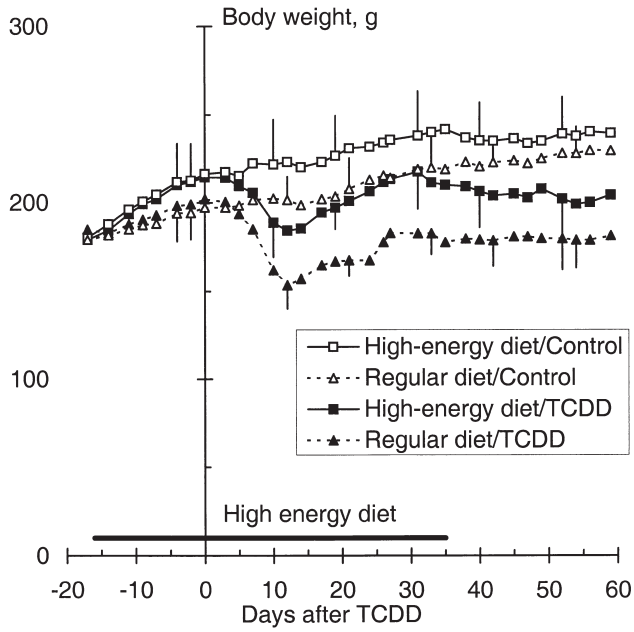

FIG. 1. Body weight (mean ± SD) of H/W rats on the high-energy diet or the regular feed (Experiment 1; $n = 5$). TCDD (1000 μg/kg IP) was administered on day 0, 16 days after the start of diet. Only a few standard deviations are shown for clarity.

TABLE 1

EFFECTS OF HIGH ENERGY AND REGULAR DIETS ON BODY WEIGHT (MEAN ± SD) IN FEMALE H/W RATS (EXPERIMENT 1)

Diet Before/After TCDD	TCDD μg/kg	Body Weight, g		
		Day −17	Day 0	Day 14
Regular/regular	Control	212 ± 13	225 ± 18	233 ± 16
	1000	211 ± 11	221 ± 10	206 ± 12*
High-energy/ regular	Control	207 ± 12	235 ± 15	224 ± 14
	1000	215 ± 17	250 ± 25†	207 ± 28
Regular/ high-energy	Control	208 ± 19	218 ± 21	238 ± 27
	1000	208 ± 10	216 ± 14	215 ± 13

TCDD was administered on day 0. The first diet was provided until day 0, the second diet thereafter; $n = 6$.

Effect of TCDD: $F(2, 60) = 40.2$, $p = 0.000$ (repeated measures ANOVA).

Effect of diet: $F(4, 60) = 22.6$, $p = 0.000$ (repeated measures ANOVA).

*Statistically significant differences vs. control group (Duncan, $p < 0105$).

†Statistically significant difference vs. regular-regular group (Duncan, $p < 0.05$).

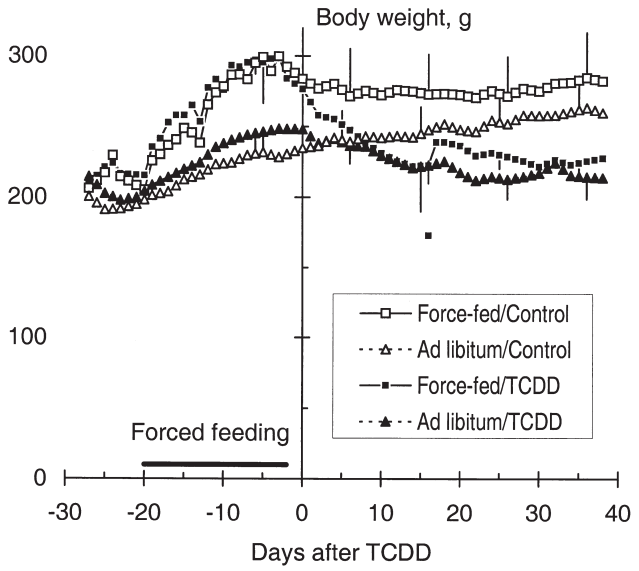

FIG. 2. Body weight (mean ± SD) of H/W rats with intragastric forced feeding or ad lib feeding of the balanced liquid diet (Experiment 2; n = 4–5). Permanent intragastric cannulas were placed on day −27. TCDD (1000 μg/kg IP) was administered on day 0. One force-fed TCDD-exposed rat died on day 16 (an individual mark on that day indicates its body weight). This also results in an increase in the mean weight of the rest of the group.

