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GENETIC VARIATION OF ALKALINE LIPOLYTIC ACTIVITY IN RABBITS

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SUMMARY

Rabbit adipose tissue extracts have been shown to have a "fast" and a "slow" phenotype of alkaline lipolytic activity which have been attributed to two genotypes, FS and SS. The "fast" phenotype is similar to the alkaline lipolytic activity of human adipose tissue in its migration during starch gel electrophoresis and elution from Sephadex G-200. The "slow" phenotype contains less activity, is electrophoretically different, and exists mainly as a smaller molecule than that which comprises the "fast" phenotype. The genetics of this alteration are not yet understood. However, some rabbits appear to inherit a subunit which is altered in charge and thus in electrophoretic mobility. Associated with this alteration is a decrease in measurable activity and a decreased tendency to dimerize.

INTRODUCTION

Alkaline lipolytic activity is a soluble esterase found in human adipose tissue[1,2]. By means of an enzymatic staining method which employs naphthol esters as substrate and Fast Blue B as coupling agent, it has been shown to exist as five isozymes (ALA-1–5) after starch gel electrophoresis[3]. Sephadex gel filtration studies have shown that it exists primarily as two fractions: ALA-II, a dimer responsible for ALA-1–4, and ALA-III, a monomer responsible for ALA-5. In addition, ALA-II can associate with lipid to produce an electrophoretically immobile molecule, ALA-I (ref. 4).

Electrophoretic studies of the adipose tissue of other mammals have demonstrated a characteristic pattern for each of the species studied*. Although screening of over 700 specimens of fresh human adipose tissue has failed to show any genetic variation[4], a genetic variant was observed in the adipose tissue of rabbits, the appropriate matings were made, and the results are reported herein.

Abbreviations: the isoenzymes of alkaline lipolytic activity are designated as ALA-1, etc.

* R. C. RIVELLO, J. A. CORTNER AND J. D. SCHNATZ, unpublished data.

METHODS

Source of tissue

The adipose tissue of adult white New Zealand rabbits was obtained from an open inguinal biopsy under light sodium pentobarbital anesthesia. The adipose tissue of newborn rabbits was obtained from the intrascapular brown fat pads, perinephric fat, and the subcutaneous tissue after sacrificing the animals by exsanguination. No difference in electrophoretic mobility of alkaline lipolytic activity was seen in the adipose tissues obtained from different sites.

Preparation of tissue

Immediately after obtaining the adipose tissue, 300 mg was homogenized with a glass tissue grinder in 1 ml of 0.15 M KCl at 4°. This homogenate was centrifuged at 30 000 × *g* for 10 min and the clear middle fraction recovered. This aqueous extract was stored at 4° until applied to the starch gel within a few hours of removal.

Tissue extracts for Sephadex gel filtration were prepared by homogenizing 500 mg of tissue per ml of 0.15 M NaCl in a Virtis-23 homogenizer at room temperature. This homogenate was centrifuged at 800–1000 × *g* for 30 min at 4°. The fat layer which rose to the top of the tube and the sediment were discarded. The remaining aqueous extract was immediately layered on a Sephadex column for filtration.

Starch gel electrophoresis

Vertical starch gel electrophoresis was performed according to the method of SMITHIES[5]. The end trays contained 0.165 M phosphate–citrate buffer (pH 7.0) and a 1:20 dilution of the same buffer was used in preparing the gel. 0.1-ml samples were placed at the origin in 12 mm × 7 mm × 1 mm slots. The gel was exposed to a voltage gradient of 8 V/cm for 17 h at 4°. After electrophoresis, the gel was sliced horizontally in two sections and the cut surfaces of the gel were examined for their ability to hydrolyze α-naphthyl butyrate[3].

Assay of alkaline lipolytic activity

1 ml of effluent from a column was added to an assay system which contained 11 μmoles of tributyrin, 50 mg of bovine albumin Fraction V, 20 μmoles of sodium phosphate buffer and 20 μmoles of Tris–HCl buffer (pH 8.0) to give a final volume of 4 ml. This was incubated at 47° for 30 min[2]. Aliquots of the assay system were obtained before and after incubation, extracted[6] and titrated[7] for free fatty acids. Activity was expressed as μequiv of free fatty acid released per ml of eluate per h, or as μequiv of free fatty acid released per mg of protein per h. In the latter case, protein was determined by the method of LOWRY *et al.*[8] with crystalline albumin as standard.

Sephadex gel filtration

Sephadex gel filtration was performed in a manner similar to that previously described[4]. 10 g of Sephadex G-200, particle size 40–120 μ, was placed in 0.008 M phosphate–citrate buffer (pH 7.0), allowed to swell, poured into a glass column 2.5 cm in diameter and allowed to settle at 4°.

5–15 ml of fresh adipose tissue extract, 500 mg/ml, was mixed with 2 ml of 0.2% dextran blue 2000 and dextrose to give a concentration of 0.125 M dextrose in the

final volume of extract. This was layered between the column of packed Sephadex and phosphate–citrate buffer and was eluted in 4-ml fractions at a rate of approx. 10 ml/h.

