Influence of chronic ethanol intake on obesity, liver steatosis and hyperlipidaemia in the Zucker *fa/fa* rat

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1. The effect of chronic alcohol intoxication on metabolic disturbances and fatty infiltration and degeneration was studied in genetically obese, hyperlipoproteinaemic, *fa/fa* Zucker rats.

2. Sixteen obese Zucker (*fa/fa*) rats, sixteen lean Zucker rats (*Fa/-*) and sixteen Wistar rats, all male rats aged 7–8 weeks, were given either a control (C) diet (13% of energy from protein, 37% from fat, 50% from carbohydrate) or an ethanol (E) diet (13% of energy from protein, 37% from fat, 14% from carbohydrate, 36% from ethanol) for 4 weeks.

3. The *fa/fa* rats given diet E consumed more energy than those given diet C, but after 4 weeks the weight gains and degrees of obesity were similar for both groups. With both diets, the developed hyperlipidaemia could be explained by the hyperinsulinaemia. Both hypertriglyceridaemia and hypercholesterolaemia were lower in *fa/fa* rats eating diet E than in those given diet C. Fatty infiltration of the liver, as assessed by hepatic triacylglycerol and cholesterol contents, was observed with both diets, but for *fa/fa* rats it was less extreme in those given diet E.

Chronic alcohol intoxication in rats leads to liver steatosis (fatty infiltration and degeneration of the liver) (Lieber *et al.* 1965; De Carli & Lieber, 1967; Baraona & Lieber, 1979; Reitz, 1979) with (Redgrave & Martin, 1977; Lederer *et al.* 1978) or without hyperlipoproteinaemia (Hirayama *et al.* 1979). As the genetically obese *fa/fa* Zucker rat has both these metabolic disorders, we have studied this species to determine if these conditions are exacerbated by chronic alcohol intoxication.

MATERIALS AND METHODS

Animals

Forty-eight male rats were used: sixteen Wistar rats (CESAL, Vigneul-sous-Montmédy), sixteen obese (*fa/fa*) and sixteen lean (*Fa/-*) Zucker rats (CNRS, Orléans-la-Source). They were housed individually in randomly assigned cages and allowed to adapt over a 2-week period, during which they were allowed free access to a standard diet (UAR no. A 04, Villemoisson-sur-Orge) containing (g/kg): protein 170, carbohydrate 587, lipid 30. At 7–8 weeks of age, equal numbers of each strain were randomly assigned to a control (C) or an ethanol-containing (E) diet, and they were allowed free access to food and tap water for a 4-week experimental period. The rats were weighed on arrival and at weekly intervals thereafter.

Diets

The compositions of diets C and E are given in Table 1. The diets were given in a semi-liquid form to simplify ethanol incorporation and preserve a homogenous appearance. The proportion of ethanol in diet E was progressively increased at the expense of maize starch over the first 5 d. Amounts of ethanol given (g/kg dry diet) were 180 for the first 2 d, 240

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Table 1. *Composition of the experimental diets (g/kg dry diet)*

Diet...	C		E	
Ingredients	g/kg dry diet	kJ (%)	g/kg dry diet	kJ (%)
Casein	155	13	193	13
Fat (ground-nut oil)	195	37	244	37
Maize starch	595	50	207	14
Ethanol	—	—	300	36
Salt mixture*	16	—	15	—
Vitamin mixture†	16	—	14	—
Cellulose‡	13	—	15	—
Carrageenan§	10	—	12	—
Water (distilled)	800	—	400	—
kJ/kg dry diet	19870	—	24750	—

* Contained (g/kg mixture): $CaHPO_4$ 430, KCl 100, NaCl 100, $MgCl_2$ 50, $MgSO_4$ 50, Fe_2O_3 3, $FeSO_4 . 7H_2O$ 5, $MnSO_4 . H_2O$ 2·45, $CuSO_4 . 5H_2O$ 0·50, $CoSO_4 . 7H_2O$ 4 mg, $ZnSO_4 . 7H_2O$ 2, stabilized KI 8 mg; no. 205b UAR Villemoisson-sur-Orge.