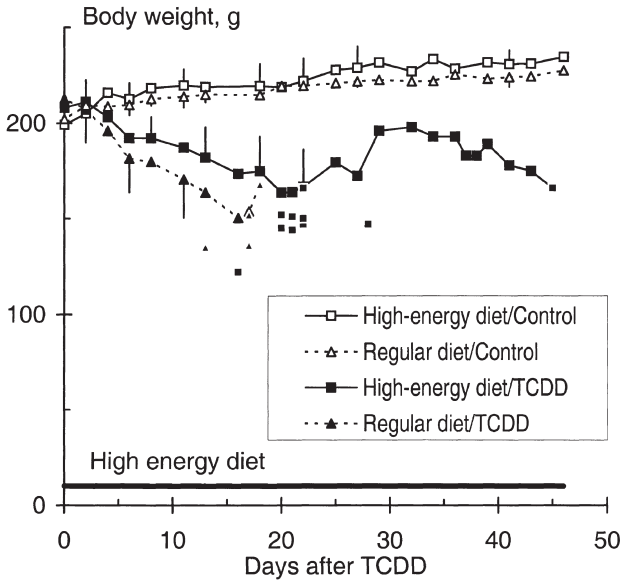

FIG. 3. Body weight (mean ± SD) of L-E rats on the high-energy diet or the regular feed (Experiment 3; n = 5 except for TCDD-exposed high-energy diet group where n = 10). TCDD (50 μg/kg IP) was administered and the feeding regiments commenced on day 0. The death of each nonsurvivor rat is shown individually with the symbol indicating the day, body weight, and group.

The high-energy diet increased body weight slightly compared with the regular diet, $F(1, 21) = 14.1$, $p = 0.001$, repeated-measures ANOVA, although the body weight difference was not significant at any single time point (Fig. 3). The high-energy-diet rats also survived significantly longer than regular-diet rats (Table 3). One high-energy diet rat survived for 45 days, the longest time ever recorded in this strain after a dose of 50 μg/kg.

Experiment 4. Female and male H/W and L-E rats were adapted to forced feeding through intragastric cannulas with small amounts of food for at least a week and exposed to 50 μg/kg TCDD. Then they were given a fixed amount of high-fat/low-protein liquid food only [200 and 183 kJ/day for female H/W and L-E rats, respectively (adjusted to initial mean metabolic body weight)], while other, intact, rats were offered a fixed amount of regular feed only (174 and 159 kJ/day, respectively). The amount of food was below the normal intake of the rats, and this led to weight loss in all groups. The liquid food reduced body weight more than the regular feed despite its higher energy content both in TCDD-treated rats and controls (Table 2). TCDD promoted weight loss slightly, $F(2, 13) = 16.0$, $p < 0.001$; repeated-measures ANOVA), although the differences were not statistically significant at any single time point. No effects of TCDD on body weight were seen in H/W rats or male L-E rats (male data not shown).

Feed-eating TCDD-treated female L-E rats survived significantly longer than rats force fed with high-fat/low-protein liquid food (Table 3).

Experiment 5. To study further the relationship between body weight loss and TCDD lethality, male L-E rats were force fed (294 kJ/day, 7 ml × 10 infusions) with a peristaltic pump for 5 weeks. No additional food was available. The amount of the balanced liquid food was large enough to increase body weight marginally (Table 2). TCDD (50 μg/kg) did not reduce body weight in this experimental setting. Despite this, all TCDD-exposed rats died between days 18 and 33 postexposure (Table 3), while no control rats died during the experiment.

Experiment 6. Male L-E rats were offered the regular feed ad lib with or without additional intragastric liquid food. The amount of the manually infused high-fat/low-protein liquid food was 220 kJ/day. In one group, forced feeding was begun only on day 8 postexposure. This group mimicked the pattern of feed intake recovery often seen in H/W rats (16). Forced feeding reduced voluntary food intake in both control and TCDD (50 μg/kg) groups, but total energy intake increased when compared with the corresponding rats given the regular diet (Fig. 4). TCDD exposure reduced body weight, $F(1, 19) = 28.3$, $p < 0.001$, ANOVA, while different diets had no significant effect. Force-fed TCDD-treated rats lost almost as much weight as regular-diet TCDD-treated rats within eight days (−19 ± 3% and −25 ± 6%, respectively), although the total energy intake had been much greater (231 ± 11 kJ/day and 88 ± 25 kJ/day, respectively). A slightly higher energy intake (280 ± 29 kJ/day) in regular-diet control rats lead to a 5 ± 2% body weight increase. All TCDD-exposed rats, but no control rats, died before day 24 postexposure (Table 3). There were no differences in survival times between the exposed groups.

