Depression, anxiety and cognitive function in patients with type 2 diabetes: an 8-year prospective observational study.

Running title: Psycho-cognitive variables and type 2 diabetes.

Marina Trento, Lorena Charrier°, Martina Salassa, Stefano Merlo, Pietro Passera, Franco Cavallo°, Massimo Porta

Laboratory of Clinical Pedagogy, Department of Medical Sciences, University of Turin

° Department of Public Health and Pediatrics, University of Turin.

Correspondence:

Dr. Marina Trento

Laboratory of Clinical Pedagogy,

Department of Medical Sciences,

University of Turin,

Corso AM Dogliotti 14

10126 Turin, Italy.

e-mail: marina.trento@unito.it.

Tel: +39 011 6709591

Fax: +39 011 6708477

Abstract word count: 200

Text Word Count: 2460

Tables: 4

Figures: 1

Abstract

Introduction and aim. Since depression, anxiety and cognitive function may be impaired in type 2 diabetes, we investigated the relationships between clinical and socio-economic variables and these psychological dimensions.

Methods. Observational 8-year prospective study of 498 patients, 249 not insulin-treated (NIT) and 249 insulin-treated (IT). Demographic, socio-economic and clinical data were monitored along with depression and anxiety (assessed by Zung questionnaire) and cognitive function (Minimal Mental State Examination, MMSE).

Results. After 8 years, 131 patients remained NIT (NIT-NIT), 179 remained IT (IT-IT), 47 switched to insulin (NIT-IT), 111 were lost to follow-up and 30 died. In all groups, HbA1c remained stable, BMI, glucose, and lipid profile improved, foot ulcers and retinopathy worsened. Mild worsening in depression and anxiety scores was observed in the IT-IT patients only. On multivariate analysis, worsening of depression was associated with female gender, disease duration and being IT-IT, and worsening of anxiety with disease duration. Decreased MMSE was associated inversely with smoking and directly with being IT-IT.

Discussion. Patients with type 2 diabetes are at relatively low risk of psycho-cognitive decline. However, being female and on long-term insulin treatment may be risk factors for psychological distress, suggesting that special attention is required for these patients.

Key words: depression, anxiety, cognitive function, type 2 diabetes, metabolic control.

Introduction.

People with type 2 diabetes are constantly involved in the management of their disease (1) and assessing depressive mood or diabetes distress may help identify patients at higher risk of poor control and complications (2, 3).

Depression, anxiety and cognitive function are among the main psychological traits influenced by, and impacting on, diabetes but the results of previous surveys are not homogeneous. Depression may be at least twice as prevalent in diabetes (3,4) whereas data on anxiety are conflicting (5), ranging from 6% (6) to 32% (7) compared to an estimated 12-21% in the general population (8-10).

The mechanisms through which these psychological traits may impact on diabetes control and complications are poorly understood. Depression may impair glycemic control through negative effects on self-care, poor adherence to medication and diet, reduced quality of life and increased health-care costs (11). Anxiety may be associated with complications, high blood glucose, reduced quality of life and increased body mass index (12).

Accelerated cognitive impairment has been described in diabetes and may be linked to cerebrovascular damage, poor glycemic control, hypoglycemia, microvascular disease, inflammation and depression (13, 14)

In a previous survey, we analyzed the possible relationships between the above psychological traits in type 2 diabetes (15). On a first cross-sectional study, we reported that depression was associated with older age, female gender and being on insulin treatment and that anxiety was associated with depression and older age, whereas we could not observe any significant cognitive impairment (15). Four years later, depression had worsened among patients on long-term insulin treatment and was associated with female gender. Anxiety increased, and cognitive function declined, with diabetes duration and lower schooling (16). Here, we further explored the evolution of the above psychological dimensions in the same cohort after another 4 years in order to identify possible longer-term associations with clinical variables in relation to metabolic control and the development of complications.

Methods

At baseline 249 patients treated by lifestyle intervention alone or with oral agents (Non Insulin Treated, NIT) and 249 also receiving insulin treatment (Insulin Treated, IT) had been enrolled. The patients were invited to a follow up visit 8 years later. They were outpatients, mostly Caucasian, with type 2 diabetes, aged 40-80 at baseline. They were routinely followed in urban diabetes clinics which could be reached easily by public transport, suggesting that the sample was representative of the local diabetic population. All patients gave their informed consent, in accordance with the Declaration of Helsinki principles. Exclusion criteria were history of psychiatric illnesses in the patients or their families, presence of cancer, renal replacement therapy or other severe chronic conditions. After 8 years, January 2014 to December 2014, on the occasion of routine visits, the patients were invited to participate in the follow-up as they attended the clinic. When no visits were programmed, the patients were contacted by telephone.

