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Prevalence of prediabetes and undiagnosed diabetes in the Mollerussa cohort study

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ABSTRACT

Objectives: To assess the prevalence of undiagnosed diabetes and prediabetes in the general population in the Mollerussa cohort. As a secondary objective, we aimed to identify the variables associated with these conditions and to describe the changes in glycaemic status after one year of follow-up in subjects with prediabetes.

Design: Prospective observational cohort study.

Setting: General population from a semi-rural area (Mollerussa, Spain).

Participants: General population ≥25 years of age without a diagnosis of diabetes.

Results: The prevalence of undiagnosed diabetes was 3.4% (95% confidence interval 2.65 - 4.15) and that of prediabetes was 39.3% (37.28 - 41.32). Among the 229 subjects with prediabetes, 18.3% had isolated impaired fasting plasma glucose (FPG) (FPG: 100 to <126 mg/dL), 58.1% had isolated impaired HbA1c (HbA1c 5.7 - <6.5), and 23.6% fulfilled both criteria. Follow-up data was available for 166 subjects; 41.6% (37.77 - 45.43) returned to normal glycaemic status, 57.6% (57.76 - 61.44)

persisted in their state of prediabetes, and 0.6% (0 -1.20) progressed to diabetes. Individuals with prediabetes had worse cardiometabolic risk profiles and sociodemographic features than normoglycaemic subjects. In the logistic regression model, variables significantly associated with prediabetes (*versus* normoglycaemia) were older age (odds ratio; 95% confidence interval) (1.033; 1.011-1.056), higher physical activity level (0.546; 0.360 - 0.827), body mass index (1.121; 1.029 - 1.222), and a family history of diabetes (1.543; 1.025 - 2.323). The variables significantly associated with glycaemic normalization were older age (0.948; 0.916 - 0.982) and body mass index (0.779; 0.651 - 0.931).

Conclusions: Among adults in our region, the estimated prevalence of undiagnosed diabetes was 3.4% and that of prediabetes was 39.3%. After a one-year follow-up, few subjects with prediabetes progressed to diabetes, while 41.6% returned to normoglycaemia. Individuals with prediabetes who returned to normoglycaemia were younger and had a lower body mass index.

KEYWORDS

Prediabetes, undiagnosed diabetes, prediabetes prevalence

ARTICLE SUMMARY

Strengths and Limitations

- This was a population-based study of a small cohort that included a representative sample of a non-previously studied population of a semi-rural area in Catalonia.
- We did not perform an oral glucose tolerance test, which is a common test in most studies but is a time-consuming and expensive procedure.
- The percentage of glucose normalization among prediabetic subjects was higher than expected compared to the percentages described in previous studies.
- The small number of cases of undiagnosed diabetes precluded further statistical analyses on this topic.

BACKGROUND

Diabetes mellitus, a major problem whose incidence is increasing worldwide, is a great threat to general health and is leading to increased morbidity and mortality. These effects are mainly occurring because diabetes is a disorder of glucose metabolism that affects multiple organ systems and is associated with various micro- and macro-vascular complications and several nonvascular

complications. Additionally, a large group of subjects do not fulfil the diabetes criteria but have intermediate glycaemic variables, between normal and diabetes, and are thus classified as having prediabetes. One of the most commonly used definitions of prediabetes is that of the 2010 American Diabetes Association (ADA) criteria[1, 2]: (a) impaired fasting plasma glucose (IFG), defined as fasting plasma glucose (FPG) between 100 and <126 mg/dL (5.6–5.9 mmol/L); (b) impaired glucose tolerance (IGT), defined as a 2-hour plasma glucose value after a 75 g oral glucose tolerance test (OGTT) between 140 and <200 mg/dL (7.8–11.0 mmol/L); or (c) glycated haemoglobin (HbA1c) levels between 5.7% and < 6.5% (39–46 mmol/mol). Prediabetes is becoming increasingly important as it represents a high risk of developing type 2 diabetes (T2D) and cardiovascular diseases.[2, 3] Moreover, individuals with prediabetes are phenotypically quite similar to patients with T2D. That is, they tend to be older, with a higher body mass index (BMI) and higher blood pressure than people with normal glucose tolerance; in addition, they tend to have insulin resistance and dyslipidaemia.[4] Additionally, multiple risk factors, such as family history, gestational diabetes, and certain ethnicities as well as combined risk factors such as metabolic syndrome, are known to predispose subjects to a higher risk for prediabetes and its progression to T2D. Based only on impaired glucose tolerance (IGT), the worldwide prevalence of prediabetes among adults has been estimated by the International Diabetes Federation to be 7.3% in 2017, with half of these individuals (49%) being younger than 50 years.[5] The National Diabetes Statistics Report in the United States reported that the total crude prevalence of diabetes was 9.4% (30.3 million, 2017 US population), with 23.8% undiagnosed and an additional 33.9% with prediabetes.[6] In Spain, according to data from the Di@bet.es study, based on OGTT, FPG and HbA1c, 13.8% of the adult population, adjusted for age and sex, had diabetes, and of these individuals up to 6% had undiagnosed diabetes. Furthermore, an additional 14.8% of individuals presented with some type of prediabetic state, 3.4% based on IFG, 9.2% based on IGT and 2.2% with disturbances in both, after adjusting for age and sex.[7, 8] According to the ADA, up to 70% of people with prediabetes will develop overt diabetes throughout their lives.[9, 10] Moreover, each year, 5-10% of subjects with prediabetes will eventually develop overt diabetes, and according to some studies, this percentage can reach up to 18% per year; however, this rate may vary with the definition of prediabetes and population characteristics.[11-14] It has been shown that over 3-5 years, approximately 25% of subjects progress to T2D, 25% return to a normal state of glucose tolerance and 50% remain in the prediabetic state.[15] Thus, the early diagnosis and screening of prediabetes are essential steps towards the prevention of its progression or at least the delay of the onset of T2D.

The primary aim of this study was to assess the prevalence of undiagnosed diabetes and prediabetes in the general population in the Mollerussa cohort. As a secondary objective, we aimed to assess the variables associated with these conditions and to describe the changes in glycaemic status after one year of follow-up in subjects with prediabetes.

METHODS

Subjects

This was a prospective population-based cohort study from a semi-rural area of Mollerussa in Catalonia (northeast Spain). The description of the cohort and the procedures performed were initially published as a cohort profile.[16] Briefly, the database of the Catalan Health Institute (ICS) through its Primary Care Electronic Clinical Station (Estació Clínica Electronica d'Atenció Primaria – eCAP) was used to select the population sample. From a total population in the health-care area that included twenty-four thousand six hundred and sixty-six potentially eligible individuals, 2,226 subjects were invited to participate by telephone contact, and 594 subjects aged ≥25 years were recruited.[16] The exclusion criteria included a previous diagnosis of diabetes (type 1 diabetes(T1D), T2D or any specific subtype of diabetes), treatment with oral antidiabetic drugs or the use of metformin for other conditions. In addition, subjects with cardiovascular disease (heart disease, heart failure, aortic stenosis), cancer, kidney disease, anaemia, hepatitis, gastrointestinal diseases, recent abdominal surgery, chronic pulmonary obstructive disease, chronic infectious diseases, use of systemic glucocorticoids or beta blockers or major psychiatric disorders with psychotic symptoms were excluded from the study. Subjects were considered to have hypertension or dyslipidaemia if they were using anti-hypertensive or lipid-lowering agents. Prediabetes was defined as any of the following abnormal glycaemic variables: FPG 100 to <126 mg/dL or HbA1c 5.7 to <6.5%; diabetes was defined as FPG >125 mg/dL or HbA1c ≥6.5%. Normal glycaemic status was defined by FPG <100 mg/dL and HbA1c <5.7 according to the 2010 ADA criteria.[1] Eleven subjects without baseline HbA1c or FPG measurements were excluded. Subjects with prediabetes underwent a second visit 12 months after the baseline visit, and 166 of them had relevant information at follow up.

