**Biochemical Urine Testing of Medication Adherence and its Association with Clinical Markers in an Outpatient Population of Type 2 Diabetes Patients: The DIAbetes and LifEstyle Cohort Twente (DIALECT) study**

**Running title:** Diabetes and medication adherence in urine

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**Abstract**

Objective: To assess adherence to the three main drug classes in real-world patients with type 2 diabetes using biochemical urine testing and to determine the association of non-adherence with baseline demographics, treatment targets and complications.

Research Design and Methods: Analyses were performed in baseline data of 457 patients in the DIAbetes and LifEstyle Cohort Twente (DIALECT) study. Adherence to OADs (oral antidiabetics), antihypertensives and statins was determined by analyzing baseline urine samples using LC-MS/MS. Primary outcomes were micro/macrovascular complications and treatment targets of LDL-cholesterol, HbA1c and blood pressure. These were all assessed cross-sectionally at baseline.

Results: Overall, 89.3% of the patients were identified as being adherent. Adherence rates to OADs, antihypertensives and statins were 95.7%, 92.0%, and 95.5%, respectively. The prevalence of microvascular (81.6% vs 66.2%, p = 0.029) and macrovascular complications (55.1% versus 37.0%, p = 0.014) was significantly higher in non-adherent patients. The percentage of patients who reached an LDL-cholesterol target of ≤2.5 mmol/L was lower (67.4% versus 81.1%, p = 0.029) in non-adherent patients. Binary logistic regression indicated that a higher BMI, current smoking, elevated serum LDL-cholesterol, high HbA1c, the presence of diabetic kidney disease and the presence of macrovascular disease were associated with non-adherence.

Conclusion: Despite medication adherence of real-world type 2 diabetes patients managed in specialist care was relatively high, the prevalence of microvascular and macrovascular complications was significantly higher in non-adherent patients and treatment targets were reached less frequently. This emphasizes the importance of objective detection and tailored interventions to improve non-adherence.

Optimal medication adherence is of utmost importance in patients with type 2 diabetes mellitus, since non-adherence can lead to disease progression, complications, mortality and increased healthcare costs.1,2 The challenge of adequate therapy in type 2 diabetes is illustrated by the lasting high incidence of diabetes complications and failure to reach treatment targets of HbA1c, LDL-cholesterol, and blood pressure.3-5 Because patients with type 2 diabetes have a high prevalence of multimorbidity, they often require to take multiple drugs, which poses challenges for adherence. Medication adherence is especially important for patients with advanced disease, who are often on multiple therapies and at high risk of non-adherence.1 Medication adherence can be assessed in several ways, such as patient self-report, healthcare professional direct observation and using pharmacy data or electronic monitoring. However, there is no method that can be qualified as the gold standard in order to accurately assess the medication adherence.6 The biggest drawback of self-report is that patients tend to overreport adherence to avoid disapproval from their healthcare professionals.7 A promising new tool to objectively assess medication adherence is biochemical urine testing using liquid chromatography-tandem mass spectrometry (LC-MS/MS).8,9 LC-MS/MS is an extremely specific and sensitive instrument with a detection limit in the low nanogram range in a spot urine or blood samples. Medications are detected for between 4-6 half-lives of the drug, thus providing an objective snapshot of drug adherence.10

While there is a previous small study on prevalence of non-adherence determined by LC-MS/MS in primary care 10, we aimed with this larger study to assess the prevalence of non-adherence to OADs, antihypertensives and statins in a real-life population of type 2 diabetes patients managed in a specialist setting using urine testing by LC-MS/MS. In addition, we determined associations of non-adherence with baseline demographics, treatment targets and diabetes complications.