The same variables collected at baseline were recorded 8 years later: age, gender, schooling, occupation, family status, smoking status, self monitoring of blood glucose, family history and duration of diabetes, body weight, glycated haemoglobin (HPLC, IFCC aligned), fasting blood glucose (glucose-oxidase), blood pressure, serum creatinine, total and HDL cholesterol, triglyceride. All patients underwent feet and fundus examination by 2-field, 45° digital colour photography. Active or previous presence of foot ulcers or amputations was collected and accounted for as a dichotomous variable. Mild retinopathy was defined as microaneurysms only or isolated blot haemorrhages/cotton wool spots. Any other presentation was classified as moderate or more severe for the purpose of this study.

Psychological evaluation

Three questionnaires were administered at baseline and after 8 years to evaluate depression, anxiety and cognitive performance. Depression and anxiety were assessed by the relevant Zung Self-rating scales (17) and cognitive status by the Mini Mental State Examination (MMSE) (18).

The Zung Self-Rating Depression Scale includes 20 items on a scale that rates four common characteristics of depression: the pervasive effect, the physiological equivalents, other disturbances, and psychomotor activities. There are 10 positively worded and 10 negatively worded questions, each scored on a scale of 1 to 4, and total scores range from 20 to 80. The four possible outcomes are: 20-49 normal range, 50-59 mildly depressed, 60-69 moderately depressed, 70 and above severely depressed.
The Zung Self-Rating Anxiety Scale is also a self-administered 20-item test, each scored on a scale of 1-4. questions are worded toward increasing and 5 toward decreasing anxiety levels. Total scores range from 20 to 80: 20-44 normal range, 45-59 mild to moderate anxiety, 60-74 marked to severe anxiety, 75-80 extreme anxiety.
The MMSE is administered as a semi-structured interview and includes 30 items assessing orientation, attention, immediate and short-term recall, language and the ability to follow simple verbal and written commands. Cognitive performance varies by age and educational level, with an inverse relationship between MMSE scores and age, ranging from a median of 29 for individuals 18 to 24 years of age, to 25 for those 80 years of age and older. The median MMSE score is 29 for individuals with at least 9 years of schooling, 26 for those with 5 to 8 years of schooling, and 22 for those with 0 to 4 years of schooling.
All three tools had been translated into Italian and revalidated. If the patients had literacy problems, the questionnaires were completed with the help of a health operator.

Statistical Methods

Descriptive data are shown as absolute frequencies of the different modalities for categorical data and as mean ± standard deviation (SD) for continuous variables.
Paired t-test for continuous variables, and McNemar test for categorical variables were carried out to evaluate differences between values at baseline and after 8 years in the three patient groups: NIT-NIT, NIT-IT and IT-IT.
T-test for continuous variables and chi-square test for qualitative variables were carried out to compare data at baseline of possible predictors of depression and anxiety; patients with no to moderate depression at 8 years and patients who developed marked-severe depression were compared. The same analyses were performed for anxiety. A score of 60 was used for both outcomes as cut-off to discriminate the development of severe depression or anxiety. We could not perform threshold analysis for MMSE due to absence of patients with scores below 20 points, the cutoff threshold discriminating the presence of cognitive impairment.
A t-test, or an analysis of variance, was performed to compare the mean scores of depression, anxiety and cognitive performance among the different modalities of categorical predictors.
The difference between scores for depression, anxiety and MMSE at baseline and after 8 years was assessed by fitting different types of multivariate linear regression models, in order to adjust for the independent effect of clinical and behavioural variables. Finally, we took into account 3 models where, for each outcome –depression, anxiety and MMSE, the difference between final score and baseline represented the dependent variable, while the relevant score at baseline, together with gender, schooling, disease duration, changes in therapy between baseline and 8 years and smoking habit were the independent variables. For the MMSE regression model the differences between 8 years and baseline for BMI and HbA1c were also included as covariates.
Patients previously treated with antidepressants were excluded from all analyses.
A p-value of less than 0.05 was taken as significant.
All analyses were performed with Stata 13.

Results

After 8 years, 131 out of 249 patients were still NIT (NIT-NIT), 47 had switched to insulin (NIT-IT) and 179 of 249 were still IT (IT-IT).
Another 111 (56 NIT and 55 IT) were lost to follow up and 30 (15 NIT and 15 IT) had died (Fig. 1).
The patients still active after 8 years had a mean age of 66.8 (± 7.6) years and a mean HbA1c of 64.7 (± 13.9) mmol/mol (8.07 ± 1.27 %) at baseline. Those lost to follow up where slightly older

(68.5 ± 8.1 years, p=0.046), had worse HbA1c (69.6 ± 18.2 mmol/mol, 8.52 ± 1.67 %, p=0.0025) and anxiety score (37.5 ± 8.3 vs 35.5 ± 8.6, p=0.043), but did not differ for any of the other clinical or psychometric variables measured at baseline. Mortality after 8 years was associated with older age (p<0.0001) and being a smoker, active or ceased, (p=0.04) at baseline.

