Diabetologia (1996) 39: 298–305

Diabetologia

Intra-individual variation of glucose, specific insulin and proinsulin concentrations measured by two oral glucose tolerance tests in a general Caucasian population: the Hoorn Study

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Summary We studied the intra-individual variation in plasma glucose, specific serum insulin and serum proinsulin concentrations, measured by two 75-g oral glucose tolerance tests in an age, sex, and glucose tolerance stratified random sample from a 50–74-year-old Caucasian population without a history of diabetes mellitus. The intra-individual variation was assessed by the standard deviation of the test-retest differences (SD_{dif}). For subjects with normal ($n = 246$), impaired glucose tolerance ($n = 198$), and newly detected diabetes ($n = 80$) classified at the first test, the following (SD_{dif}/median level of individual average scores) were found: fasting glucose: 0.4/5.4, 0.5/5.9 and 0.7/7.2 mmol/l; 2-h glucose: 1.3/5.6, 1.8/8.5 and 2.3/12.8 mmol/l; fasting insulin: 23/76, 32/89 and 30/116 pmol/l; 2-h insulin: 190/303, 278/553 and 304/626 pmol/l; fasting proinsulin: 4/8, 6/13 and 9/18 pmol/l; 2-h proinsulin: 19/49, 23/84 and 33/90 pmol/l, respectively. In both glucose, proinsulin and insulin concentrations the total intra-individual variation was predominantly determined by biological variation, whereas analytical variation made only a minor contribution. The SD_{dif} can easily be interpreted, as 95 % of the random test-retest differences will be less than $2 \cdot SD_{dif}$, or in terms of percentage, less than ($2 \cdot SD_{dif}$/median level of individual average scores) · 100. Therefore, for subjects with normal glucose tolerance, 95 % of the random test-retest differences will be less than 15 % (fasting glucose), 46 % (2-h glucose), 61 % (fasting insulin), 125 % (2-h insulin), 100 % (fasting proinsulin) and 78 % (2-h proinsulin) of the median value of the individual average scores. No substantial independent association of either age, gender or obesity with the intra-individual variation in glucose, proinsulin, or insulin concentrations was found. [Diabetologia (1996) 39: 298–305]

Keywords Intra-individual variation, glucose, specific insulin, proinsulin, oral glucose tolerance test, reproducibility.

Non-insulin-dependent diabetes mellitus (NIDDM) constitutes a massive health problem with prevalence rates of 5–10 % in elderly Caucasian populations [1].

Received: 22 May 1995 and in revised form: 8 August 1995

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Abbreviations: OGTT, Oral glucose tolerance test; NGT_{1st}, IGT_{1st}, new DM_{1st}, normal glucose tolerance, impaired glucose tolerance and newly detected diabetes mellitus, respectively, as classified at first OGTT; SD_{dif}, standard deviation of the difference scores; CV_{intra}, intra-individual coefficient of variation; CV_{bi}, biological intra-individual coefficient of variation; CV_a, analytical intra-individual coefficient of variation; CI_{95}, 95 % confidence interval.

The diagnosis of diabetes and impaired glucose tolerance (IGT), a major risk factor for diabetes, is based on levels of fasting and/or 2-h post-load glucose. Previous studies demonstrated a high intra-individual variation in the 2-h plasma glucose concentration [2–5], resulting in a low reproducibility of the IGT category in particular [6, 7]. Recently, the assessment of specific insulin and proinsulin levels has received considerable attention, as these parameters are considered to be estimates of insulin resistance and beta-cell function [8, 9], appropriate for measurement in population-based studies on glucose intolerance. To our knowledge, indices of intra-individual variation in specific insulin and proinsulin concentration within the general population have not been

presented previously. Theoretically, the intra-individual variation measured by two tests has at least three contributors: 1) a potential systematic difference between the two tests, 2) the random biological and 3) the random analytical variation. A systematic difference between glucose concentrations measured by two subsequent oral glucose tolerance tests (OGTTs) has been reported in an epidemiological survey in Tanzania, with the second OGTT producing significantly lower glucose values than the first [10]. Elevated arousal during the first test has been hypothesized to be a potential determinant of this systematic difference. As far as we know, this phenomenon has not been mentioned in connection with a general Caucasian population.

