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PRELIMINARY COMMUNICATIONS

Factors Determining Serum-Insulin Response in a Population Sample

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Summary. Serum-insulin levels were measured in 1770 subjects one hour after a 50 g glucose load. Multiple regression analyses reveal that a substantial proportion of the variability of $\log_{10}$ serum-insulin can be attributed to significant independent effect of blood-sugar, age, the food-interval, sex, parameters of body size, and serum uric acid. Females have significantly higher levels of serum-insulin than males "One-hour" blood-sugar levels exceeding 160 mg/100 ml are associated with declining insulin levels. An independent association of increasing age with increasing serum-insulin is evident at 50 years and over. The findings provide strong support for the biological significance of serum-immunoreactive insulin.

Facteurs déterminant la réponse de l'insuline sérique dans un échantillon de population

Résumé. Les taux d'insuline sérique ont été mesurés chez 1770 sujets une heure après une charge en glucose de 50 g. Des analyses de régression multiple révèlent qu'une proportion considérable de la variabilité du $\log_{10}$ de l'insuline sérique revient de façon significative aux effets indépendants entre eux de la glycémie, de l'âge, de l'intervalle entre les repas, du sexe, des paramètres de la taille corporelle et de l'acide urique sérique. Les femmes ont des niveaux d'insuline sérique significativement plus élevés que les hommes. Les taux de la glycémie une heure après, dépassant 160 mg/100 ml sont associés à une défaillance des taux d'insuline. Une association indépendante entre l'augmentation de l'âge et l'augmentation de l'insuline sérique est évidente à 50 ans et plus. Ces résultats apportent un argument solide pour l'importance biologique de l'insuline immunoréactive du sérum.

Einfluß verschiedener Faktoren auf die Seruminsulinausschüttung bei einer Reihenuntersuchung

Zusammenfassung. Die Seruminsulinspiegel 1 Std nach oraler Verabreichung von 50 g Glucose wurden bei 1770 Probanden bestimmt. Eine multiple Regressionsanalyse der Zehnerlogarithmuswerte für die Seruminsulinkonzentrationen ergab, daß ein beträchtlicher Teil der Variationsbreite durch signifikante und von einander unabhängige Einflüsse des Blutzuckers, des Alters, der Intervalle zwischen den Mahlzeiten, des Geschlechtes, des Körpergewichtes und der Harnsäure bedingt ist. Frauen wiesen significant höhere Insulinwerte als Männer auf. Häufig waren 1 Std Blutzuckerwerte über 160 mg% mit sinkenden Insulinspiegeln gekoppelt. Bei über 50jährigen stiegen mit zunehmendem Alter auch die Insulinwerte an. Die Befunde stützen die Annahme, daß den immunologisch gemessenen Seruminsulinspiegeln biologische Bedeutung zukommt.

Key-words: Serum-insulin, blood-sugar, population study, multiple regression analysis.

Introduction and Methods

Factors that relate significantly to the serum-insulin response following a glucose load are obesity (Yalow and Berson 1965), the blood-glucose concentration (Welborn et al., 1966; Buchanan and McKiddie, 1967) and age (Chlouverakis et al., 1967), but the *independent* contribution of such variables has been difficult to assess, since the factors themselves tend to be inter-related. In a population study in Busselton, Western Australia, a large number of physical and biochemical variables were measured in the adult population, including blood-sugar and serum-insulin levels approximately one hour after a 50 g glucose load. Details of the 1966 Busselton Community Health Study are reported elsewhere (Curnow et al., 1968; Welborn et al., 1968). Venous blood-sugar levels were measured immediately by Autoanalyzer macro-method, and serum-immunoreactive insulin levels were estimated on stored samples by a double-antibody technique (Morgan and Lazarow, 1963; Welborn et al., 1966). The interval between glucose load and venepuncture was documented (mean "drink-interval" 65 minutes, standard deviation ±10), as was the interval from last taking food and venepuncture (mean "food-interval" 185 min, standard deviation ±98). This interim report deals with an analysis of 1770 sera, representing an unselected sample of the 3331 sera in the process of being assayed. The age and sex distribution of the 1770 individuals and the means and ranges of the physiological variables are very similar to those of the total population studied. The serum-insulin levels were lognormal in distribution, and the $\log_{10}$-serum-immunoreactive-insulin values ($\log_{10}$-serum-IRI) are used in all statistical analyses.