**Research Design and Methods**

Study design

This study was performed in the DIAbetes and LifEstyle Cohort Twente (DIALECT) cohort.3 DIALECT is an observational prospective cohort study performed in the Ziekenhuis Groep Twente Hospital (Almelo and Hengelo, the Netherlands) that was designed to investigate the effect of lifestyle and dietary habits on outcomes in patients with complicated type 2 diabetes treated in specialist care. The primary aim of DIALECT is to identify targets for the improvement of treatment quality by a systematic assessment of both pharmacological and nutritional management. DIALECT consists of two identical inclusion periods. Patients in the DIALECT-1 population were recruited between September 2009 and January 2016 (n = 400). Recruitment of DIALECT-2 started from that moment on and will recruit until a total of 850 participants is reached. The study was performed according to the guidelines of good clinical practice and the Declaration of Helsinki. Written informed consent was obtained from all subjects before participation. The study has been approved by the local institutional review boards (METC-registration numbers NL57219.044.16 and 1009.68020) and is registered in the Netherlands Trial Register (NTR trial code 5855).

Population

The study population consists of patients with type 2 diabetes aged ≥18 years, treated in the specialist outpatient clinic as part of routine secondary care. In the Netherlands, criteria for referral from primary to secondary health care are inability to achieve adequate glycemic control (defined as failure to achieve the HbA1c target, which is usually ≤7% (53 mmol/mol)) with OADs or a standard insulin regimen, macroalbuminuria and/or estimated glomerular filtration rate (eGFR) ≤60 mL/min or multiple cardiovascular complications.4 Patients on renal replacement therapy or patients with insufficient knowledge of the Dutch language were excluded from participation.

Study procedures and baseline characteristics

Eligible patients were selected from the electronic patient file as described in detail previously.3 All the data was obtained at baseline. At the outpatient clinic, anthropometric measurements, sociodemographic characteristics, medical history, lifestyle behaviors, and current medications of participants who gave informed consent were recorded. BMI was calculated as weight divided by height squared (kg/m2). Non-fasting blood tests were taken at baseline visit in order to determine serum albumin creatinine ratio (ACR), LDL-cholesterol and HbA1c. Twenty four-hour urine was collected from 8 am to 8 am the next morning, while patients were on their usual medication to obtain non-biased data on medication adherence. Patients signed previously for future studies of frozen blood/urine samples and were not aware that their urine would be checked on medication adherence. A separate single morning void urine was used to assess the urinary ACR. Blood samples, 24-hour urine collection, and morning void urine were stored in a biobank at -80 degrees Celsius to allow for future analyses. Blood pressure (BP) was measured in a supine position by an automated device (Dinamap®; GE Medical systems, Milwaukee, WI, USA) for 15 minutes with a one-minute interval. The mean systolic and diastolic pressure of the last three measurements were used for further analysis.3 The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.11 Physical activity was assessed using the validated Short Questionnaire to Assess Health enhancing physical activity (SQUASH) questionnaire.12 An activity score was calculated based on minutes of activity per day multiplied by an intensity factor. We scored which patients met the Dutch Healthy Exercise Norm of 30 minutes moderate intense activity a day for at least 5 days a week.13 Adherence to the Dutch Guidelines for a Healthy Diet was assessed using the Dutch Healthy Diet index (DHD-index).14

Urine samples were analyzed in December 2018 of all participants that were included between the start of the study in September 2009 and December 2018, totaling a population of 632 participants. For the current study, we excluded patients who did not have a prescription of any detectable drugs (n = 11), or if no shipment for analysis was available (n = 119), or if no urine was available for secondary analysis (n = 45), leaving a total of 457 participants for analysis (Figure 1).

Treatment targets

Participants were considered to be well controlled if their HbA1c was ≤7% (53 mmol/mol), in line with the Dutch guidelines for type 2 diabetes.13 Similarly, patients were considered to be at target if the serum LDL-cholesterol was ≤2.5 mmol/L for primary prevention and <1.8 mmol/L for secondary prevention.15 BP targets were derived from the international guidelines for diabetes management.16,17 In patients with diabetic kidney disease (DKD), the BP target was set according to the Kidney Diseases Improving Global Outcomes (KDIGO) guidelines.17 Patients with DKD without albuminuria (eGFR <60, no albuminuria) had a BP target of <140/90 mmHg, while patients with albuminuria had a BP target of <130/80 mmHg. For patients with type 2 diabetes without DKD, the European Association for the Study of Diabetes (EASD) guidelines were used, which stipulate a BP target of <140/85 mmHg.16