A fasting blood sample was taken to determine glucose, HbA1c, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, renal function, and other parameters following standard protocols.[16] The fatty liver index (FLI) was calculated with the equation developed by Bedogni *et al*. [17] Insulin resistance was calculated by the homeostatic model assessment (HOMA2-IR); beta cell function (HOMA2-ß) and insulin sensitivity (HOMA2-S) data were calculated with a HOMA2 calculator released by the Diabetes Trials Unit, University of Oxford: HOMA Calculator. This

calculator is available at: http://www.dtu.ox.ac.uk/homacalculator/ (updated October 11, 2017). [18] The estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration equation.[19]

Sociodemographic variables were recorded, and a physical examination (weight, height, blood pressure and waist circumference) was carried out by researchers following a protocol for the inclusion of patients using a standardized baseline questionnaire for the clinical interview. Education level and physical activity were assessed according to the International Standard Classification of Education[20] and the Spanish-validated International Physical Activity Questionnaire,[21] respectively. We classified the education level as low level (studied until primary school) and high level (secondary high school education or higher). Physical activity was classified as sedentary or active (not regularly versus regularly active).

Ethical approval

The study protocol was approved by the Ethics Committee of the Primary Health Care University Research Institute (IDIAP) Jordi Gol (P12/043) and was conducted following the Declaration of Helsinki. All study participants signed an informed consent form.

Sample size

The sample size was determined based on an estimated prediabetes prevalence of 35.5% and 38% using HbA1c levels and the 2010 ADA criteria, respectively.[1, 22, 23] It was estimated that a random sample of 505 subjects was sufficient to assess an estimated prevalence of approximately 30% with a 95% CI and an error of ±4%.[16]

Statistical methods

Descriptive statistics of the mean (standard deviation) or median [interquartile range] were estimated for quantitative variables, while qualitative variables were assessed using absolute and relative frequencies. Comparisons between groups of all variables were performed to evaluate the differences. Student's t-test, ANOVA, the Mann-Whitney test, or the Kruskal-Wallis test were used to assess the differences between groups. The chi-squared test or Fisher's exact test were used to determine differences in qualitative variables. Tukey's correction was applied to account for multiple tests. Multivariate logistic regression models were used to determine the association of variables with prediabetes, isolated FPG, isolated HbA1c and both FPG and HbA1c at baseline and were performed using the enter method with covariables that were clinically or statistically associated. A backward conditional logistic regression model was used to predict the normalization of the glycaemic state; in all models, the goodness-of-fit assumption was tested by the Hosmer-Lemeshow test. The predictive accuracy of the logistic regression model for normalization was

checked by receiver-operating characteristic (ROC) curves and the area under the ROC curve (AUC_{ROC}). Odds ratios with corresponding 95% confidence intervals are shown, and statistical significance was established as a p-value <0.05. Data management and all analyses were performed using R statistical software, version 3.3.1, and SPSS software (version 22, IBM, SPSS, Chicago, Illinois, USA).

Patient and Public Involvement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

RESULTS

Out of the 594 individuals recruited, complete data on FPG and HbA1c were available from 583 (98.1%). The prevalence of undiagnosed diabetes was 3.4% (95% confidence interval: 2.65-4.15), and the prevalence of prediabetes was 39.3% (37.28-41.32). Furthermore, the prevalence based on isolated FPG was 7.2%, and that based on isolated HbA1c was 22.8%, while based on the criteria of both FPG and HbA1c, the prevalence was 9.3% (Figure 1).

The clinical and sociodemographic characteristics according to glycaemic status are shown in Table 1. Except for sex, family history of diabetes, current smoking status, alcohol consumption status, triglycerides and high density lipoprotein (HDL)-cholesterol levels, there were significant differences in the majority of parameters, including age and BMI, between the three groups.

We observed a positive trend in age, BMI, waist circumference, systolic and diastolic blood pressure (SBP and DBP), alcohol consumption status, hypertension, dyslipidaemia, triglycerides, total cholesterol, low density lipoprotein (LDL)-cholesterol, insulin test, FLI, and HOMA2-IR, which were higher in individuals with prediabetes than in individuals with normoglycaemia and were higher in the diabetic group than in the prediabetic group. On the other hand, physical activity, education level, eGFR, HOMA2-ß and HOMA2-S exhibited a negative trend between the same groups. In the prediabetic group, 41.9% had impaired FPG and 81.7% had impaired HbA1c. On the other hand, among the newly identified diabetic subjects, up to 80% met the FPG criteria and 85% met the HbA1c criteria. The prevalence of prediabetes increased with increasing age, with percentages of 17.4%, 28.6%, 46.4%, 50 and 52.9% in participants aged <35 years, 36-45 years, 46-55 years, 56-65

years and >65 years, respectively. Regarding BMI categories of normal weight (BMI <25 kg/m^2), overweight (BMI 25-29.9 kg/m^2), and obese (BMI>30 kg/m^2), the prevalence of prediabetes was 29%, 45.9%, and 49%, respectively (Figure S1).

Table 1. Clinical and sociodemographic characteristics of the Mollerussa cohort by glycaemic status.

	Normoglycaemia FPG <100 mg/dL and HbA1c <5.7%	**Prediabetes** FPG 100 to 125 mg/dL, or HbA1c 5.7 to 6.4%	**Diabetes** FPG >125 mg/dL or HbA1c ≥6.5%	***p.* overall**	***p.* NG vs PD**	***p.* NG vs DM**	***p.* PD vs DM**
N	334	229	20	-	-	-	-
Sex, women	193 (57.8%)	135 (59.0%)	13 (65.0%)	0.803	0.85	0.85	0.85
Age, years	45.0 [37.0;55.8]	54.0 [46.0;62.0]	62.0 [53.2;69.5]	**<0.001**	**<0.001**	**<0.001**	**0.036**
BMI, Kg/m^2	25.0 [22.5;27.3]	26.4 [24.8;29.7]	30.9 [26.4;35.6]	**<0.001**	**<0.001**	**<0.001**	**0.016**
BMI categories				**<0.001**	**<0.001**	**<0.001**	**0.01**
Normal weight	160 (50.0%)	67 (30.2%)	4 (20.0%)				
Overweight	120 (37.5%)	106 (47.7%)	5 (25.0%)				
Obesity	40 (12.5%)	49 (22.1%)	11 (55.0%)				
Waist, cm	93.0 [84.0;100]	97.0 [89.0;104]	100 [91.0;108]	**<0.001**	**<0.001**	**0.016**	0.275
SBP, mm Hg	119 [109;128]	125 [116;136]	132 [114;144]	**<0.001**	**<0.001**	**0.012**	0.248
DBP, mm Hg	75.0 [69.0;82.0]	78.0 [71.0;85.0]	79.0 [72.5;86.0]	**0.005**	**0.005**	0.281	0.886
Hypertension	37 (11.1%)	49 (21.4%)	9 (45.0%)	**<0.001**	**0.002**	**0.001**	**0.025**
Dyslipidaemia	27 (8.08%)	39 (17.0%)	5 (25.0%)	**0.001**	**0.006**	**0.038**	0.364
Family history DM	94 (29.6%)	78 (37.0%)	8 (42.1%)	0.141	0.275	0.553	0.845
Education, high level	265 (82.6%)	145 (65.0%)	11 (55.0%)	**<0.001**	**<0.001**	**0.008**	0.514
Physical activity	243 (75.9%)	141 (63.2%)	10 (50.0%)	**0.001**	**0.006**	**0.031**	0.354
Current smoker	82 (24.6%)	63 (27.5%)	3 (15.0%)	0.405	0.49	0.49	0.49
Alcohol, g/day	2.84 [0.00;10.6]	3.42 [0.04;15.9]	7.04 [1.42;11.5]	0.303	0.518	0.524	0.71
FPG, mg/dL	87.0 [82.0;92.0]	97.0 [89.0;106]	126 [110;131]	**<0.001**	**<0.001**	**<0.001**	**<0.001**
HbA1c, %	5.30 [5.10;5.50]	5.80 [5.70;6.00]	6.50 [6.07;6.62]	**<0.001**	**<0.001**	**<0.001**	**<0.001**
HbA1c, mmol/mol	34.4 [32.2;36.6]	39.9 [38.8;42.1]	47.5 [42.9;48.9]	**<0.001**	**<0.001**	**<0.001**	**<0.001**
eGFR mL/min/1.73m^2	97.5 [87.7;106]	91.5 [78.6;102]	89.0 [68.0;101]	**<0.001**	**<0.001**	**0.02**	0.287
Triglycerides, mg/dL	86.0 [65.0;119]	89.0 [72.0;132]	112 [70.8;161]	0.077	0.177	0.177	0.459
T-cholesterol, g/dL	194 [169;225]	202 [184;226]	216 [187;244]	**0.004**	**0.013**	0.054	0.234
HDL, mg/dL	57.0 [48.0;68.0]	57.0 [50.0;68.0]	64.0 [49.8;78.0]	0.331	0.849	0.246	0.246
LDL, mg/dL	116 [95.8;140]	125 [106;146]	125 [111;150]	**0.019**	**0.024**	0.292	0.696
Insulin, µU/mL	7.10 [5.30;9.70]	8.40 [6.60;11.8]	12.6 [9.25;15.6]	**<0.001**	**<0.001**	**<0.001**	**0.003**
Fatty Liver Index	26.4 [10.9;53.5]	40.4 [18.9;68.2]	61.5 [37.9;92.0]	**<0.001**	**<0.001**	**0.001**	**0.041**
HOMA2-ß	100 [82.0;124]	93.3 [73.0;115]	80.2 [61.1;97.9]	**0.003**	**0.018**	**0.018**	0.075
HOMA2-S	110 [80.1;145]	87.7 [64.4;112]	59.1 [46.0;80.9]	**<0.001**	**<0.001**	**<0.001**	**0.001**
HOMA2-IR	0.90 [0.70;1.20]	1.10 [0.90;1.60]	1.70 [1.28;2.20]	**<0.001**	**<0.001**	**<0.001**	**0.001**