† Contained (g/kg mixture): retinol 396 mg, cholecalciferol 15 mg, thiamin 2, riboflavin 1·5, pantothenic acid 3·5, pyridoxine 1, Ψ-inositol 15, cyanocobalamin 5 mg, ascorbic acid 80, α-tocopherol 17, menaphthone 4, nicotinic acid 10, choline 136, pteroylmonoglutamic acid 0·5, *p*-aminobenzoic acid 5, biotin 0·03; no. 200 UAR Villemoisson-sur-Orge.

‡ Cellulose (Schleicher Schüll – GmBH).

§ Carrageenan type I (Sigma).

for the next 2 d and 300 on the 5th day. At this stage ethanol supplied 36% of the energy. Protein and fat supplied equal proportions of the energy in the two diets. The food was given fresh daily and daily individual food intakes were recorded.

Sampling and assay techniques

At the beginning and after 2 weeks of the experiment, blood samples were taken from the tail vein under diethyl ether anaesthesia without fasting. On the last day the rats were weighed at 08.30 hours and one rat from each group was killed (in an order that was rotated over the 8 d) between 08.30 and 10.30 hours and blood was taken from the abdominal aorta.

Blood samples were assayed for triacylglycerols (Wahlefeld, 1974), cholesterol (Klose *et al.* 1975) and free cholesterol (Zlatkis *et al.* 1953), glucose (Trinder, 1969) and insulin (Herbert *et al.* 1965).

We have shown that there is no significant difference between venous and arterial blood triacylglycerol and total cholesterol levels, so the values obtained from the different blood samplings can be compared.

The liver was rapidly removed, weighed and homogenized (Ultraturrax Polytron Mixer, Type PT 10–35) in a 0·25 mM-saccharose–1 mM-Tris buffer (Beaufay *et al.* 1974). The liver homogenate was assayed for triacylglycerols and total and esterified cholesterol.

Statistics

Results were compared using Student's *t* test.

Table 2. *Effect on body-weight gain, food intake and food conversion efficiency of giving rats a diet containing alcohol (diet E) or an alcohol-free control diet (diet C)*

(Mean values with their standard errors for eight rats per group)

		Initial body-wt (g)		Body-wt gain† (g)		Food intake‡				Food conversion efficiency§	
						kJ/d		kJ/kg body-wt per d			
Phenotype	Diet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Wistar	C	254	7	81	7	334	13	124	4	8·33	0·12
	E	260	5	70	6	409*	14	174*	5	5·64*	0·04
Zucker *Fa/-*	C	240	3	86[a]	3	338[a]	8	136[b]	2	9·09[a]	0·50
	E	236[b]	6	70[a]	9	401[a,*]	12	186*	4	5.49[a,*]	0·03
Zucker *fa/fa*	C	320[a,c]	11	178[c]	10	480[c]	21	138[c]	5	13·15[c]	0·50
	E	314[a,c]	9	153[c]	6	551[c,*]	15	176*	3	9.80[c,*]	0·33

Values were significantly different (Student's *t* test) ($P < 0·05$): [a] *Fa/- v. fa/fa* on the same diet, [b] *Fa/- v.* Wistar on the same diet, [c] *fa/fa v.* Wistar on the same diet, * diet E *v.* diet C for the same phenotype.

† Body-weight gain during the 4 weeks.

‡ Mean of the food intake measured each day during the 4 weeks.

§ Food conversion efficiency $= \frac{\text{g gained}}{\text{kJ eaten}} \times 10^{-3}$.