The aim of the present analysis was, firstly, to determine whether there was a systematic lowering of the plasma glucose values when repeating the OGTT. Secondly, we quantified the biological and analytical components of the (random) intra-individual variation in the glucose, specific insulin and proinsulin response measured by two OGTTs, and studied the potential determinants of this variation. Finally, the implications of the intra-individual variation in plasma glucose for the reproducibility of the World Health Organisation (WHO) classification of glucose tolerance [11] are described. For these analyses, we used data from the Hoorn Study, a cross-sectional population-based survey on glucose tolerance in Dutch Caucasians based on two OGTTs, repeated within 2 to 6 weeks.

Subjects and methods

From 1989 to 1992 a population-based survey of glucose tolerance was carried out in the city of Hoorn, a middle-sized town of about 57,000 residents with a mixed rural-urban population. The Hoorn Study was approved by the ethical review committee of the Academic Hospital of the Vrije Universiteit of Amsterdam and informed consent was obtained from all participants. The eligible population of the Hoorn Study consisted of 3,553 men and women (aged 50–74 years), randomly selected from the municipal registry. The participation rate of the first OGTT was 71 %. The OGTTs were performed between 08.00 and 10.00 hours. Participants were instructed to abstain from alcohol from 17.00 hours and to fast (except for drinking water) from 22.00 hours the previous day. Subjects on medication unrelated to diabetes took their medication as usual. Those taking insulin, oral hypoglycaemic agents and/or blood pressure lowering agents were requested not to use these drugs prior to the test. If any of these instructions had not been followed, or if there had been any unusual physical activity or fever during the previous 3 days, the OGTT was postponed. Blood samples were collected in a sodium fluoride tube before and 2 h after the intake of 75 g lemon-flavoured glucose anhydrate in 300 ml of water over the course of 5 min. During the OGTT, the subjects relaxed in the research centre and refrained from smoking. Diabetes mellitus was diagnosed if the fasting plasma glucose level was 7.8 mmol/l or above and/or the 2-h post-load plasma glucose level was 11.1 mmol/l or above; IGT, if the fasting plasma glucose was less than 7.8 mmol/l and the 2 h plasma glucose level was between 7.8 and 11.1 mmol/l; normal glucose tolerance (NGT), if both fasting and the 2-h plasma glucose levels were less than 7.8 mmol/l [11]. Applying these WHO criteria to the data of the first OGTT yielded the categories NGT_{1st}, IGT_{1st}, and DM_{1st}. As all subjects with diabetes in the present analysis were 50 years of age and over and had no previous diagnosis of diabetes (and, consequently, were not dependent on insulin for their survival), they can be classified as having NIDDM.

Sampling procedures and exclusion criteria (Fig. 1). Non-Caucasian participants and subjects with a verified history of diabetes were excluded from the analysis. Criteria for verified known diabetes were: 1) current use of insulin or hypoglycaemic agents or 2) when diet only had been prescribed, a fasting and/or 2-h glucose measurement meeting the WHO criteria on at least one OGTT [11].

For reasons of efficiency, not all subjects were invited for the second OGTT: from those with a 2-h glucose level of less than 7.5 mmol/l, a random sample was taken, stratified by five age categories and by sex (ten strata). The remaining participants with 2-h glucose levels of 7.5 mmol/l or more were all eligible for the second test (11^{th} stratum). Subjects who underwent a second OGTT (sample 4 Fig. 1) had no knowledge of the results of the first OGTT and were invited at the same time in the morning as scheduled on their first visit. Again for economy reasons, insulin and proinsulin were measured in a subsample (sample 6) of sample 4, i.e. in all subjects with IGT_{1st} ($n = 239$) and new DM_{1st} ($n = 110$) and in a random sample of subjects with NGT_{1st} (281 of 760 subjects, Fig. 1).

In order to investigate systematic test-retest differences in the plasma glucose concentrations, we reconstructed from sample 4 a new representative sample (sample 5) in the following way: from each of the 11 strata in sample 4 (determined by 2-h glucose at first test, age and sex) a number of subjects was randomly selected, such that the distribution of subjects over these strata in the newly formed sample 5 was the same as in the original representative sample of 2,394 subjects who had completed the first OGTT (sample 3). This yielded a smaller twice-tested subsample of 555 subjects, representative of subjects without a history of diabetes (sample 5).

Blood pressure and anthropometric parameters. Prior to each OGTT, two blood pressure readings were recorded, measured on the right arm of seated subjects after at least 5 min of rest with a random zero mercury sphygmomanometer (Hawksley-Gelman, Lancing, Sussex, UK). The average of these two readings of systolic and diastolic (Korotkoff V) blood pressure were calculated. Height and weight of the subjects were measured without shoes and outer garments during the first visit, and BMI was calculated as weight (kg) divided by height (meters) square. Waist and hip measurements were taken, according to a standardized procedure [12]. Waist-hip ratio was defined as waist circumference divided by hip circumference.