Diabetes complications

Microvascular disease was defined as the presence of either DKD, neuropathy, or retinopathy. Presence of these complications was assessed cross-sectionally at baseline. DKD was defined as an eGFR <60 mL/min with or without albuminuria. Neuropathy was assessed using monofilament and VibraTip™. Retinopathy was assessed at one to two-year intervals by an ophthalmologist. Macrovascular disease was defined as the presence of either coronary heart disease (CHD), cerebrovascular disease, or peripheral artery disease. CHD was defined as the presence of one of the following in medical history: physician diagnosed unstable angina pectoris, myocardial infarction, percutaneous coronary intervention, or coronary artery bypass graft. Cerebrovascular disease was defined as a history of transient ischemic attack or cerebrovascular accident. Peripheral artery disease was defined as the presence of one of the following in medical history: proven artery disease by angiogram or magnetic resonance angiogram, percutaneous transluminal angioplasty, or peripheral artery bypass graft.

Measurement of adherence

Urine samples were obtained at baseline from collections of 24-hour urine. The samples were stored at the local site at -80°C and subsequently transported on dry ice by a courier to the biobank at the University Medical Center Groningen. Thereafter, the samples were shipped to the laboratory at the University Hospitals of Leicester NHS Trust and stored at -80°C. Urine samples were analyzed by LC-MS/MS using an Agilent 1290 HPLC interfaced with an Agilent 6490 triple quad mass spectrometer (Santa Clara, California USA).8 The LC-MS/MS assay is a qualitative yes/no method to detect the presence and absence of medications. The assay is UKAS accredited – the premier laboratory validation organization of the UK. The assay has a high sensitivity with limits of detection of the medications analyzed between 10-110 ng/mL (in house data). Also, the assay is highly specific since it uses separation by chromatograms and mass to charge ratios to identify analytes. Hence, due to its high sensitivity and specificity, LC-MS/MS based detection of analytes is a well-established technique used in forensics and detection of illegal performance enhancing drugs in elite sports.18,19 The non-detection of a prescribed medication in urine implies that it was not ingested for at least 4-6 half-lives prior to sample collection, which can vary from a few hours to a few days. Further, Lane et al. previously demonstrated in a retrospective study that pharmacokinetic parameters like half-lives, median concentration in plasma and volume of distribution do not affect the diagnosis of non-adherence.20

Urine samples were screened for OADs, antihypertensives, and statins. Detectable OADs included biguanides, sulfonylurea (SU) derivatives, dipeptidyl peptidase-4 (DPP-4)-inhibitors, and thiazolidinediones. Of the SU-derivatives, the drugs tolbutamide and glibenclamide give no rise to urinary excretion of metabolites and are therefore not detectable. Detectable antihypertensives included diuretics (thiazide, low ceiling, high ceiling, and potassium-saving), beta-blockers, calcium channel blockers, ACE-inhibitors, angiotensin II receptor blockers (ARBs), and other antihypertensives interfering in the renin-angiotensin system (RAAS). Of the antihypertensives, hydralazine, barnidipine, methyldopa, ketanserin and clonidine give no rise to urinary excretion of metabolites and are therefore not detectable. Of the statins, only atorvastatin and rosuvastatin give rise to urinary excretion of metabolites and are therefore detectable.

Statistical analysis

All statistical analyses were performed using IBM SPSS for Windows (version 24.0; IBM Corp., Armonk, NY). Differences between the groups were tested using the Independent Samples T-test for normally distributed variables, Mann-Whitney U test for skewed variables, and the χ2 test for dichotomous variables. Normally distributed data are presented as mean ± standard deviation. Skewed variables are presented as median [interquartile range]. Dichotomous variables are presented in number (percentage). A 2-tailed p-value less than 0.05 was considered statistically significant. Normality of data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality and by visually inspecting the frequency of histograms of each variable.

The population was divided in two groups according to their overall biochemical results. Patients were considered adherent if all screened medications were detected in the urine and non-adherent if at least one of the screened medications was not detected. Determinants of non-adherence were studied using binary logistic regression analysis based on complete cases with overall adherence as dependent variable. Confounders were based on relevant differences in baseline characteristics and previous literature. All univariate variables with a p-value below 0.20 were included in a forward logistic regression model together with other relevant pathophysiologic variables. Variables that remained significant were tested in each model, until full adjustment.

