Table 1. Multiple regression analysis[a]

	Fasting insulin	2-h insulin	Triglycerides
Constant	1.015 (< 0.000)	3.015 (< 0.000)	3.757 (< 0.000)
Age	− 0.004 (0.009)	− 0.000 (0.845)	0.004 (< 0.000)
Gender	− 0.095 (0.013)	− 0.434 (< 0.000)	0.123 (< 0.000)
Waist/Hip	1.495 (< 0.000)	1.284 (< 0.000)	0.907 (< 0.000)
Ethnicity	0.183 (< 0.000)	0.356 (< 0.000)	0.097 (< 0.000)
Obesity	0.516 (< 0.000)	0.358 (< 0.000)	0.236 (< 0.000)
Type 2 diabetes	0.471 (< 0.000)	− 0.097 (NS)	0.247 (< 0.000)
IGT	0.307 (< 0.000)	0.743 (< 0.000)	0.212 (< 0.000)
HBP	− 0.171 (0.001)	− 0.220 (< 0.000)	− 0.142 (< 0.000)
Hyper Tg	0.234 (< 0.000)	0.206 (< 0.000)	–
Hyper Ch	− 0.023 (NS)	0.107 (0.066)	0.355 (< 0.000)
Multiple R	0.50 (< 0.000)	0.50 (< 0.000)	0.53 (< 0.000)

[a] The values of the dependent variables (fasting and 2-h insulin levels, triglyceride concentrations) are log transformed. Age and waist/hip (ratio) are continuous variables, the others are categorical variables (gender 0 = female, 1 = male; ethnicity 0 = non-hispanic white, 1 = Mexican-American; high blood pressure (HBP) 1 = presence, 2 = absence; Type 2 (non-insulin-dependent) diabetes, obesity, impaired glucose tolerance (IGT), hypertriglyceridaemia (Hyper TG), hypercholesterolaemia (Hyper Ch) 0 = absence, 1 = presence). Diseases defined previously [1]. Numbers in parenthesis are p values. Multiple r = multiple correlation coefficient

of Mexican-Americans). These regression-predicted values are almost identical to the actual mean values for the control group selected by exclusion (Tables 2 and 3 of our paper [1]).

(c) The final point concerns the possibility that we overestimated the prevalence of Syndrome X. The purpose of our analysis was not, however, to prove the existence and gauge the frequency of Syndrome X. We simply showed that, if one takes a pool of individuals in the general population including obese, diabetic, glucose intolerant, hypertensive, and dyslipidaemic subjects, essentially similar metabolic profiles are recovered whichever categorical abnormality is used to enter the pool. One common change (or key feature, as we titled the paper) in the cluster is the presence of hyperinsulinaemia (and, by inference, insulin resistance). We did not mean to imply that hyperinsulinaemia is the causative factor in this insulin resistant pool of individuals. Although, as Dr. Turner and colleagues say, hyperinsulinaemia can have pathological consequences, the analysis of cross-sectional observations cannot provide any evidence that high insulin levels (or any other variable, for that matter) play a causal role in the appearance of the cluster of abnormalities.

As defined by Reaven [2], Syndrome X is the simultaneous presence of diagnostic glucose intolerance, high blood pressure, and dyslipidaemia (high VLDL triglycerides and low HDL cholesterol). By using this definition, the prevalence of Syndrome X in our database is only 0.24% in lean persons, and 1.2% in obese individuals. Fasting plasma insulin levels are 145 and 250 pmol/l, respectively in lean and obese Syndrome X patients, while the corresponding 2-h plasma insulin values are 870 and 1,265 pmol/l. Thus, this Syndrome X is indeed characterised by rather extreme hyperinsulinaemia (Table 3 of our paper [1]) whether it occurs in lean or obese subjects. If, on the other hand, we define the syndrome as the presence of at least one diagnostic abnormality (e.g. diabetes or hypertension), we showed [1] that the syndrome will most often manifest itself with a constellation of clinical and subclinical changes in glucose tolerance, blood pressure, and lipid metabolism. The prevalence of such a primary insulin resistance syndrome can be estimated (in our database) to range between 8 and 10% of the general population when occurring in lean subjects. By including obesity, the prevalence jumps up to 64%. Naturally, one may think, that the insulin resistance syndrome that accompanies obesity is different in origin and significance from that observed in the lean, as Dr. Turner and colleagues infer from their own data in a population of Type 2 diabetic patients [3]. We tend to agree on this, and the final point therefore is that a better understanding is needed of the origin, pathogenetic impact, and prognostic value of reduced insulin sensitivity in non-obese individuals.