Laboratory analyses. The laboratory analyses, including the determination of all analytical coefficients of variation (CV_a), were performed at the Free University Hospital of Amsterdam. Plasma glucose concentrations were determined directly and serum was stored at −20 °C for the subsequent assessment of serum insulin and proinsulin levels. Glucose (inter-assay CV_a 1.4 %) was measured with a glucose dehydrogenase method (Merck, Darmstadt, Germany). HbA_{1c}(inter-assay CV_a 0.6–3.1 %) by ion-exchange high-performance liquid chromatography, using a Modular Diabetes Monitoring System (BioRad, Veenendaal, The Netherlands). Immuno-specific insulin was

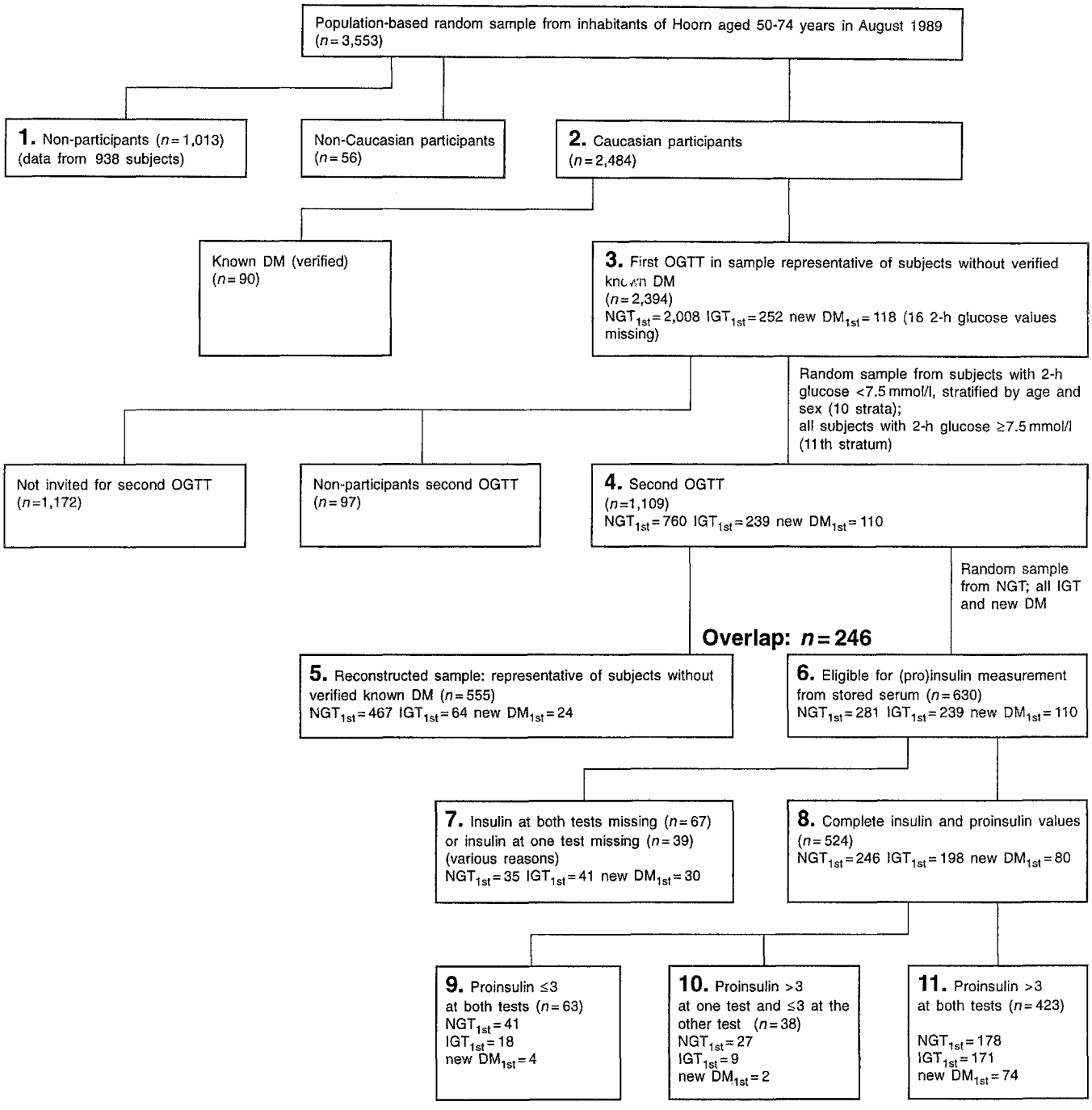

Fig. 1. Sampling procedures relevant for the present analysis. NGT_{1st}, IGT_{1st}, and new DM_{1st}, normal glucose tolerance, impaired glucose tolerance and newly detected diabetes, respectively, as classified at first OGTT

