Increased risk of Type 2 diabetes in Elderly Twins

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**Objective:** Genetic susceptibility, low birth weight (LBW) and ageing are key etiological factors in the development of type 2 diabetes (T2D). LBW is common among twins. It is unknown whether twin status *per se* is associated with risk of T2D, and valid concordance rates of T2D in twins on a life-time perspective are lacking.

**Research design and methods:** Clinical study of a population-based cohort of same-sex elderly monozygotic (MZ) and dizygotic (DZ) twins (n=297) and singleton controls (C) (n=71) including measures of anthropometry and glucose tolerance. In addition, T2D incidence cases in twins (n=626) and singletons (n=553) were identified through the National Diabetes Register.

**Results:** Twins were more abdominal obese, insulin resistant and glucose intolerant as evidenced by a higher HbA1c (%) (mean (SD)) (MZ: 6.0 (1.0), DZ: 5.8 (0.7), C: 5.6 (0.3), p=0.004) and 120-min post OGTT plasma glucose levels (mmol/l) (MZ: 8.6 (4.6), DZ: 8.4 (3.9), C: 6.8 (2.4), p=0.003) compared to singletons. Importantly, twins had a higher prevalence of T2D (MZ: 17.5% (95% CI; 14.4-20.6), DZ: 15.7% (13.1-18.3), C: 5.6% (3.0-8.2), p=0.03) together with a 60% higher incidence rate of T2D compared to singletons. Cumulative concordance rates of T2D to the age of 84 years were similar among elderly MZ (0.76 (0.68-0.84)) and DZ (0.71 (0.63-0.78)) twins.

**Conclusion:** Twin status *per se* is associated with abdominal obesity, insulin resistance and increased prevalence of T2D in elderly twins. The data supports a quantitatively significant impact of the fetal environment as opposed to genetics on risk of T2D.
Type 2 diabetes (T2D) is a complex disease with a multifactorial etiology. The finding of higher concordance rates among monozygotic (MZ) as compared to dizygotic (DZ) twins in some (1-3) - but not all (4) - twin studies has been considered as strong evidence of a significant genetic component in T2D. Further support has been provided by the recent identification of a number of T2D associated genes in the genome-wide association studies (5;6). However, the combined effect of these T2D susceptibility genes accounts for less than 10% of the population risk of the disease, and even for the most significant T2D susceptibility genes, such as the \( TCF7L2 \) gene, the predominant proportion of the carriers of risk alleles will not develop T2D on a life-time perspective (5).

Low birth weight is another known risk factor for T2D and is more common in twins as compared to singletons (7). In accordance with the "fetal origin hypothesis" (8-10) we have demonstrated elevated plasma glucose and insulin profiles during an oral glucose challenge in MZ as compared to DZ twins (11). A more recent study of non-diabetic elderly twins (12) provided some mechanistic explanation in such that monozygosity was associated with reduced peripheral insulin sensitivity. Furthermore, twin status \textit{per se} was related to increased hepatic glucose production (i.e. hepatic insulin resistance) compared to elderly age-matched singleton controls (12). Whether these metabolic differences within and between twins and singletons have any clinical importance as reflected in an increased risk of developing overt T2D is currently unknown.

Age is an important etiological factor in T2D, and has been shown to play a key role in unmasking the unfavourable metabolic effects associated with adverse fetal environment (13;14). In addition, age may be equally important in modulating the genetic influence on T2D, however, valid concordance rates of T2D among the oldest twins are lacking. We hypothesised that age may change the relative genetic effect on T2D as well as the effect of twin and zygosity status on T2D. Therefore, we performed a 10 years follow-up study of a population-based cohort of elderly twins examined in 1995 (4;11) together with an age-matched cohort of singleton control subjects.