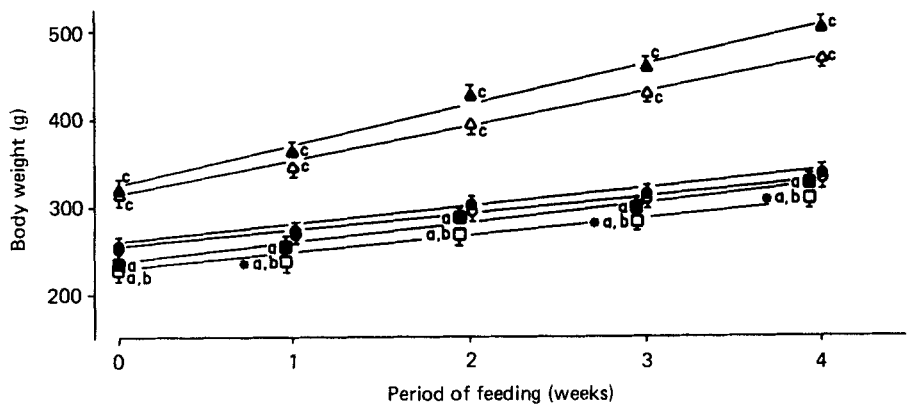

Fig. 1. Weight gain in rats receiving either a control diet (●, ▲, ■) or a diet containing alcohol (○, △, □) of three phenotypes: (○, ●), Wistar; (△, ▲), Zucker *Fa/-*; (□, ■), Zucker *fa/fa*. Points are mean values with their standard errors, represented by vertical bars, for eight rats/group.

Values are significantly different (Student's *t* test) ($P < 0·05$); [a] *Fa/- v. fa/fa* on the same diet, [b] *Fa/- v.* Wistar on the same diet, [c] *fa/fa v.* Wistar on the same diet, * diet E *v.* diet C for the same phenotype.

RESULTS

Food intake and weight gain

Daily energy consumption remained constant during the 4 weeks for any one rat. For all three phenotypes (Wistar, *Fa/-* and *fa/fa*), the rats given diet E consumed significantly more food energy than those given diet C. For the same diet, the non-obese Zucker and the Wistar rats had comparable energy intakes; the *fa/fa* Zucker rats were hyperphagic (Table 2).

The food conversion efficiency of diet C was significantly higher than that of diet E (Table 2). Indeed, although the rats given diet C consumed less energy than those given

Table 3. *Effect on plasma lipids of giving rats a diet containing alcohol (diet E) on an alcohol-free control diet (diet C)*

(Mean values with their standard errors for eight rats per group)

Phenotype...		Diet C						Diet E					
		Wistar		*Fa/-*		*fa/fa*		Wistar		*Fa/-*		*fa/fa*	
	Week	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Triacylglycerols (mmol/l)	0	1·33†	0·10	0·65[a,b]†	0·02	2·47[c,†]	0·21	1·78	0·19	0·65[a,b]	0·04	2·40[c,‡]	0·06
	2	2·79	0·30	1·00[a,b]	0·06	11·85[c]	2·39	1·98	0·29	0·87[a,b]	0·09	6·28[c,*]	0·80
	4	2.49§	0·29	1·09[a,b,§]	0·09	10·10[c,§]	1·04	1·90	0·50	0·76[a,b,*]	0·09	7·83[c,§]	0·94
Total cholesterol (mmol/l)	0	1·39†	0·05	1·65[a,†]	0·10	2·22[c,†]	0·15	1·55	0·10	1·65[a]	0·10	2·24[c,†]	0·07
	2	1·70	0·05	1·93[a]	0·12	3·41[c,‡]	0·12	1·62	0·07	1·83[a]	0·15	2·68[c,*]	0·15
	4	1·75	0·23	1·83[a]	0·07	2·89[c,§]	0·07	1·80	0·10	2·74	0·62	2·53[c,*]	0·07[*,c]
Esterified cholesterol (mmol/l)	0	0·88	0·02	1·03†	0·07	1·39†	0·20	1·03	0·10	1·03[a]	0·05	1·42[c,†]	0·05
	2	1·06	0·07	1·29[a]	0·10	2·19[c,‡]	0·10	1·06	0·05	1·16[a]	0·10	1·78[c,*]	0·12
	4	1·16	0·20	1·29[a]	0·18	1·78[c]	0·10	0·98	0·10	1·21	0·20	1·49[c,*]	0·02
Blood glucose (mmol/l)	0	10·2	0·5	9·1[b]	0·2	9·3	0·2	9·5	0·3	8·8[a]	0·2	9·9	0·3
	2	9·6‡	0·6	9·3‡	0·3	10·5‡	1·0	10·2‡	0·4	9·0[b,‡]	0·3	10·0‡	0·4
	4	13·0§	0·5	12·1§	0·8	14·8§	1·1	13·1§	0·3	12·7§	0·4	13·4§	0·9
Plasma immunoreactive insulin (after 4 weeks) (μg/l)		2·0 (*n* 6)	0·2	2·5 (*n* 3)	0·7	8·9[c] (*n* 4)	2·5	1·7 (*n* 5)	0·4	—	—	6·1[c] (*n* 2)	0·6

