Influence of Aging on Insulin Receptor Binding and Metabolic Effects of Insulin on Human Adipose Tissue

JAN BOLINDER, JAN ÖSTMAN, AND PETER ARNER

SUMMARY

The influence of aging on the peripheral action of insulin was studied using subcutaneous adipose tissue from eight young (range 22–30 yr) and seven middle-aged (40–59 yr), healthy, normal-weight subjects. Insulin binding per cell was 50% lower in the older than in the younger group ($P < 0.01$), essentially owing to a decrease in the insulin receptor number. Concomitantly, insulin sensitivity, as reflected in the degree of antilipolysis and stimulation of glucose oxidation, was 10–20 times smaller in the older subjects ($P < 0.01$). Basal lipolysis and the maximum antilipolytic effect of insulin were similar in the two groups. The basal rate of glucose oxidation in the older subjects was less than one-half that for the younger group ($P < 0.025$), and the maximum level of insulin-induced glucose oxidation was lower by about 75% ($P < 0.01$). Age was significantly and negatively correlated with insulin receptor number ($r = -0.81$), basal production of $^{14}CO_2$ ($r = -0.73$) and maximum level of insulin-induced glucose oxidation ($r = -0.68$). The decreases in the receptor number and insulin sensitivity were larger in early adulthood than in the elderly, while the decrease in insulin responsiveness was more uniform. It is concluded that aging is accompanied by impairment of the action of insulin on target cells, owing to alterations at both the receptor and the postreceptor levels. These mechanisms, and especially the postreceptor defect, may be essential factors in the development of relative glucose intolerance in the aged. DIABETES *32*:959–964, October 1983.

While it is generally recognized that aging is associated with progressive lowering of glucose tolerance,[1,2] the underlying mechanisms are not fully understood. In theory, several factors such as nutritional status, physical inactivity, reduction of lean body mass, and impairment of insulin secretion may contribute to the abnormal glucose utilization in the aged.[1,2] Employing the hyperglycemic and euglycemic insulin clamp techniques, DeFronzo has demonstrated that the tissue sensitivity to insulin in vivo decreases with age;[3] this indicates that the peripheral insulin resistance may also be important for the development of glucose intolerance in the elderly.

Resistance to the action of insulin at the cellular level can be due to impairment of insulin-receptor binding and to defects in the initiated intracellular processes located distal to the insulin-receptor interaction (postreceptor defects).[4] To distinguish between these possible mechanisms, simultaneous measurements of insulin binding and metabolic effects of the hormone must be performed,[5] preferably using a target tissue of insulin action. In the present study, this approach was applied for the first time to examine the influence of aging on the peripheral action of insulin in man. Subcutaneous adipose tissue was obtained from young and middle-aged healthy, normal-weight subjects matched for sex, body weight, and fat-cell size. Determinations were made of insulin binding to isolated adipocytes and of the effects of the hormone on adipose tissue lipolysis and glucose utilization. With increasing age, there was a progressive decline in the metabolic effectiveness of insulin, which was due to defects at both the receptor and the postreceptor levels.

MATERIAL AND METHODS

Subjects. The study was performed on eight young subjects with ages ranging from 22 to 30 yr and seven middle-aged subjects aged 40–59 yr. They were healthy and matched for sex, body weight, fasting plasma insulin level, and fat-cell size. Their clinical data are given in Table 1. The fasting blood glucose concentration and glucose tolerance (OGTT or IVGTT[6]) were normal, and there was no family history of diabetes mellitus. None had taken any drug known to affect adipose tissue metabolism. All subjects consumed a diet

From the Department of Medicine and the Research Center, Huddinge Hospital, Karolinska Institute, Huddinge, Sweden.
Address reprint requests to Jan Bolinder, M.D., Department of Medicine, Huddinge Hospital, S-141 86 Huddinge, Sweden.
Received for publication 6 December 1982 and in revised form 9 March 1983.

TABLE 1
Clinical and metabolic characteristics of the study groups

	N	Sex M/F	Age (yr)	Body weight (% of average)	Fasting blood glucose level (mmol/L)	Fasting plasma insulin level (μmol/L)	Fat cell volume ($mm^3 \times 10^{-6}$)
Young subjects	8	3/5	25 ± 1	99 ± 3	4.8 ± 0.2	9.0 ± 1.6	1023 ± 38
Middle-aged subjects	7	3/4	51 ± 3	99 ± 2	4.8 ± 0.2	9.0 ± 3.0	944 ± 93

The values are the mean ± SEM. The average body weight was obtained from Documenta Geigy.[34]

consisting of approximately 45% carbohydrates, 35% fat, 20% protein, and 7–8.5 MJ (1700–2100 kcal) according to a 24-h recall. All were fully physically active.

