Table II. Cholesterol esterification activity in rat pancreas

Group	Palmitic	Stearic	Oleic	Linoleic	Arachidonic
10% Safflower oil	35.2	38.0	980.0	773.0	575.0
20% Safflower oil	41.2	45.0	1156.0	820.0	597.0
30% Safflower oil	44.6	46.4	1158.0	924.0	758.0
50% Safflower oil	46.0	46.4	1094.0	864.0	652.0
10% Lard	48.2	52.0	1223.0	806.0	581.0
20% Lard	48.6	48.6	1318.0	924.0	724.0
30% Lard	50.0	50.0	1313.0	912.0	768.0
50% Lard	48.1	49.3	1106.0	877.0	697.0

The values are expressed as nmoles of cholesterol esterified/mg protein/h. The enzyme assay system is the same as in Table I, except that 10 mg of pancreatic powder corresponding to 3 mg protein was used as the enzyme source.

with palmitic or stearic acid. A progressive increase is noticed for unsaturated fatty acids; the more the double bonds, the greater the enzyme activity. There is a slight increase in the cholesterol esterification activities as the amount of dietary fat is increased from 10% to 30% in both oil groups. Exceptions are seen in the 20% fat-fed groups with respect to $C_{18:1}$. The activity found is slightly greater for lard groups than for the safflower oil groups when incubated with C_{16}, $C_{18:1}$, C_{18} and $C_{20:4}$. With $C_{18:2}$ a slight decrease is noticed in the lard groups compared to the safflower seed oil groups, except when the rats are on 10% fat. Since the esterification of cholesterol is higher with unsaturated fatty acids, and since, as is well known, the majority of the cholesterol is esterified during absorption, the present work supports the view that unsaturated fatty acids enhance cholesterol absorption. On the other hand, the results show little esterification activity when the intestinal enzymes are incubated with saturated fatty acids. The dietary fats do not seem to change the esterifying capacity of the intestines. This is in agreement with the findings of MURTHY et al.[10] who observed no change in the esterifying activity of the intestines as a result of change in dietary fat given at a level of 10%.

The pancreatic enzymes, like the intestinal ones, esterify the cholesterol preferentially with unsaturated fatty acids (Table II). The esterification is slightly greater with C_{18} than with C_{16} in all the groups of rats. Maximum activity is reached when the enzymes are incubated with $C_{18:1}$. This is in contrast to the enzyme specificity seen in the intestines. This specificity for fatty acids confirms the concept that the pancreatic cholesterol esterifying enzymes are different from the intestinal ones. In pancreas also, a slight increase in the esterification activities is observed as the amount of the dietary fat increases from 10% to 30%. The cholesterol esterification activities of all fatty acids are greater in rats fed lard diets than safflower seed oil diets. This activity is significantly higher in the pancrease than in the intestines – 3.5 to 5 times with respect to C_{16} and C_{18}, 20 times with $C_{18:1}$ and 12–14 times with $C_{20:4}$.

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Lipolytic Response of 'Diabetic' Mice (*db/db*) to Isoproterenol and Propranolol in vivo

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Summary. The genetically diabetic and obese *db/db* mice responded lipolytically to isoproterenol and propranolol similarly to normal mice in vivo. However, considering the large amount of triglyceride in a *db/db* mouse, we conclude that the in vivo response of *db/db* adipose tissue is deficient in magnitude.

Many in vitro studies have shown that the adipose tissue of genetic obese mice (*ob/ob*, *db/db*, A^{vy}/a, A^{y}/a) does not respond to lipolytic agents, especially catecholamines, so well as that of normal mice[1–3]. The major implication of these observations is that the obesity of these mice is caused by a defective lipolytic mechanism. However, ABRAHAM et al.[4] found that *ob/ob* mice responded to catecholamines in vivo. In that study, the *ob/ob* mice were of a strain background[5] different from that of C57BL/6J on which most of the in vitro studies were based. The phenotypic expressions of the *obese* gene and the *diabetes* gene are affected by the strain genome[6–8]. It is therefore difficult to compare the results of ABRAHAM et al.[4] with the results obtained in vitro because of the difference in the strain background. In this study, we investigated the in vivo response of *db/db* mice to isoproterenol. The mice we used were of the same strain background as those we used in previous in vitro studies[1] so that unequivocal comparisons could be made between the in vivo and the in vitro data.

Materials and methods. Genetic diabetic and corpulent *db/db* mice and lean mice (db^{+}/db^{+} normals) of the C57BL/KsJ strain were obtained from the Jackson Laboratory, Bar Harbor, Maine. The animals used were 2 to 4 months of age. They were housed in groups of 3 in filter-capped, transparent plastic breeding cages maintained at about 25 °C with lights on from 06.00 h to 18.00 h. The mice were allowed at least 1 week to become acclimated to our animal room. Purina Laboratory Chow and water were available ad libitum.

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Blood samples (about 150 µl) were collected from the tail in heparinized hematocrit capillary tubes which were then centrifuged at 4 °C in an International Hematocrit Centrifuge. A single blood sample was taken from each mouse after each injection. The time required to collect the blood sample was 1–2 min. All mice were bled between 08.30 h and 12.00 h; no mouse was treated more often than 3 times a week. Plasma samples of 50 µl were assayed for free fatty acid (FFA) concentration by the method of FALHOLT et al.[9], using oleic acid as a standard.

Isoproterenol solutions in saline were prepared just prior to s.c. injection. Isoproterenol hydrochloride was obtained from Eli Lilly and Company and propranolol from Ayerst Laboratories.