The void volume of the column (V_0) was determined by analyzing the fraction in which the maximum concentration of dextran blue occurred. Every other fraction was analyzed for alkaline lipolytic activity[2] and for electrophoretic mobility of esterase activity on starch gel[3].

MATERIALS

Hydrolyzed starch for use in electrophoresis was obtained from Connaught Medical Research Laboratories and prepared by heating 65 g per 500 ml of buffer. Sephadex G-200, particle size 40–120 μ, and dextran blue 2000 were obtained from Pharmacia Fine Chemicals Co. Tributyrin, 99%, and α-naphthyl butyrate were obtained from Mann Research Laboratories. Polyvinyl alcohol, used in preparing the tributyrin emulsion, was obtained as Grade 52-22 from E.I. DuPont DeNemours and Co. and Fast Blue 2B salt from Matheson, Coleman and Bell. Bovine plasma albumin, Fraction V, was obtained from the Pentex Co.

RESULTS

Analysis of fat from adult rabbits

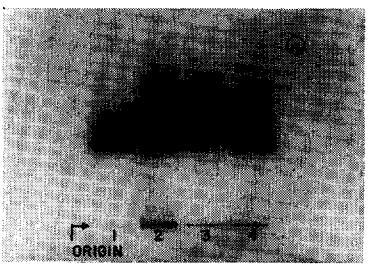
Alkaline lipolytic activity in adipose tissue of white New Zealand rabbits demonstrated two different patterns in terms of starch gel electrophoresis, Sephadex gel filtration and total activity.

Starch gel electrophoresis. Two types of electrophoretic patterns were observed in the adipose tissue extracts of these adult rabbits (Fig. 1). These phenotypes will subsequently be referred to as "fast" and "slow" according to the electrophoretic mobility of the predominant bands. Although Fig. 1 suggests minor variation between the two "fast" phenotypes, multiple comparisons of the two have shown them to be identical. In this combination of four rabbits, each phenotype is represented by a male and a female.

The staining characteristics observed in Fig. 1 suggested that the "slow" phenotype has less total activity than the "fast" phenotype.

Sephadex gel filtration. Two types of Sephadex G-200 elution patterns were observed in the adipose tissue extracts of these adult rabbits (Fig. 2). The "fast" rabbits demonstrated predominance of a fraction which was eluted relatively early and an overall pattern which resembled that of alkaline lipolytic activity Fractions II and III seen with human adipose tissue extracts[4]. The pattern obtained when these eluates were analyzed by starch gel electrophoresis also resembled that seen after Sephadex gel filtration of human adipose tissue extracts. In contrast, the "slow" rabbits demonstrated predominantly a fraction which was eluted from Sephadex relatively late, corresponding with the second fraction observed with extracts of the "fast" rabbits (Fig. 2), and resembled ALA-III of human adipose tissue[4].

As with electrophoresis, the results of Sephadex gel filtration suggested that activity was less in extracts of adipose tissue obtained from "slow" rabbits than those obtained from "fast" rabbits. This was confirmed in two ways. The activity in eluates



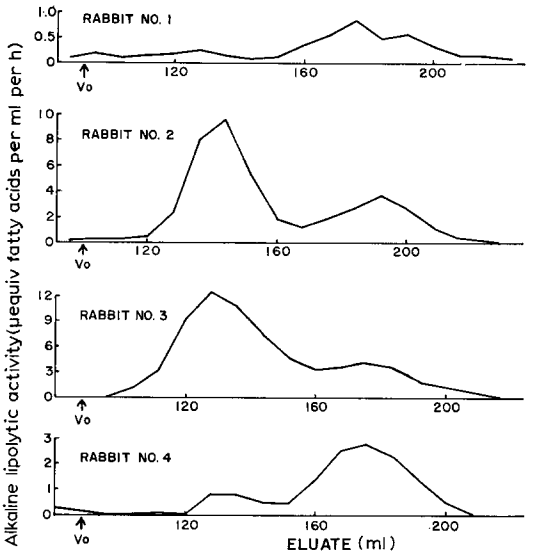


Fig. 1. Alkaline lipolytic activity zymograms of four adult rabbits. Pictured above are the starch gel electrophoretic patterns of adipose tissue extracts from the two "fast" (Lanes 2 and 3) and two "slow" (Lanes 1 and 4) adult rabbits who were subsequently mated in the four possible combinations. Extracts of adipose tissue from female rabbits were placed in Lanes 2 and 4, and from male rabbits in Lanes 1 and 3.