The subjects were examined as ambulatory volunteers at the out-patient department at 8 a.m. after an overnight fast. After a 30–60-min rest, venous blood samples were taken for determinations of the fasting levels of glucose[7] and plasma-immunoreactive insulin.[8] Specimens of subcutaneous adipose tissue were then removed surgically from the gluteal region. Field-block local anesthesia was induced as described previously.[9] The study was approved by the Ethical Committee of the Karolinska Institute. Each subject was given a detailed oral and written description of the study, and his or her consent was obtained.

Insulin binding. Fat cells were isolated[10] and incubated in triplicate or quadruplicate for 60 min at 24°C. Specific mono-^{125}I(Tyr A_{14})-insulin binding per cell and per cell surface area was measured as described elsewhere.[11,12] Insulin degradation was negligible. The binding data were transformed by the method of Scatchard.[13] Insulin receptor number and affinity were determined as described elsewhere.[11,12]

Lipolysis and glucose oxidation. Adipose tissue segments were incubated in triplicate or quadruplicate for 2 h at 37°C in a bicarbonate buffer (pH 7.4) containing dialyzed bovine albumin (40 mg/ml), glucose (1 mg/ml), (U^{14}C)-glucose (2×10^6 cpm/ml) and insulin (0–5000 μU/ml). The details of the incubation procedures have been described in detail elsewhere.[11] Glycerol release and $^{14}CO_2$ production were determined[11] and used as indices of the rates of lipolysis and glucose oxidation, respectively. The mean and the individual dose-response curves for insulin inhibition of lipolysis and stimulation of glucose oxidation were linearized as described elsewhere,[14] and the concentrations of insulin exerting half the maximum effect (ED_{50}) were determined. Insulin responsiveness was calculated as the absolute values of glycerol release or CO_2 production at the maximum effective insulin concentration minus the basal value (i.e., with no insulin present).

The reasons for using fat segments instead of isolated fat cells and for examining the effect of insulin on the basal rather than on the catecholamine-stimulated metabolism have been given elsewhere.[11,12,14]

Insulin degradation in adipose tissue segments. Adipose tissue segments (100 mg) were incubated for 2 h at 37°C in 1 ml of a bicarbonate buffer (pH 7.4) containing dialyzed bovine serum albumin (40 mg/ml), glucose (1 mg/ml) and ^{125}I-insulin (0.1 pmol/ml). After incubation, to 0.5 ml of buffer containing albumin (40 mg/ml) were added 200-μl aliquots of the buffer containing radioactive material and 0.5 ml of buffer containing 15% (vol/vol) trichloroacetic acid (TCA). The precipitate was washed once with 15% of TCA. Insulin degradation was measured as soluble radioactivity and expressed as the percentage of total radioactivity in the buffer.

Fat cell determinations. Fat cell size was measured by the method of Sjöström and co-workers.[15] Mean fat cell volume, weight, and surface area were determined from previously derived formulas.[16,17] The number of fat cells incubated was determined as the quotient of the lipid weight of the incubated sample by the mean fat cell weight.

Chemicals. Crystalline, glucagon-free porcine insulin was generously supplied by Vitrum AB (Stockholm, Sweden). Mono-^{125}I (Tyr A_{14})-insulin was purchased from Novo (Bagsvaerd, Denmark) and (U-^{14}C)-glucose (specific activity 268 mCi/mmol) was obtained from The Radiochemical Centre (Amersham, England). Bovine serum albumin (fraction V) was obtained from The Pharmaceutical Company (Eastbourne, England) and Hyamine-X from Packard, USA. Col-

