# PRIMATOLOGY

# Age-Associated Changes in Hormonal Function of the Pancreas and Regulation of Blood Glucose in Monkeys

**N. D. Goncharova, A. A. Vengerin, T. E. Oganyan, and B. A. Lapin**

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Age-associated changes in the concentrations of glucose, insulin, and DHEAS in peripheral blood plasma of female rhesus macaques were studied in intact animals and in response to a standard dose of glucose and insulin. Basal levels of insulin and glucose increased, insulin sensitivity and glucose tolerance decreased, and pancreatic reaction to glucose load was impaired in old monkeys. Insulin level positively correlated with glucose concentration, body weight, and abdomen circumference and negatively correlated with DHEAS level.

**Key Words:** *female rhesus macaques; insulin; glucose; tolerance*

The second half of life is characterized by pronounced metabolic disorders. In humans blood glucose and insulin levels typically increase with age, insulin sensitivity and glucose tolerance decrease [5,10], and chronic hyperglycemia syndrome develops (type II diabetes mellitus — non-insulin-dependent diabetes, NIDDM). The incidence of NIDDM increased in recent years and became a cause of early disability and mortality. Therefore studies aimed at investigation of mechanisms of age-associated changes in pancreatic function and regulation of blood glucose levels (including the stage of insulin interaction with target tissues), detection of endogenous and exogenous factors modulating insulin sensitivity of peripheral tissues and glucose metabolism acquire special importance. The most significant endogenous factors are neuroendocrine changes (concentrations of insulin, somatotropic hormone, somatotropin C, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS)), anthropometric changes, and changes in the antioxidant defense system. Exogenous factors of priority significance are diet and exercise [3,6-9,11]. An important problem is the choice of experimental model for these studies. Laboratory primates are studied best of all, especially rhesus macaques, in which spontaneous hyperinsulinemic diabetes mellitus was observed, similar by the clinical picture and pathogenesis to NIDDM in humans [10].

We investigated the trend of age-associated changes in the hormonal function of the pancreas and glucose regulation during physiological aging of female rhesus macaques, evaluated the correlations between the parameters of pancreatic endocrine function and anthropometric changes and changes in DHEAS concentrations.

## MATERIALS AND METHODS

Experiments were carried out on 14 young adult (6-8 years) and 14 old (20-27) clinically healthy female *Macaca mulatta* living in breeding center of Institute of Medical Primatology (Sochi-Adler). Anthropometric parameters of the animals are presented in Table 1. All experiments were carried out in June-September in 2002 and 2003. Animals were usually kept in groups in cages; for experiments they were placed into an isola-

State Institute of Medical Primatology, Russian Academy of Medical Sciences, Sochi. ***Address for correspondence:*** iprim@sochi.net. Vengerin A. A.

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ted room in individual metabolic cages. The temperature in the room was 20-25°C, with light hours at 6.00-18.00. The animals were fed balanced rations. They adapted to conditions of metabolic cages and the procedure of blood collection for at least 4 weeks.

Basal glucose and insulin levels were measured in all monkeys before functional tests. Blood was collected from the ulnar or femoral vein after overnight fasting at 9.00-10.00. Glucose tolerance was studied in 14 monkeys (7 aged 6-8 years and 7 aged 20-27 years). To this end, the monkeys were intravenously injected with 40% glucose (300 mg/kg) after overnight fasting at 9.00. Blood samples were collected before and 5, 15, 30, 60, and 90 min after glucose injection. Insulin tolerance test according to Zlaf [2] was carried out in 14 other monkeys (7 young and 7 old females). Short-acting insulin (Aktrapid, Novo Nordisk) was injected intravenously in a dose of 0.1 U/kg in 1 ml normal saline at 9.00. Blood samples were collected before and 30, 90, and 120 min after insulin injection.

Glucose concentration in the plasma was measured by the glucose oxidase method, the concentrations of insulin and DHEAS by enzyme immunoassay using DSL kits. Glucose tolerance was evaluated by the rate of disappearance of exogenous glucose injected in a dose of 300 mg/kg over the first 15 min of glucose tolerance test.

