PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 652–655 (1975)

Age Influence on the Lipolytic Effect of Glucagon in Geese[1] (38872)

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The effect of glucagon on the lipolytic activity of adipocytes isolated from rat epididymal fat is influenced by the age of the donor animal (1). Glucagon has a remarkable lipolytic activity in birds, both in the adipose tissue *in vitro* and in the whole animal (2, 3). In isolated fat cells from chickens, this effect remains constant between 1 and 15 days of age (4), but there is no other information regarding the influence of age on the lipolytic effect of glucagon in birds.

It was observed in this laboratory that the lipolytic effect of glucagon *in vitro* was greater for the tissue of "young" geese (4–8 wk) than for that of "old" geese (over 1 yr of age). However, when glucagon was injected intravenously into intact geese at doses between 1.0 and 20.0 μg/kg, the elevation of plasma free fatty acids (FFA) was greater in the old than in the young animals. The present report describes these results.

Methods. Young geese were brought to the laboratory the day after being hatched and were used between 30 and 56 (av 44.5) days of age. They weighed 0.7–2.4 (av 1.5) kg. The old geese were 1 yr of age when purchased, and were used between 3 and 6 mo thereafter. Average age for the old geese at the time of the experiment was 16.5 mo; their average weight was 4.9 (3.7–6.6) kg. both young and old geese were kept in an air-conditioned room (22–23°) lighted for 10 hr every day, and fed ad lib. a commercial diet (Purina Duck Breeder Layena, Ralston Purina Co., St. Louis, MO).

The lipolytic effect of glucagon was tested *in vitro* as previously described (3), using pieces of the lateral abdominal subcutaneous fat pad. Fat pads were removed under anesthesia with 35 mg/kg (⅔ iv, ⅓ im) of sodium pentobarbital (Somnopentyl, Pitman Moore Inc., Washington Crossing NJ) after 16–18-hr fast. The fat pads of the young animals weighed 7.4 (4.0–11.0) g.; those of the old animals weighed 21.4 (19.5–25.0) g.; and represented the entire range of ages. Tissues of young and old geese were tested on alternate days.

The diameter of the adipocytes was measured in cells isolated by digestion with collagenase, as described by Di Girolamo *et al.* (5) Number of fat cells was calculated as described by Di Girolamo *et al.* (5) Total lipid was estimated as previously described (6). Density of lipid (37°) was determined by weighing volumes of the lipid extracted from the fat pads, accurately measured with a syringe microbuter (Model No. SB2, Micro Metric Instruments, Cleveland, Ohio), calibrated at 37°.

The effect of glucagon *in vivo* was tested simultaneously in young and old nonanesthetized geese, after 16–18 hr of fasting. Solutions of crystalline glucagon (lot 258-234 B-167-1, Eli Lilly and Co., Indianapolis, IN) in glycine buffer (0.02 *M*, pH 9.5) were prepared daily just before intravenous injection. Each of the doses of glucagon was tested on several different occasions. Blood samples were taken from a wing vein 30 min before, just before injection, and 5, 15, and 30 min after injection. Plasma FFA were determined as previously described (6).

The data were analyzed by standard statistical methods. Unless otherwise stated, the significance of the differences was calculated by the *t* test for paired variables. The significance of the difference between slopes was calculated as described by Brownlee (7).

Results. Effect of glucagon in vitro. The production of FFA (μmoles/g/hr) induced by glucagon was significantly greater in the

[1] Supported by NIH Grant 1 R01 HD 07799-01, and by the Mount Sinai Hospital Research Fund.

[2] Dr. Gonzales Santos is the recipient of a Fulbright Scholarship under the Program of Cultural Cooperation between U.S.A. and Spain.

TABLE I. EFFECT OF GLUCAGON ON IN VITRO FFA AND GLYCEROL PRODUCTION BY ADIPOSE TISSUE OF YOUNG AND OLD GEESE. VALUES ARE DIFFERENCES BETWEEN GLUCAGON-TREATED AND CONTROL[a] TISSUE (NO GLUCAGON). MEAN ± SE FOR NUMBER OF TISSUES GIVEN IN PARENTHESES.