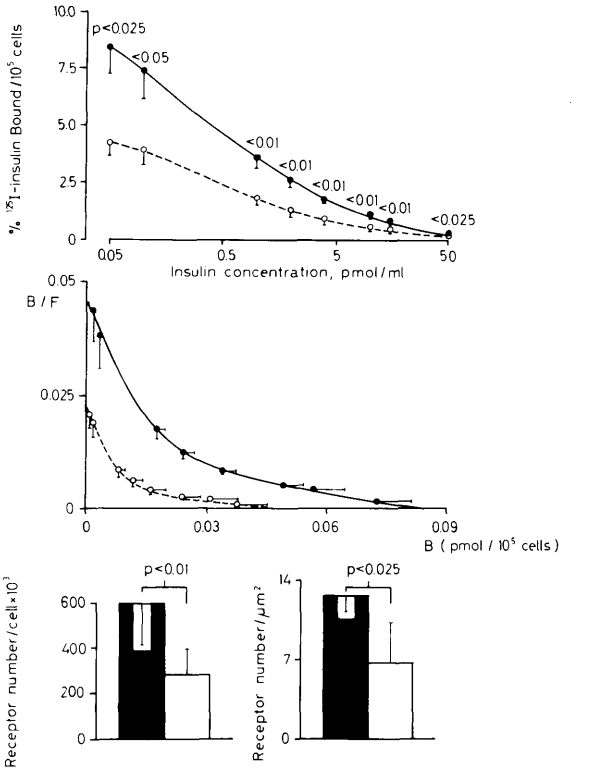

FIGURE 1. Insulin receptor binding to isolated adipocytes obtained from eight young (•) and seven middle-aged subjects (○). The percentage of ^{125}I-insulin specifically bound per 10^5 cells was plotted against the total insulin concentration (top graph). The binding data were transformed according to Scatchard[13] (middle graph). The individual Scatchard[13] plots were used for measuring the total number of insulin receptors (bottom graph). The values are the mean ± SEM. Student's *t* test was used.

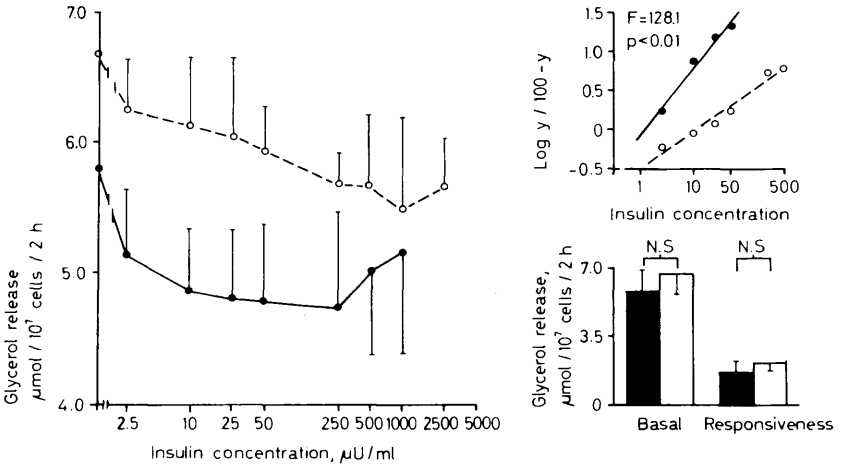

FIGURE 2. The effect of insulin on adipose tissue lipolysis. Subcutaneous adipose tissue segments obtained from seven young (•) and seven middle-aged subjects (○) were incubated with and without insulin (0–5000 μU/ml) and the glycerol release was determined. The mean dose-response relationship for insulin action was calculated (left panel). The dose-response curves were linearized using log-logit plots,[14] where Y denotes the effect of insulin at a particular insulin concentration as a percentage of the maximum insulin effect (upper right panel). The positions of the regression lines were compared by the F-distribution test.[18] Insulin responsiveness, defined as the basal rate of glycerol release minus the rate at the maximum effective insulin concentration, is given in the lower right panel. NS = not significant. For further details, see legend to Figure 1.

lagenase (type I), prepared from clostridium histolyticum, was obtained from Sigma Co. (St. Louis, Missouri).

Statistical analysis. The reported values are the mean ± the standard error of the mean (SEM). Linear regression analysis by the method of least squares was performed. Individual data were statistically compared using Student's unpaired *t* test. When the mean dose-response curves were compared, the position of the regression lines were tested for statistical difference by the F-distribution test.[18]

RESULTS

The binding data are presented in Figure 1. At each of the insulin concentrations used, adipocytes from the middle-aged subjects bound significantly less ^{125}I-insulin than did those from the young group. When the binding curves were transformed by the method of Scatchard,[13] typical curvilinear plots were obtained. The curves were similar in slope but their abscissa intercepts differed significantly; this indicates that the binding affinity was similar for the two groups but that the maximum insulin binding capacity differed. The insulin receptor number was about 600,000 sites per cell (13 sites/μm^2) for the young subjects and 300,000 sites per cell (7 sites/μm^2) for the middle-aged group (Figure 1). The insulin receptor affinity, calculated according to an occupancy-dependent model,[19] was similar in the two groups (data not presented).

As regards the inhibition of lipolysis by insulin the mean dose-response curve for the middle-aged subjects was significantly rightward shifted as compared with that for the young group (Figure 2); the respective ED_{50} values were about 1.5 and 12 μU/ml ($P < 0.01$). The basal rate of lipolysis and the maximum antilipolytic effect of insulin (responsiveness) were similar for the two groups.