In the NIT-NIT patients, BMI (p<0.0001), fasting blood glucose (p=0.043), total cholesterol and triglyceride (p<0.0001, both) had decreased and HDL cholesterol (p<0.0001) increased over 8 years. The prevalence of hypertension (p<0.0001), foot ulcers (p=0.0009) and moderate to severe retinopathy (p<0.0001) had also increased (Table 1).
In the 47 patients who had switched to insulin (NIT-IT), total cholesterol had decreased (p=0.0003); BMI (p=0.039), HDL cholesterol (p=0.0117) and prevalence of hypertension (p<0.0001) had increased along with the prevalence of foot ulcers (p=0.0253) and moderate to severe retinopathy (p=0.0005).
In the IT-IT patients, total cholesterol had decreased (p<0.0001) and the prevalence of hypertension (p<0.0001), foot ulcers (p<0.0001) and moderate to severe retinopathy (p=0.0001) increased.
There were no significant changes for the other variables, including HbA1c, in any of the above 3 groups. Psychometric variables did not change in the NIT-NIT or the NIT-IT. Mild but significant worsening in the depression (p<0.0001) and anxiety (p=0.0001) scores was observed in the IT-IT whereas MMSE scores did not change in any of the 3 groups.
On univariate analysis (Tables 2 and 3), depression Zung scores above 60 at 8 years were predicted by higher age (p=0.0311), longer disease duration (p=0.0344) and lower LDL levels (p=0.0380). Female gender (p<0.0001), lower schooling (p=0.0070), and never vs active smokers (p=0.025) at baseline were associated with significantly higher depression scores. Worse anxiety scores at 8 years were predicted by female gender (p<0.0001), lower schooling (p=0.0025), higher BMI (p=0.0068), and never vs active smokers (p=0.004) at baseline. Lower MMSE scores at 8 years were predicted only by being never versus former smoker at baseline (p=0.005).
On multivariate analysis (Table 4), worsening of depression over 8 years remained associated with female gender (p=0.037), disease duration (p=0.046) and being on insulin treatment since the beginning of the study (p=0.033). Worsening of anxiety was only associated with disease duration (p=0.027). A statistically significant decrease in MMSE was inversely associated with smoking (p=0.001 for past vs never smoking) and directly associated with being on insulin treatment since the beginning of the study (p=0.010) but not with known disease duration.

Conclusions

This study confirms and extends our previous 4-year follow up observations. In particular, it suggests that the association of type 2 diabetes with depression, anxiety and cognitive impairment may be less profound than expected from previous reports in the literature. Specifically, we did not observe progressive worsening of depression and anxiety, except for female patients with longer duration of disease and on insulin treatment for many years.
Cognitive function did not decline significantly in any of the three treatment groups and minor worsening of the MMSE score, not reaching pathological levels, was again associated with being on insulin treatment for years, suggesting that it may take a long time to affect cognitive status.
In our previous 4-year follow-up, we found that depression had worsened among patients on long-term insulin treatment and was associated with female gender (16). At 8 years, the only patients who experienced mild but significant worsening in depression and anxiety scores were again those on insulin from baseline (IT-IT), suggesting that higher levels of depression and anxiety may be linked, among other factors, with negative feelings towards therapy with insulin (19-21) and long disease duration (22). Our findings confirm that being female is also a risk factor for depression and anxiety in type 2 diabetes, supporting the role of gender and the notion that, in the long run, social and clinical determinants concur in making the psychological burden of diabetes heavier. The risk posed by an unfavorable socioeconomic profile, a proxy for gender, age, schooling and other related variables, for people with type 2 diabetes to develop a depressive-anxious trait is not new to the literature (23) and confirms our own findings at 4 years (16).

Unexpectedly, non-smokers had the worse outcome in terms of cognitive decline. A possible protective effect of smoking on retaining cognitive function is difficult to interpret. Smoking is a well accepted risk factor for vascular dementia and mild cognitive impairment (24, 25), although a 10-year follow up in Taiwan suggested that smoking could be protective (26). On the other hand, smoking is unrelated to depression and anxiety (27) and may protect from other neurodegenerative conditions, such as Parkinson's disease, and nicotine may reduce toxicity of storage proteins, amyloid precursor and presenilin, in Alzheimer's disease (28). However, one should consider the risk of exclusion bias resulting from higher mortality rate in patients who were older and smokers at baseline, as in our study. Recall bias from difficulty for demented patients to remember and declare their smoking status is unlikely because none in our study reached clinically relevant cognitive decline.

Interestingly, HbA1c did not worsen over 8 years, whereas BMI and lipid profile even showed a trend to improve, possibly as a result of intensified pharmacological and support strategies and improved patient self-management. This appears in contrast with progressive worsening of beta-cell function and metabolic control, as reported in the UKPDS (29). However, all UKPDS patients were newly diagnosed while ours already had years of known diabetes duration at baseline. Attrition bias is unlikely because patients lost to follow up had clinical variables similar to those who remained in the study, apart from aging and smoking status in those who deceased. Data on non glucose lowering medication was not fully available from clinical records and this may limit interpretation of the reasons for this observation.

Strengths of this study include a large outpatient population carefully followed for a long period of time and the use of standardized procedures to monitor clinical and psycho-cognitive variables. The study cohort was homogeneous and not contaminated by new patient enrolment during follow-up.