	Glucagon concentration, μg/ml		
	0.05	0.5	5.0
		FFA μMoles/g/hr	
Young geese (14)	16.3 ±1.92*	18.7 ±2.16*	19.4 ±2.00*
Old geese (19)	6.0 ±0.79*	8.3 ±1.13*	10.2 ±1.38*
Difference young minus old (*t* test for nonpaired variates)	10.3 ±1.88*	10.4 ±2.27*	9.2 ±2.34*
		Glycerol, μMoles/g/hr	
Young geese (14)	6.0 ±0.60*	6.6 ±0.61*	6.8 ±0.49*
Old geese (19)	2.1 ±0.30*	2.9 ±0.39*	3.5 ±0.52*
Difference young minus old (*t* test for nonpaired variates)	3.9 ±0.62*	3.7 ±0.70*	3.3 ±0.73*

[a] Control values were: FFA 1.3 ± 0.20 for the old and 1.7 ± 0.30 for the young; glycerol 1.2 ± 0.24 for the old and 1.1 ± 0.26 for the young.

* $P < 0.01$.

adipose tissue of young geese than in that of old geese, for each of the doses tested (Table I). On the average, the effect of glucagon on FFA production by young geese adipose tissue was 2.2 times that observed in the tissue of the old animals. Similar results are shown by the glycerol production data.

Effect of glucagon in vivo. The elevations of plasma FFA above the corresponding preinjection levels, observed 5 min after injection of various glucagon doses, are shown in Table II. There was no significant difference between young and old geese for the dose of 0.5 μg/kg. However, each of the doses between 1.0 and 20.0 μg/kg caused significantly greater elevations of plasma FFA in the old than in the young geese. As previously observed (2), FFA elevations 5 min after injection increased linearly with the logarithm of the dose. There was a significant correlation between $\log_{10}$ of the glucagon dose and FFA elevation. Correlation coefficients were +0.824 for 105 old geese and +0.668 for 78 young geese ($P < 0.01$ for both). The regression equations relating $\log_{10}$ of glucagon dose in ng/kg (x), and FFA elevation in meq/liter (y) were $y = 0.956x - 2.143$, for 105 old geese, and $y = 0.325x - 0.477$, for 78 young geese. The difference between the slopes (0.631) was highly significant (SE ± 0.081, $t = 7.79$ $P < 0.0001$).

The increase in FFA response associated with an increase of glucagon dose was, therefore, significantly greater in the old than in the young animals.

Fat cell number. The number of cells in the adipose tissue of young and old geese was estimated using the distribution of diameters of the isolated fat cells, the total lipid content of the adipose tissue, and the density of the lipid (5). The average cell diameter and the lipid content of young geese adipose tissue were significantly lower than the corresponding values for the old geese. The number of cells per g of adipose tissue of young geese was three times that estimated for the old geese (Table III).

Discussion. The lipolytic effect of glucagon, expressed as *in vitro* production of either FFA or glycerol per g of tissue per hour, was greater in the adipose tissue of young geese than in that of old geese, for each of the doses tested. These results are similar to those reported for the isolated adipose cells of the rat (1). In this preparation the reduction of glucagon effect correlates with the decreased glucagon binding observed in large fat cells (8). It has been

TABLE II. EFFECT[a] OF INTRAVENOUS GLUCAGON INJECTION ON PLASMA FREE FATTY ACIDS (FFA) IN OLD AND YOUNG GEESE. Means ± SE for Number of Geese Given In Parentheses.

	Glucagon dose μg/kg							
	0.2	0.3	0.5	1.0	2.0	3.0	5.0	10.0	20.0
	ΔFFA, meq/liter								
Old geese (105)	—	(7)	(12)	(11)	(15)	(15)	(16)	(15)	(14)
		0.20	0.39	0.75	1.10	1.11	1.39	1.72	1.93
		±0.05*	±0.05*	±0.06*	±0.11*	±0.09*	±0.09*	±0.12*	±0.14*
Young geese	(5)	—	(7)	(10)	(9)	(7)	(14)	(11)	(15)
(78)	0.22	—	0.43	0.56	0.55	0.67	0.67	0.84	0.93
	±0.07**		±0.04*	±0.04*	±0.05*	±0.11*	±0.04*	±0.09*	±0.07*
Difference old minus young (*t* test for nonpaired variates)			−0.04	0.19	0.55	0.44	0.72	0.88	1.00
			±0.073	±0.076**	±0.149*	±0.151*	±0.106*	±0.158*	±0.159*
			NS						

[a] Glucagon effect, ΔFFA: Difference between FFA value 5 min after injection and corresponding mean preinjection value (control). Mean control value (meq/liter) was 1.00 (SE ± 0.29) for the 105 old geese and 0.95 (SE ± 0.23) for the 78 young geese.

* $P < 0.01$.

** $0.05 > P > 0.01$.

NS = $P > 0.1$.

postulated that the resistance to glucagon of the large fat cells of older rats is adequately explained by their elevated phosphodiesterase activity and reduced glucagon binding (9).