The basal rate of (U-^{14}C)-glucose oxidation was more than twice as great for the younger than for the older subjects ($P < 0.025$; Figure 3). Although there was a dose-dependent insulin-induced stimulation of $^{14}CO_2$ production in both age groups, it was much more pronounced in the younger than in the older subjects, and there was also a considerable difference in the level of sensitivity, the respective ED_{50} values being about 30 and 700 μU/ml ($P < 0.01$) when pooled data were analyzed. The groups also differed significantly as regards the maximum stimulation of glucose oxidation by insulin, the insulin responsiveness being four times as great in the young as in the middle-aged subjects ($P < 0.01$; Figure 3). Comparison of the mean dose-response curves for lipolysis and glucose oxidation showed that in both age groups the ED_{50} values for antilipolysis were lower than the

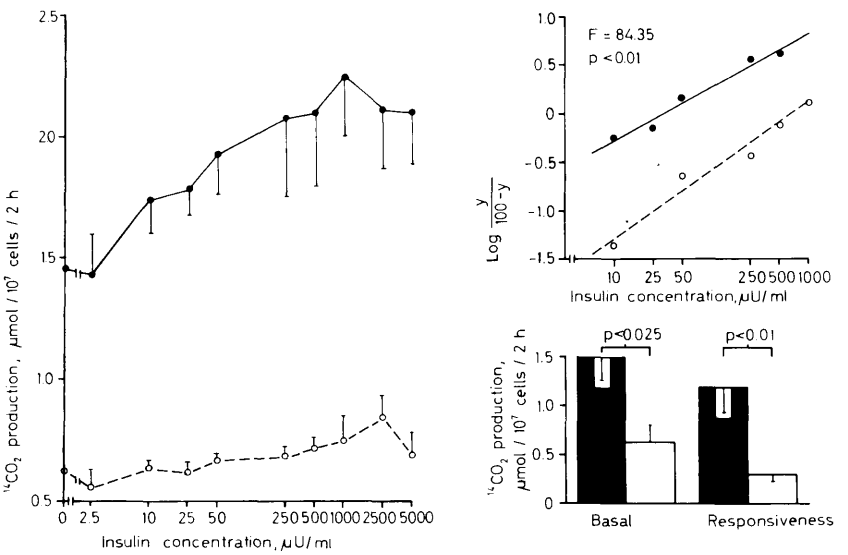

FIGURE 3. Insulin-induced stimulation of glucose oxidation in subcutaneous adipose tissue segments obtained from seven young (•) and seven middle-aged subjects (○). The incorporation of (U-^{14}C)-glucose into $^{14}CO_2$ was determined in the experiments to which Figure 2 relates. The mean dose-response relationship was determined. Insulin responsiveness was defined as the maximum insulin-induced production of $^{14}CO_2$ minus the basal production of $^{14}CO_2$. For further details, see legends to Figures 1 and 2.

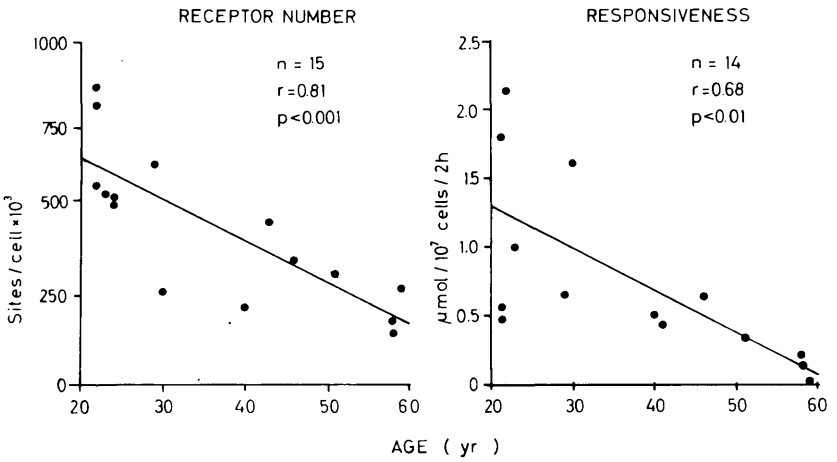

FIGURE 4. The relationships between age and insulin receptor number (left panel) and insulin responsiveness (right panel). Adipocyte insulin receptor number and insulin-induced $^{14}CO_2$ production were determined in each subject and the correlation with age was determined. Linear regression analysis was used for statistical evaluation. For further details, see legends to Figures 1 and 3.