measured in serum by a double-antibody radioimmunoassay (lot SP21; Linco Research, St. Louis, USA), in which proinsulin and des(31,32)proinsulin cross-reacts by less than 0.2 %. The cross-reactivity with des(64,65)proinsulin in this assay is 76 %, but this will barely contribute to the measured insulin values because des(64,65)proinsulin is only a minor component in human serum [13]. The inter-assay CV_a was 6 % at insulin levels in the range of 40–1000 pmol/l. The lower limit of sensitivity was 12 pmol/l. Proinsulin was measured by a double-antibody radioimmunoassay, based on reagentia from Dr. R. Bowsher (Lilly Laboratory for Clinical Research, Indianapolis, Ind., USA) [14]. In this assay, using the polyclonal antibody 168 AB, des(31,32)proinsulin cross-reacts by 63 %, and des(64–65)proinsulin by 19 %. The inter-assay CV_a was 6 % at levels of 100 pmol/l and increased to 15 % at lower levels. The lower limit of sensitivity was 3 pmol/l. Each hormone measurement was performed in duplicate.

Statistical analysis

As in the stratified sample (sample 8 Fig. 1) a systematic test-retest lowering in glucose concentrations can also be attributed to regression to the mean, we studied this phenomenon within the representative sample only (sample 5 Fig. 1). Assuming

Table 1. Characteristics of the various samples from the general Dutch population

Subjects	Sample number from Figure 1	*n*	Age (years)	Male (%)	Known diabetes[a] self-reported (%)	Positive family diabetes history (%)	HbA_{1c}	BMI male; female (kg/m^2)	Waist-hip ratio male; female
Non-participants	1	938	63 ± 8	47	4.5	20	–	25 ± 3 25 ± 3[b]	–
Cauc. part. (incl. known DM)	2	2,484	62 ± 7	46	4.2	27	5.5 ± 0.9	26 ± 3 27 ± 3	0.95 ± 0.07 0.85 ± 0.07
Cauc. part. (excl. known DM)	3	2,394[c]	62 ± 7	47	–	26	5.4 ± 0.7	26 ± 3 27 ± 4	0.95 ± 0.06 0.84 ± 0.07
NGT_{1st}		2,008	61 ± 7	47	–	25	5.3 ± 0.5	26 ± 3 26 ± 4	0.95 ± 0.06 0.84 ± 0.07
IGT_{1st}		252	65 ± 7	42	–	30	5.5 ± 0.5	28 ± 4 28 ± 4	0.99 ± 0.06 0.87 ± 0.07
new DM_{1st}		118	66 ± 7	47	–	36	6.7 ± 1.8	28 ± 3 29 ± 5	1.00 ± 0.07 0.91 ± 0.08
Cauc. part. (reconstructed) twice-tested	5	555	62 ± 7	45	–	23	5.4 ± 0.5	26 ± 3 27 ± 4	0.95 ± 0.07 0.84 ± 0.07
Cauc. part. (stratified) twice-tested	8	524	64 ± 7	50	–	27	5.5 ± 0.7	27 ± 3 27 ± 4	0.97 ± 0.07 0.86 ± 0.07
NGT_{1st}		246	63 ± 7	56	–	22	5.3 ± 0.5	26 ± 3 26 ± 4	0.95 ± 0.06 0.84 ± 0.07
IGT_{1st}		198	65 ± 7	43	–	29	5.5 ± 0.5	28 ± 4 28 ± 4	0.99 ± 0.06 0.87 ± 0.06
new DM_{1st}		80	66 ± 6	49	–	39	6.2 ± 1.0	28 ± 3 29 ± 5	1.00 ± 0.07 0.90 ± 0.06

Values are means ± SD or percentages. DM, diabetes mellitus; Cauc., Caucasian; part., participants; for other abbreviations: see Figure 1.

[a] 'Known diabetes' was defined as 'self-reported diabetes', because for non-participants only this information was available.

