agents[16]. Accordingly, the enhanced production of H_2O_2, and the substantially high level of MPO in tuftsin-M stimulated macrophages, make these cells very aggressive and potent, so that they could kill any infectious agent located intracellularly or in their vicinity. Nonetheless, the peptide could not produce a prophylactic action, as the activation of the cells was not long-lasting (fig.). The stimulation of a respiratory burst in peritoneal exudate cells appears to be a direct effect of tuftsin-M, as like PMA this peptide also instantly enhances the generation of both O_2^- and H_2O_2 (table 2). Furthermore, the enhancement pattern shown by this peptide is consistent with that of tuftsin which stimulates macrophages to release O_2^- within a range of 125–625 nM with a maximum at 350 nM[17].

Entrapment of sodium stibogluconate in tuftsin-M bearing liposomes prepared from egg lecithin and cholesterol (2:1 molar ratio) has been shown to enhance the antileishmanial activity of the drug[4]. However, the incorporation of the peptide into the bilayer of similarly-prepared liposomes caused only an insignificant (10–20%) (fig.) increase in the production of O_2^- and H_2O_2 by the peritoneal exudate cells compared with the stimulation caused by the free peptide. This suggests that the improvement in the efficacy of sodium stibogluconate brought about by liposomization may result from the respiratory burst-inducing activity of tuftsin-M, in addition to the effect of the targeted delivery of the drug to the macrophages as discussed in the report[4].

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Effect of a restricted diet on the in vitro glucose-induced insulin release of aging rats

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Abstract. To study the effect of a sudden loss of body weight on the β-cell function of aging rats, basal and glucose-induced insulin secretion was measured in pancreatic islets obtained from young (2-month-old), adult (12-month-old) and aging (24-month-old) rats, either fed ad libitum or fed a restricted diet (50% caloric restriction). Basal insulin secretion was similar in islets of young, adult and older rats. Glucose stimulated insulin release was significantly reduced in aging rats as compared to young animals. Animals fed a restricted diet showed a prolonged and higher secretory rate during first phase release when compared to animals fed ad libitum.
Key words. Aging; insulin release; pancreatic islets; dieting.

Previous reports have shown that pancreatic beta cells from collagenase-isolated and incubated or perifused islets from aging rats secrete less insulin in response to glucose or leucine stimulation than islets from younger rats[1–3]. In contrast, the total islet content of both proinsulin and insulin has been found to be increased in older animals[4] which is in accordance with the fact that average islets from older rats are bigger and contain more beta cells than average islets from young rats[5]. In the rat, the loss, with age, of insulin secretory function appears to be independent of obesity[6], and is not alleviated if obesity is prevented by long-term caloric restriction[7]. Nevertheless, a connection may exist between age-associated obesity and decreased β-cell function: female Sprague-

Dawley rats, which normally gain less weight with age than their male counterparts, secrete insulin more efficiently and show better peripheral sensitivity to insulin than male rats [7]. The long-term (10.5 months) caloric restriction used, in the studies reported, to prevent obesity in aging animals may by itself have adversely affected the insulin secretory function [7]. Therefore we investigated, in isolated perifused rat pancreatic islets, the beta cell response to glucose stimulation in islets from aging animals either fed ad libitum or fed on a limited diet just for a short period (1 month) in order to induce a rapid decrease in the amount of adipose tissue. The results were compared with those obtained from younger control animals.

Methods

Albino male Wistar rats were used. Their initial body weight at 2 months ranged between 240 and 279 g (260 ± 23 g). The animals had unlimited access to tap water and were maintained on a 12-h light-dark cycle. A standard pellet chow was provided ad libitum to all the animals until they were 23 months old. For the next month, one group of animals (n = 5) remained on ad libitum feeding while another group of animals (n = 6) had a 50% reduction in their daily diet. The pancreatic islets from adult and old rats were obtained when the animals were 12 and 24 months old, respectively; pancreatic islets from young rats were obtained when they were 2 months old. Immediately before the pancreas was removed, blood was collected from the abdominal aorta for measurement of plasma glucose and plasma insulin levels.

Pancreatic islets were isolated by collagenase (Worthington 169 U/mg) and perifused as previously described [8]. The medium used for perifusion was a modified Krebs-Henseleit bicarbonate buffer, containing glucose 2.7 (basal) or 16.7 (stimulated) mmol/l, supplemented with 0.5% bovine albumin and equilibrated with a mixture of 95% O_2 – 5% CO_2 and adjusted to pH 7.4. The perifusion flow rate was 1 ml/min and the effluent was collected at 1-min intervals. The insulin content was measured by radioimmunoassay using rat insulin as standard.