Clinical Chemistry

Experiment 1. Blood glucose was measured in the H/W rats from the tip of the tail before TCDD exposure and on days 4, 7, and 13 postexposure. There were no significant differences between TCDD-exposed rats and controls (mean ± SD: 5.8 ± 0.8 mM vs. 6.2 ± 0.7 mM, respectively). Time and high-energy diet did not influence the blood glucose levels (data not shown).

Experiment 7. Female L-E and H/W rats were exposed to TCDD (50 and 1000 μg/kg, respectively) with high-energy

TABLE 2

EFFECTS OF INTRAGASTRIC FORCED FEEDING ON BODY WEIGHT (MEAN ± SD) IN TWO RAT STRAINS

Experiment, Strain, Sex	Nutrition	TCDD μg/kg	Body Weight, g		
			Day −16	Day 0	Day 14*
Experiment 4, H/W female†	Liquid food	Control	224 ± 48	206 ± 35	194 ± 29
		50	209 ± 16	197 ± 14	169 ± 10‡
	Regular feed	Control	213 ± 34	206 ± 25	206 ± 19
		50	207 ± 17	204 ± 12	206 ± 5
Experiment 4, L-E female†§	Liquid food	Control	206 ± 7	179 ± 7	160 ± 7‡
		50	212 ± 14	182 ± 11	152 ± 13‡
	Regular feed	Control	211 ± 17	186 ± 13	183 ± 10
		50	210 ± 13	186 ± 11	171 ± 11
Experiment 5, L-E male	Liquid food	Control	—	313 ± 19	334 ± 20
		50	—	313 ± 14	327 ± 9

Rats were fed with a fixed amount of regular feed or force-fed with liquid food.

Day 0 = time of TCDD exposure. Liquid food = liquid food through a permanent intragastric cannula. Regular feed = fixed amount of regular feed offered; $n = 4$ in experiment 5, otherwise $n = 5$–6.

*In Experiment 5, day 15.

†Statistically significant nutritional effect (repeated measures ANOVA, $p < 0.05$).

‡Statistically significant difference vs. Regular feed group (Duncan, $P < 0.05$).

§Statistically significant exposure effect (repeated measures ANOVA, $p < 0.05$).

diet (started on day 0) or regular diet. Serum-free fatty acids, triglycerides, and total bilirubin were measured at 11 (L-E) or 14 (H/W) days postexposure. The mean values of free fatty acids were similar in all groups being 0.53 ± 0.51 and 0.34 ± 0.13 mM in L-E and H/W rats, respectively.

In H/W rats, serum triglycerides were elevated only in control animals on the high-energy diet (1.57 ± 0.32 mM vs. 0.81 ± 0.21–1.06 ± 0.25 mM in other groups; $p = 0.001$, Duncan). These rats consumed large amounts of high-energy food unlike their TCDD-exposed counterparts. In L-E rats, all other groups had elevated triglyceride values compared with the control/regular feed group (1.16 ± 0.57–1.36 ± 0.32 mM vs. 0.60 ± 0.19 mM, respectively; $p < 0.05$, Duncan).

Bilirubin was elevated in TCDD-exposed animals in both strains, but the change was much higher in L-E rats (194 ± 41 μM vs. 1.7 ± 0.4 μM; $p < 0.0001$, Mann–Whitney *U*) than in H/W rats (2.9 ± 0.7 μM vs. 1.4 ± 0.4 μM; $p = 0.0001$, Mann–Whitney *U*). Bilirubin was higher in L-E rats on regular diet than L-E rats on high-energy diet (225 ± 31 M vs. 167 ± 28 μM, respectively; $p < 0.05$, Mann–Whitney *U*). In H/W rats, diet did not affect bilirubin levels.