Yours sincerely,

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Height and glucose tolerance

Dear Sir,

We read with interest the article by Brown and colleagues [1]. In their study, they found that subjects of both sexes with impaired glucose tolerance (IGT) were shorter than matched control subjects with normal glucose tolerance (NGT). The small sumber of patients evaluated (58 IGT subjects) and the lack of similar findings in the literature encouraged us to carry out a similar comparison study in a larger population of patients living at a different latitude.

A total of 163 subjects with IGT (standard 75 g oral glucose tolerance test, National Diabetes Data Group criteria) were compared to NGT control subjects matched for age, sex and waist/hip ratio. We intentionally excluded body mass index as a matching variable since height is actually used to calculate body mass index. Besides anthropometric measures, we also measured plasma lipids (cholesterol and triglycerides) by conventional laboratory methods, serum C-peptide by radioimmunoassay [2] and HbA1c by column chromatography [2]. Comparison of the IGT and NGT groups was made with the unpaired t-test after preliminary analysis of variance (ANOVA).

As shown in Table 1, the male subjects were satisfactorily matched for age, weight and waist/hip ratio. However, we were unable to find any difference between IGT and NGT groups in height, which was almost identical. The IGT patients had significantly higher HbA1c, triglyceride and C-peptide concentrations than the NGT group. Contrary to the male subjects, the IGT females were significantly shorter than control NGT females. As a consequence body mass index was slightly, but significantly ($p < 0.05$) higher in IGT females. As for males, HbA1c, triglycerides and C-peptide levels were significantly higher in the IGT group.

The results can be divided in two discrete parts. As a group, the IGT patients have elevated concentrations of HbA1c and C-peptide: thus, the IGT can significantly affect a reliable index of the overall glucose metabolism and produce augmented insulin secretion from the pancreas in an attempt to overcome insulin resis-

Table 1. Characteristics (mean ± SD) of the subjects

Males	IGT $n = 73$	NGT $n = 100$
Age (years)	54.6 ±11	53.6 ±13.2
Weight (kg)	81.3 ±14.4	79.9 ±12.6
Height (cm)	169.0 ± 7.1	168.7 ± 7.9
BMI (kg/m^2)	28.4 ± 4.6	28.1 ± 4.1
Waist/hip ratio	0.95 ± 0.06	0.94 ± 0.06
HbA_{1c} (%)	5.47 ± 0.75[b]	4.99 ± 0.46
Triglyceride (mmol/l)	2.07 ± 1.07[a]	1.63 ± 0.92
Cholesterol (mmol/l)	5.38 ± 1.37	5.59 ± 1.23
C-peptide (µg/l)	2.56 ± 0.77[a]	2.39 ± 0.64
Females	**IGT $n = 89$**	**NGT $n = 94$**
Age (years)	49.2 ±13.4	47.5 ±12.3
Weight (kg)	77.2 ±14.6	76.1 ±14.1
Height (cm)	156.3 ± 6.27[b]	159.3 ± 6.1
BMI (kg/m^2)	31.5 ± 5.6[a]	30.0 ± 5.2
Waist/hip ratio	0.82 ± 0.06	0.81 ± 0.06
HbA_{1c} (%)	5.34 ± 0.6[b]	4.97 ± 0.55
Triglyceride (mmol/l)	1.65 ± 0.77[a]	1.42 ± 0.73
Cholesterol (mmol/l)	5.45 ± 1.08	5.35 ± 1.2
C-peptide (µg/l)	2.80 ± 1.3[c]	2.32 ± 0.63

[a] $p < 0.05$; [b] $p < 0.001$; c $p < 0.005$ compared to NGT. IGT, Impaired glucose tolerance; NGT, normal glucose tolerance

tance. We therefore confirm even in this large population of IGT patients the clustering of metabolic abnormalities [3]: hypertriglyceridaemia, hyperinsulinaemia, obesity and insulin resistance (not specifically addressed in this study but likely to be present). Interestingly, total cholesterol concentrations did not differ between IGT and NGT groups.