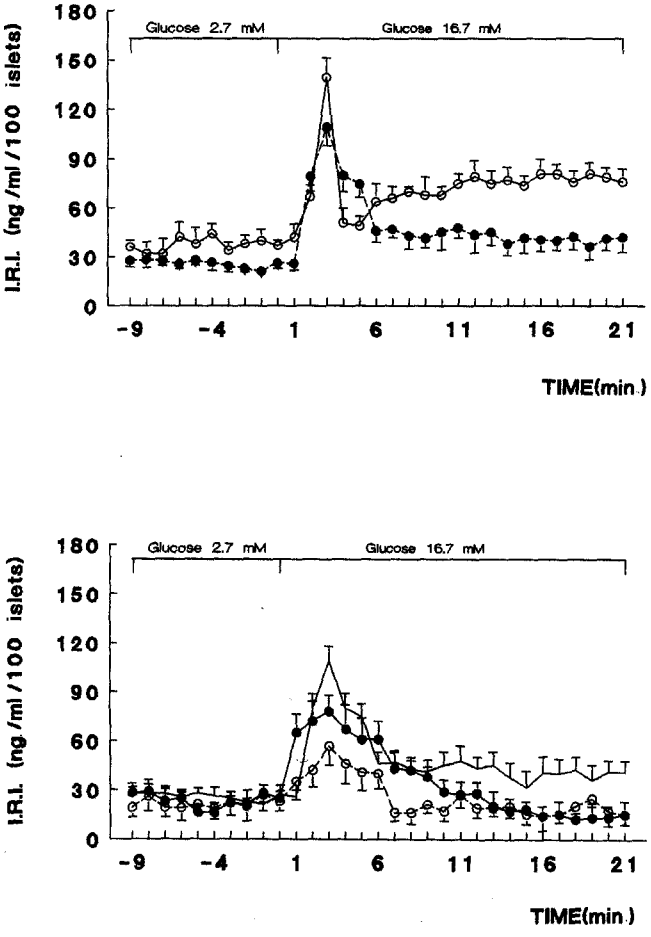
The Newman-Keuls test was applied after a previous analysis of variance.

Results

At 23 months, the animals (n = 9) had a mean (± SEM) body weight of 487 ± 49 g. After 1 month of food restriction, dieting animals (n = 6) showed a 28% reduction in body weight as compared to animals fed ad libitum (493 ± 51 g vs 354 ± 48 g). No major alteration in fasting glucose homeostasis was evident in old rats (24 months old) as compared to young (2 months old) or adult (12 months old) rats, as assessed by either plasma glucose or insulin levels. Similarly, diet restriction did not influence either parameter (table).

Plasma glucose and plasma insulin levels at basal state in young and both groups of aging rats. Results are presented as mean ± SD.

	Plasma glucose (mg/dl)	Plasma insulin (μU/ml)	
Young rats	147.77 ± 17.09	38.96 ± 15.24	n = 6
Adult rats	149.53 ± 12.34	35.41 ± 13.79	n = 5
Aging rats fed ad libitum	146.12 ± 17.25	36.41 ± 14.59	n = 5
Aging rats fed restricted diet	153.66 ± 11.32	34.14 ± 17.21	n = 6



Top. Kinetics of glucose-induced insulin release (I.R.I.) of pancreatic islets from young (2-month-old) and adult rats (12-month-old). -⊖-, Young rats; -●-, 12-month-old rats.
Bottom. Kinetics of glucose-induced insulin release of pancreatic islets from adult or aging rats (24-month-old) ($p < 0.01$). —, 12-month-old rats; -●-, 24-month-old dieting rats; —⊖—, 24-month-old rats.
All results are expressed as mean ± SEM.

The kinetics of insulin release in response to glucose stimulation was altered in adult and aging rats, showing a marked reduction of the second phase release with a relative preservation of the first phase secretion (fig.). This effect was not completely reversed by weight reduction in aging rats, but animals fed a restricted diet showed a prolonged and higher secretory rate during first phase release as compared to animals fed ad libitum (fig.).

Discussion

Reports from in vitro studies using either the isolated perfused rat pancreas, or the incubation of isolated pancreatic islets, suggest that aging induces a certain degree of impairment of insulin release, but the mechanism involved is still unclear. In experiments using isolated incubated pancreatic islets, it has been observed that islets from old (24-month) rats exhibited a 50% lower glucose-stimulated insulin release than pancreatic islets from young rats[9]. These data are in accordance with the results presented in the present study, clearly showing that glucose-induced insulin release is significantly impaired in islets from old rats as compared to islets from young animals. It has to be noted that in the above-mentioned studies the decrease in insulin secretory function is only observed when the maximal glucose-induced insulin release is corrected for the pancreas size and the β-cell content of the islets, while no differences are observed in absolute terms. Our results, using isolated perifused rat islets, have not been corrected for β-cell content, and also show decreased insulin secretion in absolute terms. A relevant finding of our study is that the age-associated impairment of glucose-induced insulin release is an impairment of the second phase secretion while first phase release is preserved. This fact could account for impaired glucose tolerance during a glucose overload without hyperglycemia in the fasting state, as is the case in our study.

Leitter et al. have suggested that impaired glucose homeostasis in aging is not an intrinsic consequence of impaired function of pancreatic β-cells[10]. An impairment in the mechanism of islet paracrine control, for example by somatostatin[11] and/or glucagon[12], may be involved.

From the results presented here it appears clear that obesity or caloric restriction has a significant influence on changes in the glucose-induced first phase but not on second phase insulin release pattern from pancreatic islets of aging rats. This is in accordance with other authors[7], who showed that in 12-month-old rats, insulin secretion was significantly reduced as compared to that in young (2-month) rats, and the decrease is still present when the animals have had a diet restricted in calories for the previous 10.5 months. However, this reduction was only sham when the results were expressed as a function of the islet volume. If the insulin secretion was not corrected for the islet volume, young rats and adult rats (12 months old) fed ad libitum, had a similar insulin secretion rate in response to glucose. Only the animals maintained on a caloric restricted diet for the previous 10.5 months showed an unequivocally decreased insulin secretion, together with a reduced content of β-cells in the islets. The two phenomena were probably linked, and were probably induced by the long-term caloric restriction. A short-term caloric restriction of only one month might affect the glucose-induced insulin secretion to a different extent. The results presented in this study show that although a decrease in glucose-induced insulin release is an intrinsic feature of the aging process, a rapid loss of weight due to one month of caloric restriction does not affect the second phase release, but may enhance and prolong the first phase secretion in response to glucose stimulation.

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