

Insulin Resistance as a Predictor of Age-Related Diseases

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The current study was initiated to evaluate the ability of insulin resistance to predict a variety of age-related diseases. Baseline measurements of insulin resistance and related variables were made between 1988–1995 in 208 apparently healthy, nonobese (body mass index < 30 kg/m²) individuals, who were then evaluated 4–11 yr later (mean ± SEM = 6.3 ± 0.2 yr) for the appearance of the following age-related diseases: hypertension, coronary heart disease, stroke, cancer, and type 2 diabetes. The effect of insulin resistance on the development of clinical events was evaluated by dividing the study group into tertiles of insulin resistance at baseline and comparing the events in these 3 groups. Clinical endpoints (n = 40) were identified in 37 individuals (18%) of those evaluated, including 12 with hypertension, 3 with hypertension + type 2 diabetes, 9 with cancer, 7 with coronary heart disease, 4 with

stroke, and 2 with type 2 diabetes. Twenty-eight out of the total 40 clinical events were seen in 25 individuals (36%) in the most insulin-resistant tertile, with the other 12 occurring in the group with an intermediate degree of insulin resistance. Furthermore, insulin resistance was an independent predictor of all clinical events, using both multiple logistic regression and Cox's proportional hazards analysis. The fact that an age-related clinical event developed in approximately 1 out of 3 healthy individuals in the upper tertile of insulin resistance at baseline, followed for an average of 6 yr, whereas no clinical events were observed in the most insulin-sensitive tertile, should serve as a strong stimulus to further efforts to define the role of insulin resistance in the genesis of age-related diseases. (*J Clin Endocrinol Metab* 86: 3574–3578, 2001)

WE HAVE RECENTLY shown (1), in a prospective study, that resistance to insulin-mediated glucose disposal predicted cardiovascular morbidity in a group of 147 healthy volunteers, with an average age of 50 yr at baseline, followed for 4.7 yr. The focus of the initial study was on cardiovascular disease, in light of the many observations linking insulin resistance and/or its manifestations to this clinical endpoint (2–9). The current study was initiated to extend our earlier observations, expanded to include a total of 208 individuals evaluated between 1988–1995, and the clinical endpoints enlarged to include type 2 diabetes and cancer as additional outcome events to be evaluated. Although evidence from human and animal studies has led to the suggestion that insulin resistance, or hyperinsulinemia, may play a role in the development of cancer (10–13), we are not aware of any prospective studies that have examined this view. The results to be presented are based upon study of 208 individuals, with no apparent disease at baseline, evaluated 4–11 yr after their initial assessment of degree of insulin resistance.

Materials and Methods

To select apparently healthy individuals for this prospective study, the population to be evaluated was limited to volunteers recruited during the period from 1988–1995, who met the following criteria: more than 30 yr of age; body mass index (BMI) less than 30 kg/m²; no history of hypertension (HT), and blood pressure less than 145/90 mm Hg, normal physical examination and routine clinical chemistries, and a normal oral glucose tolerance test (OGTT) (14).

Abbreviations: BMI, Body mass index; CHD, coronary heart disease; CVA, cerebrovascular accident; HDL, high-density lipoprotein; HT, hypertension; LDL, low-density lipoprotein; SSPG, steady-state plasma glucose; OGTT, oral glucose tolerance test; TG, triglycerides.

Measurements at baseline included weight, height, sitting blood pressure, and fasting lipid and lipoprotein concentrations (1). In addition, level of habitual physical activity was assessed by questionnaire based on reporting the number of activities per week that resulted in sweating (15).