The socioeconomic profile of the patients was investigated in relation to specific factors (age, gender, schooling, living alone), although direct data on income were unavailable.

Limitations include the high number of dropouts but this is not unexpected, given the length of observation. Reduced observations may potentially limit the overall statistical power, but the number of patients still in the study was sufficient for meaningful multivariate analysis to be carried out. In addition, in insulin treated patients, psychological insulin resistance may reflect broader distress about diabetes and concerns about its treatment (19).

Finally, the study population may have been too young at baseline to capture a significant cognitive impairment after 8 years. Indeed, we could not perform threshold analysis for MMSE due to absence of patients with scores below 20. Anyway, this study shows the complexity of the disease and emphasizes the existential fatigue that can afflict a person with type 2 diabetes. Recent studies report that family history correlates with increased body weight and diabetes in women and highlight the need to verify available options to treat depression and eating disorders, as well as the use of health-care resources, their cost-effectiveness and the problems posed by different cultural and health-care systems in people with type 2 diabetes (30-33).

In conclusion, this study confirms that type 2 diabetes, on top of having an important clinical impact is a disease that may change the meaning of life. People with type 2 diabetes must constantly check their lifestyle habits and perform regular controls, and women in particular appear more fragile in the daily management of life with the disease (34).

Fundinsgs
The work described in this paper was supported by funds from Ricerca Sanitaria Finalizzata Regione Piemonte.

Duality of interest. Marina Trento, Lorena Charrier, Martina Salassa, Stefano Merlo, Pietro Passera, Franco Cavallo, Massimo Porta declare that there is no duality of interest associated with this manuscript.

Ethical standard The study protocol conformed to the principles of the Declaration of Helsinki.

Human and animal rights disclosure All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent disclosure Informed consent was obtained from all patients for being included in the study.

References

1. Potter L, Wallston K, Trief P, Ulbrecht J, Juth V, Smyth J. (2015) Attributing discrimination to weight: associations with well-being, self-care, and disease status in patients with type 2 diabetes mellitus. J Behav Med. Epub ahead of print
2. Co MA, Tan LS, Tai ES, et al (2015). Factors associated with psychological distress, behavioral impact and health-related quality of life among patients with type 2 diabetes mellitus. J Diabetes Complications. 29: 378-83.
3. Snoek FJ, Bremmer MA, Hermanns N. (2015) Constructs of depression and distress in diabetes: time for an appraisal. Lancet Diabetes Endocrinol. 3: 450-460.
4. Semenkovich K, Brown ME, Svrakic DM, Lustman PJ. (2015) Depression in type 2 diabetes mellitus: prevalence, impact, and treatment. Drugs. 75: 577-87.
5. Fisher L, Skaff M, Mullan JT, et al. (2008) A longitudinal study of affective and anxiety disorders, depressive affect and diabetes distress in adults with Type 2 diabetes. Diabet. Med. 25: 1096–1101.
6. Roy T, Lloyd CE. (2012) Epidemiology of depression and diabetes: a systematic review. J Affect Disord. 142: S8-21
7. Grigsby AB, Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. (2002) Prevalence of anxiety in adults with diabetes: a systematic review. Journal of Psychosomatic Research. 53: 1053–60.
8. Kessler RC, Berglund P, Demler O, et al. (2005) Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Archives of General Psychiatry. 62: 593–602.
9. Leray E, Camara A, Drapier D et al. (2011) Prevalence, characteristics and comorbidities of anxiety disorders in France: results from the Mental Health in General Population survey (MHGP). European Psychiatry. 26: 339–45.
10. Minelli A, Pedrini L, Magni LR, Rotondo A. (2009) Personality traits in an italian sample: relationship with anxiety and depression. Clin Pract Epidemiol Ment Health. 5: 26-30
11. Egede LE. (2005) Effect of depression on self-management behaviors and health outcomes in adults with type 2 diabetes.Curr Diabetes Rev. 1: 235-43.
12. Smith KJ, Béland M, Clyde M, et al. (2013) Association of diabetes with anxiety: a systematic review and meta-analysis. J Psychosom Res. 74: 89-99.
13. Degmecic D, Bacun T, Kovac V et al. (2014) Depression, Anxiety and Cognitive Dysfunction in patients with type 2 Diabetes Mellitus. A study of adult patients with type 2 diabetes mellitus in Osijek, Croatia. Coll Antropol. 38: 711-16.
14. Feinkohl I, Price JF, Strachan MW, Frier BM. (2015) The impact of diabetes on cognitive decline: potential vascular, metabolic, and psychosocial risk factors. Alzheimers Res Ther. 10: 1-46.
15. Trento M. Raballo M, Trevisan M, et al. (2012) A cross-sectional survey of depression, anxiety, and cognitive function in patients with type 2 diabetes. Acta Diabetol. 49:199-203
16. Trento M, Raballo M, Trevisan M et al. (2014) Depression, anxiety, cognitive impairment and their association with clinical and demographic variables in people with type 2 diabetes: a 4-year prospective study. J Endocrinol Invest. 37: 79-85
17. Zung WW. A self-rating depression scale. Archives of General Psychiatry (1965) 12: 63-70
18. Folstein MF, Folstein, SE and McHugh PR. Mini-Mental State: A practical method for grading the state of patients for the clinician, Journal of Psychiatric Research (1975) 12:189-198
19. Holmes-Truscott E, Skinner T, F. Pouwer and J. (2015) Speight. Negative appraisals of insulin therapy are common among adults with Type 2 diabetes using insulin: Results from Diabetes MILES – Australia cross-sectional survey. Diabet Med. Epub ahead of print