The results were statistically processed using analysis of correlations. The significance of differences wad evaluated by Student's *t* test.

## RESULTS

Basal glucose level was higher in old rhesus macaque females in comparison with young animals and varied in a narrow range (2.48-5.82 mmol/liter). Basal glucose concentrations positively correlated with body weight ($r$=0.525) and abdomen circumference ($r$=0.706 for all monkeys examined and $r$=0.542 for old monkeys).

Similarly to glucose, basal insulin level was higher in old animals (35.8±8.6 mU/liter) in comparison with young monkeys (23.7±5.0 mU/liter; Fig. 1) and, in contrast to glucose, the individual levels varied within a wide range (10-83 mU/liter). Basal insulin level positively correlated with body weight ($r$=0.665) and abdominal circumference ($r$=0.764). These correlations were most demonstrative in the group of old animals ($r$=0.902 for insulin and body weight and $r$=0.991 for insulin and abdominal circumference). Basal levels of insulin and glucose were also in positive correlation ($r$=0.600 for all animals examined and $r$=0.805 for the group of old animals).

Glucose tolerance test showed appreciably higher glucose concentrations 5, 15, 30, and 60 min after injection of a standard glucose dose in old animals compared to young monkey (Fig. 2, *a*).

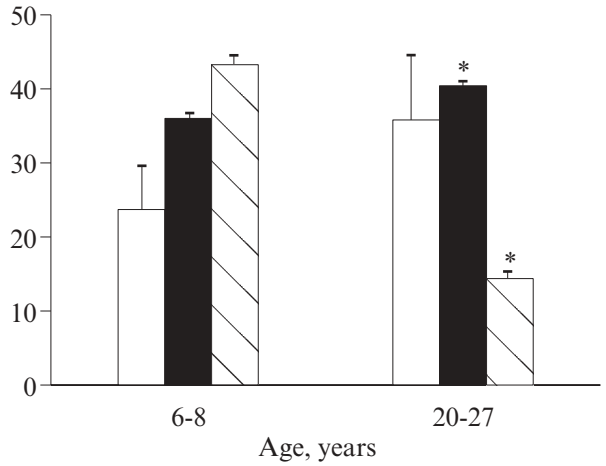


**Fig. 1.** Basal levels of insulin, glucose, and DHEAS in peripheral blood plasma of *Macaca mulatta* females of different age. Light bars: insulin (mU/liter); dark bars: glucose (mmol/liter*10); cross-hatched bars: DHEAS (ng/ml/10). *$p$<0.01 compared to young animals.

The dynamics of insulin levels in response to a standard dose of glucose was also different in the two age groups (Fig. 2, *b*). In young monkeys the dynamics of insulin concentrations was similar to the dynamics of glucose levels ($r$=0.919). The peak values of insulin concentration were observed 5 min after injection, after which it gradually decreased and returned to the initial level after 30-60 min. In old animals changes in insulin level after a standard glucose dose less correlated with changes in glucose level ($r$=0.823), and the dynamics of insulin concentrations differed from that in young animals. Insulin level reached the peak 5 min after injection, did not decrease 15 and 30 min postinjection, and returned to normal only 60 min postinjection. Hence, in old monkeys insulin concentrations 30 and 60 min after injection were appreciably higher than in young animals ($p$<0.05).

**TABLE 1.** Anthropometric Parameters of *Macaca mulatta* Females of Different Age Groups ($M$±$m$)

| Parameter | Age, years | |
|---|---|---|
| | 6-8 | 20-27 |
| Body weight, kg | 5.1±0.2 | 4.9±0.4 |
| Body length, cm | 74.3±1.2 | 69.5±0.9** |
| Trunk length, cm | 44.2±0.7 | 42.3±0.6 |
| Lower limb length, cm | 30.2±0.9 | 26.5±0.6** |
| Head circumference, cm | 27.7±1.6 | 28.7±1.3 |
| Chest circumference, cm | 33.2±0.6 | 33.9±0.7 |
| Abdomen circumference, cm | 26.4±0.5 | 31.1±1.5** |