DISCUSSION

Obesity and TCDD

Diet-induced obesity and TCDD exposure had independent effects on body weight and feed intake in H/W rats after the high-energy diet, as well as after forced feeding. A high-energy diet kept the body weight higher than regular feed also after TCDD exposure. Thus, diet and TCDD exposure seemed to have additive effects on body weight.

TCDD-treated rats respond to dietary challenges similarly to control rats, except that they maintain their body weight at a subnormal level (14,21). When the high-energy diet was withdrawn, the body weights started to converge with those in the respective groups on the regular diet. The same was true of forced feeding. In contrast, the weight loss caused by TCDD remained until the end of experiments. This suggests that TCDD, unlike dietary manipulations, permanently altered body weight and the putative set point (20,21).

We have previously found that rats made obese by ventromedial hypothalamic lesions exhibit a more severe anorexia and lose more weight after TCDD than sham-operated rats

TABLE 3

EFFECTS OF DIETARY MANIPULATIONS ON SURVIVAL TIMES AFTER TCDD (50 μg/kg) IN L-E RATS

Experiment, Sex	Nutrition	Availability of Food	Survival Time, Days Median (Range)
Experiemnt 3, female	Regular feed	Freely	17 (14–18)
	High-energy diet	Freely	21.5 (16–45)*
Experiement 4, female	Regular feed	Fixed	19 (16–)†
	Liquid food (high-fat/low-protein)	Fixed	10.5 (9–18)*
Experiment 5, male	Liquid food (balanced)	Fixed	26.5 (18–33)
Experiment 6, male	Regular feed	Freely	15 (11–21)
	Regular feed and liquid food (high-fat/low protein)	Feed freely; liquid fixed	18 (12–23)
	Same as above; liquid food from day 8 on	Feed freely, liquid fixed	18 (8–20)

$n = 4$–10.

*Statistically significant difference vs. regular feed group (Mann–Whitney U-test, $p = 0.01$).

†Two of five rats survived 19 days.

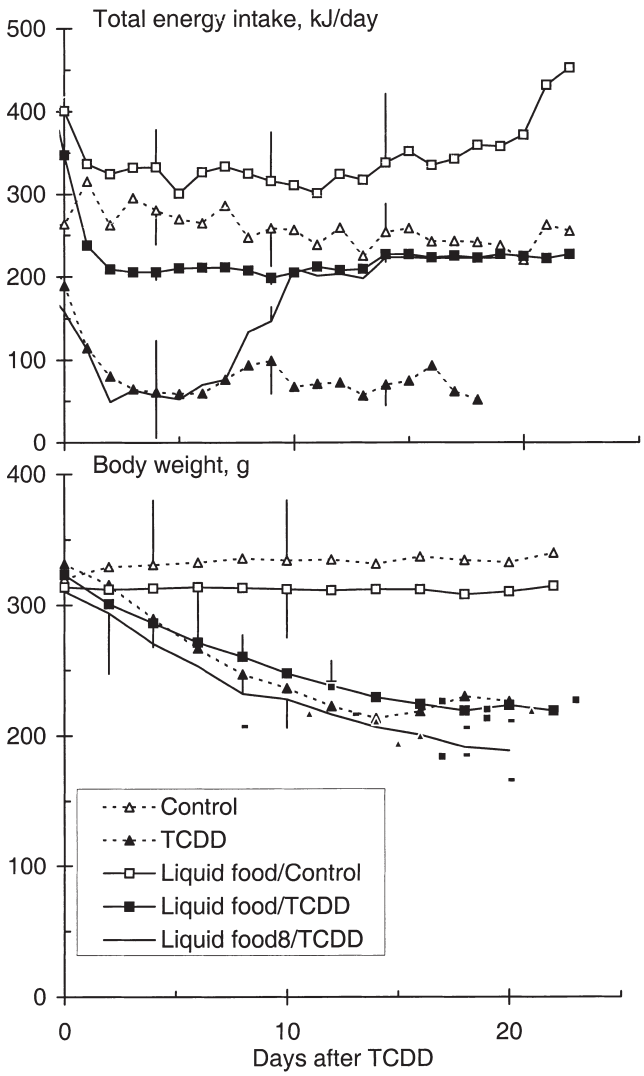

FIG. 4. Total energy intake and body weight (mean ± SD) of L-E rats on ad lib feeding and, in certain groups, with additional forced-fed liquid food through intragastric cannulas (Experiment 6; n = 5–6). TCDD (50 μg/kg IP) was administered on day 0. In one liquid food group, the forced feeding was begun on day 8 (Liquid food8/TCDD, line without symbols). The death of each nonsurvivor rat is shown individually with the symbol indicating the day, body weight, and group.