Significant values are shown in bold. Median [interquartile range] and n (%). NG, normoglycaemia; PD, prediabetes; DM, diabetes; Ed level, education level; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; T-cholesterol, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA2-IR, Homeostatic Model Assessment-2_Insulin Resistance; HOMA2-ß, Homeostatic Model Assessment-2 Beta cell function; HOMA2-S, Homeostatic Model Assessment-2 Insulin Sensitivity.

Table 2 shows the characteristics of prediabetic individuals by glycaemic state: isolated FPG, isolated HbA1c and both altered FPG and HbA1c. Thus, among the 229 subjects with prediabetes, 42 (18.3%) had abnormal isolated FPG, 133 (58.1%) had abnormal isolated HbA1c, and 54 (23.6%) had both abnormal FPG and HbA1c. Patients with both abnormal FPG and HbA1c were older; had larger waist circumferences; had increased FLI and HOMA2-IR; were more likely to be overweight or obese and have hypertension; and had lower HOMA2-S. The isolated FPG group had a higher proportion of subjects with a family history of diabetes, higher alcohol consumption, higher levels of total cholesterol and LDL-cholesterol and lower levels of HDL-cholesterol, although none of these differences were statistically significant. Finally, the isolated HbA1c group had an elevated HOMA2-ß. Although there were no statistically significant differences, the proportion of men was higher in the isolated FPG group, whereas the proportion of women was higher in the isolated HbA1c and both FPG and HbA1c groups. Among the three groups, no statistically significant differences were found regarding the following variables: sex, dyslipidaemia, family history of diabetes, education level, physical activity, current smoking status, alcohol consumption, triglycerides, total cholesterol, HDL-cholesterol or LDL-cholesterol.

Table 2. Clinical and sociodemographic characteristics by glycaemic status of the individuals with prediabetes.

	Impaired HbA1c 5.7%-6.4%	**Impaired FPG 100-125 mg/dL**	**HbA1c 5.7%-6.4% and FPG 100-125 mg/dL**	***p* overall**	***p* HbA1c vs. FPG**	***p* HbA1c vs. Both**	***p*. FPG vs. Both**
N	133	42	54	-	-	-	-
Sex, Women	84 (63.2%)	19 (45.2%)	32 (59.3%)	0.12	0.181	0.74	0.369
Age, years	53.4 (12.4)	50.6 (11.8)	60.6 (10.5)	**<0.001**	0.388	**0.001**	**<0.001**
BMI, Kg/m2	25.8 [24.5;28.9]	27.8 [24.5;30.6]	27.5 [25.6;30.5]	0.056	0.534	**0.036**	0.534
BMI categories				**0.018**	0.107	**0.05**	**0.032**
Normal weight	43 (33.1%)	16 (41.0%)	8 (15.1%)				
Overweight	64 (49.2%)	12 (30.8%)	30 (56.6%)				
Obesity	23 (17.7%)	11 (28.2%)	15 (28.3%)				
Waist, cm	95.0 [88.0;102]	98.0 [90.0;106]	101 [95.0;107]	**0.008**	0.232	**0.006**	0.333
SBP, mm Hg	124 (16.1)	129 (15.5)	128 (18.4)	0.169	0.296	0.29	0.991
DBP, mm Hg	78.0 (9.44)	79.5 (12.0)	77.9 (9.39)	0.674	0.675	0.999	0.723
Hypertension	21 (15.8%)	9 (21.4%)	19 (35.2%)	**0.014**	0.542	**0.019**	0.32
Dyslipidaemia	25 (18.8%)	4 (9.52%)	10 (18.5%)	0.358	0.515	1	0.515
Family history DM	43 (34.1%)	18 (48.6%)	17 (35.4%)	0.265	0.471	1	0.471
Education, high level	91 (69.5%)	23 (59.0%)	31 (58.5%)	0.252	0.455	0.455	1
Physical activity	88 (67.2%)	21 (53.8%)	32 (60.4%)	0.281	0.547	0.68	0.68
Current smoker	38 (28.6%)	14 (33.3%)	11 (20.4%)	0.338	0.693	0.496	0.496
Alcohol, g/day	2.92 [0.00;15.2]	7.42 [0.90;16.3]	1.53 [0.00;17.9]	0.369	0.336	0.735	0.336
FPG, mg/dL	89.2 (6.89)	106 (4.97)	109 (5.96)	**<0.001**	**<0.001**	**<0.001**	0.14
HbA1c, %	5.80 [5.70;6.00]	5.40 [5.40;5.57]	5.95 [5.80;6.10]	**<0.001**	**<0.001**	**<0.001**	**<0.001**
HbA1c, mmol/mol	39.9 [38.8;42.1]	35.5 [34.7;37.4]	41.5 [39.9;43.2]	**<0.001**	**<0.001**	**<0.001**	**<0.001**

eGFR, mL/min/1.73m^2	93.6 [79.6;103]	93.2 [79.7;107]	89.3 [73.1;97.2]	0.076	0.556	0.073	0.073
Triglycerides, mg/dL	88.0 [72.0;134]	86.5 [67.0;130]	106 [74.5;132]	0.332	0.729	0.304	0.304
Total cholesterol, mg/dL	205 (34.5)	209 (28.6)	203 (29.8)	0.689	0.767	0.947	0.677
HDL-cholesterol, mg/dL	58.0 [51.0;69.0]	52.0 [45.0;65.8]	57.0 [51.0;66.0]	0.128	0.141	0.755	0.18
LDL-cholesterol, mg/dL	125 (32.2)	133 (25.5)	120 (23.5)	0.114	0.278	0.593	0.096
Insulin, µU/mL	8.00 [6.10;10.0]	9.90 [6.90;15.9]	10.9 [7.90;15.6]	**<0.001**	**0.01**	**<0.001**	0.577
Fatty Liver Index	34.4 [16.9;59.2]	42.2 [17.7;73.6]	53.8 [32.2;73.0]	**0.016**	0.373	**0.011**	0.378
HOMA2-ß	96.6 [81.5;122]	81.7 [64.5;118]	82.8 [63.0;108]	**0.001**	**0.034**	**0.002**	0.693
HOMA2-S	98.0 [77.2;127]	75.0 [47.2;107]	67.5 [47.5;91.3]	**<0.001**	**0.003**	**<0.001**	0.564
HOMA2-IR	1.00 [0.80;1.30]	1.30 [0.90;2.15]	1.50 [1.10;2.10]	**<0.001**	**0.005**	**<0.001**	0.545

Significant values are shown in bold. Median [interquartile range] and n (%). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA2-IR, Homeostatic Model Assessment-2_Insulin Resistance; HOMA2-ß, Homeostatic Model Assessment-2 Beta cell function; HOMA2-S, Homeostatic Model Assessment-2 Insulin Sensitivity.

Prediabetes follow-up

Of the 229 individuals with prediabetes at baseline, 166 (72.49%) had clinical and laboratory data after 12 months of follow-up. Of them, 52 (41.6%) returned to a normal glycaemic status, 112 (57.6%) persisted in their state of prediabetes, and only 2 (0.6%) progressed to diabetes. Table 3 shows the outcome of the follow-up of the isolated FPG, HbA1c and both FPG and HbA1c groups.

Table 3. Outcomes at follow-up of patients with different altered glucose metabolism statuses at baseline.