Results and Discussion

Table 1 shows the mean levels of $\log_{10}$-serum-IRI and mean blood-sugar levels for males and females in 10-year age groups. Females have consistently higher insulin levels than males, statistically significant for the 21—29 year age group ($p < 0.01$), the 50—59 year age group ($p < 0.05$), and for all ages pooled ($p < 0.001$). Log_{10}-serum-IRI rises progressively with increasing age in both sexes. Blood-sugar levels also rise with age, but there is no significant difference between the sexes.

Table 2 gives the mean levels (± 1 standard deviation) of the other variables measured in the sample, and shows the simple correlations that obtain between

Table 1. *Mean levels (± standard error) of $\log_{10}$ serum-IRI and blood-sugar by sex and 10-year age groups for 1,770 individuals*

Age (years)	$\log_{10}$ serum-insulin Males	 Females	 Significance	Blood-sugar (mg per 100 ml) Males	 Females	 Significance	Number of subjects Males	 Females
21–29:	1.506 ± 0.025	1.603 ± 0.019	($p < 0.01$)	86 ± 2	85 ± 2	(N.S.)	110	143
30–39:	1.534 ± 0.024	1.591 ± 0.021	(N.S.)	92 ± 2	91 ± 2	(N.S.)	142	185
40–49:	1.573 ± 0.021	1.624 ± 0.017	(N.S.)	97 ± 2	102 ± 2	(N.S.)	194	199
50–59:	1.618 ± 0.021	1.678 ± 0.018	($p < 0.05$)	107 ± 3	106 ± 2	(N.S.)	164	177
60–69:	1.729 ± 0.022	1.771 ± 0.019	(N.S.)	114 ± 4	121 ± 4	(N.S.)	138	138
70+:	1.750 ± 0.029	1.816 ± 0.025	(N.S.)	131 ± 6	138 ± 6	(N.S.)	82	98
All Ages:	1.610 ± 0.010	1.667 ± 0.008	($p < 0.001$)	103 ± 1.3	105 ± 1.3	(N.S.)	830	940

Table 2. *Physiological variables related to $\log_{10}$ serum-IRI. The mean value (± 1 standard deviation) is shown for each variable and its correlation co-efficient with $\log_{10}$ serum-IRI*

Variable	Mean ± 1 S.D.		Correlation Coefficient
$\log_{10}$ IRI	1.6401	(± 0.270)	1.000 ...
Blood sugar (mg/100 ml)	104	(± 38)	0.321 ($p < 0.001$)
Age (years)	48	(± 16)	0.286 ($p < 0.001$)
Sex (male = 1; female = 2)	....		0.104 ($p < 0.001$)
Height (cm)	166	(± 9)	– 0.180 ($p < 0.001$)
Weight (kg)	68	(± 12)	0.123 ($p < 0.001$)
Mid-triceps fatfold (mm)	16	(± 8)	0.206 ($p < 0.001$)
Systolic B.P. (mm Hg)	149	(± 27)	0.226 ($p < 0.001$)
Diastolic B.P. (mm Hg)	80	(± 14)	0.080 ($p < 0.001$)
S. Urea (mg/100 ml)	31	(± 9)	0.022 (N.S.)
S. Uric Acid (mg/100 ml)	4.9	(± 1.2)	0.132 ($p < 0.001$)
S. Cholesterol (mg/100 ml)	256	(± 48)	0.120 ($p < 0.001$)
S. Sodium (mEq/L)	143	(± 3)	0.016 (N.S.)
S. Potassium (mEq/L)	4.3	(± 0.5)	– 0.038 (N.S.)
S. Chloride (mEq/L)	104	(± 3)	– 0.007 (N.S.)
S. Bicarbonate (mEq/L)	25	(± 2)	– 0.025 (N.S.)
S. Calcium (mEq/L)	9.9	(± 0.4)	0.016 (N.S.)
"Drink-interval" (minutes)	66	(± 9)	– 0.019 (N.S.)
"Food-interval" (minutes)	185	(± 98)	– 0.134 ($p < 0.001$)

these and $\log_{10}$-serum-IRI. There are positive correlation coefficients that are highly significant for $\log_{10}$-serum-IRI with blood-sugar, age, weight, mid-triceps fatfold, systolic and diastolic blood-pressure, serum uric acid and serum cholesterol. Sex is treated as a dichotomy (male = 1, female = 2) and shows a highly significant correlation with serum-insulin. Significant negative correlations occur with height and with the "food-interval". No association of significance is evident with serum-electrolytes, serum urea, nor with the "drink-interval". These data demonstrate the overall relationships that exist with $\log_{10}$-serum-IRI, but give no indication of the *independent* contribution of various factors to the serum-insulin levels. For example, the correlation of $\log_{10}$-serum-IRI with age could be mediated by the common association of these variables with the blood-sugar levels.