Results and discussion. The response of *db/db* and *db*$^+$/*db*$^+$ mice to 0.5 mg/kg isoproterenol, s.c. over 60 min after injection is shown in Figure 1. On an ml plasma basis,

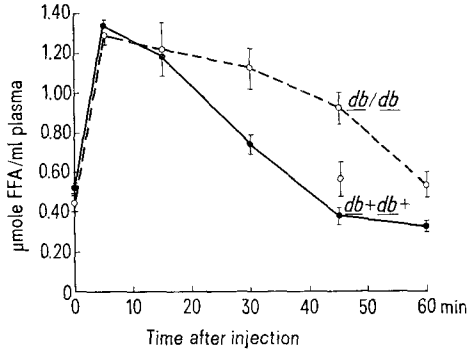

Fig. 1. A comparison of time course of plasma FFA level between *db/db* and normal mice after isoproterenol, 0.5 mg/kg, s.c. The values for each point are mean ± SE from 6 animals. The *db/db* values on the curve are from mice of 40 to 50 g body weight. Their levels at 30, 45 and 60 min were significantly different from the levels of lean mice at corresponding times ($p < 0.005$). The single value at 45 min is from *db/db* mice of about 24 g body weight; their FFA level was not significantly different from the level of lean mice at the same time ($0.10 > p > 0.05$).

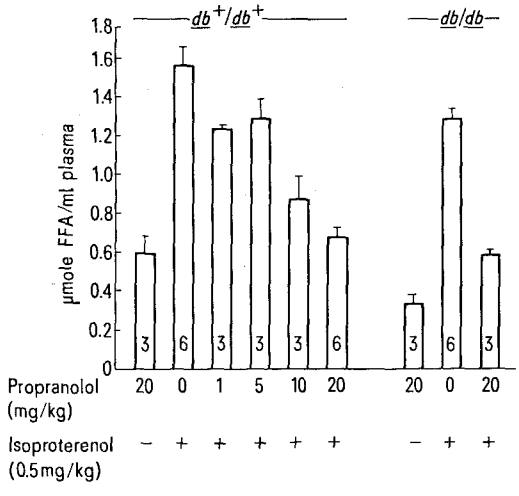

Fig. 2. Effect of propranolol on isoproterenol-stimulated lipolysis. Propranolol was given s.c. 30 or 45 min before isoproterenol. Isoproterenol doses were all 0.5 mg/kg, s.c. Blood was sampled by tail bleeding 5 min after isoproterenol injection. Values are mean ± SE. *N* is noted within each bar.

FFA levels of *db/db* mice were equal to those of normal mice at the peak response time but higher than those of normal mice after the peak. The slow decay of plasma FFA in *db/db* mice could result from: 1. a longer response due to the abundance of substrate triglyceride; 2. a longer response due to the slower degradation of isoproterenol in the large mass of adipose tissue; 3. a slower release of FFA from the larger mass of adipose tissue; and/or 4. a slower clearance of FFA from the plasma, which is governed by the rate of oxidation of FFA in various tissues and the rate of re-esterification of FFA in liver and in adipose tissue. The single value at 45 min in Figure 1 which is not part of the time study is from *db/db* mice that had not developed overt obesity. That value is not significantly different from the plasma FFA concentration of *db*$^+$/*db*$^+$ mice at the same time, suggesting that the slow decay of plasma FFA in corpulent *db/db* mice may be related to their excessive adiposity. By giving a fixed dose (0.5 mg) of epinephrine to people of different weights from normal to very obese, PORTUGAL-ALVAREZ et al.[10] have also observed a more prolonged lipolytic response in obese subjects.

The response of *db/db* mice to propranolol is similar to that of normal mice. Propranolol, an anti-lipolytic, β-adrenergic blocker, given at 20 mg/kg, s.c., 30 or 40 min prior to isoproterenol blocked most of the response of both *db/db* and normal mice to isoproterenol (Figure 2).

Our isoproterenol data on *db/db* mice agree with those of ABRAHAM et al.[4] on the noradrenaline response of *ob/ob* mice in that both *db/db* mice and *ob/ob* mice definitely respond to catecholamines in vivo. However, we do not believe that the in vivo data are in contradiction to the in vitro data, which show very little response of the adipose tissue of these mice to catecholamines[1–3]. These corpulent mice contain from 42% (for an *ob/ob* mouse) to 56% (for a *db/db* mouse) of their body weight as triglyceride whereas normal mice of the same strains have only 12–14% of their body weight in triglyceride (unpublished). A fat mouse that weighs 50 g has 25 g of triglyceride and a normal mouse that weighs 25 g has only about 3 g of triglyceride. The blood volume of a fat mouse does not increase proportionately with its weight. An *ob/ob* mouse or a *db/db* mouse that weighs 50 g has 2.7 ml of blood and a normal mouse that weighs 25 g has 2.1 ml of blood[11]. Therefore, the triglyceride/blood volume ratio is 25 g/2.7 ml = 9 g/ml for a fat mouse and 3 g/2.1 ml = 1.4 g/ml for a normal mouse. In other words, if the tissue of a fat mouse responded to a lipolytic agent to the same extent as a normal mouse, the plasma FFA level of a fat mouse would be approximately 6.5 times (9/1.4) that of a normal mouse. In this study, we observed the same peak response from *db/db* mice and from normal mice (Figure 1). Assuming that the slower decay of plasma FFA in *db/db* mice is entirely due to a longer and continued response because of the abundance of substrate-triglyceride, and has nothing to do with a slower clearance, one could then integrate the area under the time curve as an expression of total FFA released during that time period. With that kind of maximum estimate, the concentration of plasma FFA in *db/db* mice is only 1.5 times that of normal mice. We would thus conclude that both in vivo and in vitro data indicate that *db/db* mice, and for that matter, *ob/ob* mice also, are quantitatively but not qualitatively deficient in lipolysis.

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