\textbf{Study population:} The study represents a 10 year follow-up study of a population-based cohort of elderly MZ and same-sex DZ twins (n=626) previously investigated in 1995 (4;11). Among these twins, 122 (MZ, n=43 (16.6%); DZ, n=79 (21.5%), \( \chi^2=0.17 \)) were deceased in 2005 when the follow-up study was performed. Of the remaining 504 twins, 207 twins (MZ, n=91; DZ, n=116) did either not wish or were refrained to participate by the investigator due to severe current or chronic illness including cancer or severe dysfunction of major organ systems. Although the clinical examination was sought performed in close proximity to the homes of the participants, and transportation was provided, some subjects were not able to participate solely due to reduced mobility. A total of 297 twins (MZ, n=125, of which 49 full pairs; DZ, n=172, of which 55 full pairs) equivalent to 57.9% and 59.7% of eligible MZ and DZ twins, respectively, participated in a subsequent clinical examination. One additional MZ twin (co-twin to a twin participating in the baseline 1994-95 study) was included in the follow-up study. The final sample of twins undergoing the clinical examination was 298 twins. The singleton control subjects (n=71) were recruited among spouses to the participating twins. All spouses (n=553) of the 626 twins included in the baseline study were identified through
the Civil Registration System (CRS). Both twins and spouses were followed for death through the CRS and occurrence of T2D through Danish National Diabetes Register (NDR) (15). The register-based diagnosis of T2D is based upon hospital diagnosis (in- or outpatient) and/or consecutive plasma glucose measures performed in the primary health care sector (15).

The study was approved by the regional Ethical Committees and was conducted according to the principles of the Helsinki Declaration.

**METHODS**

The clinical examination included a standardised 75 g oral glucose tolerance test (OGTT) performed after an overnight fast. Blood samples were taken before and 30, 60 and 120 minutes after glucose ingestion. Plasma glucose and insulin concentrations were analysed as previously described (4;11). The fasting blood samples were furthermore analysed for serum triglycerides and HDL-cholesterol using commercial kits from Boehringer Mannheim, Mannheim, Germany.

Weight and height were measured with the subject in lightweight clothes without shoes and the body mass index (BMI) (weight (kg)/height\(^2\) (m\(^2\))) was calculated. Waist circumference was measured using a soft tape on standing subjects midway between the lowest rib and the iliac crest. Hip circumference was measured over the widest part of the gluteal region, and the waist to hip ratio (WHR) was calculated accordingly.

The same T2D diagnostic criteria was used in the both the baseline and follow-up study; 1) known T2D, that is diagnosis of diabetes after the age of 40 years and current treatment with antidiabetic agents or diet and/or 2) a fasting plasma glucose concentration \(\geq 7.8\) mmol/l and/or two-hour post OGTT plasma glucose concentration \(\geq 11.1\) mmol/l. Impaired glucose tolerance (IGT) was defined as fasting plasma glucose concentrations below 7.8 mmol/l, and two-hour post OGTT plasma glucose concentrations between 7.8 mmol/l and 11.1 mmol/l.

Areas under the curve (AUC) for plasma glucose, insulin and c-peptide were calculated for the initial 30 min period and for the entire 120 min period using the trapezoidal method. Insulin resistance was assessed using the homeostasis model assessment (HOMA) (16) and whole-body insulin sensitivity indices (17). As measurements for insulin secretion we calculated the HOMA \(\beta\)-cell function and insulinogenic indices i.e. ratio between the 30 min increment in insulin and glucose concentration after oral glucose loading.

**Statistical methods:** The distributions of zygosity and sex among non-participating and participating twin pairs as well as the prevalence of T2D and IGT were compared by chi-square tests. The comparisons of continuous variables between participating and non-participating MZ and DZ twins and singletons were performed with a mixed model (proc mixed in SAS, Version 9.1, SAS Institute). We adjusted for the intra-twin-pair relationship in the analyses by including a random effect term for twin-pair membership with zygosity-specific variance and a fixed effect term for zygosity in the model. Raw data are presented as mean (SD).

Cumulative probandwise concordance rates of T2D from age 45 to 84 years were estimated for MZ and DZ twins and compared with a chi-square test (18;19). Probandwise concordance rate expresses the risk of disease among co-twins of affected twins and is comparable to the recurrence risk in other groups of relatives and in the general population.

The incidence rates of diabetes from the NDR (15) were tabulated by zygosity (MZ, DZ, spouse), sex and age and calendar time in one-year classes using the SAS-macro %Lexis (20) for splitting follow-up time, and modelled using a Poisson regression
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RESULTS

Clinical characteristics of MZ and DZ twins at baseline according to participation in follow-up study—Both MZ and DZ twins participating in the follow-up study were younger, taller, and had lower post OGTT plasma glucose and insulin levels at baseline than those not participating. Moreover, the participating DZ had lower WHR as compared to non-participating DZ twins (Table 1). Finally, the distribution of T2D, IGT and NGT baseline was different in the twins participating in the follow-up study among both MZ (p=0.008) and DZ (p<0.001) twins (Table 1). There was no difference in participation according to glucose tolerance status between MZ and DZ, p=0.76.