**Note.** *$p$<0.05, **$p$<0.01 compared to young animals.

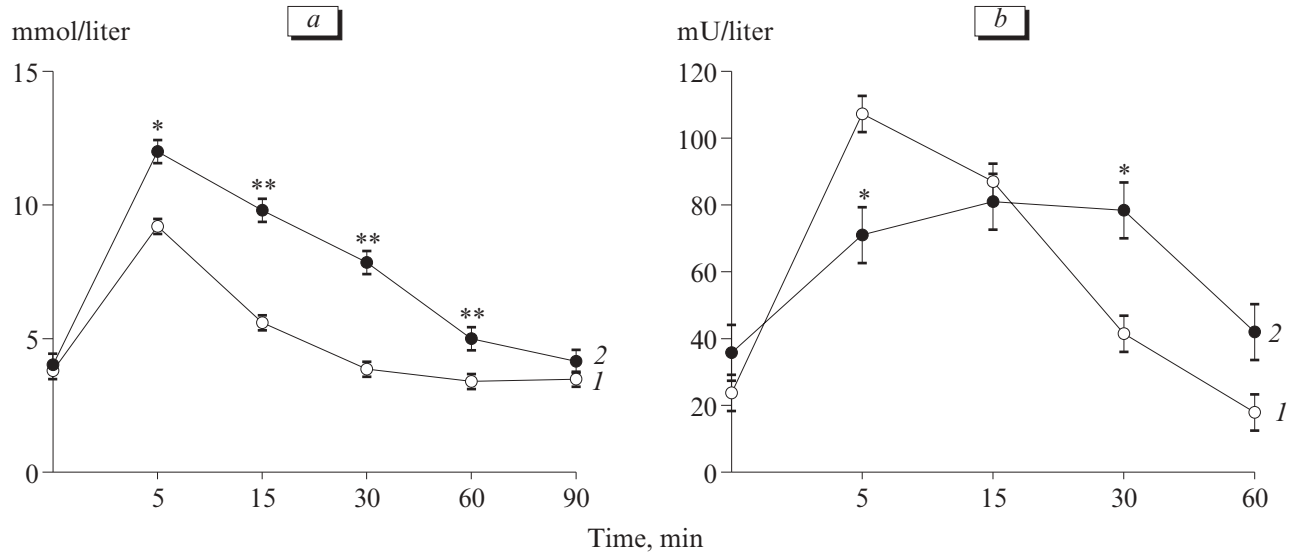


**Fig. 2.** Dynamics of glucose (*a*) and insulin (*b*) concentrations in peripheral blood plasma in response to a standard dose of glucose (300 mg/kg) in rhesus macaque females aged 6-8 (*1*) and 20-27 (*2*) years. **p*<0.05, ***p*<0.001 compared to young animals.