The glucagon-induced lipolysis per g of adipose tissue from young geese was 2.2 times that observed in the tissue of the old geese for the three doses of glucagon used (Table I). Our estimates indicate that the number of fat cells per g of adipose tissue in the young geese was three times that of the old geese tissue. Accordingly, the lipolytic effect of glucagon per individual cell, was no greater for the young than for the old geese used. These findings are compatible with the view that the greater lipolytic effect of glucagon per g of tissue, observed in the tissues of young geese, might be explained by the greater number of adipose cells present in this tissue, as compared to that of old geese. Since the tissues were taken under nembutal anesthesia, it is possible that this barbiturate influences the lipolytic effect of glucagon. If so, the differences between the tissues from old and young geese could be due to different rates of removal of the anesthetic.

The adipokinetic effect of glucagon in the intact animal for doses between 1.0 and 20.0 μg/kg, was greater in the old than in the young geese. The results suggest that, in the goose, age influences the lipolytic effect of glucagon through factors operating in the whole animal, rather than by a primary change of the fat cell. The old animals had a larger adipose tissue mass than the young. Thus, the average weight of the whole fat pad used in our experiments was 21.4 g for the old and 7.4 g for the young geese. With the data given in Table III, it can be calculated that the total number of adipose cells per pad was 63.6×10^6 for the young geese and 62.1×10^6 for the old. There are obvious limitations in the estimations of the number of fat cells (5), but our data do suggest that the total number of adipocytes per pad tends

TABLE III. CELL DIAMETER, TOTAL LIPID, AND ESTIMATED NUMBER OF FAT CELLS IN ADIPOSE TISSUE OF YOUNG AND OLD GEESE.

	Cell diameter (μm)	Total lipid (%)	Estimated cell number per g[a] (×10⁶)
Young geese (6)	54.8	74.6	8.6
	±4.3	±1.1	±1.5
Old geese (6)	81.2	88.7	2.9
	±5.1	±0.8	±0.6
Difference old minus young	26.4	14.1	−5.7
	±6.7	±1.3	±1.6
P (*t* test for nonpaired variates)	<0.01	<0.01	<0.01

[a] Lipid density (g/ml at 37°), 0.9073 (SE ± 0.0004) for the 12 samples.

Means ± SE for Number of Geese Given in Parentheses.

to be the same in the pads of the old and the young geese, in spite of their different sizes. To the extent that these fat pads are representative of the whole adipose tissue mass of the animals, this would indicate that the total number of adipose cells per animal is likely to be the same in the old and the young geese. Accordingly, the greater elevation of plasma FFA produced by glucagon in the old geese can not be ascribed to the presence of a greater total number of adipose cells in these animals, as compared to the young.

The greater elevation of plasma FFA observed in the old geese could be due to slower removal of circulating fatty acids by these animals, as compared to the younger geese. This possibility, however, has not been explored.

A discrepancy between the results *in vitro* and *in vivo*, with respect to the influence of age on the lipolytic effect of norepinephrine, has been reported by Jelinkova-Tenorova and Hruza (10). These authors noted that the elevation of plasma FFA produced in rats by the intraperitoneal injection of norepinephrine became progressively smaller with increasing age. No difference was found, however, when the effect of norepinephrine was tested *in vitro* with adipose tissue from rats of different age.

Summary. The lipolytic effect of glucagon was measured *in vitro* with adipose tissue of "young" (4–8 wk) and "old" (over 1 yr) geese. The response of the young geese tissue was about twice that observed with tissue of old geese, for glucagon concentrations of 0.05, 0.5, and 5.0 μg/ml. Our estimates indicate that the number of adipose cells per g of adipose tissue of young geese was three times that of the old geese tissue. This suggests that the greater lipolytic response to glucagon, observed in young geese adipose tissue, may possibly be due to its greater cellularity, rather than to a greater lipolytic response of the individual adipocyte.

The lipolytic effect of glucagon *in vivo*, for each of the doses between 1.0 and 20.0 μg/kg, was significantly greater in the old than in the young geese. The slope of the linear equation relating $\log_{10}$ of glucagon dose and elevation of plasma FFA 5 min after injection, was significantly greater for the old than for the young geese. In the goose, therefore, the influence of age on the adipokinetic effect of glucagon appears to be mediated by factors operating in the whole animal, more than by changes in the adipose cell itself. A slower removal rate of circulating FFA by the old geese, could be one of these factors.

The excellent technical help of Mrs. D. E. Raphael, Mr. W. F. Prigge, and Mr. E. J. Levens is gratefully acknowledged. We thank Dr. M. Di Girolamo for his valuable suggestions regarding the fat cells' measurements.

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Received Nov. 6, 1974. P.S.E.B.M., 1975, Vol. 149.