Evaluation of glucose and insulin metabolism at baseline included a standard 75-g OGTT, with blood samples for measurement of plasma glucose and insulin concentration obtained before, and 30, 60, 120, and 180 min after the oral glucose load. The area under the curve was calculated by the trapezoidal formula to estimate the postload glucose and insulin areas (1). Insulin resistance was measured at baseline by the insulin suppression test as in our earlier study. Briefly, subjects were continuously infused for 180 min with somatostatin (250 µg/h), insulin (25 mU/m²·min), and glucose (240 mg/m²·min). Blood was drawn for measurement of plasma glucose and insulin concentrations every 10 min during the last 30 min of the infusion, and the average of these four values (150, 160, 170, 180 min) was used to define the steady-state plasma glucose (SSPG) and steady-state plasma insulin concentrations. Plasma glucose and insulin concentrations reached a plateau by 120 min, and the steady-state plasma insulin concentrations were essentially identical in all individuals. Therefore, the SSPG concentrations provide an estimate of how effective the same amount of insulin is in mediating disposal of the infused glucose load; the higher the SSPG, the more insulin resistant the individual. Insulin resistance determined with this method correlates almost perfectly ($r > 0.9$) with values obtained by the insulin clamp technique (16).

Follow-up evaluation was performed 4–11 yr after the baseline studies, with a mean (±SEM) duration of 6.3 ± 0.2 yr. All subjects were asked about their current status of health; medication usage; whether or not they had developed cancer, diabetes, or high blood pressure; and to complete the Rose questionnaire on chest pain (17). All positive reportings were verified by examination of the medical record, with the cooperation of the primary care physician in each instance, and included tissue evidence in the case of a diagnosis of cancer. For those who could not be contacted, it was assumed that either they had moved away or died. The names of these individuals were submitted to the Office of State Registrar in California for search against the death registry.

The study endpoints were the development of HT, coronary heart disease (CHD), stroke, type 2 diabetes, or cancer. HT was defined as the

use of antihypertensive medication; CHD included chest pain with positive stress test, coronary angiography (with or without revascularization), coronary bypass surgery, or documented myocardial infarction; stroke included documented clinical neurological deficit lasting over 24 h, with or without confirmatory neuroimaging [cerebrovascular accident (CVA)]; diabetes (type 2) was assumed to be present in subjects treated with at least one oral hypoglycemic agent; and cancer was defined by history of specific treatment (radio-, chemo-, or palliative therapy or a combination of the above), and tissue diagnosis.

Results are expressed as mean ± SEM, and statistical significance was evaluated by ANOVA and contingency table, as appropriate. Nonparametric variables: triglycerides (TG), postload insulin area, and SSPG concentration were log-transformed before analysis. Univariate, multivariate, and logistic regression analysis were used to assess the relationships and interactions of baseline variables, with age-related diseases considered as categorical outcome variable(s). The proportional hazards model (Cox regression) was also used to evaluate relationships among study variables and clinical outcomes in a time-independent manner. All the calculations were performed with a commercial statistical software (Statistica, Statsoft Inc., Tulsa, OK) for the Macintosh computer (mod. iBook, Apple Computers, Cupertino, CA).

Results

During the period of 1988–1995, 290 healthy volunteers met all of the criteria for inclusion into this study, and we were able to obtain follow-up data on 208 (98 males, 110 females) of these individuals (72%). Baseline demographic characteristics of those available for evaluation were quite similar to those individuals lost to follow-up (age, 50 vs. 48 yr; BMI, approximately 24.7 vs. 24.5 kg/m²).

Clinical endpoints (n = 40) were identified in 37 individuals of those evaluated, including 12 with HT, 9 with cancer, 7 with CHD, and 5 with type 2 diabetes, 3 of which also had high blood pressure, and 4 with stroke. The cancers were distributed as follows: 3 prostate, 2 gastric, 1 breast, 1 colon, 1 bladder, and 1 renal. There were six deaths reported: 2 cardiovascular, 3 cancer related, and 1 due to infection. Search through the State of California Death Registry was negative for those individuals lost to follow-up. It should be emphasized that the period of observation after baseline was 6.2 ± 0.2 yr, 6.3 ± 0.2 yr, and 6.5 ± 0.2 yr in tertile 1, 2, and 3, respectively.

The 208 subjects were divided into tertiles on the basis of their SSPG concentrations. The baseline clinical characteristics of the 3 groups are given in Table 1. Subjects in the highest

SSPG tertile were older and had a higher BMI, diastolic blood pressure, plasma TG, total cholesterol, and low-density lipoprotein (LDL) cholesterol concentrations. In addition, high-density lipoprotein (HDL) cholesterol concentrations were lower, and these individuals were less physically active. The male-to-female ratios were similar in each tertile (31/38, 34/35, and 33/37, respectively, for the low, mid, and top tertile), as well as the number of smokers (~10%).

