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Chronologic and Physiologic Age Affect Replicative Life-Span of Fibroblasts from Diabetic, Prediabetic, and Normal Donors

Abstract. *Cultured skin fibroblasts from subjects with clinically apparent diabetes mellitus and from subjects genetically predisposed to diabetes have a replicative life-span that is inversely related to donor age. Fibroblasts from carefully defined normal subjects not predisposed to diabetes fail to show this correlation. The data support the idea that physiologic status of the tissue donor is a more precise determinant of fibroblast replicative lifespan than chronologic age.*

The cultured human fibroblast has a finite replicative lifespan (*1–3*) which is inversely proportional to the age of the donor (*3–7*). Moreover, this negative correlation appears to hold true whether the tissue of origin is lung (*3*), liver (*6*), or skin of various anatomical sites (*4, 5, 7*). Most tissue donors in such studies have, as a rule, been randomly chosen from living subjects and from subjects at postmortem, many of them with overt pathology (*1–3, 5–7*). But other donors, affected by specific inherited disorders of premature and severe aging, give rise to fibroblast strains with significantly decreased replicative lifespans in comparison to age-matched controls (*5, 8*). At several laboratories it has now been demonstrated that diabetes mellitus, a common genetically determined disorder that reduces life expectancy (*9*), also has an adverse, although more subtle, influence on fibroblast growth capacity (*4, 5, 10*). The present results indicate that both clinically apparent diabetics and subjects genetically predisposed to diabetes show the inverse correlation between donor age and replicative lifespan of cultured fibroblasts, whereas carefully selected normal individuals fail to show this phenomenon.

Three groups of subjects, 25 normal controls, 26 diabetics, and 21 "prediabetics" (both parents of each prediabetic had maturity-onset diabetes) volunteered skin biopsies for this study. Fibroblast strains were developed from 4-mm punch biopsies of anterior forearm skin and grown in a humidifed atmosphere (95 percent air, 5 percent CO_2 at 37.0 ± 0.3°C) (*4*). The normal subjects were in excellent health, had a negative family history for diabetes, and showed normal glucose tolerance after repeated testing (*11*). Diabetic subjects were predominantly free of clinical complications and were not affected by other specific genetic syndromes that are frequently associated with diabetes (*5, 8*). Of the 26 diabetics, 24 were on daily insulin therapy, while the other two were regulated on oral hypoglycemic agents. The prediabetics, although they show no clinical evidence of diabetes, presumably carry an increased risk of developing diabetes, although it is now believed that these subjects may only have a 10 to 20 percent risk of developing clinically apparent diabetes by the age of 45 (*11*).

Samples were assigned code numbers at the time of biopsy and handled in random order to avoid systematic bias. All tissue culture operations were then carried out by one of us (E.J.M.) without knowledge of the specific or group identities of each donor. Cells were harvested from explants and subcultured at a 1 : 8 ratio as soon as they attained confluence, three mean population doublings (MPD) being counted each time (*2–7*). The number of cells that became attached to the petri dish after each subculture was not routinely determined. But in random tests on all strains, plating efficiency 6 hours after subculture ranged from 80 to 100 percent at early passage and 50 to 80 percent at late passage; no significant differences were found between the cells of normal subjects, prediabetics, or diabetics at these two passage levels. Thus, while the cumulative number of MPD is clearly an underestimate in all cases (*2–7*), values in the three groups can still be compared with validity.

The onset of senescence in cultures was marked by a slowing of growth so that longer periods were required between subculture. Cultures not confluent by day 7 were given a complete change of medium then and each week thereafter until they became confluent, whereupon they were subcultured. If a cell strain was unable to grow to confluence after 28 days with three changes of medium (on days 7, 14, and 21), it was declared dead and the cumulative number of MPD at the last confluence was desig-

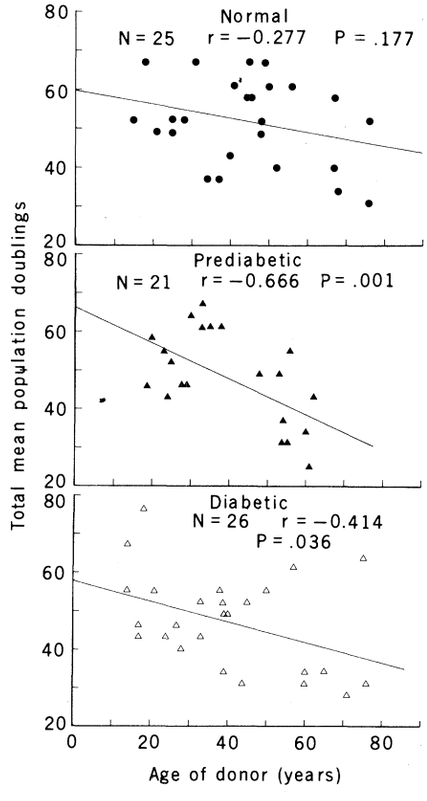

Fig. 1. Correlation between donor age and replicative life-span of cultured fibroblasts (total mean population doublings) in normal, prediabetic, and diabetic individuals. The linear regression equations are: for normals, $y = 59.24 - 0.25\ x$; prediabetics, $y = 66.57 - 0.46\ x$; and diabetics, $y = 57.80 - 0.27\ x$. The mean age (± S.D.) of diabetic patients was 40.15 ± 19.25 (range 14 to 76) years; prediabetics, 40.00 ± 15.06 (15 to 62) years; and normals, 44.28 ± 17.50 (15 to 76) years. The mean weight in the three groups according to Metropolitan Life Insurance tables was between 100 to 108 percent of ideal body weight.