Table 3 shows the multiple regression analysis (STENHOUSE, 1965), for factors determining $\log_{10}$-serum-IRI. In this form of analysis the partial effect of each variable is calculated after the effects of all the other determining variables have been taken into account. The form of the regression equation is:

$$Y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + \ldots b_p X_p$$

Table 3. *Results of multiple regression analysis of factors determining $\log_{10}$ serum-IRI. The form of the equation is shown in the text. The units for the stated factors are as shown in Table 2. The Student's "t" value indicates the significance of the associated regression coefficient*

Multiple Regression Analysis Factor	 Regression Coefficient	 Student's "*t*" Value
Blood-sugar	$+ 1.292 \times 10^{-2}$	10.3 ($p < 0.001$)
(Blood-sugar)^2	$- 5.451 \times 10^{-5}$	7.4 ($p < 0.001$)
(Blood-sugar)^3	$+ 6.386 \times 10^{-8}$	5.4 ($p < 0.001$)
Age	$- 2.117 \times 10^{-2}$	2.7 ($p < 0.01$)
(Age)^2	$+ 4.220 \times 10^{-4}$	2.7 ($p < 0.01$)
(Age)^3	$- 2.243 \times 10^{-6}$	2.3 ($p < 0.05$)
Food-interval	$- 1.575 \times 10^{-3}$	9.0 ($p < 0.001$)
(Food-interval)^2	$+ 1.995 \times 10^{-6}$	6.1 ($p < 0.001$)
Sex	$+ 5.830 \times 10^{-2}$	3.6 ($p < 0.001$)
Height	$- 5.247 \times 10^{-3}$	5.5 ($p < 0.001$)
Weight	$+ 5.848 \times 10^{-3}$	10.4 ($p < 0.001$)
S. Uric Acid	$+ 1.992 \times 10^{-2}$	3.7 ($p < 0.001$)
S. Urea	$- 1.819 \times 10^{-3}$	2.7 ($p < 0.01$)
S. Potassium	$- 2.574 \times 10^{-2}$	2.6 ($p < 0.05$)
S. Calcium	$+ 2.922 \times 10^{-2}$	2.1 ($p < 0.05$)
Diastolic B.P.	$- 1.096 \times 10^{-3}$	2.6 ($p < 0.05$)
Intercept on Y-axis	= 1.4556	
Mean $\log_{10}$ Serum-IRI	= 1.6401	
Coefficient of Multiple Correlation	= 0.5892	

where Y is $\log_{10}$-serum-IRI, b_0 is the intercept on the Y axis, and b_1, b_2, ... b_p are the partial regression coefficients of the variables x_1, x_2, ... x_p. The significance of the effect of each variable is assessed by the associated Student's "t" value. Non-significant variables are removed from the analysis serially, and the multiple regression analysis repeated until all remaining variables achieve significance in the final equation. A non-linear relationship between $\log_{10}$-serum-IRI and blood-sugar, age, and the "food-interval" was anticipated, and therefore the square and the cube of these variables were also included in the analysis.

The blood-sugar level is a major determinant of serum-insulin. The partial regression of $\log_{10}$-serum-IRI on blood-sugar levels is shown in Fig. 1 (a). Mean serum-insulin levels rise with increasing blood-sugar over the range 25 to 160 mg/100 ml, but at blood-sugar levels greater than 160 mg/100 ml there is a progressive decline in mean serum-insulin. *Thus a "one-hour" blood-sugar level that exceeds 160 mg/100 ml indicates insulin deficiency as a general rule in this population sample.* It is of interest to note that the levels of venous blood-glucose selected on an empirical basis by experienced physicians to discriminate abnormal glucose tolerance are; — 160 mg/100 ml for a casual postprandial blood sample (Malins, 1968); 160 mg/100 ml for the one-hour sample after 1.75 g/kg oral glucose load (Conn, 1958); and 170 mg/100 ml for the one-hour blood-sugar after a 100 g glucose load (Joslin, 1959).