Follow-up study – glucose profiles: During the 10 year follow-up period fasting plasma glucose levels (mean (SD) mmol/l) were unchanged within both MZ (MZ_{1995}: 6.1 (1.9) vs. MZ_{2005}: 6.1 (1.8), p=0.87) and DZ (DZ_{1995}: 5.9 (1.4) vs. DZ_{2005}: 5.8 (1.0), p=0.78) twins. In MZ twins, the 30 min plasma glucose level was unchanged (MZ_{1995}: 9.8 (2.5) vs. MZ_{2005}: 9.6 (2.6), p=0.39), whereas there was a significant increase over time within DZ twins (DZ_{1995}: 9.0 (2.3) vs. DZ_{2005}: 9.6 (2.1), p<0.0001). Accordingly, the increment in 30 min plasma glucose levels over the 10 years follow-up period was significantly higher in DZ twins (MZ: -0.18 (2.2); DZ: 0.6 (1.8), p=0.006). There was a significant deterioration in glucose tolerance with age with an increase in 120 min plasma glucose levels in both MZ (MZ_{1995}: 7.4 (4.1) vs. MZ_{2005}: 8.5 (4.7), p<0.0001) and DZ twins (DZ_{1995}: 7.0 (3.6) vs. DZ_{2005}: 8.4 (3.9), p<0.0001). The increment in 120 min plasma glucose levels was of similar magnitude in MZ (1.1 (2.7)) and DZ (1.6 (2.4)) twins (p=0.22).

Cumulative concordance rates: The age-adjusted cumulative probandwise risk at age 84 which approximates the lifetime risk of T2D was similar in MZ (0.76 (0.68-0.84)) and DZ (0.71 (0.63-0.78)) twin pairs, Figure 1.

Influence of zygosity and twin status on glucose metabolism: Seventy-one singletons were included as control subjects in the 2005 follow-up study. The singleton controls were slightly younger than the twins (p=0.004), and the distribution of sex differed significantly between the groups of singletons and twins (p=0.006). Therefore, adjustments were performed for sex and age in the subsequent analyses. Adult height, weight and BMI were similar in twins and singletons, whereas twins were significantly more abdominally obese with a higher WHR than singletons (p=0.02), Table 2. Plasma glucose levels during the OGTT were similar in MZ and DZ twins, whereas post OGTT plasma glucose levels at time points 30 (p=0.05), 60 (p=0.04) and 120 (p=0.004) minutes were significantly higher in twins than in singletons (Figure 2A) as were the incremental AUC_{glucose}’s during the initial 30 min and the total 120 min of the OGTT. Accordingly, HbA1c concentration was higher (p=0.004) in twins than singletons, and most importantly, the prevalence of T2D was higher in twins as compared to singletons (MZ: 17.5 %; DZ: 15.7 %; singletons: 5.6 %, p=0.03), whereas the prevalence of IGT was similar (MZ: 27.8 %; DZ: 25.0 %; singletons: 22.5 %).

Plasma insulin levels were similar in twins and singletons before and during the OGTT except at time point 120 min were twins had significantly higher levels compared to singletons (p=0.01), Figure 2B. Plasma c-peptide levels were significantly increased in twins compared to singletons at all time points as were the incremental AUC_{c-}
peptide’s (all p<0.001), Figure 2C. Twins tended to be more insulin resistant than singletons expressed as the HOMA (p=0.12) and whole body insulin sensitivity indices (p=0.04). Finally, systolic and diastolic blood pressure as well as serum lipids (i.e. triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol) were similar in twins and singletons (data not shown).

Incidence rate of diabetes by zygosity and twin status: The singleton controls exhibited a significant lower prevalence of T2D subsequent to oral glucose testing as compared to twins. In order to determine whether this was a true finding or merely due to selection we investigated incidence rates of T2D as recorded in NDR during the 10 year follow-up period. The rate ratio of T2D adjusted for sex and age between the groups was 1.51 (0.93; 2.44, p=0.09) for MZ vs. singletons, 1.69 (1.10; 2.59, p=0.02) for DZ vs. singletons and 1.12 (0.71; 1.77, p=0.63) for MZ vs. DZ. The rate ratio between all twins and singletons was 1.62 (1.10; 2.38, p=0.02). The register-based diabetes prevalence by the end of 2005 was 4.0% in singletons and 9.1% (MZ: 8.2%; DZ: 9.8%) in twins (all participants in the baseline examination), p=0.004.