[b] Calculated from self-reported body weight and height.

[c] 16 subjects had missing 2-h plasma glucose values

that there is no systematic test-retest difference, the total intra-individual variation can be assessed by the standard deviation (SD_{dif}) of the difference scores (test 1 minus test 2) with its 95 % confidence interval (CI_{95}) [15, 16]. We checked that the distributions of the difference scores were approximately normal.

To estimate the contribution of the biological and analytical variations to the total intra-individual variation, we used the following formula: $CV^2_{intra} = CV^2_{bi} + CV^2_{a}$, where CV_{intra} equals the intra-individual coefficient of variation, and CV_{bi} and CV_a stand for the biological and analytical intra-individual coefficient of variation, respectively [17]. The CV_{intra} was estimated in our data by ($SD_{dif}/\sqrt{2}$) divided by the median of the individual average scores and multiplied by 100. The CV_a of the relevant parameters is given in the Laboratory analyses section.

To test whether the intra-individual variation of glucose, insulin and proinsulin concentration was associated with sex, age or obesity, independent of glucose tolerance, we divided each category of glucose tolerance (NGT_{1st}, IGT_{1st}, new DM_{1st}) into two strata: men/women, young/old (cut-off point: the category-specific median level of age), non-obese/obese (cut-off point: category and sex-specific median level of BMI or waist-hip ratio). Stratum-specific SD_{dif} values within each category of glucose tolerance were compared with each other and considered to be substantially different if their confidence intervals were not overlapping.

Results

Cases with missing insulin values for both tests ($n = 67$) or for one test ($n = 39$) were excluded from the analysis (Fig. 1). There were no proinsulin values missing. From those with complete insulin values, 423 subjects (sample 11) had proinsulin values above 3 pmol/l at both tests, and 101 subjects had proinsulin values 3 pmol/l or less for at least one test (samples 9 and 10 combined). Since the recorded differences in proinsulin values below 3 pmol/l are meaningless, being below the lower limit of sensitivity of the assay, we replaced proinsulin values below 3 by 3 pmol/l. There were no subjects with insulin values below the sensitivity limit of the insulin assay.

Table 1 describes the characteristics of the various samples from Figure 1. Comparing non-participants with Caucasian participants (samples 1 and 2), no substantial differences were found; participants had a more frequent positive family history of diabetes. Table 1 also shows that the reconstructed representative sample 5 had similar values on the relevant parameters compared to the total representative sample 3. Due to the sampling strategy for the second OGTT, we found higher percentages of subjects with IGT and diabetes in sample 8, compared to the total represen-

Table 2. Intra-individual variation in glucose concentration measured by two OGTTs in a representative sample of elderly Caucasians without a history of diabetes ($n = 555$)

	Difference scores (test 1 minus test 2)		reference: Individual average scores	CV_{intra} (%)	CV_{bi} (%)
	$mean_{dif}$ [Cl_{95}] (mmol/l)	SD_{dif}[Cl_{95}] (mmol/l)	median (20th, 80th percentile) (mmol/l)		
FPG	0.03 [−0.01, 0.07]	0.49 [0.46, 0.52]	5.4 (5.0, 5.9)	6.4	6.3
$_2$hPG	0.12 [0.01, 0.23]	1.3 [1.22, 1.38]	5.5 (4.3, 7.2)	16.7	16.6

Cl_{95}, 95 % confidence interval: CV_{intra}, intra-individual coefficient of variation; CV_{bi}, biological coefficient of variation

Table 3. Prevalence of categories of glucose tolerance in a representative sample of the general elderly Caucasian population without a history of diabetes ($n = 555$)

	1st OGTT % [Cl_{95}]	2nd OGTT % [C_{95}]	Based on individual average scores of both OGTTs % [Cl_{95}]	Based on meeting the WHO criteria at both OGTTs % [Cl_{95}]
Normal glucose tolerance	84.1 [81, 87]	84.5 [82, 88]	85.2 [82, 88]	79.6 [76, 83]
Impaired	11.5 [8.9, 14.2]	10.6 [8.1, 13.2]	10.5 [7.9, 13.0]	5.6 [3.8, 7.8]
new diabetes	4.3 [2.8, 6.4]	4.9 [3.2, 7.0]	4.3 [2.8, 6.4]	3.4 [2.1, 5.3]

tative sample (sample 3); in addition, among NGT subjects in sample 8, there were more men and more older subjects compared to the NGT subjects of the total representative sample (sample 3), also due to the sampling strategy for the second OGTT.