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nated as the replicative life-span. Each strain was monitored for mycoplasma contamination (*12*) two or three times during the life-span and again on termination; results were uniformly negative.

Normal strains showed significantly better growth capacity than diabetic and prediabetic cells for various parameters in primary and secondary culture (*10*); these data will be reported in detail elsewhere (*13*). In no case did growth failure occur in primary cultures, and all cell strains were capable of at least 25 MPD following initial harvest. The replicative life-span of normal strains was 51.76 ± 10.92 MPD (mean ± standard deviation) compared to prediabetic strains, 48.92 ± 11.88, and diabetics, 47.54 ± 13.10. Although these means were not statistically different, a significant linear trend was observed ($P < .05$). That is, with increasing predisposition to diabetes (diabetic > prediabetic > normal) there is a progressive decrease in replicative capacity.

When donor age was plotted against total MPD, diabetics and prediabetics showed a significant negative correlation which was not evident for normal subjects (Fig. 1). When all three groups were combined (Fig. 2) a highly significant negative correlation appeared between donor age and replicative life-span. The slope of this regression line indicated a mean decrement of 0.25 MPD for each year of life, similar to the value reported by Martin *et al.* (*5*).

The results confirm and extend earlier observations on the inverse correlation between donor age and replicative life-span but emphasize the necessity to identify thoroughly the physiologic status of the donors. Thus, individuals with clinically apparent diabetes mellitus, a common genetically determined disorder associated with reduced life expectancy (*9*), conform to the expected inverse correlation. Similar results are found in subjects with a genetic predisposition to diabetes as well as in these two groups combined with normals, and this last situation probably provides a closer approximation to the more random population samplings of earlier studies (*1–3, 5–7*). That carefully selected normal subjects fail to show the negative correlation may be explained by two perhaps interrelated factors. First, the minimal risk of developing diabetes in our normal subjects may be associated with greater genetic heterogeneity than in the other two groups, and therefore, greater variance in fibroblast growth potential. Second, in the normal subjects defined here, particularly those in and beyond middle age, there is a greater likelihood of longevity (*9*), and this may be expressed in vitro as increased replicative capacity of fibroblasts. The idea that cell strains derived from older subjects free of detectable abnormality perform as well as many strains from chronologically younger individuals remains unproved but now warrants further study. However, the converse, that donors with progeria, Werner's syndrome, and other hereditary disorders of premature aging give rise to fibroblast cultures with a downward skew in growth potential (and other cellular defects) is now increasingly evident (*5, 8*).

Our data indicate that in any studies of the effect of donor age on growth or any other parameter in cultured cells the genetic and metabolic identity of each subject must be meticulously defined; likewise, the presence or absence of specific pathology must be established. Taken with other observations (*4, 5, 8, 10, 13*), the data also show that the genetic defects in diabetes mellitus can be expressed in vitro as decreased growth potential in cells of extrapancreatic origin.

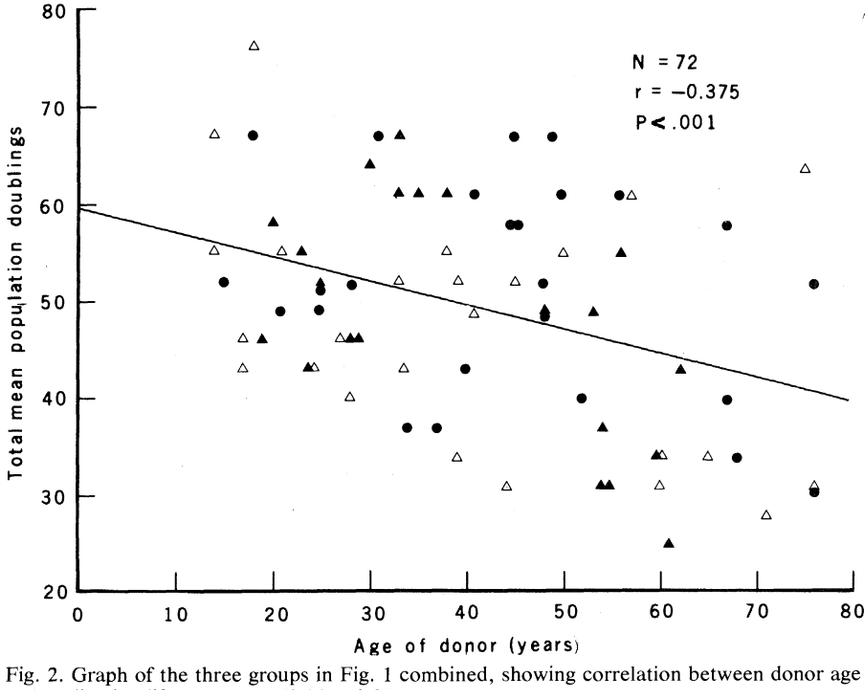

Fig. 2. Graph of the three groups in Fig. 1 combined, showing correlation between donor age and replicative life-span: $y = 59.24 - 0.25\,x$.

SAMUEL GOLDSTEIN
ELENA J. MOERMAN
Departments of Medicine and *Biochemistry,*
McMaster University, Hamilton, Ontario, Canada L8S 4J9
J. STUART SOELDNER
RAY E. GLEASON
DONALD M. BARNETT
E. P. Joslin Research Laboratory, Department of Medicine, Harvard Medical School, Boston, Massachusetts 02215

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