Fig. 1 illustrates the number of clinical events in the 3 SSPG tertiles. The most striking observation is that none of the 5 endpoints occurred in the most insulin-sensitive tertile. In marked contrast, 25 individuals in the most insulin-resistant tertile (36% of the group) had a total of 28 clinical events. Clinical endpoints were also observed in 12 individuals in the middle SSPG tertile, but it should be noted that only 1 individual in this group (1.4%) had CHD, compared with 6 of those in the most insulin-resistant tertile (8.6%). The rate of developing age-related diseases was significantly different in the 3 tertiles (P < 0.002). Deaths were also confined to the 2 most resistant tertiles, with 2 observed in the middle (infection and cancer), and 4 in the upper (2 cardiovascular and 2 cancer-related).

Because insulin resistance (SSPG) is related to a cluster of demographic, hemodynamic, and metabolic variables, multiple regression analysis was performed to estimate which relationships would remain significant after adjustment for age, gender, BMI, level of activity, and smoking. The results of this analysis are seen in Table 2, and it is apparent that the relationships between SSPG concentration and diastolic blood pressure concentration of cholesterol, LDL cholesterol, HDL cholesterol, and TG, and both the plasma glucose and insulin responses during the OGTT, were all statistically significant and were independent of age, gender, BMI, smoking status, and level of physical activity.

The results in Table 2 emphasize the well-established covariance of the multiple variables related to insulin resistance and compensatory hyperinsulinemia (4, 6, 9), and the difficulty of establishing the nature of the relationship between them and various clinical outcomes. In approaching this problem, we initially used univariate logistic regression analysis. These results are shown in Table 3; and it can be seen

TABLE 1. Baseline characteristics of the 208 subjects divided into tertiles according to their baseline steady-state plasma glucose concentration; P values for low vs. top tertile

Variable	Low tertile	Mid tertile (SEM)	Top tertile (SEM)	P value
SSPG (mM)	3.2 ± 0.1	5.9 ± 0.1	11.3 ± 0.3	0.0001
Age (yr)	48 ± 1	51 ± 1	53 ± 1	0.002
Body mass index (kg/m ²)	23.1 ± 0.3	24.9 ± 0.3	25.6 ± 0.3	0.0001
Systolic BP (mm Hg)	116 ± 2	119 ± 2	123 ± 2	0.02
Diastolic BP (mm Hg)	69 ± 1	72 ± 1	77 ± 1	0.0001
Triglycerides (mM)	0.82 ± 0.03	1.07 ± 0.04	1.45 ± 0.04	0.0001
Cholesterol (mM)	4.68 ± 0.09	5.01 ± 0.1	5.07 ± 0.01	0.03
LDL cholesterol (mM)	2.62 ± 0.08	2.86 ± 0.1	2.91 ± 0.1	0.005
HDL cholesterol (mM)	1.51 ± 0.04	1.38 ± 0.04	1.20 ± 0.04	0.0001
Ratio of total/HDL cholesterol	3.2 ± 0.11	3.9 ± 0.14	4.5 ± 0.16	0.0001
Glucose area (mM/h)	16.3 ± 0.4	18.4 ± 0.4	21.3 ± 0.5	0.0001
Insulin area (pM/h)	602 ± 29	847 ± 39	1674 ± 119	0.0001
Exercise (sweats/wk)	3.5 ± 0.3	2.9 ± 0.3	2.5 ± 0.3	0.04
Smoker (%)	9	8	11	NS

BP, Blood pressure; NS, not significant.