corresponding values for stimulation of glucose oxidation. Similar differences in ED_{50}, reflecting the effect of insulin on lipolysis and glucose utilization, have previously been observed in human adipose tissue.[11,20]

When individual ED_{50} values for the effects of insulin on lipolysis and glucose oxidation were calculated, similar findings as those observed for the group data were obtained. Thus, ED_{50} for the antilipolytic effect of insulin was 12-fold higher in the older than in the younger subjects; 62.7 ± 13.6 and 4.8 ± 2.2 µU/ml, respectively ($P < 0.0025$). The ED_{50} value for glucose oxidation was also 12-fold higher in middle-aged than in young individuals; 610.6 ± 291.5 and 72.1 ± 28.7 µU/ml, respectively ($P < 0.05$). Furthermore, the individual ED_{50} values for antilipolysis, as well as for glucose oxidation, were positively correlated with age ($r = 0.62$ and 0.54, respectively, $P < 0.05$). However, when the individual dose-response curves were linearized, the regression coefficients for antilipolysis and glucose oxidation were somewhat low (0.87 ± 0.05 and 0.81 ± 0.05, respectively). When calculated on pooled data (Figures 2 and 3), on the other hand, the corresponding r values were much higher (0.98–0.99). This may be due to scattered individual dose-response curves which are overcome when group data are analyzed. Preference should therefore be given to determine insulin sensitivity on pooled rather than on individual data.

Insulin degradation by adipose tissue segments during the course of the metabolic experiments was determined in five young and five middle-aged subjects. It was 7.8 ± 2.6% in the former and 4.2 ± 0.7% in the latter group (NS).

As is seen in Figure 4, both the number of receptors per cell and the maximum insulin-induced production of $^{14}CO_2$ were significantly and negatively correlated with age ($r = -0.81$ and -0.68, respectively). A similar relationship was found between the number of receptors per cell surface area and age ($r = -0.72$, $P < 0.01$). A strong negative correlation was also found between the basal rate of glucose oxidation and age ($r = -0.73$, $P < 0.01$). As regards lipolysis, no correlation was found between either basal glycerol release and age or insulin responsiveness and age ($r < 0.1$). The fasting insulin level was not correlated with the insulin receptor number ($r < 0.14$), nor with insulin responsiveness ($r < 0.1$). Neither fat-cell volume nor relative body weight was correlated with insulin receptor number ($r < 0.04$).

To study more closely the relationship between aging and the action of insulin on adipose tissue all the subjects were divided into three age groups with the ranges 22–24, 30–46, and 51–59 yr (Table 2). The difference in the insulin receptor number was larger between the lower two groups (251,000 sites per cell) than between the upper two groups (164,000 sites per cell). The differences between the groups, as regards the ED_{50} values for both antilipolysis and stimulation of glucose oxidation, showed a similar pattern. Thus, the difference in the ED_{50} values was larger between the lower two groups (22-fold for lipolysis, 33-fold for $^{14}CO_2$ pro-

TABLE 2
Insulin action in human adipose tissue of three age groups

	Lower	Middle	Higher
N	6	5	4
Mean age (yr)	23	38	57
Range	23–24	30–46	51–59
Receptor number (sites/cell × 10^3)	647 ± 62	396 ± 76	232 ± 42
ED_{50}, lipolysis (µU/ml)	0.3	6.5	45
ED_{50}, $^{14}CO_2$ production (µU/ml)	5	165	1500
Insulin responsiveness, $^{14}CO_2$ production (µmol/10^7 cells/2 h)	1.20 ± 0.34	0.77 ± 0.22	0.17 ± 0.06

The subjects were divided into three groups according to age. Insulin receptor number, ED_{50} values for lipolysis and glucose oxidation, and the maximum insulin-induced $^{14}CO_2$ production were calculated. Values are mean ± SEM. N = number of subjects. For further details, see legends to Figures 1–3.

duction) than between the upper two groups (sevenfold for lipolysis, ninefold $^{14}CO_2$ production). The age-related difference in the maximum insulin-induced glucose oxidation was similar for the upper and the lower two age groups with values of 0.43 and 0.60 μmol/10^7 cells/2 h, respectively.

The presently observed ED_{50} value for glucose oxidation in the middle-aged group (700 μU/ml) is much higher than that previously reported (30 μU/ml)[11] for seven healthy subjects aging 31–60 yr (mean age 45 ± 4 yr). If, however, three subjects in the latter group were excluded, the remaining four subjects had an almost identical age (range 40–59 yr, mean age 47 ± 4 yr) as the middle-aged subjects presently studied. When the mean dose-response curve for glucose oxidation relating to this newly formed group was linearized, the ED_{50} value was calculated to be 400 μU/ml. More important, the position of the regression line was not statistically different from that relating to the middle-aged group presently studied (F = 2.05, NS). On the basis of this finding it may be suggested that the low ED_{50} value for glucose oxidation in the control subjects previously studied[11] was due to the fact that also younger subjects were included in this study group. When subjects from the two experimental series are better matched for age, there is no statistical difference in the level of insulin sensitivity.