Values were significantly different (Student's *t* test) ($P < 0·05$); [a] *Fa/- v. fa/fa* on the same diet, [b] *Fa/- v.* Wistar on the same diet, [c] *fa/fa v.* Wistar on the same diet, * alcohol *v.* control for the same phenotype; for the same phenotype on the same diet: † 0 *v.* 2 weeks, ‡ 2 *v.* 4 weeks, § 0 *v.* 4 weeks.

diet E, they gained more weight, although this was not significant. The Zucker *fa/fa* rats were more efficient converters of food energy than either the *Fa/-* or the Wistar rats, and these two latter groups were equally efficient when eating the same diet. Energy consumption: body-weight was the same for *fa/fa* and *Fa/-* rats.

At the start of the experiment, rats of the same phenotype assigned to the two dietary treatments were of similar weight. The *fa/fa* rats were heavier than the other two types.

Weight gain was not significantly affected by acute ethanol consumption. The *fa/fa* rats grew faster than the other rats on the same diet and the weights of the *Fa/-* and Wistar rats changed similarly (Fig. 1).

Blood biochemical values

Triglyceridaemia increased in all three types of rat during the 4 weeks, and to a greater extent in those given diet E than in those given diet C. For the *fa/fa* rats, values for those eating the two diets were significantly different at the end of the 2nd and 4th weeks (Table 3).

At all times and for both diets the triglyceridaemia in *Fa/-* rats was less than that in Wistar rats.

Hypertriglyceridaemia was present in *fa/fa* rats from the start, and increased significantly (three- to four-fold) over the 4-week period for both diets.

Hypercholesterolaemia (total and esterified cholesterol) in *fa/fa* rats followed the same pattern, although the variations were less than those for the triacylglycerols, and the values for the other strains of rat were close to those obtained for the *fa/fa* rats. The ratio, esterified cholesterol:total cholesterol was similar in the three phenotypes.

All three phenotypes on both diets had comparable blood glucose concentrations and these increased during the experiment.

Blood insulin concentrations were lower in the *fa/fa* rats when alcohol was substituted for starch. However, blood insulin was higher in *fa/fa* rats than in the other phenotypes for both diets.

Liver weight and hepatic lipid assays

At the end of the experiment, total liver weight was greater in *fa/fa* rats than in the other two phenotypes, and within this group those given diet C had heavier livers than those given diet E (Table 4). When expressed as liver weight: body-weight, only the value for *fa/fa* rats given diet C was higher than those for the other experimental groups.

Hepatic triacylglycerol contents were significantly higher in the *fa/fa* rats than in the *Fa/-* and Wistar rats whether expressed as per liver or per g liver. Liver triacylglycerol levels for the *fa/fa* rats were higher when diet E was given than when diet C was given; for the other phenotypes the opposite was the case.

In general, similar results were obtained for cholesterol contents, although the differences were smaller. However, the cholesterol contents of *Fa/-* rat livers (both total and esterified) were not significantly different when the two diets were given.

DISCUSSION

Most published work in this area has been based on the use of Wistar and Sprague-Dawley rats and the effects of alcohol on Zucker *fa/fa* and *Fa/-* rats have not been studied previously. In this discussion, the obese (*fa/fa*) Zucker rat is compared with the lean (*Fa/-*) Zucker rat and the Wistar rat which proved to be closely similar.