DISCUSSION

We have demonstrated that elderly twins exhibit a higher degree of abdominal obesity, glucose intolerance and insulin resistance, and most importantly, a higher prevalence and incidence rate of T2D than singletons. This is in accordance with the idea of pre – and early postnatal programming as an underlying key player in the development of the dysmetabolic syndrome later in life.

Singletons were included in the follow-up study due to our previous observation that elderly non-diabetic twins exhibited higher rates of hepatic glucose production (12) as compared to age-matched singletons, and therefore may be at increased risk of developing overt T2D. In this study we found a higher prevalence of T2D in twins than singletons. Unfortunately, the methodology does not allow mechanistic explanations of this phenomenon, although the indirect OGTT measures of insulin resistance and secretion indicates reduced insulin sensitivity in twins relative to singletons. Notably, despite the somewhat subtle influence of twin status on OGTT plasma insulin levels, plasma c-peptide concentrations at all time-points were increased in twins compared to singletons consistent with a state of compensatory increased pancreatic insulin secretion in the presence of a higher hepatic insulin extraction in twins as compared to singletons. Indeed, studies have suggested an elevated hepatic extraction rate in diabetic subjects (22).

We have previously demonstrated significantly elevated 30 min concentrations and AUC’s of plasma glucose and insulin in MZ as compared to DZ twins (11). We were able to replicate these differences in plasma glucose profiles at baseline in the subpopulation of MZ and DZ twins participating in both studies. However, the 10 year follow-up study revealed similar OGTT plasma glucose profiles in MZ and DZ twins. The convergence of these metabolic profiles during the 10 year follow-up period was primarily due to a somewhat “delayed” increase in 30 min plasma glucose levels in the DZ twins. MZ and DZ twins had similar 2 hour plasma glucose levels at baseline and follow-up, and had similar increments with advancing age. These findings suggest that the deterioration in plasma glucose profiles after a glucose challenge associated with advancing age (23) may be more accelerated in MZ twins, whereas DZ twins preserve a conspicuous degree of insulin sensitivity to a higher age resulting in lower postprandial glucose profiles.
The twins participating in the follow-up study were younger and more glucose tolerant at baseline compared to the twins that were either deceased or non-participants. Nevertheless, we demonstrated an approximately 2 fold higher prevalence of T2D in these surviving and healthy twins as compared to singleton controls. Although the National Diabetes Registry (NDR) may not be complete, there is no reason to suspect any dissimilar or skewed recordings between twins and singletons. The NDR incidence rates of a 60% higher T2D risk in twins compared with singletons, including all twins and spouses irrespective of participation in follow-up study or death, indeed confirmed the data obtained from the clinical (OGTT) examinations.

The effect of ageing on the relative genetic influence on the development of T2D was assessed by means of cumulative concordance rates. Interestingly, the lifetime risk of T2D (to age 84 years) among healthy co-twins to diabetic twins was similar in MZ and DZ twins, suggesting that the relatively modest influence of genetic variation on the development of T2D per se does not change significantly within the range of 60 to 80 years of age. Most interestingly, the cumulative concordance rate – and hence recurrence risk of - T2D of approximately 70% in DZ twins is considerably higher than the life-time risk of ~35% (24) for first degree relatives (i.e. siblings) who share the same amount of genes with their diabetic or non-diabetic proband as DZ twins. Furthermore, the cumulative concordance rate for T2D to age 84 exceeds 0.50, which represents the rate that should be expected in DZ twins for a dominant inherited monogenic disease. These results indeed support that twin status per se represents a risk factor for the development of T2D. Although the birth weights differed markedly between twins and singletons (Table 2), we did not find any statistical significant association between birth weight in the subset of twins (n=188) or singletons (n=59) with know birth weights. This may to some extent be due to lack of statistical power in each of the subgroups. Furthermore, it supports our previous conclusion of the impact of fetal programming by twin and zygosity status going beyond the impact of birth weight per se in twins (12).