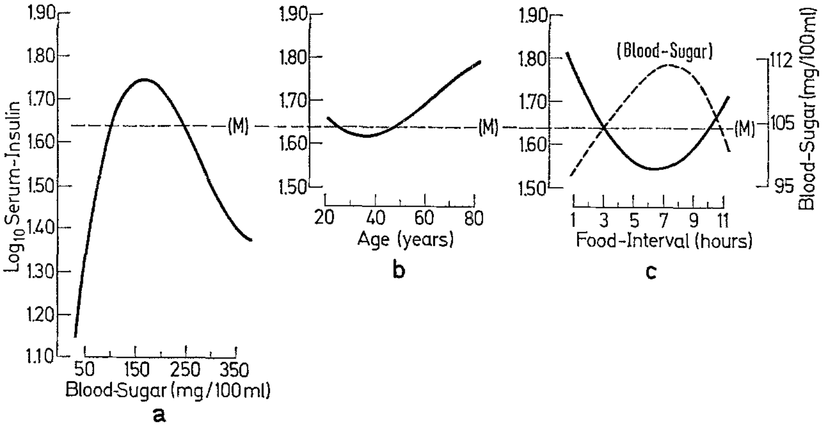

Fig. 1. The independent associations of $\log_{10}$ serum-IRI with
(a) Blood-sugar
(b) Age
(c) The "food-interval"
as obtained from the multiple regression analysis data in Table 3. M = mean value for $\log_{10}$ serum-IRI. Fig. 1 (c) shows in addition the independent, association of blood sugar with the food-interval from a separate analysis

The partial regression of $\log_{10}$-serum-IRI on age is shown in Fig. 1 (b). Apart from marginally elevated serum-insulin levels in the early twenties, there is no substantial independent effect of age until 50 years and over, when a steep and progressive rise in serum-insulin is evident. This is an important confirmation of the findings of Chlouverakis et al., (1967), who reported a rise in serum-insulin with age in the absence of a mean rise in blood-sugar in a normoglycaemic sample of the Bedford population. Since the age at which serum-insulin commences its progressive rise corresponds with the age at which manifestations of generalized vascular disease are more commonly encountered, some support accrues for the hypothesis that impaired egress of insulin from the circulation is the underlying common factor for the deteriorating glucose tolerance and the hyperinsulinism of aging subjects.

The food-interval affects serum-insulin and blood-sugar levels in a paradoxical manner (Fig. 1 (c)). Thus if the "one-hour" blood sample is obtained within 3 h of taking food, comparatively low blood-sugars and high serum-insulin levels are found. Between 3 and 10 h after food, the general trends reverse, so that blood-sugar levels exceed and serum-insulin levels are lower than the mean values for the whole sample. Beyond 10 h, in the small number of subjects contributing to these parts of the curves, a lowering of blood-sugar levels and a tendency for rising serum-insulin occur.

Sex contributes independently as a highly significant determinant of serum-insulin, even though height and weight are also significant determining variables. More detailed analysis has indicated that sex (male = 1; female = 2) and the mid-triceps fatfold are virtually interchangeable variables in the multiple regression equation, and thus the observed sex difference in serum-insulin may be explained essentially by differences in adiposity. Height has a negative relationship with serum-insulin; presumably height reflects lean body mass, and a standard glucose load administered to subjects of varying lean body mass can be expected to produce reciprocal changes in the levels of circulating insulin. The substantial positive effect of weight on $\log_{10}$-serum-IRI represents the effect of obesity, since all other factors including height are taken into account.

The relationship of serum uric acid to serum-insulin levels is probably of biological importance since the simple correlation between the two variables (Table 2) is highly significant, *and* multiple regression analysis demonstrates a highly significant independent association. Serum uric acid levels relate to parameters of lipid metabolism (Bercowitz, 1964) and to manifestations of vascular disease (Hall, 1964; Breckenridge, 1966), and either of these possibilities may account for the observed association. Any substantial alloxan-like effect of uric acid on pancreatic beta cells (Conn et al., 1949) would seem to be discounted.

Multiple regression analysis reveals significant associations of $\log_{10}$-serum-IRI and serum urea, potassium, calcium and diastolic blood pressure that do not correspond with the simple correlations observed. Interpretation of these findings must await more detailed analysis when data on the entire population sample is available.

The coefficient of multiple correlation in Table 3 is 0.5892, and it is calculated that 34.1 percent of the total variability of serum-insulin levels is accounted for by the contribution of the factors studied. This provides further support for the validity of the radioimmunoassay of insulin levels in serum.

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