20. Krall J, Gabbay R, Zickmund S, Hamm ME, Williams KR, Siminerio L. (2015) Current perspectives on psychological insulin resistance: primary care provider and patient views. Diabetes Technol Ther. 17: 268-74.
21. Mashitani T, Hayashino Y, Okamura S, Kitatani M, Furuya M, Iburi T, Kuwata H, Tsujii S, Ishii H (2015). Diabetes treatment-related quality of life is associated with levels of self-care activities in insulin injection among Japanese patients with type 2 diabetes: Diabetes Distress and Care Registry at Tenri (DDCRT 8). Acta Diabetol. 52: 639-47.
22. Bruce DG, Davis WA, Cetrullo V, Starkstein SE, Davis TM. (2013) Clinical impact of the temporal relationship between depression and type 2 diabetes: the Fremantle diabetes study phase II. PLoS One. 8: e81254.
24. Jiang X, Ma H, Wang Y, Liu Y. (2013) Early life factors and type 2 diabetes mellitus. J Diabetes Res. 485082.
25. Reitz C, den Heijer T, van Duijn C, et al. (2007) Relation between smoking and risk of dementia and Alzheimer disease. The Rotterdam Study. Neurology. 69: 998–1005
26. Orsitto G, Turi V, Venezia A, Fulvio F and Manca C. (2012) Relation of Secondhand Smoking to Mild Cognitive Impairment in Older Inpatients. The Scientific World Journal Volume. 2012: ID 726948
26. Cheng-Ching W, Tsung-Hsueh L, Wen-Chun L, et al. (2010) Cigarette smoking and cognitive impairment: a 10-year cohort study in Taiwan. Archives of Gerontology and Geriatrics. 51: 143–148
27. Taylor AE Fluharty ME, Bjørngaard JH, et al. (2014) Investigating the possible causal association of smoking with depression and anxiety using Mendelian randomisation meta-analysis: the CARTA consortium. BMJ Open 4:e006141
28. Brown D, Ramlochansingh C, Kebreten F, Tizabi Y. (2013) Nicotine promotes survival of cells expressing amyloid precursor protein and presenilin: implication for Alzheimer's disease. Neuroscience Letters. 535: 57- 61
29. UKPDS Group. (200) Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study. Br Med J. 321: 412-19
30. Bianco A, Pomara F, Raccuglia M, et al. (2014) The relationship between type 2 diabetes family history, body composition and blood basal glycemia in sedentary people. Acta Diabetologica. 51:79-84
31. Petrak F, Baumeister H, Skinner TC, Brown A, Holt I. (2015) Depression and diabetes: treatment and health-care delivery. Lancet Diabetes & Endocrinology. 3: 472-485
32. Nicolau J, Simó R, Sanchís P, Ayala L, Fortuny R, Zubillaga I, Masmiquel L. (2015) Eating disorders are frequent among type 2 diabetic patients and are associated with worse metabolic and psychological outcomes: results from a cross-sectional study in primary and secondary care settings. Acta Diabetol. (Epub ahead of print)
33. Browne JL, Nefs G, Pouwer F, Speight J. (2015) Depression, anxiety and self-care behaviours of young adults with Type 2 diabetes: results from the International Diabetes Management and Impact for Long-term Empowerment and Success (MILES) Study. Diabet Med.32:133-40.
34. Dalsgaard EM, Skriver MV, Sandbaek A, Vestergaard M. (2015) Socioeconomic position, type 2 diabetes and long-term risk of death. PLoS One. 5: e0124829.