Discrepancies

Since medication lists were obtained electronically, discrepancies were noted between prescribed medication in the electronic health record and medication detected in urine. Of the total number of prescribed drugs which gave rise to urinary excretion of metabolites (n = 1631), 24 discrepancies were found (Supplemental Figure S1).

**Results**

Baseline characteristics

Overall, the study population consisted of 457 participants with type 2 diabetes (Table 1). The average age was 64.2 ± 9.0 years and the average diabetes duration was 11 [7-19] years, reflecting a population with advanced type 2 diabetes, as can be anticipated in a referred care population like DIALECT.

Adherence

Of the total population, 408 patients were adherent (89.3%). The two groups were comparable and had similar mean age, gender composition, BMI, waist hip ratio, duration of diabetes, alcohol intake, physical activity, DHD-index and urine ACR. There were more current smokers (28.6% vs 15.0%; p = 0.015), HbA1c levels were higher (7.9 ± 3.5% (62.9 ± 14.5 mmol/mol) versus 7.4 ± 3.2% (57.4 ± 11.2 mmol/mol), p <0.01) and LDL-cholesterol levels were also higher (2.2 ± 0.9 versus 2.0 ± 0.7 mmol/L, p = 0.022) in the non-adherent group compared with the adherent group. Furthermore, a significantly greater number of total prescribed medications was seen in the non-adherent group (8 [7-9] vs 7 [5-8]; p <0.01). Adherence to OADs, antihypertensives, and statins was 95.7%, 92.0%, and 95.5%, respectively.

Target achievement

The percentage of people who reached an LDL-cholesterol target of ≤2.5 mmol/L was significantly lower (p = 0.029) in the non-adherent group compared to the adherent group (67.4% vs 81.1%) (Figure 2). However, no significant association was found between adherence and an LDL-cholesterol target of <1.8 mmol/L (p = 0.40). There were no statistically significant differences between the groups in the percentages of people who reached the HbA1c and blood pressure targets (26.5% vs 38.8%; p = 0.09 and 41.7% vs 44.4%; p = 0.46, respectively).

Diabetes complications

The percentages of both microvascular and macrovascular complications were significantly higher in the non-adherent group (81.6% vs 66.2%; p = 0.029 and 55.1% vs 37.0%; p = 0.014, respectively). Within the individual components of microvascular disease, the prevalence of DKD was 18.8% higher in the non-adherent versus the adherent group (p = 0.012). There were no statistically significant differences in the prevalence of neuropathy and retinopathy between the groups (51.0% vs 37.6%; p = 0.07 and 30.6% vs 25.2%; p = 0.41, respectively).

Determinants of non-adherence

Binary logistic regression (Table 2) indicated that a higher BMI, current smoking, elevated serum LDL-cholesterol, high HbA1, the presence of DKD, and the presence of macrovascular disease were significantly associated with non-adherence. No significant association was found for number of screened drugs, former smoking, and neuropathy. Univariate analyses of all the variables that were considered for the multivariable model are shown in Supplemental table S1. Furthermore, the sensitivity analysis with patients of whom all data were available of the variables used in the multivariable model is shown in Supplemental table S2.

**Conclusions**

In this paper, we present the assessment of adherence to OADs, antihypertensives, and statins in a real-life population with type 2 diabetes managed in routine specialist clinical practice using urine testing by LC-MS/MS. We feel this is the first large study to report adherence in the real-world setting to measure adherence objectively and report the association of adherence to microvascular and macrovascular complications. Generally, both the overall medication adherence as the adherence to the specific drug classes was relatively high compared to other studies. However, in non-adherent patients, treatment targets were reached less frequently and the prevalence of microvascular and macrovascular complications at baseline was higher. This demonstrates a window of opportunity for early detection and interventions in case of non-adherence.