DISCUSSION

It has been proposed that the well documented lowering of glucose tolerance with age is due in some measure to the development of resistance to the action of insulin at the cellular level.[3] To identify the mechanisms underlying this resistance to insulin, it is necessary to study simultaneously receptor binding and the metabolic effects of the hormone in a target tissue of insulin action. The results of this study show clearly that aging is accompanied by a significant decline in the action of insulin in human adipose tissue. The observed progressive reduction of insulin binding with age was essentially due to a decrease in the number of insulin receptors. Since the maximum effect of insulin is elicited when only a fraction of the available receptors is occupied,[21] a decrease in the insulin binding capacity would presumably result in a corresponding loss of sensitivity to the hormone.[4] That insulin sensitivity was much lower in the older subjects is reflected in the large rightward shift of the insulin dose-response curves both for antilipolysis and stimulation of glucose oxidation. Thus, a comparison of the middle-aged and the young subjects disclosed that a reduction of the number of insulin receptors by half corresponded to a 10–20-fold increase in the ED_{50} values. The maximum insulin-induced production of $^{14}CO_2$ was also significantly lower in the older than in the younger subjects—this confirms earlier observations[22]—and there was a negative correlation between the maximum insulin-induced glucose oxidation and age. Since a decline in insulin responsiveness has been considered to reflect a decrease in the effectiveness of the hormone distal to the activated receptor[4] these findings suggest an age-related postreceptor defect in the action of insulin on the utilization of glucose by adipose tissue. However, the possibility of a primary effect of aging on the ability of adipocytes to metabolize glucose cannot be ruled out, since the basal production of $^{14}CO_2$ was significantly lower in the older than in the younger subjects, and the basal rate of glucose oxidation was negatively correlated with age. It is possible that age has a bearing on rate-limiting steps in intracellular pathways of glucose metabolism or the number of glucose transport carriers available. As regards the antilipolytic effect of insulin, the fact that the levels of responsiveness to the hormone in the middle-aged and the young groups were similar indicates that this property of the hormone was unimpaired at the postreceptor level. This suggests that age exerts a selective influence on a separate intracellular pathway for the action of insulin.

Insulin degradation during the course of the metabolic experiments was similar and low (4–7%) in the two study groups. Thus, it seems unlikely that this factor had any bearing on the present findings.

Previous findings relating to age and insulin binding to human fat cells are contradictory. Pagano and co-workers demonstrated[23] that insulin binding was decreased in elderly subjects owing to a reduction in the number of insulin receptors. However, the possible influence of body weight was disregarded and no measurements of the metabolic effects of insulin were made. In a recent abstract Fink and co-workers[24] present results obtained from middle-aged (38 ± 3 yr) and elderly subjects (71 ± 2 yr). The peripheral insulin sensitivity, as measured by the euglycemic clamp technique, was found to be lower in the older subjects, whereas there was no difference between the two age groups as regards the tracer ^{125}I-insulin binding to isolated adipocytes. While reasons for the disparity between their results and ours are obscure some possible explanations may be discussed. Since we observed the largest reduction in the insulin receptor number between the ages of 20 and 40 years (Table 2), it is possible that in the study by Fink and co-workers,[24] a major part of the age-related decrease in insulin binding that apparently occurs in early adulthood was overlooked. Furthermore, as the insulin receptor binding was determined at only a tracer concentration of ^{125}I-insulin the number of insulin receptors cannot be estimated. Except for the above-mentioned studies, the relationship between aging and insulin binding in man has been investigated only in cultured skin fibroblasts[25–27] and leukocytes[28,29] and the results are conflicting; the insulin binding capacity being variously reported as decreasing,[25] remaining constant,[26,28,29] or increasing[27] with age. With these disparate results in mind, it is evident that in any study of the relationship between age and insulin action in man a primary target tissue of the hormone should be used.

The quantity of body fat[30] and the fat cell size[31] both increase with age, and these factors may have a bearing on the action of insulin. It is, however, improbable that they influenced the present findings, since the young and middle-aged subjects were normal in weight and matched for fat cell size and sex.