Our choice of diet should be briefly justified. It is difficult to administer high concentrations of ethanol to rats over a long period, and the liquid diet of De Carli & Lieber (1967) is frequently used. However, this diet employs dextrin-maltose as a carbohydrate source

Table 4. *Effect on liver weight and hepatic lipid composition of giving rats a diet containing alcohol (diet E) or an alcohol-free control diet (diet C)*

(Mean values with their standard errors for eight rats per group)

Phenotype...	Diet C						Diet E					
	Wistar		*Fa/-*		*fa/fa*		Wistar		*Fa/-*		*fa/fa*	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Liver wt (g)	11·2	0·4	10·9[a]	0·6	20·5[c]	1·0	11·6	0·4	10·2[a, b]	0·3	16·1[c, *]	1·2
Liver wt/body-wt	3·3	0·1	3·3[a]	0·2	4·1[c]	0·1	3·4	0·2	3·3	0·1	3·4*	1·2
Triacylglycerols												
μmol/g liver	23·7	1·6	12·2[a, b]	0·8	109·3[c]	13·4	54·8*	8·0	17·8[a, b, *]	1·2	82·1	12·4
μmol/liver	265·6	19·0	132·0[a, b]	6·8	2223·0[c]	285·0	637·2*	103·7	182·4[a, b, *]	14·8	1291·6[c, *]	189·2
Total cholesterol												
μmol/g liver	21·7	1·3	16·2[a, b]	0·9	34·3[c]	3·3	32·9*	1·8	16·9[a, b]	0·6	30·2	3·2
μmol/liver	244·6	20·7	172·5[a, b]	17·3	695·6[c]	62·0	380·0	27·9	174·3[a, b]	11·9	478·4*	52·7
Esterified cholesterol												
μmol/g liver	14·6	1·4	9·5[a, b]	0·8	26·3[c]	4·5	25·2*	1·4	10·5[a, b]	1·6	22·9	2·3
μmol/liver	166·3	21·7	109·6[a, b]	13·9	523·6[c]	80·9	292·5	21·4	109·1[a, b]	20·2	363·6	41·6

Values were significantly different (Student's *t* test) ($P < 0·05$): [a] *Fa/- v. fa/fa* on the same diet, [b] *Fa/- v.* Wistar on the same diet, [c] *fa/fa v.* Wistar on the same diet, * diet E *v.* diet C for the same phenotype.

because of its high solubility, and many studies have shown that, compared with starch, soluble sugars such as sucrose or glucose and fructose promote increased storage of lipid in the liver of Wistar (Laube *et al.* 1973; Reiser & Hallfrisch, 1977; Michaelis *et al.* 1980) and obese (*fa/fa*) Zucker rats (Michaelis *et al.* 1980). In consequence, it seemed wise to avoid rapidly-assimilated sugars in favour of starch, the more usual carbohydrate source for the rat. The starch-based diets used in the present work supplied 36% of the total energy of the diet as ethanol, as did the De Carli & Lieber (1967) diet. Although rats normally eat a solid diet, we considered that the use of a semi-liquid diet would be acceptable as Ozelci *et al.* (1978) have demonstrated that lipid storage in Sprague–Dawley rats is unaffected by the physical form of the diet.

Food intakes and development of obesity in the fa/fa *Zucker rat after alcohol intoxication*

In the *fa/fa* Zucker rat, hyperphagia promotes, although it is not indispensable to, the development of obesity and hyperlipidaemia (Bray & York, 1979). Energy consumption was higher when diet E was given than when diet C was given for each phenotype studied, but the energy intake was higher for the *fa/fa* rats than the other strains for both diets. The ratio, daily energy consumption: body-weight was higher for diet E than for diet C and did not vary among the phenotypes (*Fa/-* and *fa/fa*), which confirms the report of Michaelis *et al.* (1980).

The rats given diet E had smaller weight gains than rats given diet C, although this was not significant.

The ratio, weight gain: energy consumed, an expression of food conversion efficiency, was higher for the obese (*fa/fa*) rats than for the *Fa/-* or Wistar rats. This greater efficiency can be attributed to the obese rat's increased hepatic and adipose lipogenesis, which leads to increased body fat storage (Bray & York, 1979).

Rats eating diet E all had lower food conversion efficiencies compared with rats given diet C. Indeed, even though these rats consumed more energy, their weight gain did not differ significantly from that of rats given diet C. After alcohol ingestion, energy is lost as heat during the oxidation of alcohol (Pirola & Lieber, 1972; Baraona & Lieber, 1979) and since this energy cannot be used for lipogenesis (Baraona & Lieber, 1979) a lower efficiency of utilization of food energy results. This effect could explain why the alcohol and alcohol-free diets resulted in the same level of obesity for the *fa/fa* rats, despite the different energy consumptions.