Much of the evidence regarding poor medication adherence in diabetes is based on retrospective or observational studies that collect data from claim databases using a broad range of definitions. The reported incidence of poor medication adherence in patients with type 2 diabetes varies widely, primarily due to different underlying (sub)populations and different methodological approaches to measure adherence.1 Previously, in a small observational study in type 2 diabetes patients attending different primary care practices, Patel et al. reported a LC-MS/MS non-adherence rate of 28.1% to antidiabetic, antihypertensive, and/or lipid-lowering medications. Non-adherence to statins was the highest at 23.7% and non-adherence to OADs was 9.3%. Side effects such as myalgia or the poor perception of statins in the general population were given as possible explanations for the high rates of non-adherence to statins.10 In contrast to these high rates of non-adherence to statins, we found non-adherence rates of only 4.5%. This difference can be partly explained by the more severe population managed in a specialist center, as compared to the primary care setting in the Patel et al study. Additionally, we should note that the statin subgroup in our population covered less than half of our cohort patients because the most prescribed statin subtype simvastatin does not give rise to urinary excretion of metabolites and was therefore note detectable.

In a post-hoc analysis of a small trial (n = 98), De Jager et al. assessed medication adherence using LC-MS/MS to analyze serum samples in patients with apparent resistant hypertension using ≥3 antihypertensives. They reported that 68% was partly non-adherent and 16% was completely non-adherent, of which the latter (aligning with our definition) is still twice as high compared with the 8% non-adherence to antihypertensives we found.21 We speculate that two mechanisms may be responsible for this difference. On the one hand, our additional underlying condition (diabetes) could enhance adherence. On the other hand, the high number of antihypertensives in the trial conducted by De Jager et al could worsen non-adherence.

A possible explanation for the relatively high degree of medication adherence in our population could be that patients treated in specialist care may feel more urgency to adhere to their treatment in comparison to patients treated in primary care. This is supported by comparing our study results with the results of the Patel et al. study, where a LC-MS/MS non-adherence rate of 28.1% to antidiabetic, antihypertensive and/or lipid-lowering medications was reported.10 Another hypothesis regarding the high rates of medication adherence in general could be the presence of microvascular and macrovascular complications, which could be a motivation for patients to take their medication. However, this is not supported with data from this study, as non-adherent patients had higher proportions of microvascular and macrovascular complications. Furthermore, the well-organized pharmacy service in the Netherlands, where medication is often delivered automatically, could also improve medication adherence. Further research is needed in order to assess the role of diabetes complications and automatic refills versus self-initiated refills in medication adherence.

In a cohort like DIALECT, it is important to take the possibility of selection bias into account, i.e. selection of highly motivated patients. We can refute this if we take the criteria for referral from primary to secondary health care in the Netherlands into consideration. Patients in DIALECT are treated in a specialist setting because their type 2 diabetes was not optimally controlled in primary care. In addition, the vast majority of the patients developed diabetes complications. Moreover, the average BMI of 32.9 kg/m2 reflects a predominant obese population. These data are not in line with patients who are highly motivated. However, despite the high prevalence of diabetes complications, high BMI, serum LDL-cholesterol and HbA1c, this population is motivated in their own way and they show this by taking their medication properly, with high rates of adherence as result.

Usually, by determining non-adherence using an objective method like LC-MS/MS bias can be introduced if patients take their medication just before baseline visit, the so-called ‘white coat adherence’. However, patients in this study were not aware that their urine would be checked on medication adherence. Therefore, the results in our study are a true reflection of adherence, which is a major strength of this study. A limitation of this study is that no analyses could be made on the associations between specific drug classes/individual drugs and adherence because the sample size was not adequate to make meaningful analyses (data not shown).

Apparently, non-adherence in our population was not recognized by the patient’s healthcare professionals. However, it is important to recognize that poor medication adherence contributes to suboptimal clinical benefits and to actively search for non-adherence. Healthcare professionals should in particularly be apprehensive for non-adherence if a patient smokes or fails to reach LDL-cholesterol and HbA1c targets. The multifactorial nature of poor medication adherence implies that a broader strategy is needed to manage non-adherence. It is important to note that the utility of the LC-MS/MS assay is an evolving and rapidly developing field since the last 3-4 years. Currently, it is possible to analyze the most common cardiovascular medications, except aspirin and simvastatin. However, the prescription of simvastatin is decreasing since more other potent statins have become generic medications and therefore there is no cost difference that used to make simvastatin the cheaper option to prescribe. In the course of time, this could increase the utility of the LC-MS/MS assay in both clinical trials and real-world settings. Further prospective studies are needed to compare the effects of pharmacokinetic parameters of individual medications on the diagnosis of non-adherence. The role of using prescription refill information to estimate medication adherence is well established and broadly used. Future research should be aimed at examining the agreement between this method (LC-MS/MS) and other established methods for identifying poor adherence, like prescription refill-based methods.