Variables	Baseline	N with follow-up	Follow up		
			Normalized	Persisted	Progressed
Prediabetes	229 (39.3%)	166 (90.7%)	52 (41.6%)	112 (57.8%)	2 (0.6%)
Isolated FPG	42 (7.2%)	3 (1.8%)	1 (33.3%)	1 (33.3%)	1 (33.3%)
Isolated HbA1c	133 (22.8%)	114 (68.7%)	47 (41.3%)	67 (58.7%)	0 (0%)
Both altered	54 (9.3%)	49 (29.5%)	4 (8.2%)	44 (89.8%)	1 (2%)

FPG, fasting plasma glucose

Association of prediabetes with glycaemic status

The multivariate logistic regression model of prediabetes *versus* normoglycaemia showed that the variables associated with prediabetes were older age (odds ratio; 95% confidence interval) (1.033; 1.011-1.056), higher physical activity levels (0.546; 0.360-0.827), higher BMI (1.121; 1.029-1.222), and a family history of diabetes (1.543; 1.025-2.323) (Figure 2a). The models for isolated FPG alterations, isolated HbA1c alterations and both FPG and HbA1c alterations are shown in

Supplementary tables S1, S2 and S3, respectively. The variables associated with isolated FPG were older age (1.032; 1.008-1.057), higher physical activity levels (0.535; 0.318-0.899), and a family history of diabetes (1.798; 1.067-3.028). On the other hand, the only variable associated with impaired HbA1c was older age (1.048; 1.029-1.067). Finally, in the model for altered FPG and HbA1c, the variables associated were older age (1.056; 1.026-1.086) and high FLI (1.031; 1.002-1.061).

Prediction of normalization

Backward conditional logistic regression, as described in the methods section, starting with the variables age, sex, waist circumference, BMI, hypertension, physical activity, family history of diabetes, education level, total cholesterol, HDL-cholesterol, FLI and HOMA2-IR, was performed to identify factors independently associated with the prediction of glycaemic status normalization. The variables that predicted glycaemic normalization were older age (0.948; 0.916-0.982) and BMI (0.779; 0.651-0.931) (Figure 2b); this model had a good predictive ability (AUC_{ROC} 0.77; p<0.001) (Figure S2).

DISCUSSION

We found that the prevalence of undiagnosed diabetes was 3.4%, and the prevalence of prediabetes was 39.3% in this semi-rural population in Catalonia (northeast Spain). The prevalence of prediabetes was three-fold higher based on HbA1c than that based on FPG. Subjects with prediabetes defined by both HbA1c and FPG criteria had unfavourable clinical and sociodemographic profiles related to increased cardiovascular risk. These factors were older age; abdominal obesity; higher triglycerides; increased FLI; and a higher proportion of overweight, obesity and hypertension. In our population, age was the variable most strongly associated with prediabetes based on all specific glycaemic status variables: isolated impaired FPG, isolated impaired HbA1c or both impaired FPG and HbA1c. Other variables associated with prediabetes were lower physical activity levels, a family history of diabetes, and obesity. Finally, the characteristics related to normalization at follow-up were younger age and lower BMI.

The prevalence of prediabetes and undiagnosed diabetes in our general population were within the ranges found in other population studies defining prediabetes based on the 2010 ADA criteria, using FPG and/or HbA1c. Among these studies, a large national Chinese study (with 170,287 subjects) showed a prevalence of prediabetes of 35.7% and a prevalence of undiagnosed diabetes of 6.9%.[24] In a study of the Caribbean population, the corresponding figures were 44.1% for

prediabetes and 7.3% for undiagnosed diabetes.[25] In England, based on HbA1c levels, the prediabetes prevalence was 35.5% in the adult population in 2011.[22] In these studies, the prevalence of prediabetes was higher in older, overweight and obese participants.[22, 24, 25] Many other studies found this relationship of age and obesity with the risk and incidence of diabetes.[26-29]

In the 1999-2002 National Health and Nutrition Examination Survey (NHANES), the prevalence of undiagnosed diabetes was 2.8%, and up to 26% of the participants had IFG.[30] However, the age-standardized prevalence of prediabetes based on HbA1c and FPG combined was similar in the periods between 1999 and 2002 and 2003 and 2006 at 29.2% and 29.3%, respectively, but increased significantly to 36.2% in the period between 2007 and 2010.[31] This prevalence continued to increase to as high as 38% in 2012 among adults from the USA.[23] The change in the prevalence of prediabetes over time occurred because of a significant change in elevated HbA1c, whereas the prevalence based on elevated FPG was similar over this period.[31] Thus, in our population, as in the NHANES study, HbA1c was the most significant contributor to prediabetes prevalence, followed by FPG, which is in concordance with the findings in the Caribbean population[25] and discordant with the reports from the NHANES study between 2011 and 2014 in which they reported that FPG was the most significant contributor to prediabetes prevalence followed by HbA1c.[32] Our results show that individuals with isolated impaired HbA1c when diagnosed with prediabetes might have a slightly better cardiometabolic risk profile than those with isolated FPG, while those individuals with both impaired FPG and HbA1c had the worst CV risk. These results are in line with the findings of the prospective observational study in the primary care setting of a Spanish cohort with prediabetes (PREDAPS) of our group.[33, 34]

Additionally, two meta-analyses found that among individuals with prediabetes based on the ADA criteria, all-cause and CVD mortality were increased[35] and that the risk of cardiovascular disease increased independently of the glucose assessment in comparison to the risk of normoglycaemic subjects.[36] Moreover, a recent study concluded that those who returned to normoglycaemia from FPG- or HbA1c-defined prediabetes were not at reduced risk of future CVD or death.[37] Studies of shorter duration, over 3-5 years, have shown that approximately 25% of subjects progress to diabetes, 25% return to a normal state of glucose tolerance and 50% remain in the prediabetic state;[15] after 1 year, 18.8% of subjects with prediabetes returned to normoglycaemia and approximately 30% with abnormal FPG, 29.1% with abnormal HbA1c and 7.6% with abnormalities

in both FPG and HbA1c returned to a normal state of glucose tolerance.[38] In our findings from a one-year follow-up, the rate of reversion from prediabetes to normoglycaemia was approximately 40%, and approximately 60% of participants remained in the prediabetic state. On the other hand, lifestyle modifications, such as weight loss and increased physical activity, among other factors associated with prediabetes, reduced the risk of diabetes among these subjects.[12, 39] According to these reports, in our study, lower BMI was a factor that was independently associated with the normalization of the glycaemic state, and an active lifestyle decreased the risk of having prediabetes.

The results of this study need to be interpreted in light of its strengths and weaknesses. First, we included a small number of participants in comparison to the sample sizes of other studies. However, the Mollerussa cohort is a representative sample of the region, which is a specific semi-rural area that has never been specifically investigated. Second, we did not assess glucose tolerance through an oral glucose tolerance test, which is common in most population studies. Although this assay is sensitive, it is also less specific for identifying subjects who could develop diabetes.[40] Furthermore, the oral glucose tolerance test has a low reproducibility and is a rather time-consuming and expensive procedure.[8, 41] Conversely, HbA1c and FPG are cost-effective and more convenient for patients. Currently, FPG is an accepted screening method to detect diabetes and prediabetes. HbA1c improves the sensitivity of FPG in the detection of early T2D in high-risk subjects[30, 42] and is a better predictor of CV events than FPG.[43] Third, although traditional factors such as hypertension, dyslipidaemia and obesity were included in the analysis models, the existence of unmeasured confounding variables cannot be entirely ruled out. Finally, it is probable that the use of the World Health Organization prediabetes criteria in our study would have resulted in a smaller proportion of subjects who returned to a normal glycaemic state. The World Health Organization established a normal concentration of FPG between 110 and <126 mg/dl.[44]

Conclusions

For the first time, our study provides information on the prevalence of diabetes and prediabetes in the Mollerussa health care area, a Mediterranean semi-rural area in northeast Spain. Individuals with prediabetes had a more unfavourable cardiometabolic risk profile than normoglycaemic subjects. Moreover, individuals with abnormalities in both criteria used to diagnose prediabetes had the worst risk profile. Finally, after one year of follow-up, few people progressed to diabetes, while more than 40% returned to a normal glycaemic state, and nearly 60% persisted in the prediabetic state. These results suggest that the use of both FPG and HbA1c criteria in clinical practice could

help identify people with high diabetes and cardiovascular risk. Moreover, the identification of individuals with prediabetes provides an opportunity for intervention through lifestyle modification and pharmacological treatments not only to reduce the development of diabetes but also to prevent the development of chronic complications.