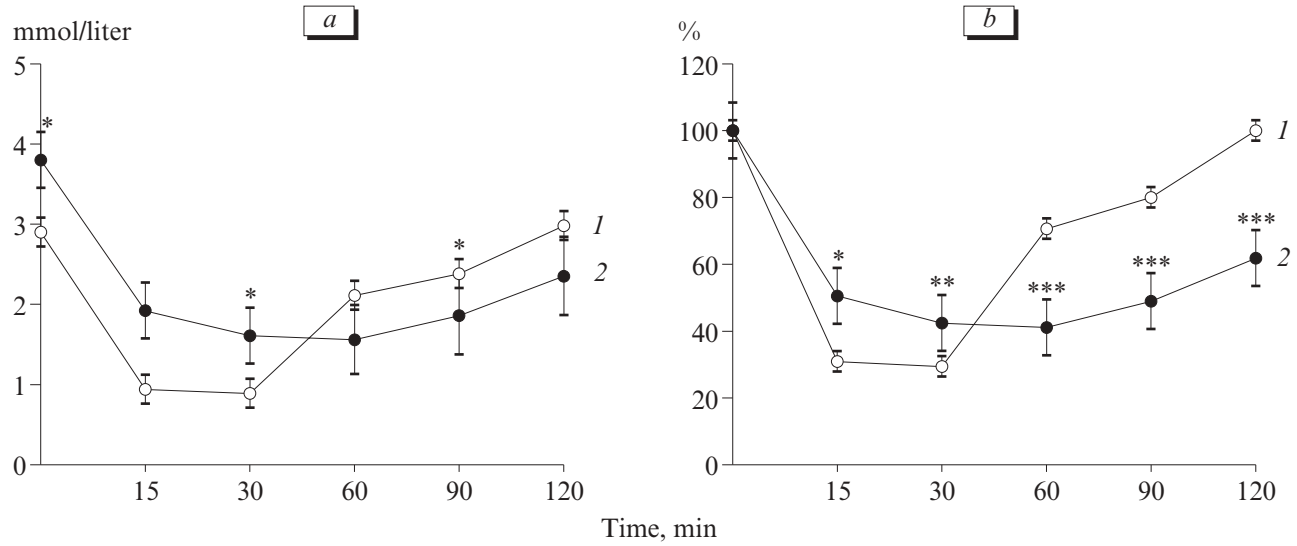


**Fig. 3.** Time course of glucose content in peripheral blood plasma in rhesus macaque females aged 6-8 (*1*) and 20-27 years (*2*) in response to insulin injection (0.1 U/kg). **p*<0.05, ***p*<0.01, ****p*<0.001 compared to young animals.

Age-associated differences in the dynamics of insulin and glucose levels in response to a standard glucose dose indicate that, despite the absence of pronounced age-specific changes in the basal levels of insulin, age-associated disorders in the pancreatic hormonal function are detected under conditions of activation of this function. It seems that the sensitivity of peripheral tissues to insulin is reduced in old animals. This is seen from decelerated elimination of the injected standard dose of glucose in old animals in comparison with young monkeys (Table 2).

However, it seems that decrease of peripheral tissue sensitivity to insulin in normal aging is paralleled by impairment of Langerhans islet β-cells sensitivity to glucose. This is seen from opposite changes in glucose and insulin concentrations in old animals 5 min after glucose injection (Fig. 2).

In young animals glucose level decreased 15 and 30 min after insulin injection more markedly than in old animals and returned to normal sooner than in young animals (Fig. 3). In young animals glucose concentrations returned to the initial level after 120 min, while in old animals it was only 61.8±2.6% of the basal level at the same term. The less pronounced decrease in glucose level in old rhesus macaques in response to insulin injection confirms the above results of glucose tolerance test. It attests to the development of relative tissues resistance to insulin during physiological aging.

The concentration of DHEAS in old rhesus macaque females was on average 3-fold lower than in

**TABLE 2.** Rate of elimination (%/min) of Standard Glucose Dose (300 mg/kg) from Peripheral Blood of Female Rhesus Macaques of Different Age Groups ($n$=7, $M \pm m$)

| Age, years | Time after glucose injection, min | | |
|---|---|---|---|
| | 0-5 | 5-15 | 0-15 |
| 6-8 | 8.7±0.7 | 2.26±0.20 | 4.40±0.09 |
| 20-27 | 5.36±0.70* | 1.52±0.10* | 2.80±0.15** |

**Note.** *$p$<0.01, **$p$<0.001 compared to young animals.

young animals (Fig. 1), which is in good correlation with our findings and previous data for monkeys and humans [1].

A favorable effect of DHEA on insulin binding to receptors was demonstrated [6]; DHEA therapy increased the sensitivity of peripheral tissues to insulin in postmenopausal women [4]. Therefore the decrease in DHEA level with age can underlay the detected age-specific differences in insulin tolerance of peripheral tissues.

Hence, basal glucose and insulin levels are higher in old rhesus macaque females than in young animals and these parameters are in positive correlation. The most significant correlation was observed in the group of old animals. Basal insulin level positively correlated with body weight and abdomen circumference. The highest correlation was observed in the group of old animals.

The reaction of the pancreas to glucose load depends on age: the peak insulin values are lower and normalization of insulin level is slower in old monkeys. Relative resistance of peripheral tissues to insulin and disorders in glucose tolerance developed in these animals with age.

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