When the subjects were further divided into three groups according to age the reduction in the number of insulin receptors was found to be greatest in early adulthood. Thus, between 20 and 40 yr, the number of insulin receptor decreased by about 250,000 per cell, whereas between 40 and 60 yr, the difference was only 150,000. Insulin sensitivity followed a similar pattern; the ED_{50} values for lipolysis and glucose oxidation increased 20–30-fold between 20 and 40 yr of age, but only about eightfold between 40 and 60 yr.

This indicates that the impairment of insulin action at the receptor level is greatest in early adult life. These findings, admittedly in small age groups, are consistent with DeFronzo's observation[3] that the decline in the in vivo peripheral insulin sensitivity was most prominent between ages 20 and 45 yr, after which there was a tendency to level off. As regards the development of the postreceptor defect, however, a different pattern was noted. Thus, the observed decrease in the responsiveness to the stimulatory effect of insulin on glucose oxidation was similar between ages 40 and 60 yr and between 20 and 40 yr (0.5–0.6 μmol/10^7 cells/2 h).

According to the theory of "spare receptors," impairment of insulin action at the receptor level can be compensated for by an increase in the level of circulating insulin, whereas a deterioration at the postreceptor level cannot be offset.[4,32] Since the loss of insulin receptors is apparently greatest in early adulthood, and since insulin secretion after glucose challenge is not decreased in the elderly,[1,2] the receptor defect may not be of critical importance for the development of insulin resistance in the aged. The postreceptor defect, however, seems to develop uniformly with age, and would appear to have a greater bearing on the pathogenesis of glucose intolerance due to insulin resistance in the elderly. Further evidence for this interpretation is found in the observation that insulin-resistant, normoinsulinemic, non-insulin-dependent diabetics have been found to have a severe postreceptor defect of insulin action on glucose metabolism in adipose tissue, whereas insulin binding to fat cells was normal.[11] It must be born in mind, however, that only a minute part of orally administered glucose is utilized by human adipose tissue.[33] Impairment of the action of insulin on other major target tissues, such as liver and skeletal muscle, may be a more important factor in the development of glucose intolerance and hyperglycemia in the elderly. It remains to be established in man whether the influence of age on the action of insulin in these target tissues differs from that presently observed in adipose tissue.

ACKNOWLEDGMENTS

The present study was supported by grants from the Swedish Medical Research Council, Karolinska Institute, The Swedish Medical Association, the Nordic Insulin Foundation, The Swedish Diabetes Association, The Swedish Society for Medical Sciences, The Förenade Liv Mutual Group Life Insurance Co., and The Research Foundations of Osterman, Hierta, Groschinsky, Tore Nilsson, and Folksam.

REFERENCES

[1] Davidson, M. B.: The effect of aging on carbohydrate metabolism: a review of the english literature and a practical approach to the diagnosis of diabetes mellitus in the elderly. Metabolism 1979; 28:688–705.

[2] DeFronzo, R. A.: Glucose intolerance and aging. Diabetes Care 1981; 4:493–501.

[3] DeFronzo, R. A.: Glucose intolerance and aging. Evidence for tissue insensitivity to insulin. Diabetes 1979; 28:1095–1101.

[4] Kahn, C. R.: Insulin resistance, insulin sensitivity and insulin unresponsiveness: a necessary distinction. Metabolism 1978; 27:1893–1902.

[5] Rizza, R. A., Mandarino, L. J., and Gerish, J. E.: Mechanisms of insulin resistance in man. Assessment using the insulin dose-response curve in conjunction with insulin-receptor binding. Am. J. Med. 1981; 70:169–76.

[6] Ikkos, D., and Luft, R.: On the intravenous glucose tolerance test. Acta Endocrinol. (Copenh.) 1957; 25:312–34.

[7] Coburn, H. J., and Carrol, J. J.: Improved manual and automated calorimetric determination of serum glucose with use of hexokinase and glucose-6-phosphate dehydrogenase. Clin. Chem. 1973; 19:127–30.

[8] Wide, L., Axen, R., and Porath, J.: Radioimmunosorbent assay for proteins. Chemical couplings of antibodies to insoluble dextran. Immunochemistry 1967; 4:381–86.

[9] Arner, P., Arner, O., and Östman, J.: The effect of local anaesthetic agents on lipolysis by human adipose tissue. Life Sci. 1973; 13:166–69.

[10] Rodbell, M.: Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J. Biol. Chem. 1964; 239:375–80.

[11] Bolinder, J., Arner, P., and Östman, J.: Postreceptor defects causing insulin resistance in normoinsulinemic non-insulin-dependent diabetes mellitus. Diabetes 1982; 31:911–16.