(24). Thus, the effects of ventromedial hypothalamic lesion and TCDD seem to be interactive rather than additive. This phenomenon was also seen in nonobese lesioned rats (although it was somewhat less pronounced), suggesting that the aggravation of anorexia after TCDD exposure was not due to obesity as such. The present results with dietary manipulations emphasize this view. The difference between the specific hypothalamic lesion and dietary manipulations can be utilized to pinpoint the mechanism and site of action of TCDD on body weight regulation.

Effects of Nutrition

Weight loss. Total energy intake only poorly predicted body weight loss in these experiments. When a restricted amount of food was offered to L-E rats, TCDD-exposed rats lost weight faster than control rats. In addition, the high-fat/low-protein liquid food was force fed to normalize the energy intake, but still weight loss was similar in all TCDD groups. In contrast, there were no differences in body weight compared with control animals when the balanced liquid diet was used to maintain energy intake.

The results with forced feeding imply that the ability to maintain body weight with a certain energy intake is impaired in TCDD rats compared with pair-fed controls, at least when high-fat/low-protein liquid diet is offered. A few previous studies have also implied that TCDD-treated rats lose slightly more weight or reduce slightly less their energy expenditure than their pair-fed counterparts, although the differences have usually not been statistically significant (19,22). However, this difference is seen more clearly after high-fat diet than after high-protein or high-carbohydrate diets (9,10). The low protein content in the liquid food might account for the increased weight loss in the present study, but in the previous studies, the high-fat diets contained normal amounts of protein (9,10). Taken together, these data imply that fat is not a favorable source of energy in preventing weight loss in TCDD-rats.

Mortality. Total parenteral nutrition did not prevent or postpone TCDD lethality in rats (3) or guinea pigs (8), although it could prevent weight loss in rats (3). However, L-E rats on the high-energy diet did survive slightly longer than rats on the regular diet after a lethal dose of TCDD. In addition, median survival time was longer with the balanced liquid food than with additional high-fat/low-protein liquid food, although one must be careful when different experiments are compared. When the high-fat/low-protein liquid diet was the only energy source, the rats succumbed sooner, although they received more energy than the regular diet group.

It seems clear that the increase in the total energy intake alone does not prevent mortality. However, the significant differences in survival times suggest that nutrition may have some influence on this parameter. The survival time correlated better with body weight than with energy intake. The body weights of most L-E rats were below the group average at the time of death (Figs. 3 and 4; otherwise, data not shown). It seemed that a high-fat/low-protein liquid diet, which was unable to prevent weight loss after TCDD, was also unable to delay death. Some macronutrients may be more favorable than others: a high-fat or low-protein diet might shorten survival time, and a diet with sufficient carbohydrate or protein might prolong it. Indeed, Muzi et al. (9,10) reported that a high-carbohydrate diet postponed lethality compared with high-fat or high-protein diets in rats; none of the diets, however, were low in protein (9).

Clinical chemistry. Serum triglyceride levels are increased by nutritional fat, and also slightly by TCDD exposure in L-E rats but not in H/W rats (11,17). In addition, control levels are lower in L-E rats than in H/W rats (11,17). These factors can explain the present results. TCDD elevated triglycerides in L-E rats but not in H/W rats; and high-energy food increased triglycerides only in control groups, as TCDD decreased food intake in both strains irrespective of diet (data not shown).

Bilirubins were elevated in L-E and H/W rats as reported previously (17). However, high-energy diet seemed to slightly attenuate bilirubin increase in L-E rats. The result suggests that this toxic end point may also be slightly modifiable by dietary manipulations, despite the fact that its mechanism is probably not causally linked to that of anorexia.

Free fatty acids showed surprisingly little changes, although TCDD is known to increase this parameter (11,17).

Energy Expenditure and Metabolism

Although the correlation between severe weight loss and mortality after TCDD exposure is well described, it is still not clear if weight loss is causally related to mortality, what biochemical changes underlie the wasting syndrome, and what the mechanisms of the putative changes in fat metabolism are (fat being an unsuitable form of energy).