While the BMI was similar in twins and singletons at the clinical follow-up examination, both MZ and DZ twins had an elevated waist-to-hip ratio (WHR), and thereby increased abdominal obesity, as compared with the singleton controls. Indeed, we and other have previously shown that low birth weight and an adverse intrauterine environment is associated with abdominal obesity (25-26). Abdominal obesity is a hallmark of insulin resistance, T2D and the metabolic syndrome, contributing to some unknown extent to the documented insulin resistance and glucose intolerance in the twins as compared with singletons in this study. The possibility remains that an accelerated postnatal catch-up growth in the twins rather than their documented lower birth weights per se may explain the increased abdominal obesity in the twins. Furthermore, other effects of twinning not directly related to fetal growth rate including periconceptional nutrition might theoretically explain the finding of increased abdominal obesity and risk of T2D in twins compared with singletons (27).

In conclusion, twin status is evidently associated to an increased risk of T2D with elderly twins exhibiting a higher degree of abdominal obesity, glucose intolerance and insulin resistance and most importantly a higher prevalence of T2D as compared to singletons. These findings together with the indication of a predominant role of non-genetic factors in the aetiology of T2D support the notion of pre- and early postnatal programming as a key player in the development of T2D and various components of the metabolic syndrome in adult life.
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Table 1 Baseline clinical characteristics of 626 MZ and DZ elderly twins stratified according to whether they were deceased or alive and eligible for the follow-up study conducted in 2005. Data presented as mean (SD). P value expresses the level of statistical significance for the comparison between the 3 groups (deceased, no follow-up & follow-up) (ANOVA) within MZ ($p_{MZ}$) and DZ ($p_{DZ}$) twins, respectively. The analyses have been adjusted for sex.

|                  | MZ                | DZ                | $p_{MZ}$ | $p_{DZ}$ |
|------------------|-------------------|-------------------|----------|----------|
|                  | Deceased          | No follow-up      | Follow-up| Deceased | No follow-up | Follow-up |
| N (m/f)          | 43 (23/20)        | 91 (37/54)        | 125 (71/54) | 79 (50/29) | 116 (51/65) | 172 (75/97) |        |
| Age at baseline (yrs) | 69.1 (3.7)     | 68.1 (3.8)        | 65.5 (4.9) | 68.3 (3.5) | 66.9 (5.1) | 64.7 (5.3) |        |
| **Anthropometry** |                   |                   |          |          |          |
| Height (cm)      | 162.8 (8.5)       | 164.5 (9.7)       | 167.9 (9.7) | 167.2 (10.4) | 164.8 (9.1) | 167.2 (8.4) | 0.02 |
| Weight (kg)      | 67.9 (14.0)       | 70.0 (13.9)       | 74.3 (13.6) | 71.6 (15.2) | 71.2 (13.3) | 72.4 (12.9) | 0.27 |
| BMI (kg/m²)      | 25.5 (4.2)        | 25.8 (4.6)        | 26.3 (4.3) | 25.6 (4.8) | 26.2 (4.7) | 25.8 (3.9) | 0.28 |
| Waist (cm)       | 90.1 (14.1)       | 88.9 (12.6)       | 91.1 (11.5) | 92.2 (13.8) | 89.7 (12.3) | 87.7 (12.4) | 0.13 |
| Hip (cm)         | 100.3 (8.7)       | 102.5 (9.0)       | 102.7 (7.8) | 101.6 (8.9) | 102.8 (9.5) | 103.0 (8.1) | 0.52 |
| WHR              | 0.90 (0.10)       | 0.87 (0.08)       | 0.89 (0.08) | 0.91 (0.08) | 0.87 (0.09) | 0.85 (0.10) | 0.01 |
| **OGTT –glucose (mmol/l)** |             |                   |          |          |          |
| 0 min            | 6.3 (1.4)         | 6.1 (1.5)         | 6.1 (1.9) | 6.3 (1.9) | 6.2 (1.8) | 5.9 (1.4) | 0.10 |
| 30 min           | 10.4 (2.3)        | 9.8 (2.3)         | 9.8 (2.5) | 10.6 (3.3) | 9.8 (2.7) | 9.0 (2.3) | 0.001 |
| 120 min          | 9.0 (4.3)         | 8.5 (4.6)         | 7.4 (4.1) | 8.7 (4.7) | 8.3 (4.6) | 7.0 (3.6) | 0.004 |
| **OGTT –insulin (pmol/l)** |             |                   |          |          |          |
| 0 min            | 52.9 (31.9)       | 45.3 (26.3)       | 45.9 (24.2) | 44.8 (25.3) | 48.9 (37.6) | 41.7 (22.5) | 0.05 |
| 30 min           | 309.4 (197.3)     | 324.2 (353.7)     | 324.5 (196.1) | 304.0 (273.1) | 320.8 (285.4) | 259.5 (167.9) | 0.08 |
| 120 min          | 418.6 (389.4)     | 371.1 (513.1)     | 253.7 (194.3) | 301.8 (306.0) | 345.7 (338.8) | 245.7 (223.3) | 0.01 |
| **Glucose tolerance status** |             |                   |          |          |          |
| (T2D/IGT/NGT) (%) | 23.3/27.9/48.8    | 17.6/23.1/59.3    | 8.1/16.0/75.9 | 21.5/30.4/48.1 | 13.8/25.9/60.3 | 5.8/16.9/77.3 | <0.001 |
Increased risk of Type 2 diabetes in Twins