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Author Contributions MF, EC and DM conceived and designed the study; MBV, JFN, and MMC participated in the study design; MBV, MF, NA, MGC, NM and CC collected the data; EC and JRM performed the statistical analyses; MF, EC, MMC and DM wrote the manuscript. All authors critically reviewed the manuscript and approved the final version to be published.

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Competing interests None declared.

Ethics approval The project was approved by the Ethics Committee of the Primary Health Care University Research Institute (IDIAP) Jordi Gol (PI12/043) Barcelona, Spain.

Data sharing statement Readers may contact Dr Didac Mauricio (didacmauricio@gmail.com) regarding the data.

Patient consent for publication Not required.

The original protocol for the study Readers may found the Cohort description as a Supplementary file 3.

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FIGURE LEGENDS

Figure 1. Flow diagram of subjects at baseline and after follow-up.

Figure 2. Multivariate logistic regression models **a)** model of prediabetes *versus* normoglycaemic state in the Mollerussa cohort at baseline. Significant p values are shown in bold. eGFR, estimated glomerular filtration rate. Hosmer and Lemeshow Test p=0.295. **b)** model of normalized *versus* persisted in subjects with follow-up data. Significant p values are shown in bold. Hosmer and Lemeshow Test p= 0.931.

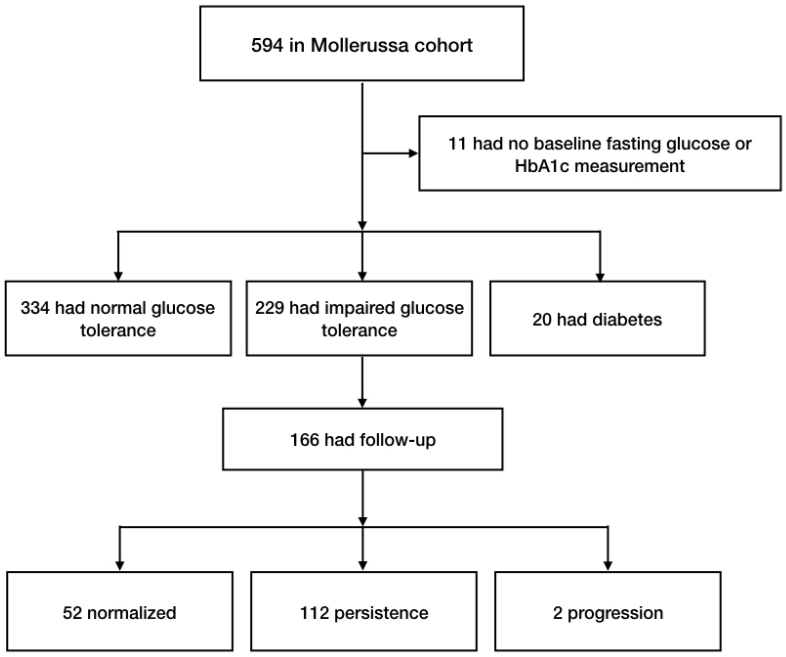

Figure 1. Flow diagram of subjects at baseline and after follow-up.

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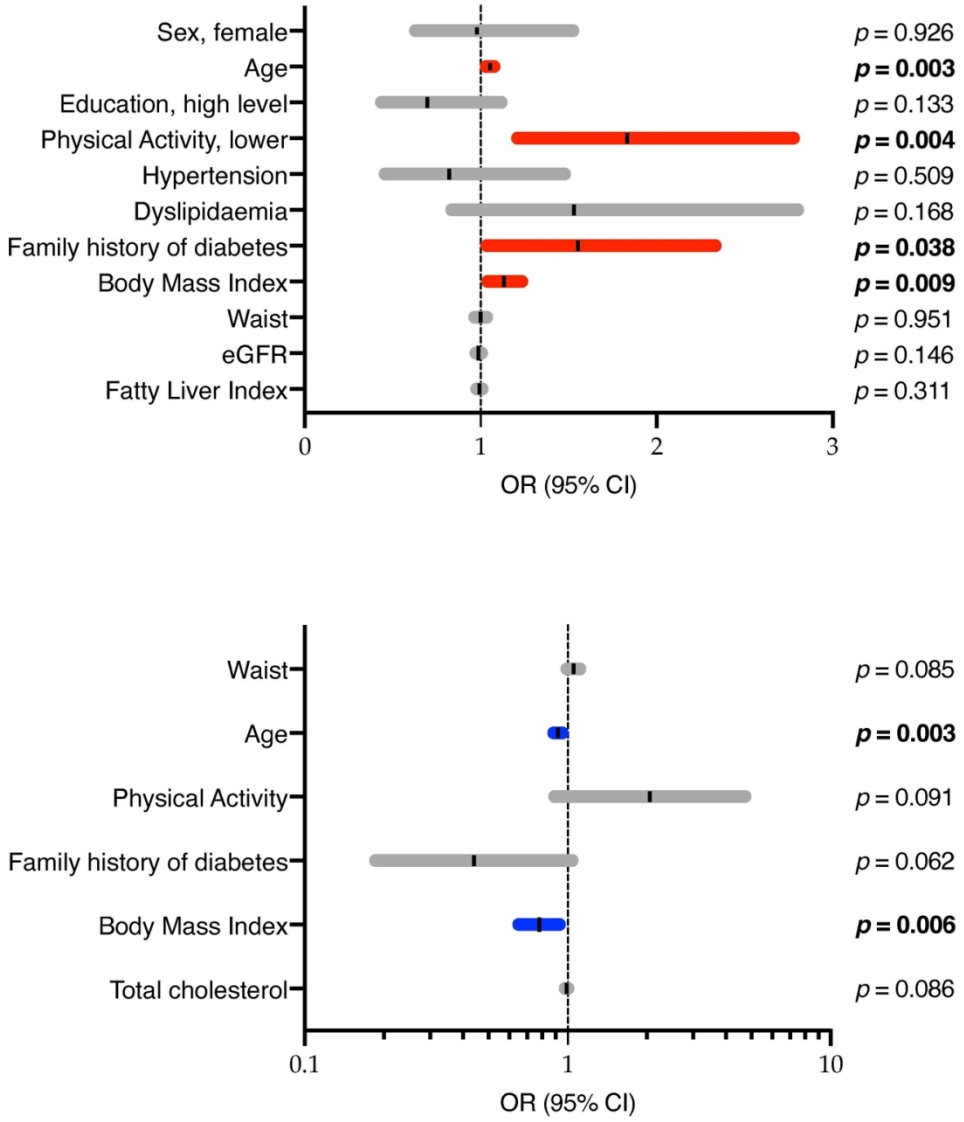