Plasma glucose. Table 2 shows that the CI_{95} of the mean difference score ($mean_{dif}$) in 2-h glucose does not include the zero value, implying that the difference between both tests is statistically significant.

We studied whether there was also a systematic test-retest difference in potential indicators of arousal. This was, indeed, the case for systolic blood pressure ($mean_{dif}$: 5, CI_{95}: 3.9 to 6.1 mm Hg), diastolic blood pressure ($mean_{dif}$: 2, CI_{95}: 1.4 to 2.6 mm Hg), and heart rate ($mean_{dif}$: 2, CI_{95}: 1.1–2.9 beats/min). Only the test-retest difference scores in heart rate were significantly associated with the test-retest difference scores in 2-h glucose: the regression coefficient with heart rate differences as independent and 2-h glucose differences as dependent variable was 0.01 ($p < 0.05$, two-tailed test). No systematic test-retest differences in body weight were found. The width of the test-retest time interval was not associated with the difference scores in 2-h plasma glucose, as judged by the result of a regression analysis with the number of days between both tests as independent, and difference scores in 2-h plasma glucose as dependent variable ($p = 0.47$).

Table 3 demonstrates that this systematic decrease in 2-h glucose values at retest had no substantial influence on the prevalence of IGT and new diabetes at the second OGTT.

Since the $mean_{dif}$ in 2-h glucose is only slightly different from zero, the SD_{dif} can be used to estimate the total intra-individual variation in plasma glucose. The scatter of the difference scores in fasting, and notably in 2-h glucose, widened in the higher part of the range of the values (data not shown). Therefore, we present the SD_{dif} values not only for the total representative population (Table 2), but also separately for the three diagnostic categories of glucose tolerance (Table 4).

Tables 2 and 4 show that the contribution of the CV_{bi} to the CV_{intra} in fasting and 2-h glucose concentration was much greater than that of the CV_a (see Methods section). Stratification for age, sex, BMI or waist-hip ratio within each category of glucose tolerance did not result in substantial differences in SD_{dif} values (data not shown). Figure 2 shows the effect of the intra-individual variation in fasting and 2-h glucose on the WHO classification. Reproducibility of NGT, IGT and new diabetes was 91 % (224/246), 48 % (95/198) and 78 % (62/80), respectively.

Specific insulin and proinsulin. Table 4 shows that the SD_{dif}, but not the $CV_{intra}$of insulin and proinsulin increased substantially in the higher range of the values. The CV_{bi} component of the CV_{intra} of insulin and proinsulin was again much greater than the CV_a component. Stratification for age, sex, BMI or waist-hip ratio within each category of glucose tolerance did not result in substantial differences in SD_{dif} values (data not shown). The data in Table 4 are from all subjects with complete proinsulin and insulin values, including 63 and 38 subjects for whom we replaced proinsulin values below 3 by 3 pmol/l (samples 9 and 10 Fig. 1). In sample 9, proinsulin differences could not be computed, and in sample 10 they could only be assessed crudely. Therefore, we repeated the analysis for proinsulin, excluding these subjects (Table 5).

Table 4. Intra-individual variation in glucose, specific insulin and proinsulin concentration measured by two OGTTs in Caucasian subjects with NGT_{1st} ($n = 246$), IGT_{1st} ($n = 198$) and new DM_{1st} ($n = 80$). (Sample 8 in Fig. 1)