**Article Information**

Author Contributions

J.M.B. performed statistical analyses and wrote the manuscript. M.O. supervised the statistical analysis, interpreted the outcome measures, reviewed/edited the manuscript and contributed to the discussion. G.L. set up and coordinated the study, performed study procedures, coordinated practical research assistant, reviewed/edited the manuscript, contributed to the discussion and is the principal investigator of this study. K.K., P.G., P.P., J.v.B., H.L., S.B., G.N. and R.N. reviewed/edited the manuscript and contributed to the discussion.

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Data Access and Responsibility

J.M.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Duality of Interest

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**References**

1. Polonsky WH, Henry RR. Poor medication adherence in type 2 diabetes: recognizing the scope of the problem and its key contributors. Patient Prefer Adherence. 2016; 10: 1299-1307.
2. Khunti K, Seidu S, Kunutsor S, Davies M. Association Between Adherence To Pharmacotherapy and Outcomes in Type 2 Diabetes: A Meta-analysis. Diabetes care. 2017 Nov; 40(11): 1588-1596.
3. Gant CM, Binnenmars SH, van den Berg E, Bakker SJL, Navis G, Laverman GD. Integrated Assessment of Pharmacological and Nutritional Cardiovascular Risk Management: Blood Pressure Control in the DIAbetes and LifEstyle Cohort Twente (DIALECT). Nutrients. 2017 Jul; 9(7): 709.
4. Jalving AC, Gant CM, Binnenmars SH, Soedamah-Muthu SS, Bakker SJL, Navis G, Laverman GD. Glycaemic control in the diabetes and Lifestyle Cohort Twente: A cross-sectional assessment of lifestyle and pharmacological management of HbA1c target achievement. Diabetes Obes Metab. 2018 Oct; 20(10): 2494-2499.
5. Gant CM, Binnenmars SH, Harmelink M, Soedamah-Muthu SS, Bakker SJL, Navis G, Laverman GD. Real-life achievement of lipid-lowering treatment targets in the DIAbetes and LifEstyle Cohort Twente: systemic assessment of pharmacological and nutritional factors. Nutr Diabetes. 2018; 8: 24.
6. Anghel LA, Farcas AM, Opream RN. An overview of the common methods used to measure treatment adherence. Med Pharm Rep. 2019 Apr; 92(2): 117-122.
7. Lam WY, Fresco P. Medication Adherence Measures: An Overview. Biomed Res Int. 2015.
8. Tomaszewski M, White C, Patel P, Masca N, Damani R, Hepworth J, et al. High rates of non-adherence to antihypertensive treatment revealed by high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) urine analysis. Heart. 2014 Jun; 100(11): 855-61.
9. Moffat A, Osselton D, Widdop B, Watts J. Clarke’s analysis of drugs and poisons. 4. London, UK: Pharmaceutical Press; 2011.
10. Patel P, Gupta P, Burns A, Mohamed AA, Cole R, Lane D, et al. Biochemical Urine Testing of Adherence to Cardiovascular Medications Reveals High Rates of Nonadherence in People Attending Their Annual Review for Type 2 Diabetes. Diabetes Care. 2019 Jun; 42(6): 1132-1135.
11. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009 May 5; 150(9): 604-12.
12. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. J Clin Epidemiol. 2003 Dec; 56(12): 1163-9.
13. Kwaliteitsinstituut voor de Gezondheidszorg CBO and Nederlands Huisartsen Genootschap (NHG). Multidisciplinaire richtlijn diabetes mellitus type 2 (revision 2018). 2018.
14. van Lee L, Geelen A, Hooft van Huysduynen EJC, de Vries JHM, van ’t Veer P, Freskens EJM. The Dutch Healthy Diet index (DHD-index): an instrument to measure adherence to the Dutch Guidelines for a Healthy Diet. Nutr J. 2012; 11: 49.
15. Kwaliteitsinstituut voor de Gezondheidszorg CBO and Nederlands Huisartsen Genootschap (NHG). Multidisciplinaire richtlijn cardiovasculair risicomanagement (revision 2019). 2019.
16. Authors/Task Force Members. Ryden L., Grant P.J., Anker S.D., Berne C., Cosentino F., Danchin N., Deaton C., Escaned J., Hammes H.P., et al. ESC Guidelines on Diabetes, Pre-Diabetes, and Cardiovascular Diseases Developed in Collaboration with the EASD: The Task Force on Diabetes, Pre-Diabetes, and Cardiovascular Diseases of the European Society of Cardiology (ESC) and Developed in Collaboration with the European Association for the Study of Diabetes (EASD) Eur. Heart J. 2013;34:3035–3087.
17. Kidney Disease: Improving Global Outcomes (KDIGO) Blood Pressure Work Group KDIGO Clinical Practice Guideline for the Management of Blood Pressure in Chronic Kidney Disease. Kidney Int. Suppl. 2012;2: 337–414.
18. Wada. Minimum Criteria for Chromatographic Mass Spectrometric Confirmation of the Identity of Analytes for Doping Control Purpose (TD2015IDCR). In; 2015.
19. Grebe SK, Singh RJ. LC-MS/MS in the Clinical Laboratory - Where to From Here? The Clinical biochemist.Reviews / Australian Association of Clinical Biochemists 2011; 32(1): 5-31.
20. Lane D, Alghamdi R, Muscat M, Kaur MS, Davis T, Cole R, Patel P, Tomaszewski M, Gupta P. The diagnosis of non-adherence in hypertension using a urine biochemical screen is unaffected by drug pharmacokinetics. European Heart Journal 2019;40:ehz748.0070.
21. De Jager RL, van Maarseveen EM, Bots ML, Blankestijn PJ. Medication adherence in patients with apparent resistant hypertension: findings from the SYMPATHY trial. Br J Clin Pharmacol. 2018 Jan; 84(1): 18-24.