Development of liver steatosis in the fa/fa *Zucker rat after alcohol intoxication*

Our results with Wistar rats are in agreement with those of other authors (Lieber *et al.* 1965; De Carli & Lieber, 1967; Lefevre *et al.* 1972; Joly *et al.* 1973; Hirayama *et al.* 1979): hepatic esterified cholesterol and triacylglycerol levels were increased after chronic alcohol intoxication. In metabolically normal rats, the accumulation of hepatic cholesterol and its esters found when diet E was given, seems to be linked to a cholesterol catabolism malfunction (Baraona & Lieber, 1979; Bray & York, 1979) rather than to an increase in cholesterol biosynthesis. Although the mechanism of action of alcohol is not completely understood, this malfunction could be caused by a decreased bile acid excretion linked to lowered cholesterol-7-α-hydroyxlase (*EC* 1.14.13.17) activity (Laksmanan & Veech, 1977).

Chronic alcohol intoxication elicited different responses in the livers of *fa/fa* Zucker rats as compared with those of the Wistar strain: for the obese rat, giving diet E resulted in lower hepatic total cholesterol and triacylglycerol levels than giving diet C, but the reverse effect was found in Wistar rats. This must be interpreted with reference to diet composition: the 36% of energy supplied by alcohol in diet E corresponded to 36% of the energy supplied

by starch in diet C, while protein and lipid levels remained constant at 50% of the total energy supply. Thus, several workers (De Waard, 1978; Bach *et al.* 1980) and the present authors (unpublished results) have shown that a low-starch diet (330–440 g/kg diet), compared with a control diet (600 g starch/kg diet), decreases the hepatic lipid overload in *fa/fa* rats even though the energy intake levels do not differ significantly.

Hyperlipidaemia in the fa/fa *Zucker rat after alcohol intoxication*

The *fa/fa* rat always had significantly higher plasma triacylglycerol levels than rats of the other strains studied, regardless of diet. Giving diet E resulted in smaller increases in plasma triacylglycerol levels than giving diet C for all three phenotypes.

The *fa/fa* rats remained hyperlipidaemic. It has been reported that after alcohol intoxication the Wistar rat's plasma triacylglycerol levels remain unchanged (Hirayama *et al.* 1979) or increase (Redgrave & Martin, 1977; Lederer *et al.* 1978). Nonetheless, the present results are not directly comparable as in Lederer *et al.* (1978) diet composition was not specified and Redgrave & Martin (1977) used younger rats than those in the present study (body-weight 190–220 g).

The total and esterified plasma cholesterol levels remained constant for the *fa/fa* rats given diet E. Most workers (Lefevre *et al.* 1972; Redgrave & Martin, 1977; Lederer *et al.* 1978; Hirayama *et al.* 1979) have found that total cholesterol is unchanged after chronic alcohol intoxication, although Laksmanan & Veech (1977) reported an increase. Possible explanations for this discrepancy could be that the rats used by Laksmanan & Veech (1977) were young (140–180 g) and that their alcohol was administered by gastric intubation and in drinking-water, although at comparable doses.

The *fa/fa* rat responded differently to alcohol than the two other phenotypes: for the obese rat, giving diet E led to a slight decrease in triacylglycerol levels and a significant decrease in total and esterified cholesterol levels, and the obese rat remained hyperlipoproteinaemic when given diet E.

Neither diet had an effect on the blood insulin contents; it remained relatively unaltered and homeostasis was very efficient since, as other workers have shown (De Waard, 1978; Bray & York, 1979), the obese rats and lean rats had similar blood glucose concentrations.

The present results show that liver steatosis in the obese rats was less when diet E was given than when diet C was given. In a subsequent study (Karsenty *et al.* 1985), an investigation of liver biochemical indices (glycogenesis, enzyme activities) led to the determination of one of the causes of this decrease: lowered hepatic lipogenesis activity in *fa/fa* rats eating diet E. The decrease in liver and adipose tissue lipogenesis and lipid synthesis is insufficient to affect the development of obesity and hyperlipidaemia, so these syndromes remain comparable for both diets.