13. Figure 1. Flow-chart of the patients enrolled in the study

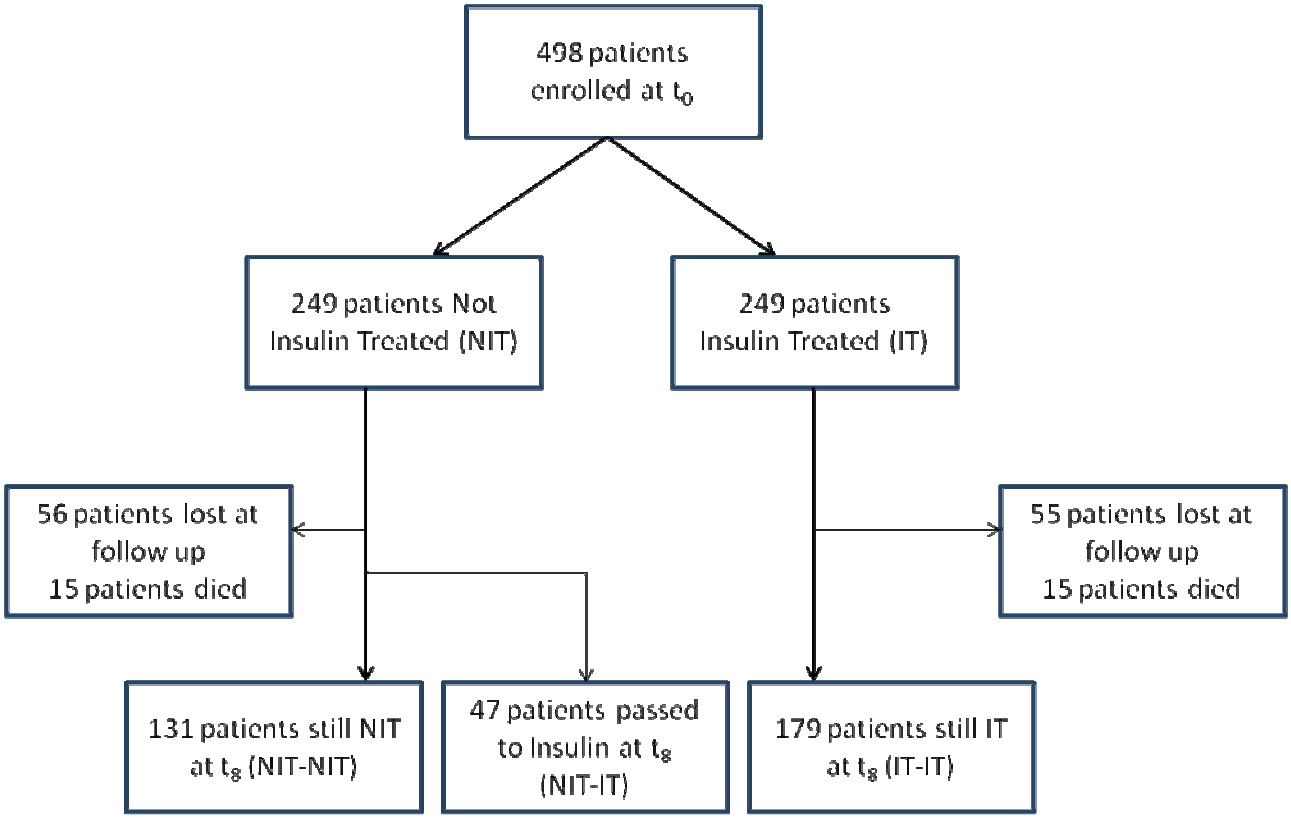

Table 1. Differences in time among variables per treatment group

Categorical variables	**NIT (t_0)** (n=249)	**NIT (t_8)** (n=131)	**p-value**	**NIT (t_0)** (n=47)	**IT (t_8)** (n=47)	**p-value**	**IT (t_0)** (n=249)	**IT (t_8)** (n=179)	**p-value**
Smoke (no/yes/former)	139/33/77	65/12/52	**p=0.0074**	25/6/16	21/4/22	**0.05**	124/24/101	81/15/81	NS
Hypertension (yes/no)	52/197	128/3	**p<0.0001**	10/37	47/0	**p<0.0001**	34/215	175/4	**p<0.0001**
Self glucose monitoring (no/ <once daily/ ≥once daily)	56/170/23	31/81/15	NS	7/34/6	2/10/33	**p<0.0001**	3/67/179	3/51/124	NS
Foot ulcers (never/actual or former ulcers or amputation)	246/3	119/12	**p=0.0009**	47/0	42/5	**p=0.0253**	221/27	142/37	**p<0.0001**
Retinopathy (no-mild/ moderate-severe or blindness)	208/24	98/33	**p<0.0001**	38/5	29/18	**p=0.0005**	142/103	63/115	**p<0.0001**
Quantitative variables	**NIT (t_0)** (n=249)	**NIT (t_8)** (n=131)	**Mean difference t_0 vs t_8** (CI95%) p-value	**NIT (t_0)** (n=47)	**IT (t_8)** (n=47)	**Mean difference t_0 vs t_8** (CI95%) p-value	**IT (t_0)** (n=249)	**IT (t_8)** (n=179)	**Mean difference t_0 vs t_8** (CI95%) p-value
BMI (mean ±SD)	28.6 ± 5.4	27.2 ± 4.6	0.8 (0.4; 1.2) **p<0.0001**	30.4 ± 6.9	31.0 ± 8.4	-1.0 (-1.9; -0.05) **p=0.039**	28.2 ± 4.8	28.2 ± 5.2	-0.06 (-0.4; 0.3) NS
Total cholesterol (mean ±SD, mg/dl, mmol/l)	193.6 ± 36.2 5.0 ± 0.9	166.2 ± 35.2 4.3 ± 0.9	26.3 (19.8; 32.9) 0.7 (0.5; 0.8) **p<0.0001**	191.2 ± 39.4 4.9 ± 1.0	168.2 ± 40.4 4.4 ± 1.0	23.5 (11.6; 35.5) 0.6 (0.3; 0.9) **p=0.0003**	179.7 ± 36.0 4.7 ± 0.9	166.8 ± 39.1 4.3 ± 1.0	13.65 (7.6; 19.7) 0.3 (0.2; 0.5) **p<0.0001**
HDL cholesterol (mean ±SD, mg/dl, mmol/l)	48.1 ± 13.2 1.2 ± 0.3	54.0 ± 15.0 1.4 ± 0.4	-5.16 (-6.9; -3.4) -0.13 (-0.2; -0.09) **p<0.0001**	45.2 ± 12.8 1.2 ± 0.3	50.5 ± 17.3 1.3 ± 0.5	-4.3 (-7.5; -1.0) -0.1 (-0.2; -0.02) **p=0.0117**	49.5 ± 15.8 1.3 ± 0.4	51.4 ± 16.3 1.3 ± 0.4	-1.36 (-3.1; 0.4) -0.04 (-0.08; 0.03) NS