[12] Bolinder, J., Kager, L., Östman, J., and Arner, P.: Differences at the receptor and postreceptor levels between human omental and subcutaneous adipose tissue in the action of insulin on lipolysis. Diabetes. 1983; 32:117–23.

[13] Scatchard, G.: The attraction of protein for small molecules and ions. Ann. NY Acad. Sci. 1949; 51:660–72.

[14] Arner, P., Bolinder, J., Engfeldt, P., and Östman, J.: The antilipolytic effect of insulin in human adipose tissue in obesity, diabetes mellitus, hyperinsulinemia, and starvation. Metabolism 1981; 30:753–60.

[15] Sjöström, L., Björntorp, P., and Vrana, J.: Microscopic fat cell size measurements of frozen-cut adipose tissue in comparison with automatic determinations of osmium-fixed fat cells. J. Lipid Res. 1971; 12:521–30.

[16] Hirsch, J., and Gallian, E.: Methods for the determinations of adipose cell size and cell number in man and animals. J. Lipid Res. 1968; 9:110–19.

[17] Zinder, O., and Shapiro, B.: Effect of cell size on epinephrine- and ACTH-induced fatty acid release from isolated fat cells. J. Lipid Res. 1971; 12:91–95.

[18] Neter, J., and Wasserman, W.: Comparison of two regression lines. *In* Applied Linear Statistical Models, Illinois. Georgetown, Irwin, 1974:160–61.

[19] DeMeyts, P., and Roth, J.: Cooperativity in ligand binding: a new graphic analysis. Biochem. Biophys. Res. Commun. 1975; 66:1118–26.

[20] Jacobsson, B., Holm, G., Björntorp, P., and Smith, U.: Influence of cell size on the effects of insulin and noradrenaline on human adipose tissue. Diabetologia 1976; 12:69–72.

[21] Kono, T., and Barham, F. W.: The relationship between the insulin binding capacity of fat cells and the cellular response to insulin: studies with intact and trypsin-treated fat cells. J. Biol. Chem. 1971; 246:6210–16.

[22] Gries, F. A., and Steinke, J.: Comparative effects of insulin on adipose tissue segments and isolated fat cells of rat and man. J. Clin. Invest. 1967; 46:1413–21.

[23] Pagano, G., Cassader, M., Diana, A., Pisu, E., Bozzo, C., Ferrero, F., and Lenti, G.: Insulin resistance in the aged: the role of the peripheral insulin receptors. Metabolism 1981; 30:46–49.

[24] Fink, R. I., Griffin, J., and Olefsky, J. M.: Effect of age upon carbohydrate metabolism in man. Abstract. Diabetes 1982; 31:64A.

[25] Ito, H., Orino, H., and Shimoda, H.: Reduced insulin action in vitro and binding of ^{125}I-insulin to cultured human skin fibroblasts with aging. Abstracts V. Int Congress Endocrinol. 1976:261.

[26] Hollenberg, M. D., and Schneider, E.: Receptors for insulin and epidermal growth factor-urogastrone in adult human fibroblasts do not change with donor age. Mech. Ageing Dev. 1979; 11:37–43.

[27] Rosenbloom, A., and Goldstein, S.: Insulin binding to cultured human fibroblasts increases with normal and precocious aging. Science 1976; 193:412–15.

[28] McConnell, J. G., Buchannan, K. D., Ardill, J., and Stout, R. W.: Glucose tolerance in the elderly: the role of insulin and its receptor. Eur. J. Clin. Invest. 1982; 12:55–61.

[29] Jackson, R. A., Blix, P. M., Matthews, J. A., Hamling, J. B., Din, B. M., Brown, D. C., Belin, J., Rubenstein, A. H., and Nabarro, J. D. N.: Influence of ageing on glucose homeostasis. J. Clin. Endocrinol. Metab. 1982; 55:840–48.

[30] Kekwick, A.: Adiposity. *In* Handbook of Physiology. Section V. Adipose tissue. Renold, A. E., and Cahill, G. F., Eds. Washington D.C., American Physiologic Society, 1965:617–24.

[31] Björntorp, P.: Effects of age, sex and clinical conditions on adipose tissue cellularity in man. Metabolism 1974; 23:1091–1102.

[32] Olefsky, J. M., and Kolterman, O. G.: Mechanisms of insulin resistance in obesity and non-insulin-dependent (type II) diabetes. Am. J. Med. 1981; 70:151–68.

[33] Björntorp, P., and Sjöström, L.: Carbohydrate storage in man: speculations and some quantitative considerations. Metabolism 1978; 27:1853–65.

[34] Documenta Geigy: Scientific tables. 7th Edit. Basel, Geigy Pharmaceuticals, 1970.