**Table 1. Baseline characteristics by overall adherence in the DIAbetes and LifEstyle Cohort Twente-1+2 population.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Total population** | **Adherent** | **Non-adherent** | **p-value** |
| **n (%)** | 457 | 408 (89.3%) | 49 (10.7%) | - |
| **Age (yr)** | 64.2 ± 9.0 | 64.3 ± 8.9 | 63.7 ± 10.2 | 0.68 |
| **Male, n (%)** | 281 (61.5) | 252 (61.8) | 29 (59.2) | 0.73 |
| **Prescribed drugs (n)** | 7 [5-9] | 7 [5-8] | 8 [7-9] | <0.01\* |
| **Screened drugs (n)** | 4 [2-5] | 4 [2-5] | 4 [3-5] | 0.025\* |
| **Detected drugs (n)** | 3 [2-5] | 4 [2-5] | 3 [1-4] | <0.01\* |
| **Diabetes duration (yr)** | 11 [7-19] | 12 [7-19] | 11 [5-19] | 0.52 |
| **BMI (kg/m2)a** | 32.9 ± 6.0 | 32.7 ± 5.9 | 33.9 ± 6.4 | 0.19 |
| **Waist-hip ratioa** | 1.01 ± 0.09 | 1.01 ± 0.09 | 1.02 ± 0.08 | 0.26 |
| **Smoking, n (%)** | | | | 0.047\* |
| Current smoker | 75 (16.4) | 61 (15.0) | 14 (28.6) |  |
| Former smoker | 243 (53.2) | 222 (54.4) | 21 (42.9) |  |
| Never smoker | 139 (30.4) | 125 (30.6) | 14 (28.6) |  |
| **Alcohol intake per week, n (%)a** | | | | 0.39 |
| None | 153 (35.5) | 133 (34.6) | 20 (42.6) |  |
| 1-13 units | 218 (50.6) | 195 (50.8) | 23 (48.9) |  |
| ≥14 units | 60 (13.9) | 56 (14.6) | 4 (8.5) |  |
| **Physical activity – adherence to Dutch Healthy Exercise norm, n (%)a** | 239 (53.6) | 217 (54.7) | 22 (44.9) | 0.20 |
| **DHD-indexa** | 70.5 ± 13.5 | 70.5 ± 13.7 | 70.5 ± 12.1 | 0.98 |
| **ACR (mg/mmol)a** | 12.5 ± 49.4 | 11.7 ± 48.7 | 20.0 ± 54.8 | 0.30 |
| **LDL-cholesterol (mmol/L)a** | 2.0 ± 0.7 | 2.0 ± 0.7 | 2.2 ± 0.9 | 0.022\* |
| **LDL-cholesterol <2.5 mmol/L, n (%)** | 344 (79.6) | 313 (81.1) | 31 (67.4) | 0.029\* |
| **LDL-cholesterol <1.8 mmol/L, n (%)** | 185 (40.5) | 168 (41.2) | 17 (34.7) | 0.40 |
| **HbA1c, % (mmol/mol)a** | 7.5 ± 3.2 (58.0 ± 11.7) | 7.4 ± 3.2 (57.4 ± 11.2) | 7.9 ± 3.5 (62.9 ± 14.5) | <0.01\* |
| **HbA1c on target, n (%)** | 171 (37.5) | 158 (38.8) | 13 (26.5) | 0.09 |
| **Systolic blood pressure (mmHg)a** | 139 ± 16 | 138 ± 16 | 141 ± 17 | 0.24 |
| **Diastolic blood pressure (mmHg)a** | 76 ± 9 | 76 ± 9 | 77 ± 9 | 0.47 |
| **BP on target, n (%)a** | 200 (44.2) | 180 (44.4) | 20 (41.7) | 0.46 |
| **Complications, n (%)** | | | | |
| Microvascular diseases | 310 (67.8) | 270 (66.2) | 40 (81.6) | 0.029\* |
| Retinopathya | 117 (25.8) | 102 (25.2) | 15 (30.6) | 0.41 |
| Neuropathya | 178 (39.0) | 153 (37.6) | 25 (51.0) | 0.07 |
| Diabetic kidney disease | 194 (42.5) | 165 (40.4) | 29 (59.2) | 0.012\* |
| Macrovascular diseases | 178 (38.9) | 151 (37.0) | 27 (55.1) | 0.014\* |
| **Insulin use, n (%)** | 300 (65.6) | 264 (64.7) | 36 (73.5) | 0.22 |