Therefore, contrary to expected results, the present study shows that liver steatosis and hyperlipidaemia in *fa/fa* Zucker rats are not exacerbated by chronic alcohol intoxication, but rather develop more slowly than with an alcohol-free diet. Obesity, however, develops similarly with both diets.

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REFERENCES

Bach, A., Schirardin, H., Chanussot, F., Bauer, M. & Weryha, A. (1980). *Journal of Nutrition* **110**, 686–696.
Baraona, E. & Lieber, C. S. (1979). *Journal of Lipid Research* **20**, 289–315.
Beaufay, H., Amar-Costesec, A., Feytmans, E., Thines-Sempoux, D., Wibo, M., Robi, M. & Berthet, J. (1974). *Journal of Cell Biology* **61**, 188–212.
Bray, G. A. & York, D. A. (1979). *Physiological Reviews* **59**, 719–809.

De Carli, L. M. & Lieber, C. S. (1967). *Journal of Nutrition* **91**, 331–336.
De Waard, H. (1978). *Nederlands Instituut Voor Zuivelonderzoek*. Ede. 112 pp.
Herbert, Y., Lau, K., Gottlieb, C. W. & Bleicher, S. J. (1965). *Journal of Clinical Endocrinology* **25**, 1375–1384.
Hirayama, C., Nosaka, Y., Yamada, S. & Yamanishi, Y. (1979). *Research Communications in Chemical Pathology and Pharmacology* **26**, 563–569.
Joly, J. G., Feinman, L., Ishii, H. & Lieber, C. S. (1973). *Journal of Lipid Research* **14**, 337–343.
Karsenty, C., Ulmer, M., Chanussot, F., Ratanasavanh, R. & Debry, G. (1985). *British Journal of Nutrition* **54**, 15–20.
Klose, S., Hagen, A. & Greif, H. (1975). Organisation des Laboratoires. Biologie Prospective. Troisième colloque de Pont-à-Mousson, pp. 505–507 [G. Siest, editor]. Paris: L'Expansion Scientifique Française.
Laksmanan, M. R. & Veech, R. L. (1977). *Journal of Lipid Research* **18**, 325–330.
Laube, H., Klöz, H. U., Fussgänger, R. & Pfeiffer, E. F. (1973). *Nutrition and Metabolism* **15**, 273–280.
Lederer, J., Niethals, E., Pottier-Arnould, A. M. & Delhaye-Pottier, C. (1978). *Revue de l'Alcoholisme* **24**, 165–171.
Lefevre, A. F., De Carli, L. M. & Lieber, C. S. (1972). *Journal of Lipid Research* **13**, 48–55.
Lieber, C. S., Jones, D. P. & De Carli, L. M. (1965). *Journal of Clinical Investigation* **44**, 1009–1021.
Michaelis, D. E., Scholfield, D. J., Gardner, L. B. & Cataland, S. (1980). *Journal of Nutrition* **110**, 1409–1420.
Ozelci, A., Romsos, D. R. & Leveille, G. A. (1978). *Journal of Nutrition* **108**, 1128–1136.
Pirola, R. C. & Lieber, C. S. (1972). *Pharmacology* **7**, 185–196.
Redgrave, T. G. & Martin, G. (1977). *Atherosclerosis* **28**, 69–80.
Reiser, S. & Hallfrisch, J. (1977). *Journal of Nutrition* **107**, 147–155.
Reitz, R. C. (1979). *Progress in Lipid Research* **18**, 87–115.
Trinder, P. (1969). *Annals of Clinical Biochemistry* **6**, 24–27.
Wahlefeld, A. W. (1974). *Methods of Enzymatic Analysis*, vol. IV, 2nd ed., pp. 1831–1835. [H. U. Bergmeyer, editor]. London and New York: Academic Press.
Zlatkis, A., Zak, B. & Boyle, A. S. (1953). *Journal of Laboratory and Clinical Medicine* **41**, 486–492.

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