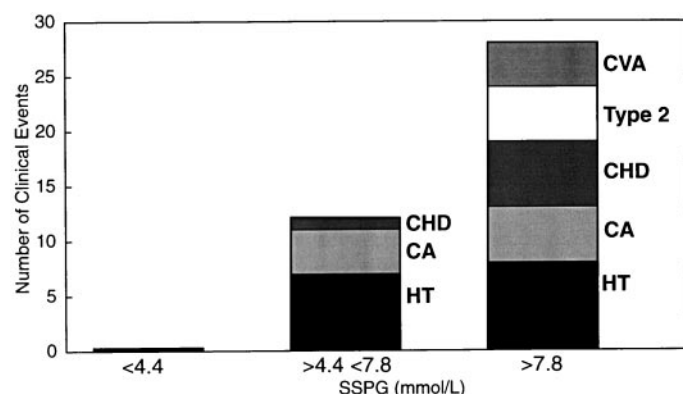


FIG. 1. The number of clinical events observed, as a function of insulin resistance tertile at baseline. CA, Cancer; Type 2, type 2 diabetes. These were 28 events in the highest tertile (SSPG > 7.8 mM), 12 in the intermediate tertile (SSPG > 4.4 < 7.8 mM), and none in the most insulin-sensitive tertile (SSPG < 4.4 mM).

TABLE 2. Multiple regression analyses between SSPG and its cluster of covariables, adjusted for differences in age, gender, BMI, smoking status, and level of physical activity

Variable	β coefficient	SE	P value
Systolic blood pressure	0.14	0.08	0.08
Diastolic blood pressure	0.31	0.07	0.0001
Cholesterol	0.21	0.07	0.03
LDL cholesterol	0.22	0.06	0.0002
HDL cholesterol	-0.29	0.08	0.0001
Triglycerides	0.40	0.07	0.00001
Glucose area	0.44	0.06	0.00001
Insulin area	0.65	0.05	0.00000

TABLE 3. Univariate logistic regression analysis between SSPG, its associated variables, and all age-related diseases, adjusted for differences in age, gender, BMI, smoking status, and level of physical activity

Variable	Estimate	SE	P
SSPG	1.03	0.19	0.0001
Systolic BP	0.04	0.03	0.07
Diastolic BP	0.08	0.02	0.01
Triglycerides	0.54	0.18	0.003
Cholesterol	0.03	0.05	0.63
LDL cholesterol	0.008	0.006	0.25
HDL cholesterol	-0.73	0.23	0.002
Glucose area	0.6	0.20	0.003
Insulin area	0.8	0.18	0.0001

that SSPG concentrations, diastolic blood pressure, HDL cholesterol and TG concentrations, and the plasma glucose and insulin responses were all statistically related to the aggregate of clinical events. However, neither systolic blood pressure nor LDL cholesterol concentrations retained statistical significance.

Table 4 presents the results of multiple logistic regression analysis evaluating the independence of the relationship between the variables identified in the univariate analysis as being associated with the appearance of all age-related diseases. It is apparent that, when taken as an aggregate, only insulin resistance (SSPG concentration) was an independent predictor of the 40 age-related clinical events. Although the number of individual events were relatively small, we also defined the relationship between them and the various risk

TABLE 4. Summary of multiple logistic regression analyses evaluating the independence of SSPG and associated variables for each clinical endpoint

Endpoint	Independent variable	P
Hypertension (n = 15)	TG	0.02
	Diastolic blood pressure	0.03
CHD + CVA (n = 11)	SSPG	0.008
	SSPG	0.01
Cancer (n = 9)	SSPG	0.0003 ^a
	OGTT-glucose	0.0004 ^a
Type 2 diabetes (n = 5)	OGTT-insulin	0.0007 ^a

^a Univariate relationship. When all three were entered into the multiple logistic regression analysis, the variable closest to being statistically significant was SSPG ($P = 0.12$).

factors identified in Table 3. Because stroke was observed only in 4 subjects, it was combined with CHD for this analysis. These results show that insulin resistance (SSPG concentration) was also the only independent predictor of CHD + stroke and cancer. SSPG concentration and both glucose and insulin responses were correlated with the development of type 2 diabetes in a univariate analysis, but when all 3 were entered into the multiple regression analysis, none of them emerged as being independent. This finding was not surprising, given how closely these 3 variables are related. In the case of HT, both diastolic blood pressure and plasma TG concentration were independent predictors.