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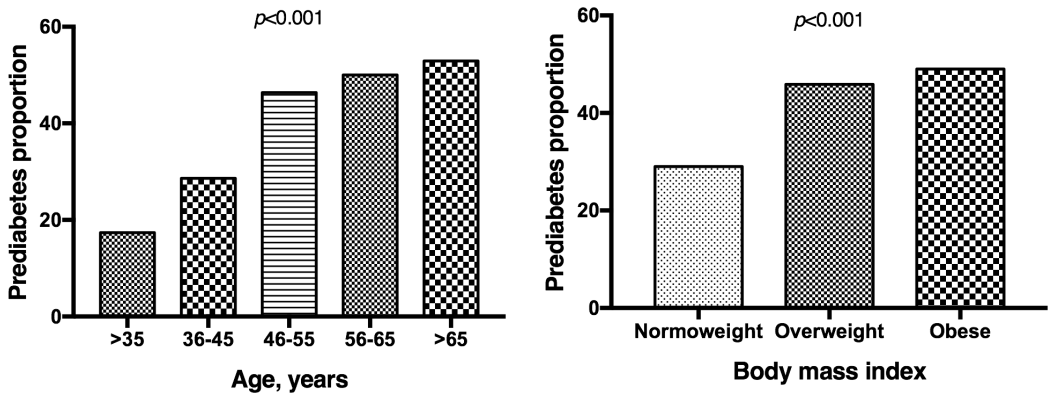
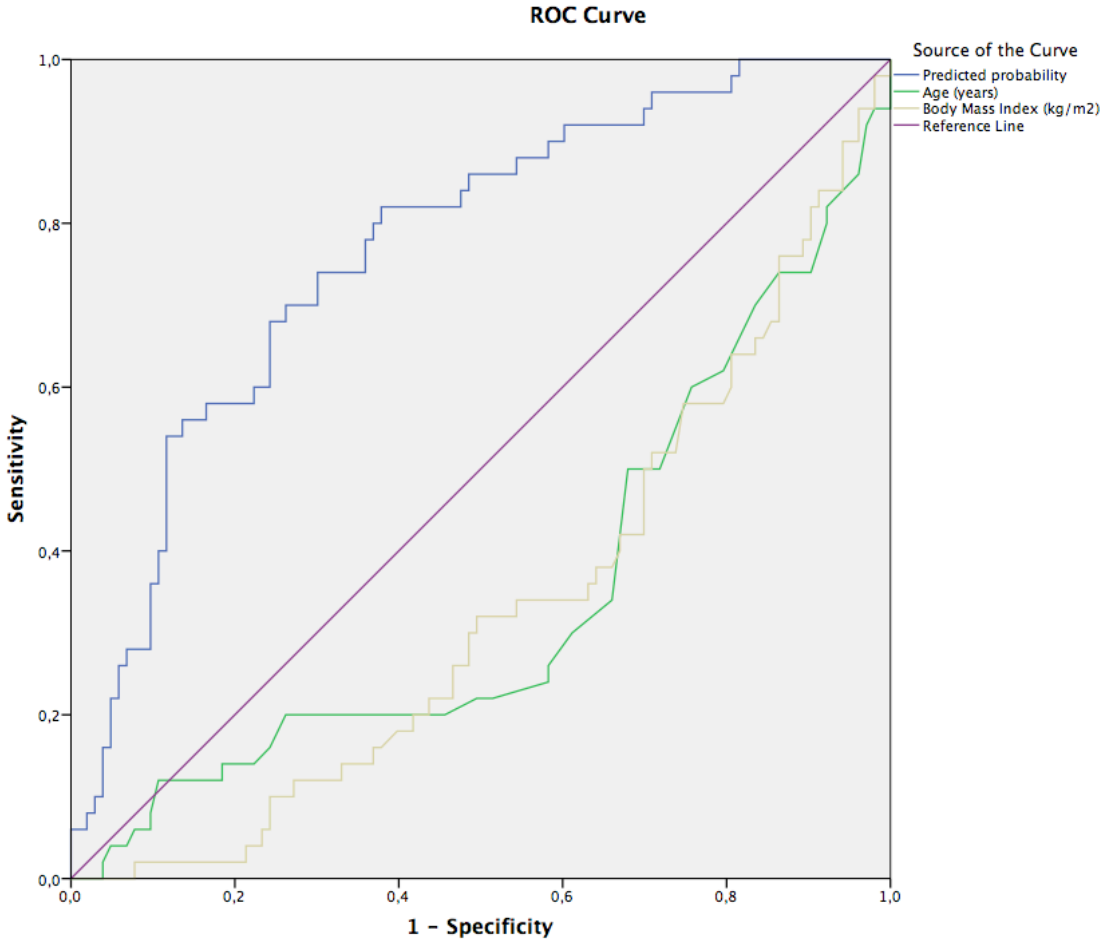


Supplementary Figure 1. Proportion of patients with prediabetes. A) Stratified by age. B) Stratified by body mass index.



Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Predicted probability	0.771	0.040	0.000	0.693	0.849
Age	0.347	0.049	0.002	0.251	0.442
Body Mass Index	0.332	0.045	0.001	0.244	0.421

Supplementary Figure 2. Receiver operating characteristics (ROC) curve showing the relationship between sensitivity and 1-specificity in determining the discriminatory ability of the logistic regression model and the variables age and body mass index separately.

Open Access Cohort profile

BMJ Open

Prevalence, clinical features and risk assessment of pre-diabetes in Spain: the prospective Mollerussa cohort study

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ABSTRACT

Purpose The Mollerussa prospective cohort was created to study pre-diabetes in a population-based sample from the primary care setting in the semirural area of Pla d'Urgell in Catalonia (Spain). The aims of the study were to assess the prevalence of pre-diabetes in our population, the likelihood to develop overt diabetes over time and to identify risk factors associated with the progression of the condition.

Participants The cohort includes 594 subjects randomly selected between March 2011 and July 2014 from our primary care population, who were older than 25 years, consented to participate and did not have a recorded diagnosis of diabetes.

Findings to date At baseline, we performed a clinical interview to collect demographic, clinical and lifestyle (including a nutritional survey) characteristics; carotid ultrasound imaging to assess subclinical cardiovascular disease was also performed, and a blood sample was collected, with an overall <5% rate of missing data. An additional blood draw was performed 12 months after initial recruitment to reassess laboratory results in patients initially identified as having pre-diabetes, with an 89.6% retention rate. Several studies investigating various hypotheses are currently ongoing.

Future plans All subjects recruited during the cohort creation will be followed long-term through annual extraction of data from health records stored in the electronic Clinical station in Primary Care database. The Mollerussa cohort will thus be a sound population-based sample for multiple future research projects to generate insights into the epidemiology and natural history of pre-diabetes in Spain.

Strengths and limitations of this study

- The Mollerusssa cohort contains data from subjects with pre-diabetes identified in a primary care setting that were collected prospectively during 12 months, minimising recall bias.
- There was a potential selection bias, with higher rates for women and middle-aged among enlisted subjects than among eligible but not enlisted subjects, which will be minimised through a weighting process during the analyses.
- Subjects will be followed long-term through annual extraction of data included in the individuals' electronic medical records, a design that will minimise losses to follow-up.
- The long-term follow-up will allow the accurate estimation of time trends and clinical features associated with progression from pre-diabetes to overt diabetes
- A potential limitation of the long-term follow-up is that it will rely on data that may be incomplete or inconsistently measured between subjects

INTRODUCTION

According to the American Diabetes Association (ADA), diabetes is broadly classified into four categories: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes and specific types of diabetes due to other causes.[1] However, there is a group of individuals that, in spite of having higher than normal glucose levels, do not meet criteria for diabetes, a condition referred to as pre-diabetes.

There are different definitions of pre-diabetes, but the most common one, the ADA criteria, considers one of the following instances[1]: (a) impaired fasting plasma glucose (IFG), defined as fasting plasma glucose (FPG) between 100 and 125 mg/dL (5.6–5.9 mmol/L); or (b) impaired glucose tolerance (IGT), defined as a 2-hour plasma glucose value after a 75g oral glucose tolerance test (OGTT) between 140 and 199 mg/dL (7.8–11.0 mmol/L); or (c) glycated haemoglobin (HbA1c) levels between 5.7% and 6.4% (39–46 mmol/mol).

The prevalence of pre-diabetes varies across countries and depending on the parameter used for the estimations. Based solely on IGT, its worldwide prevalence among adults has been estimated by the International Diabetes

Federation to be 6.7% in 2015, with half of them (50.1%) younger than 50 years.[2] In England, solely based on HbA1c levels, the prevalence was 35.5% among the adult population in 2011[3]; in Spain, isolated IFG and isolated IGT were present in 3.4% and 2.9%, respectively, and combined IFG–IGT in 2.2% of the adult population in 2010[4]; and in the USA, using the ADA definition (HbA1c levels or IFG or IGT) the prevalence was as high as 38% in 2012.[5]

Understanding the epidemiology and natural history of pre-diabetes has become a health priority, in particular at the primary care setting, because it is a source of avoidable morbidity and mortality. First, individuals with IFG and/or IGT have a clinical phenotype that resembles patients with T2DM, as they tend to be older, have a higher body mass index (BMI), have more frequent insulin resistance and dyslipidaemia and have higher arterial blood pressure (BP) than people with normal glucose tolerance.[6] Second, people with pre-diabetes are at increased risk of developing diabetes: according to the ADA, up to 70% of them will eventually develop overt diabetes[7]; the annual incidence of progression to diabetes is around 5%–10% depending on the population characteristics and the definition of pre-diabetes; 6%–9% in subjects with isolated IFG, 4%–6% in those with isolated IGT, up to 15%–19% among those with both IFG and IGT[8 9] and subjects with HbA1c levels from 5.7% to <6.5% have a 7.5-year predicted risk of 43.1% for incident diabetes.[10] Finally, individuals with pre-diabetes are at increased risk of cardiovascular disease (CVD) and premature mortality: a meta-analysis found that the risk of CVD is increased regardless of type of blood glucose assessment in comparison to subjects with normoglycaemia[11]; and another recent meta-analysis found that risks of all-cause and CVD mortality compared with normoglycaemic subjects were increased in individuals with pre-diabetes with ADA defined IFG, IGT or both criteria combined, although not with isolated IFG.[12]

Based on epidemiological and clinical evidence, it is important from a prediction and prevention perspective to target segments of the population with metabolic and cardiovascular (CV) signatures associated with an increased risk of developing diabetes and CVD.[1] The Mollerussa cohort was designed to identify undiagnosed diabetes or pre-diabetes in the adult primary care population from a semirural area of Catalonia (Spain), and to further obtain extensive epidemiological, clinical (including subclinical atherosclerotic disease) and lifestyle data. In the following phases, the cohort will be run as prospective observational studies involving identified at-risk individuals to determine the progress over time regarding risk factors, incident diabetes, incidence of CV events, rates of hospitalisation and global mortality.