Fig. 2. Sephadex G-200 gel filtration. Adipose tissue extracts were prepared and filtered through Sephadex G-200 as described in the text. The elution patterns for extracts obtained from "fast" rabbits (Nos. 2 and 3) and "slow" rabbits (Nos. 1 and 4) are pictured above. V_0 indicates the void volume for the column at the time that it was run.

obtained from Sephadex gel filtration was expressed in terms of the protein eluted from the column and extracts of adipose tissue obtained from each rabbit were assayed directly. By each method, adipose tissue extracts from "fast" rabbits contained three times as much activity as extracts from "slow" rabbits.

Analysis of fat from baby rabbits

The four rabbits whose alkaline lipolytic activity pattern is demonstrated in Fig. 1 were mated in four combinations, the results of which are depicted in Table I.

TABLE I

ALKALINE LIPOLYTIC ACTIVITY PHENOTYPES OF FOUR ADULT RABBITS AND THEIR OFFSPRING

The four adult rabbits whose adipose tissue extracts were analyzed by starch gel electrophoresis (Fig. 1) and by Sephadex gel filtration (Fig. 2) were mated in the various combinations possible. The phenotypes of the offspring were determined by starch gel electrophoresis and are noted in the table. Number of matings in parentheses.

Matings	*Offspring*	
Male × *female*	*Fast*	*Slow*
Fast Fast (2)	8	8
Fast Slow (1)	4	4
Slow Fast (2)	8	9
Slow Slow (2)	0	9

Three "fast" by "slow" matings resulted in 25 offspring with a 50:50 ratio of "fast" and "slow". Two "slow" by "slow" matings resulted in 9 offspring with only "slow" progeny. It was of interest to note a decreased ability for this "slow" female to become pregnant and the occurrence of smaller litters when mating was carried out with a male of similar phenotype. Unexpectedly, the "fast" by "fast" combination produced an equal distribution of "fast" and "slow" phenotypes among the 16 offspring. In all matings, the sex ratio approximated one with both phenotypes.

Starch gel electrophoresis. The starch gel electrophoretic results of one of the "fast" by "fast" matings are pictured in Fig. 3. These patterns are characteristic of those seen for all "fast" and "slow" offspring of each mating.

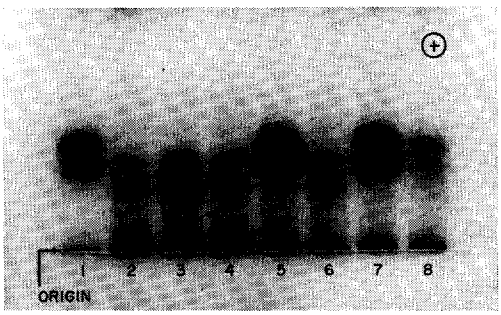


Fig. 3. Alkaline lipolytic activity zymograms of offspring from one mating. On two occasions, the mating of the "fast" male by the "fast" female resulted in four "fast" and four "slow" progeny. Their starch gel patterns are pictured above. Offspring 1, 5, 7 and 8 are examples of the "fast" phenotype and offspring 2, 3, 4 and 6 examples of the slow phenotype.

DISCUSSION

Human adipose tissue extracts have been shown to contain an esterase activity which has been fractionated electrophoretically into five bands and by gel filtration into three fractions. The faster migrating bands (ALA-1–4) are associated with the second fraction eluted from Sephadex, ALA-II, and can become bound to lipid to form the first fraction, ALA-I. The slowest migrating of the five electrophoretic bands (ALA-5) is associated with the last fraction to be eluted from Sephadex, ALA-III. To date, no genetic variants have been noted in the five electrophoretic bands or the three gel filtration fractions.

The present study has examined adipose tissue extracts of white New Zealand rabbits which were prepared in a manner similar to those described for human tissue. Two distinct patterns were observed when these extracts were subjected to starch gel electrophoresis and Sephadex gel filtration. The faster migrating band was associated with predominance of a fraction which was eluted from Sephadex G-200 relatively early and the slower migrating band was associated with predominance of a fraction which was eluted from Sephadex G-200 relatively late. A genetic variant was suggested by the occurrence of these two patterns of alkaline lipolytic activity.

Seven matings of the four adult rabbits (Table I) have lead to the following conclusions: Since the "slow" by "slow" mating resulted in only "slow" progeny, the genotype of each parent is slow (SS). Since the mating of "fast" by "slow" resulted in

both "fast" and "slow" progeny in a 50:50 ratio, the genotype of each "fast" rabbit is (FS). Thus, the expected findings of a "fast" by "fast" mating would be genotypes FF, FS and SS in a ratio of 1:2:1. In actual fact, this mating resulted in a 50:50 distribution of phenotypically "fast" and "slow" progeny. If the genotype FF were phenotypically the same as FS, one would expect a ratio of three "fast" to one "slow" which is close to the 2:2 distribution that was found. On the other hand, the FF genotype may be lethal and thus undetected. An alternative means of detecting the FF genotype, if present, might be by quantitation of activity or by its elution pattern from Sephadex. Each of these are being utilized with current matings which should help to clarify the genetics of alkaline lipolytic activity in rabbit adipose tissue.

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