		Difference scores (test 1 minus test 2)	reference: Individual average scores	CV_{intra} (%)	CV_{bi} (%)
		$SD_{dif}[Cl_{95}]$ (mmol/l or pmol/l[a])	median (20th, 80th percentile) (mmol/l or pmol/l[a])		
FPG	NGT_{1st}	0.37 [0.34, 0.41]	5.4 (5.0, 5.8)	4.8	4.6
	IGT_{1st}	0.50 [0.46, 0.55]	5.9 (5.4, 6.6)	6.1	5.9
	new DM_{1st}	0.72 [0.62, 0.85]	7.2 (6.1, 8.2)	7.1	7.0
2hPG	NGT_{1st}	1.3 [1.2, 1.4]	5.6 (4.5, 6.8)	16.4	16.3
	IGT_{1st}	1.8 [1.6, 2.0]	8.5 (7.7, 9.9)	15.0	14.9
	new DM_{1st}	2.3 [2.0, 2.7]	12.8 (11.0, 16.2)	12.7	12.6
FSI	NGT_{1st}	23 [21, 25]	76 (52, 99)	21.4	20.5
	IGT_{1st}	32 [29, 35]	89 (63, 138)	25.4	24.7
	new DM_{1st}	30 [26, 35]	116 (75, 168)	18.3	17.3
2hSI	NGT_{1st}	190 [175, 208]	303 (149, 478)	44.3	43.9
	IGT_{1st}	278 [253, 308]	553 (349, 1074)	35.5	35.0
	new DM_{1st}	304 [263, 360]	626 (344, 1114)	34.3	33.8
Fpro	NGT_{1st}	4.4 [4.0, 4.8]	8.2 (3.1, 14)	37.9	[b]
	IGT_{1st}	6.3 [5.7, 7.0]	13 (5.6, 24)	34.3	30.8[c]
	new DM_{1st}	8.9 [7.7, 10]	18 (11, 34)	35.0	31.6[c]
2hpro	NGT_{1st}	19 [17, 21]	49 (26, 80)	27.4	22.9[c]
	IGT_{1st}	23 [21, 26]	84 (52, 139)	19.4	18.4[d]
	new DM_{1st}	33 [29, 39]	90 (55, 140)	25.9	25.2[d]

FSI, Fasting specific insulin; 2hSI, 2-h specific insulin; Fpro, fasting proinsulin; 2hpro, 2-h proinsulin; for other abbreviations, see legends Table 2 and Figure 1.

[a] glucose values in mmol/l and proinsulin and insulin values in pmol/l.

[b] CV_a, and, consequently, CV_{bi} cannot be computed, as many proinsulin scores in this subgroup were below the lower limit of sensitivity.

[c] CV_a: 15 % [d] CV_a: 6 %

Due to this exclusion of subjects with low proinsulin values, the median of the individual average scores was higher in sample 11, in particular for subjects with NGT. The SD_{dif} values were not very different. This resulted in a lower CV_{intra} of fasting proinsulin in NGT subjects. All other results were similar to those found in the total sample, shown in Table 4.

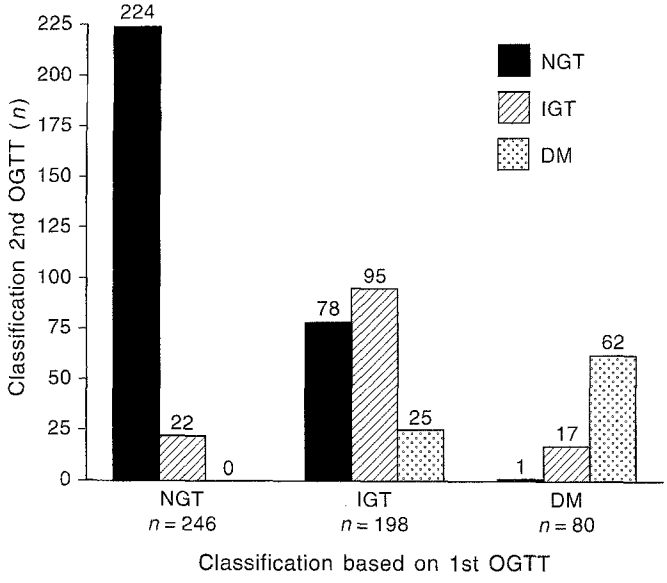

Fig. 2. Reproducibility of the three diagnostic categories of glucose tolerance in a stratified sample from the general elderly Caucasian population without a history of diabetes ($n = 524$)

Discussion

We found slightly lower 2-h glucose concentrations when repeating the OGTT after 2 to 6 weeks. Given the association with a lower heart rate at retest, it may have resulted from diminished psychological stress at the second test, comparable to the acclimatizing reaction observed in the measurement of blood pressure. In contrast with the findings in the Tanzanian Study [10], this 'settling effect' in our Caucasian population was only very small and did not result in a substantial decrease in the prevalence of IGT or diabetes measured at the second OGTT.

The (random) intra-individual variation was assessed by the standard deviation of the test-retest differences (SD_{dif}). This statistic can easily be interpreted, as 95 % of the random test-retest differences will be less than $2 \cdot SD_{dif}$, or, in terms of percentage, less than ($2 \cdot SD_{dif}$/median level of individual average scores) · 100. (As some of the distributions of the individual average scores in glucose, insulin and proinsulin concentration were skewed to the right, we described them by median instead of mean values.)