COHORT DESCRIPTION

Mollerussa is a prospective observational cohort study conducted in Pla d'Urgell, a semirural area of Catalonia (Spain), among subjects from the general population with healthcare coverage from the Catalan Institute of Health (Institut Català de la Salut).

Based on an estimated prevalence of pre-diabetes in the area of Lleida of 11% (10%–19%) in 2011,[13] we initially calculated that we would need a representative sample of 940 adults considering a 95% CI and a margin of error of ±2%. However, literature published after the initiation of the recruitment phase, using HbA1c levels and ADA criteria, reported pre-diabetes prevalence between 35.5% and 38%.[3 5] Using this datum, a random sample of 505 subjects was sufficient to assess an estimated prevalence of about 30% with a 95% CI and a margin of error of ±4%.

Recruitment

Using the electronic Clinical station in Primary Care (eCAP) health records database implemented in all primary care centres in Catalonia, a code number was given to each registered adult. During the recruitment phase, 24 666 registered health records met inclusion criteria, namely subjects older than 25 years and attending any Primary Healthcare Centre in the same health area in Pla d'Urgell (box 1; figure 1); among them, 2226 random individuals (about 5% of the total number of individuals registered at each centre) were contacted by telephone (up to three attempts) and invited to participate (figure 1). Randomisation was carried out using a randomiser programme (SPSS software V.16.0 for Windows; SPSS), following the principles of simple random sampling.

Main exclusion criteria (box 1) included a previous diagnosis of diabetes (T1DM, T2DM and any specific subtype of diabetes), and subjects on treatment with oral antidiabetic drugs to avoid the inclusion of individuals with actual diabetes but optimal glycaemic control, or even without diabetes but using metformin as treatment for other conditions. Based on their willingness to join the study and exclusion criteria, a total of 602 subjects were recruited and subsequently invited for an appointment, of whom four withdrew consent and, for four, we did not obtain any baseline laboratory data, therefore giving a final study population of 594 subjects.

Data collection

The research plan included a first phase involving two visits to the primary healthcare centre for baseline screening, a second phase conducted 12 months after the baseline visits and a third phase of long-term follow-up of the cohort.

First phase or baseline screening

The following variables were collected/explored by trained research staff in the first visit to the subject's primary healthcare centre: (a) Sociodemographic variables: age; gender; education level according to the International Standard Classification of Education[14]; sector of working activity (primary, secondary or manufacturing, tertiary or services); self-perceived work activity (minimum, light, moderate, heavy); report on

Box 1 Summary of inclusion and exclusion criteria

Inclusion criteria

Age ≥25 years

Attended a primary health care centre in the area

Exclusion criteria

Patient information about having diabetes provided in the first contact or existing ICD-10 code of diabetes (E11, E14 or E13) registered by a physician or confirmed based on clinical data:

- HbA1c ≥6.5%
- IGT: 2-hour plasma glucose in the 75g OGTT ≥200 mg/dL (11.1 mmol/L)
- IFG: FPG ≥126 mg/dL (7 mmol/L)

Specific subtypes of diabetes other than T1DM and T2DM:

- Gestational diabetes
- Genetic defect of beta-cell action
- Genetic defect in insulin action
- Diseases of the exocrine pancreas (eg, pancreatitis, haemochromatosis, pancreatic cancer, cystic fibrosis)
- Endocrinopathies (eg, Cushing's syndrome, glucagonoma, somatostatinoma, hyperthyroidism, pheochromocytoma, acromegaly)
- Chemical-induced diabetes
- Diabetes secondary to infections
- Autoimmune diabetes

Use of oral antidiabetic drugs: metformin, dipeptidyl peptidase-4 inhibitors, sulfonylureas and glitazones

Presence of cardiovascular disease:

- Previous hospitalisation to treat heart disease
- Heart failure
- Left bundle branch block or second degree atrioventricular block
- Aortic stenosis
- Systolic BP >180 mm Hg or diastolic BP >105 mm Hg

Cancer treated in the preceding 5 years, except non-melanoma skin cancers (basal-cell and squamous-cell carcinoma)

Kidney disease, defined as plasma creatinine ≥1.4 mg/dL in men and ≥1.3 mg/dL in women or proteinuria >2+

Anaemia, defined as haematocrit <36% in men and <33% in women

Hepatitis, defined as transaminases more than 10 times the upper the limit of normal

Gastrointestinal diseases (pancreatitis, irritable bowel disease and inflammatory bowel disease)

Recent abdominal surgery

Chronic pulmonary obstructive disease requiring domiciliary oxygen therapy

Chronic infectious diseases (eg, HIV, active tuberculosis, HBV and HCV)

Use of systemic glucocorticoids or beta blockers

Major psychiatric disorder with psychotic symptoms

BP, blood pressure; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin; HBV, hepatitis B virus; HCV, hepatitis C virus; ICD, International Classification of Diseases; IFG, impaired fasting plasma glucose; IGT, impaired glucose tolerance; OGGT, oral glucose tolerance test; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

physical activity according to the Spanish-validated International Physical Activity Questionnaire[15]; family history of hypertension, dyslipidaemia, hypertriglyceridaemia, diabetes mellitus and acute myocardial infarction or angina pectoris; personal history of hypertension, dyslipidaemia, hypertriglyceridaemia, alcohol consumption and smoking habit as reported by the patient; and current medication. (b) Anthropometric measures and physical examination: BP recorded according to the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure guidelines[16]; heart rate; body weight; waist circumference; and calculation of BMI. (c) Nutritional survey: food intake was assessed using a Spanish-validated version of the semiquantitative Food Frequency Qquestionnaire[17]; and we estimated the degree of adherence to the traditional Mediterranean diet through the Mediterranean Diet score.[18] (d) Carotid ultrasound imaging to measure intima-medial thickness and to assess the presence of carotid atherosclerotic plaques as previously described.[19] Briefly, both carotids were examined following a standardised operational procedure and the Mannheim consensus,[20] consisting of a cross-sectional view of the common, bulb and internal segments of the carotid arteries to identify atherosclerotic plaques (defined as a focal encroachment into the lumen of the carotid), and an online average measurement of the carotid intima-media thickness (c-IMT) of these three areas, with values of c-IMT above 1.5 mm considered as plaques.[21]

In a second appointment, the following laboratory measurements were obtained in fasting conditions: complete blood count, lipid, liver, kidney and thyroid profile. The study of glycaemia included the measurement of FPG and HbA1c. According to the ADA HbA1c criteria,[1] patients were classified into three groups: (1) without glucose metabolism disorders (HbA1c <5.7%), (2) with pre-diabetes (HbA1c between 5.7% and 6.4%) or with undiagnosed diabetes (HbA1c ≥6.5%). When the investigator detected a case of undiagnosed diabetes, the individual was re-contacted and advised to visit a general practitioner at his/her corresponding primary healthcare centre.