Finally, although the period of observation was quite similar in the three SSPG tertiles, we thought it important to also evaluate the potential confounding effect of time by performing Cox's proportional hazards analysis, using all clinical events, HT, CHD + stroke, and cancer as the four endpoints. These results are seen in Table 5, and they again document the highly significant relationship between insulin resistance (SSPG concentration) and the aggregate of clinical events ($P < 0.02$), CHD + stroke ($P < 0.02$), and cancer ($P < 0.05$). It is also clear, from these data, that the impact of insulin resistance on all of these outcomes is independent of BMI.

Discussion

This study was initiated to evaluate the hypothesis that insulin resistance would predict the development, over time, of clinical syndrome that might be best subsumed under the heading of age-related diseases (HT, CHD, stroke, cancer, and type 2 diabetes). Although the results provide substantial support for this point of view, perhaps the most striking finding was that none of these events were seen in the third of the population that was most insulin-sensitive. Given the fact that the period of observation ranged from 4–11 yr, with an average follow-up of 6.3 yr, the fact that not one clinical event took place in the insulin-sensitive tertile seems to be truly remarkable. If the ability of insulin sensitivity to decrease risk of developing age-related diseases can be confirmed in subsequent studies, the public health implications are enormous. For example, it has been shown (18) that approximately 50% of the variability in insulin-mediated glucose disposal between apparently healthy individuals is related to life-style (25% to differences in weight and 25% to differences in level of habitual physical activity). As a cor-

TABLE 5. Cox regression analysis between predictor and outcome variables

Variable	All Events		Hypertension	
	HRR	(95% CI)	HRR	(95% CI)
Age	1.05 ^a	(.998 ÷ 1.10)	1.11 ^a	(.91 ÷ 1.31)
Gender	0.995	(.956 ÷ 1.02)	1.005	(.996 ÷ 1.02)
BMI	1.13	(.934 ÷ 1.32)	0.967	(.78 ÷ 1.34)
Activity	0.97	(0.71 ÷ 1.25)	0.891	(0.67 ÷ 1.35)
Triglycerides	0.554	(.253 ÷ 855)	1.555 ^a	(.353 ÷ 2.766)
LDL cholesterol	1.000	(.982 ÷ 1.018)	1.001	(.993 ÷ 1.009)
HDL cholesterol	0.871	(0.541 ÷ 1.20)	0.719	(0.687 ÷ 0.76)
Glucose area	1.03	(0.430 ÷ 1.63)	1.31	(0.65 ÷ 2.01)
Insulin area	1.24	(.680 ÷ 1.80)	1.47	(.89 ÷ 2.112)
SSPG	40.0 ^b	(35.0 ÷ 45.04)	1.81	(0.61 ÷ 2.42)
	CHD + Stroke		Cancer	
	HRR	(95% CI)	HRR	(95% CI)
Age	1.04 ^a	(.97 ÷ 1.11)	1.23 ^a	(.89 ÷ 1.57)
Gender	0.958	(.90 ÷ 1.02)	1.00	(.90 ÷ 1.10)
BMI	1.12	(.87 ÷ 1.37)	1.22	(.90 ÷ 1.54)
Activity	0.945	(.54 ÷ 1.35)	0.909	(.79 ÷ 1.03)
Triglycerides	0.899	(.20 ÷ 1.60)	1.00	(.23 ÷ 1.77)
LDL cholesterol	0.992	(.97 ÷ 1.01)	0.999	(.98 ÷ 1.00)
HDL cholesterol	1.10	(.33 ÷ 1.83)	1.25	(0.88 ÷ 1.37)
Glucose area	0.87	(.19 ÷ 1.55)	1.07	(0.99 ÷ 1.15)
Insulin area	1.26	(.62 ÷ 1.90)	2.12	(0.99 ÷ 3.29)
SSPG	33.3 ^b	(27.3 ÷ 39.3)	5.53 ^a	(2.21 ÷ 8.73)

^a $P < 0.05$.^b $P < 0.02$.

ollary, the ability of life-style interventions to improve insulin sensitivity and to reduce risk of age-related diseases is self-evident. If 50% of the variability in insulin-mediated glucose disposal is related to life-style, it is likely that the remaining 50% is attributable to genetic differences. In support of this view is evidence that this variable is certainly familial (19). Thus, recognition of families sharing insulin resistance would permit an even more intensive attempt at life-style intervention to improve insulin sensitivity.