Trygliceride (mean ±SD, mg/dl, mmol/l)	148.9 ± 91.6 1.7 ± 1.0	116.6 ± 44.4 1.9 ± 0.5	27.1 (15.7; 38.5) 0.3 (0.2; 0.4) **p<0.0001**	165.4 ± 133.0 1.9 ± 1.5	143.0 ± 88.3 1.6 ± 1.0	20.6 (-18.3; 59.4) 0.2 (-0.2; 0.7) NS	150.4 ± 93.8 1.7 ± 1.1	137.3 ± 75.2 1.6 ± 0.9	8.56 (-3.1; 20.2) 0.1 (-0.03; 0.2) NS
Fasting glucose (mean ±SD, mg/dl, mmol/l)	151.0 ± 39.3 8.4 ± 2.2	139.2 ± 33.5 7.7 ± 1.9	7.0 (0.2; 13.8) 0.4 (0.01; 0.8) **p=0.043**	159.8 ± 44.6 8.9 ± 2.5	162.2 ± 72.7 9.0 ± 4.0	-2.38 (-26.2; 21.5) -0.1 (-1.5; 1.2) NS	173.6 ± 67.4 9.6 ± 3.7	162.1 ± 56.8 9.0 ± 3.2	5.38 (-6.3; 17.1) 0.3 (-0.4; 1.0) NS
HbA1c (mmol/mol, % total Hb)	62.3 ± 13.4 7.8 ± 1.2	56.9 ± 10.3 7.4 ± 0.9	2.0 (-0.17; 4.2) 0.18 (-0.02; 0.4) NS	70.5 ± 13.1 8.6 ± 1.2	70.1 ± 19.5 8.6 ± 1.8	0.35 (-5.3; 5.9) 0.03 (-0.5; 0.5) NS	68.9 ± 16.2 8.5 ± 1.5	65.4 ± 12.9 8.1 ± 1.2	2.03 (-0.21; 4.3) 0.19 (-0.02; 0.4) NS
Depression (means ± SD)	39.7 ± 8.3	38.8 ± 7.9	0.01 (-1.2; 1.2) NS	39.9 ± 9.0	39.2 ± 8.4	0.2 (-2.6; 3.0) NS	37.5 ± 9.3	39.4 ± 9.5	-5.0 (-6.5; -3.5) **p<0.0001**
Anxiety (means ± SD)	35.9 ± 8.7	36.1 ± 7.6	-0.5 (-1.9; 0.8) NS	35.5 ± 9.8	36.1 ± 7.8	-1.2 (-3.9; 1.5) NS	36.2 ± 8.3	36.5 ± 9.5	-2.4 (-3.6; -1.2) **p=0.0001**
MMSE (means ± SD)	25.1 ± 3.4	25.5 ± 3.6	-0.3 (-1.1; 0.6) NS	25.2 ± 4.0	24.5 ± 2.5	0.7 (-0.5; 1.9) NS	24.6 ± 3.2	24.5 ± 3.7	0.6 (-0.02; 1.1) NS

t_0 = Baseline; t_8 = Final; NIT = Not on Insulin Treatment; IT = Insulin Treatment;

Table 2. Univariate threshold analysis for depression and anxiety

Analyses of psychological variables as outcomes were conducted for scores above/below a predefined cutoff threshold; for MMSE, threshold analysis was not performed due to absence of patients below the predefined score of 20 points, that is, no patients with cognitive impairment. Threshold outcome analysis accounts for specific values of continuous putative predictors, and for frequencies of categorical putative predictors.