Table 5. Intra-individual variation in proinsulin concentration measured by two OGT in Caucasian subjects with NGT_{1st} ($n = 178$), IGT_{1st} ($n = 171$) and new DM_{1st} ($n = 74$)

		Difference scores (test 1 minus test 2)	reference: Individual average scores	CV_{intra} (%)	CV_{bi} (%)
		$SD_{dif}[Cl_{95}]$ (pmol/l)	median (20th, 80th percentile) (pmol/l)		
Fpro	NGT_{1st}	4.9 [4.4, 5.5]	11 (6.9, 17)	31.5	27.7[a]
	IGT_{1st}	6.8 [6.1, 7.6]	15 (8.8, 25)	32.1	28.4[a]
	new DM_{1st}	9.2 [7.9, 11]	20 (12, 34)	32.5	28.8[a]
2hpro	NGT_{1st}	20 [18, 22]	56 (37, 94)	26.2	21.5[a]
	IGT_{1st}	23 [21, 26]	94 (59, 146)	17.3	16.2[b]
	new DM_{1st}	35 [32, 42]	92 (63, 140)	26.9	26.2[b]

(Sample 11 in Fig. 1; subjects with proinsulin levels ≤ 3 pmol/l at either test were excluded)
Fpro, Fasting proinsulin; 2hpro, 2-h proinsulin; for other abbreviations, see legends Table 2 and Figure 1
[a] CV_a: 15 % [b] CV_a: 6 %

The random intra-individual variation in fasting and 2-h glucose had little effect on the classification of newly detected diabetes: prevalence based on one OGTT was approximately equal to the prevalence of confirmed diabetes. However, as reported previously, a high proportion of IGT subjects had a different classification at repeat test [2–7]. Since the intra-individual variation in 2-h glucose (in absolute terms) was highest in diabetic, and not in IGT subjects, this phenomenon can at least partly, be attributed to the narrow range of glucose values defining the IGT category. We could not demonstrate a substantial influence of either age, gender or obesity on the intra-individual variation in glucose concentrations. In the Tanzanian study, investigating subjects aged 15 years and over, older age was found to be associated with less intra-individual variation in 2-h glucose [10]. The discrepancy of our results may be due to the relatively small age range within our sample.

Our study showed that the intra-individual variation in specific insulin and proinsulin concentration is about 1.5 to 2 times higher than in 2-h glucose concentration. A limitation of the present analysis was that we could not determine test-retest differences in subjects with proinsulin values below the value of 3 pmol/l, due to the low sensitivity of the radioimmunoassay proinsulin assay used for this study. Therefore, for the proinsulin analysis, we had to exclude these subjects. This may have biased the fasting proinsulin results for NGT subjects in particular, as most subjects with values below 3 pmol/l belonged to this category. In general, immunoradiometric proinsulin assays have a higher sensitivity, but these assays were not available at the time of this study.

Compared to glucose, the CV_a of both insulin and proinsulin was high. In spite of this, the intra-individual variation of these hormones was also mainly determined by biological variation, and therefore will only be slightly lower when better measurement techniques become available. For example, if the CV_a of fasting proinsulin, the most imprecise measurement, would decrease from 15 % to 6 %, the CV_{intra} in NGT subjects would decrease from 32 % to 28 %.

In epidemiological studies, the measurement of insulin and proinsulin concentrations may prove useful when distinguishing between low and high risk in IGT subjects, as suggested by Yudkin et al. [6]. Our data can serve as reference to distinguish between random and non-random differences in insulin or proinsulin concentration between subgroups. Also, fasting insulin and proinsulin, combined with fasting glucose and HbA_{1c} can possibly serve as an alternative test for the (rather time-consuming) OGTT in diagnosing diabetes in large populations. Others reported that the combination of fasting plasma glucose with HbA_{1c} or serum fructosamine did not result in a test with sufficient sensitivity compared to the full OGTT [18–20]. This issue will be investigated, including the proinsulin and insulin parameters, in a future analysis of the Hoorn Study data.

In conclusion, when comparing a classification of glucose tolerance based on the first OGTT and based on meeting the WHO criteria at both OGTTs, the prevalence of IGT and newly detected diabetes in a general Caucasian population decreased from 11.5 to 5.6 and from 4.3 to 3.4, respectively. The intra-individual variation in specific insulin and proinsulin concentration is about 1.5 to 2 times higher than in the 2-h glucose concentration.

Acknowledgements. We thank Dr. R.R. Bowsher for kindly providing the assay materials for the measurement of proinsulin levels and B. Huÿser-Greenen and F. Martens for performing the hormone measurements.

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