The observation that development of type 2 diabetes, HT, and cardiovascular disease was more common in the most insulin-resistant tertile is not surprising. The presence of insulin resistance has been well recognized for approximately 25 yr (20, 21), and insulin resistance and/or compensatory hyperinsulinemia have been shown to predict type 2 diabetes in several prospective studies (22–25). Similarly, the existence of insulin resistance and/or compensatory hyperinsulinemia in patients with essential HT has been extensively documented (26), and hyperinsulinemia as a surrogate measure of insulin resistance has also been shown to be an independent predictor of essential HT (27–30). There is also considerable evidence that insulin resistance and/or hyperinsulinemia predict the development of cardiovascular disease (1–6), but controversy continues as to the validity of this association (31, 32).

In contrast to the association between insulin resistance and the clinical syndromes discussed above, the possibility that abnormalities of insulin metabolism might be linked to the risk of cancer has received much less attention. However, the fact that its prevalence increases with age, and the existence of published reports suggesting that hyperinsulinemia might increase cancer risk (10–13), led to its inclusion in this

study as one of the endpoints to be evaluated. The results showed that cancer was diagnosed in nine subjects over an average period of 6 yr. Not surprisingly, the subjects who developed cancer were somewhat older at baseline (61 ± 2 yr), compared with those who had either a noncancer (53 ± 2 yr) or no (50 ± 1 yr) events, but the three groups were similar in terms of period of observation, BMI, or history of smoking. Furthermore, the relationship between insulin resistance and cancer defined in Table 4 was present, when age-adjusted, and the results of the Cox proportional hazard analysis in Table 5 also document a statistically significant and independent relationship between insulin resistance and cancer. However, it should be emphasized that the cases of cancer were confined to the upper two tertiles, and a wide variety of different cancers were observed. Thus, in the case of cancer, it could be argued that the more appropriate conclusion is that insulin sensitivity decreases cancer risk, rather than insulin resistance increasing it. The fact that we cannot differentiate between these two alternatives at this time does not negate the potential importance of our findings nor the need to pursue this issue more vigorously.

Although the experimental findings we have presented are relatively straight-forward, we can only speculate as to why a variety of age-related diseases might be linked to insulin resistance and/or hyperinsulinemia. In this context, it is worth noting that caloric restriction, in both invertebrates and mammals, leads to a decrease in age-related morbidities and enhanced life span, associated with enhanced insulin sensitivity, and lower glucose and insulin concentrations (33–37). An age-related decline in insulin sensitivity in rats allowed free access to food would result in an elevation in ambient glucose concentrations, which could lead to higher rates of protein glycation and glycooxidation, with production of carbonyls, advanced glycation end-products, and protein cross-linking (38–42). Such products of carbohydrate and protein modification can initiate lipid peroxidation (43), with production of genotoxic, atherogenic, and diabetogenic aldehydes (44–48). It should be noted that other explanations have been proposed to account for the observation that caloric restriction can prolong life span. For example, in a recent review article, it was suggested that the benefit of caloric restriction on life span is attributable to the associated reduced fat mass, and a consequent decrease in the secretion of various peptides, cytokines, complement factors, and substrate (49). Finally, evidence has also recently been summarized implicating an increase in levels of IGF-1 as playing a role as a risk factor for several forms of cancer (50), and this offers another possible mechanistic link between insulin resistance/hyperinsulinemia and cancer. Whether any of these possibilities have relevance to our results is clearly conjectural, but they may serve to provide some framework with which to pursue future studies of the association between insulin resistance and age-related diseases.

In conclusion, an age-related disease developed in approximately one out of three healthy individuals who were in the upper tertile of insulin resistance at baseline, followed for an average of 6 yr. In contrast, no clinical event was seen in the most insulin-sensitive tertile. These data should serve as a strong stimulus for further efforts to define the role of insulin resistance in the genesis of age-related diseases.

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