Quantitative predictors	**Depression** (<60 points vs ≥ 60)	**Mean diff. (CI95%)** p-value	**Anxiety** (<60 points vs ≥ 60)	**Mean diff. (CI95%)** p-value
Age (years, mean ± SD)	65.9±7.2 vs 72.3±3.3	-6.4 (-12.2; -0.6) **0.0311**	66.1±7.2 vs 62.5±9.3	3.6 (-3.5;10.8) 0.3179
Disease duration (years, mean ± SD)	16.4±7.9 vs 23.3±9.0	-7.0 (-13.4;-0.5) **0.0344**	16.4±7.9 vs 22.0±14.8	-5.5 (-13.5; 2.4) 0.1675
LDL cholesterol (mg/dl, mmol/l, mean ± SD)	109.4±33.0 vs 81.1±22.3 2.8±0.9 vs 2.1±0.6	28.3 (1.6; 55.0) 0.7 (0.04; 1.4) **0.0380**	109.0±33.0 vs 92.3±32.3 2.8±0.9 vs 2.4±0.8	16.7 (-16.1; 49.5) 0.4 (-0.4; 1.3) 0.3164
HDL cholesterol (mg/dl, mmol/l, mean ± SD)	47.9±14.1 vs 58.0±25.2 1.2±0.4 vs 1.5±0.7	-10.2 (-21.9; 1.6) -0.3 (-0.6; 0.04) 0.0901	48.1±14.6 vs 47.3±11.9 1.2±0.4 vs 1.2±0.3	0.9 (-13.6; 15.3) 0.02 (-0.4; 0.4) 0.9078
Trygliceride (mg/dl, mmol/l, mean ± SD)	146.4±89.8 vs 149.2±88.6 1.7±1.0 vs 1.7±1.0	-2.8 (-75.8; 70.3) -0.03 (-0.9; 0.8) 0.9403	146.2±90.1 vs 161.5±49.5 1.7±1.0 vs 1.8±0.6	-15.3 (-104.4; 73.8) -0.2 (-1.2; 0.8) 0.7357
BMI (Kg/m^2, mean ± SD)	28.2±4.8 vs 27.6±7.3	0.52 (-3.4; 4.5) 0.7948	28.0±4.7 vs 34.6±9.2	-6.6 (-11.3; -1.8) **0.0068**
Categorical predictors	**Depression** (<60 points vs ≥ 60)	**p-value**	**Anxiety** (<60 points vs ≥ 60)	**p-value**
Gender (F/M frequencies)	108/148 vs 3/3	0.700	108/150 vs 3/1	0.314
Schooling (low school/high school frequencies)	185/70 vs 6/0	0.196	187/70 vs 4/0	0.576
Smoke (no/active/former frequencies)	128/29/99 vs 4/0/2	0.593	128/29/101 vs 4/0/0	0.228

Table 3. Univariate analysis for depression, anxiety and MMSE as continuous outcomes
Continuous outcome analysis accounts for specific scores in categorical putative predictors.

	Depression (continuous)	**p-value**	**Anxiety** (continuous)	**p-value**	**MMSE** (continuous)	**p-value**
Gender (F/M mean scores for continuous outcomes)	42.5±8.4/ 36.6±8.1	**<0.0001**	39.3±9.7/ 34.1±6.8	**<0.0001**	24.4±3.9/ 25.2±3.3	0.0670
Schooling (low school/high school mean scores for continuous outcomes)	40.0±9.1/ 36.7±7.0	**0.0070**	37.2±8.9/ 33.7±6.8	**0.0025**	24.7±3.6/ 25.3±3.5	0.2520
Smoke (no/active/former mean scores for continuous outcomes)	**40.2±9.0/ 35.6±8.6/** 38.6±7.9	**0.025** for never vs active smokers	**37.9±9.4**/ **32.3±5.7/** 35.3±7.3	**0.004** for never vs active smokers	**24.2±4.1/** 25.1±2.7/ **25.7±2.8**	**0.005** for never vs former smokers

Table 4. Multivariate analysis

Relevant results from 3 linear regression models, one for each outcome –depression, anxiety and MMSE, where the difference between final score and baseline is the dependent variable, and the relevant score at baseline, gender, schooling, disease duration, changes in therapy between baseline and 8 years and smoking habit are the independent variables.

	Δ Depression	**p-value**	**Δ Anxiety**	**p-value**	**Δ MMSE^**	**p-value**
Treatment (baseline-8 yrs later)						
NIT-NIT	Reference	--	Reference	--	Reference	--
NIT-IT	0.13	0.919	0.18	0.880	-0.96	0.135
IT-IT	2.31	**0.033**	0.69	0.474	-1.27	**0.010**
Gender						
F	Reference	--	Reference	--	Reference	--
M	-2.40	**0.037**	-0.58	0.576	-0.43	0.408
Disease duration (per year)	0.12	**0.046**	0.12	**0.027**	-0.01	0.725
Smoking habit						
Never	Reference	--	Reference	--	Reference	--
Active	-1.19	0.440	-2.22	0.120	1.43	0.055
Former	0.61	0.586	-1.26	0.219	1.76	**0.001**

^ For the MMSE regression model the differences between 8 years and baseline for BMI and HbA1c were also included as covariates.