a Missing values for BMI (n = 2), waist-hip ratio (n = 8), alcohol intake (n = 26), physical activity (n = 11), DHD-index (n = 19), ACR (n = 31), LDL-cholesterol (n = 25), HbA1c (n = 1), systolic blood pressure (n = 1), diastolic blood pressure (n = 1), blood pressure target (n = 4), retinopathy (n = 3), neuropathy (n = 1).

\* Statistically significant difference between the groups (p-value <0.05).

Data are presented as n (%), mean ± standard deviation or median [interquartile range] for nominal, normally distributed, and non-normally distributed data, respectively.

Abbreviations: DHD-index, Dutch Healthy Diet-index; ACR, Albumin to Creatinine Ratio; BP, Blood Pressure.

Patients of whom every screened drug was detected in the urine were considered adherent. All other patients were considered non-adherent, i.e. absence of at least one detectable drug in the urine sample.

**Table 2. Determinants of overall medication non-adherence.**

|  |  |  |
| --- | --- | --- |
| **Variable** | **OR (95%CI)** | **p-value** |
| BMI | 1.054 (1.001 - 1.110) | 0.046 |
| Current smoking | 2.471 (1.138 - 5.364) | 0.022 |
| HbA1c | 1.042 (1.015 - 1.069) | 0.002 |
| Serum LDL-cholesterol | 1.714 (1.134 - 2.591) | 0.011 |
| Diabetic kidney disease | 2.286 (1.162 - 4.497) | 0.017 |
| Macrovascular disease | 2.233 (1.145 - 4.343) | 0.018 |

Fully adjusted logistic regression model with non-adherence as study outcome.