Food restriction and weight loss cause a compensatory decrease in energy expenditure (5,19). There was a tendency, although not statistically significant, of higher oxygen consumption in TCDD-exposed than pair-fed rats (19). According to the set-point model, the compensatory reduction in energy expenditure may be smaller in TCDD animals until they have reached their lowered body weight set point. Indeed, the difference in weight loss was seen in the sensitive L-E rats, but not in the resistant H/W rats, with a fixed amount of feed or liquid food.

Fat metabolism. TCDD alters intermediary metabolism, but the primary effects are poorly understood. Previously, increased fat mobilization (12), fat combustion (10), and ketogenic rate (7), and decreased de novo fat synthesis (6) have been observed. In addition, TCDD increases liver triglycerides (2,15), and decreases cholesterol synthesis because it can decrease the activities of several lipogenic enzymes (6).

All these findings point to a negative fat balance in TCDD-exposed rats, i.e., increased output (mobilization or metabolism) compared with input (intake in food or storage in cells). Macroscopical observations include a loss of adipose tissue and fat accumulation in liver (15).The primary cause may be, for example, pathologically increased fat mobilization, which is consequently seen as increased fat in blood, liver, and metabolic pathways. The negative fat balance could also explain the present results with high-fat food helping little in preventing weight loss or postponing mortality.

In contrast, there are also data indicating decreased fatty acid oxidation in liver (7), although the whole body oxidation rate was not measured in that study. The exact role of fat metabolism in the wasting syndrome remains to be characterized.

Malabsorption and increased excretion of energy to feces or urine are not major mechanisms involved in the wasting syndrome (22). However, even a slightly restricted fat absorption in intestine could lead to differences in total energy absorption in rats on a high-fat diet.

Carbohydrate metabolism. TCDD increases liver glycogen after a low dose causing a mild reduction in feed intake (2), and after higher doses, reduces the rate of glycogenolysis compared with pair-fed rats (28). This suggests that carbohydrate oxidation or conversion to fat (or protein) are being decreased. The respiratory quotient of TCDD-exposed rats on a high-carbohydrate diet was lowered, indicating increased fat or decreased carbohydrate combustion compared with pair-fed controls (10). However, TCDD may inhibit the key enzyme phosphoenolpyruvate carboxykinase (PEPCK) and reduces gluconeogenesis from alanine (4), which upsets the metabolic balance away from the carbohydrate increase. This could be a compensatory action for a positive carbohydrate balance.

Protein metabolism. Also, protein metabolism may be altered, because urinary ammonia increased 1 week postexposure (19), and some amino acids that are metabolized in liver were increased in serum, while some others were decreased (2). The effect of TCDD on body weight and the liver was more severe in rats given a low-protein diet, but this diet itself decreased weight and caused changes in liver histology, and its role on TCDD toxicity remained unclear (27). Nonetheless, the high-protein diet had no favorable effects on TCDD toxicity (27) or survival time (9). Protein depletion could be a cause of reduced gluconeogenesis, but supplemental protein in the diet had no effect on liver PEPCK activity in TCDD-exposed rats (although it was increased in high-protein diet and high-energy diet control rats), which does not favor this hypothesis (our unpublished observations). Taken together, no major changes related to protein metabolism have been observed, indicating that it is not a major contributor to the wasting syndrome.

It is not clear which effects of TCDD on energy metabolism are primary and which are secondary. However, it seems that dietary manipulations can modify energy metabolism or expenditure in a way that is observable with robust parameters such as body weight or survival time. Further information is needed to understand how intermediary metabolism is linked to TCDD toxicity and what causal relationships exist.

CONCLUSIONS

These results support the hypothesis that dietary obesity does not aggravate the wasting syndrome, but dietary manipulations can have additive effects on body weight changes seen in the TCDD-treated rat. These features are contrary to the effects of lesion of ventromedial hypothalamic nucleus. TCDD lethality could not be prevented with additional energy or other dietary manipulations, although certain diets or manipulations could postpone or hasten death. Carbohydrate or protein may have a positive impact, while fat seems to have a negative impact in this respect. The results are in agreement with a view that TCDD-exposed rats have a negative fat balance favoring fat loss, but a positive carbohydrate balance. Dietary manipulations against this balance would lead to aggravated toxicity. Within this context, many of the presently and previously observed metabolic effects of TCDD could be understood.

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