Figure 1 Age-related cumulative concordance rates (SE) for T2D in MZ and DZ elderly twins.

Table 2 Clinical characteristics in elderly MZ and DZ twins and singletons included in the follow-up study in 2005. Data are presented as mean (SD). P value expresses the level of statistical significance for the comparison between the 3 groups (MZ, DZ and singletons) (ANOVA). The analyses have been adjusted for sex and age.

|                  | MZ          | DZ          | Singleton  | p     |
|------------------|-------------|-------------|------------|-------|
| N (m/f)          | 126 (71/55) | 172 (74/97) | 71 (24/47) | 0.006 |
| Age at follow-up | 74.4 (9.5)  | 73.7 (0.5)  | 71.4 (0.7) | 0.004 |
| Birth anthropology |           |             |            |       |
| Weight (g)       | 2656 (478)  | 2672 (459)  | 3513 (529) | <0.0001 |
| Length (cm)      | 46.9 (3.5)  | 48.1 (2.1)  | 51.4 (2.1) | <0.0001 |
| Ponderal index (g/cm³) | 24.7 (6.2) | 23.7 (3.9)  | 26.2 (3.2) | 0.001 |
| Adult anthropology |           |             |            |       |
| Weight (kg)      | 73.2 (13.6) | 72.2 (12.7) | 71.3 (12.8) | 0.91  |
| Height (cm)      | 166.8 (9.8) | 166.3 (8.6) | 165.4 (8.9) | 0.71  |
| BMI (kg/m²)      | 26.3 (4.7)  | 26.1 (3.9)  | 26.1 (4.1)  | 0.97  |
| WHR              | 0.92 (0.09) | 0.89 (0.10) | 0.86 (0.09) | 0.02  |
| HbA1c            | 6.0 (1.0)   | 5.8 (0.7)   | 5.6 (0.3)   | 0.004 |
| OGGT – glucose   |             |             |            |       |
| iAUC0-30 min     | 54.1 (22.1) | 56.3 (23.8) | 47.2 (21.1) | 0.05  |
| iAUC0-120 min    | 361.9 (232.7) | 376.1 (229.5) | 264.7 (185.4) | 0.03  |
| OGGT – insulin   |             |             |            |       |
| iAUC0-30 min     | 4410 (2871) | 4004 (2830) | 4331 (2827) | 0.32  |
| iAUC0-120 min    | 33531 (18978) | 32425 (22589) | 31010 (19985) | 0.40  |
| OGGT – c-peptide |             |             |            |       |
| iAUC0-30 min     | 22251 (10397) | 20748 (10024) | 17304 (10356) | <0.0001 |
| iAUC0-120 min    | 222194 (80846) | 214865 (81998) | 162184 (90572) | <0.0001 |
| HOMA-IR          | 2.2 (2.4)   | 1.8 (1.1)   | 1.5 (0.9)   | 0.12  |
| Insulin sensitivity index | 15.7 (8.7) | 16.5 (7.9)  | 19.4 (8.8)  | 0.04  |
| HOMA-IS          | 64.7 (43.3) | 63.2 (35.2) | 56.1 (25.5) | 0.29  |
| Insulinogenic index | 103.1 (129.4) | 79.2 (74.4)  | 90.5 (175.5) | 0.07  |

iAUC – incremental area under curve
Figure 2. Plasma glucose (A), insulin (B) and c-peptide (C) concentrations during an oral glucose tolerance test (OGTT) in elderly MZ and DZ twins and singletons included in the follow-up study. Data are presented as mean (SD). *p<0.05, ** p<0.01, ***p<0.001 twins vs. singleton.

A

B

C

MZ

DZ

Singleton

Plasma glucose (mmol/l)

Plasma insulin (pmol/l)

Plasma C-peptide (pmol/l)