Female, but not male subjects with IGT are shorter than matched NGT control subjects. At present, we have no plausible explanation for the finding. Obviously, the ethnic differences may be important, as we were unable to document a difference in height between males. Since height in post-menopausal women is negatively age-related, we wondered whether the difference found could be limited to the post-menopausal age. The difference between the height of IGT and NGT females (3 cm) remained significant ($p < 0.02$) even after dividing them into pre- and post-menopausal. The measures for the female IGT group were in pre-menopause: $n = 42$; age = 37.4 ± 8.3 years; height = 158.2 ± 6 cm; in post-menopause: $n = 47$; age = 59.7 ± 6.8 years; height = 154.6 ± 6 cm.

The corresponding measures for the female NGT group were in pre-menopause: $n = 48$; age = 37.6 ± 7.9 years; height = 161.1 ± 5.6 cm; in post-menopause: $n = 46$; age = 57.9 ± 5.9 years; height = 157.4 ± 6.1 cm.

At this point, it seems best to simply state that the height of IGT females at our latitude is shorter than NGT females, but the biological significance, if any, of this finding is unclear. However, the elimination of height as an independent variable when evaluating glucose tolerance or comparing groups deserves consideration.

Yours sincerely
A. Quatraro, A. Minei, N. De Rosa and D. Giugliano

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HPLC assay for serum glycated albumin

Dear Sir,

A note of caution [1] has recently been issued about the HPLC assay for glycated albumin [2] because it yields substantially higher values than a monoclonal antibody assay and conventional affinity chromatography assays.

Unfortunately the validity of the methods which establish glycation of about 1–3% of albumin in normal serum has not been discussed. This seems worthwhile because widely different results have been reported for glycated albumin affinity chromatography. A commercial assay kit, for example, yields much higher results (normal range 5.9–10.7%) [3] than the reference range of 1.5–2.6% which was (incorrectly) cited for this method. Others report reference ranges as low as 0.4–1.1, again for affinity chromatographic separation [4]. Such discordant results show that glycated albumin affinity chromatography is only a semiquantitative method.

Densitometric scanning [5] which has been mentioned as one of those methods which establish the anticipated target range might erroneously be taken for an additional method to determine glycated albumin. In fact, densitometric scanning was used only to quantitate albumin which had previously been fractionated by boronate affinity chromatography.

The mechanism by which conventional affinity chromatography operates has been studied with ^{14}C glycated albumin [6, 7]. Both studies showed that this method underestimates the degree of albumin glycation, apparently because only molecules carrying more than one glycated site show sufficient affinity to be tightly bound. This is in agreement with the elution of substantial amounts of glycated albumin in the unbound fraction as determined by the thiobarbiturate assay [8].

The argument that the number of bound glucose residues is not identical to the number of glycated albumin molecules is not helpful to clarify which method yields most accurate results. The only statement which can be safely made is that the amount of glycated albumin cannot exceed the amount of bound glucose. Because in non-diabetic humans about 0.37 mole glucose is attached to 1 mole serum albumin [9], not more than 37% of the albumin molecules should be glycated. Both the HPLC method and the other cited methods meet this criteria.

Lys-^{525} which is the most abundantly glycated residue in human albumin carries about half of the overall glycation [10]. In normal serum 0.18 mole glucose/mole albumin would then be expected to be derived from Lys-^{525} glycation alone. Because each albumin molecule contains only one Lys-^{525}, the glucose bound to this residue must reside on separate albumin molecules. This corresponds to a minimum estimate of about 18% glycated albumin in normal serum.

Because there is little reason to assume that the remaining glycation would be restricted to those molecules which are already glycated at Lys-^{525} the true amount of glycated albumin molecules will presumably be higher than the minimum estimate. The HPLC method, yielding about 20% glycated albumin in normal serum, appears to be much more in line with these figures than those methods which estimate only about 1–3% glycated albumin.

The fructosamine assay [11], which has also been discussed in this context, measures protein glycation, not glycated albumin. Nevertheless a close relationship can be expected because albumin is the most abundant serum protein and is readily glycated. It is also known