The baseline assessment will allow a first estimation of the prevalence of pre-diabetes in our population, its associated factors, and whether these subjects also have a higher prevalence of subclinical carotid atherosclerosis (and are therefore at high risk of CVD) compared with normoglycaemic subjects. Moreover, it will build on previous estimates of the prevalence of undiagnosed diabetes in primary healthcare in Catalonia.[22]

Second phase or short-term follow-up

Twelve months after the baseline visits, subjects initially fulfilling pre-diabetes criterion underwent a second visit to perform another blood draw to reassess the laboratory results. This was based on the ADA recommendation to repeat testing in the absence of unequivocal hyperglycaemia.[1] Based on re-evaluated HbA1c levels, those subjects with HbA1c levels between 5.7% and 6.4% were confirmed as pre-diabetes; those with a subsequent increase from pre-diabetes values at baseline to HbA1c ≥6.5% after 12 months were considered as incident diabetes (and as well re-contacted and advised to visit a general practitioner); and those with a further decrease

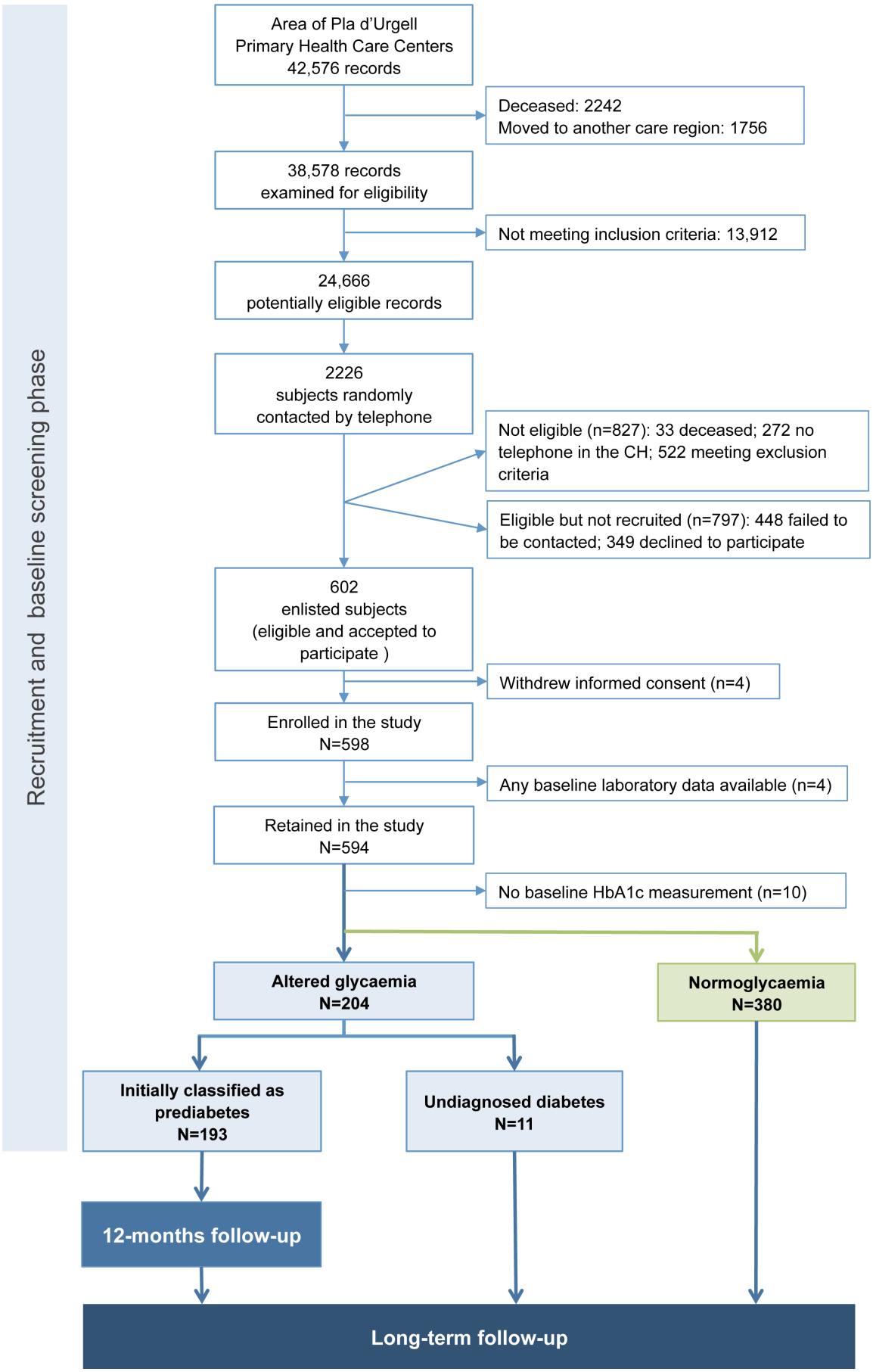

Figure 1 Mollerussa study flow chart. CH, clinical history; HbA1c, glycated haemoglobin.

from pre-diabetes values at baseline to HbA1c <5.7% after 12 months as regression to normoglycaemia, although they will be followed up to rule out a temporary improvement, and thus a false case of regression to normal mean HbA1c values.

The 12-month follow-up will give a real estimation of pre-diabetes prevalence, valuable information on the 1-year probability of progression to diabetes and which risk factors were relevant to the further development of the disease (eg, metabolic traits and lifestyle).

Third phase or long-term follow-up

In addition to the 12-month follow-up, we also plan to follow all subjects enrolled in the Mollerussa cohort annually through cross-sectional extraction of data stored in the primary care electronic medical records of the eCAP database.[23] This is based on the ADA recommendation to follow pre-diabetes in asymptomatic adults yearly.[1] We will extract data for the following variables: any diagnosis of T2DM (International Classification of Disease (ICD-10) codes E11 or E14)[22]; time since diagnosis; estimated glomerular filtration rate using the Modified Diet in Renal Disease formula; standardised HbA1c values, using the most recent value of the preceding 12 months; presence of CVD, including coronary artery disease (ICD-10 codes I20, I21, I22, I23 or I24), stroke (ICD-10 codes I63, I64, G45 or G46) and peripheral artery disease (ICD-10 code I73.9); risk factors, including BMI (most recent value in the last 12 months), cholesterol levels (total, low-density lipoproteins or LDL-cholesterol and high-density lipoproteins or HDL-cholesterol; most recent value in the last 12 months), BP (systolic and diastolic mean value in the last 12 months); and data on prescribed glucose-lowering, lipid-lowering, antihypertensive and antithrombotic medications. This information will be supplemented with data registered in the Conjunto Mínimo Básico de Datos de Altas Hospitalarias (Set of Minimum Basic Data Set of Hospital Admissions),[24] which records all admissions to public and private hospitals in the region and contains information on diagnostics, procedures and discharge reports.

With this additional longitudinal approach, we will be able to obtain the patient's data on progression to overt diabetes and/or initiation of antidiabetic treatment over time (if directly related to diabetes). This is important because besides the annualised incidence rate of progression to diabetes, the time course progression of dysglycaemia has not been studied at large. From the few available studies, the mode of onset of diabetes in subjects with pre-diabetes follows a non-linear pattern, with a rapid rather than gradual onset of diabetes over a 3-year time.[25] Moreover, we will be able to obtain data on the incidence of other diabetes-associated chronic conditions also present at the pre-diabetes stages, such as nephropathy, neuropathy or retinopathy; the incidence of macrovascular complications over time; the likelihood of initiation of hypoglycaemic agents among progressors; rates and cause of hospitalisations; and overall mortality.

This study was approved by the Ethics Committee of the Primary Healthcare University Research Institute (Institut d'Investigació en Atenció Primària, IDIAP) Jordi Gol (P12/043), and all patients signed a written informed consent form prior to participation. The study was conducted in accordance with the Declaration of Helsinki (1964).

FINDINGS TO DATE

The Mollerussa study completed its recruitment phase between August 2011 and July 2014, and the 12-month short-term follow-up in July 2015. The enlisted sample (n=602) was different from the eligible but not enlisted population (n=784) in terms of gender and age (table 1); the enlisted sample had a significantly higher proportion of women (58.6% vs 44.0%; p<0.001), and was older (mean age 48.1 years vs 45.7 years; p<0.001).

Demographic characteristics of participants who provided a blood sample during the first phase (baseline visit) are summarised in table 2.

After the first phase, the rate of missing data was less than 5% across variables of interest (table 3), which is far below the 20% maximum recommended lost to follow-up

Table 1 Age and gender characteristics between subjects eligible but not enlisted and subjects eventually enlisted in the Mollerussa cohort

	Eligible but not enlisted			Enlisted (n=602)
	Failed to be contacted (n=448)	Declined to participate (n=349)	All (n=797)	
Gender (female), n (%)	181 (40.4)	170 (48.7)	351 (44.0)	353 (58.6)*
Age, years, mean (SD)	44 (15.0)	47.9 (16.3)	45.7 (15.7)	48.1 (13.4)†
Age group, years, n (%)				
<40	218 (48.7)	140 (40.1)	358 (44.9)	168 (28.2)
40–60	160 (35.7)	125 (35.8)	285 (35.8)	315 (52.9)
>60	70 (15.6)	84 (24.1)	154 (19.3)	113 (19.0)

*χ^2 test, p<0.001.
†Kruskal-Wallis non-parametric test, p<0.001.

Table 2 Demographic characteristics of study population enrolled in the Mollerussa cohort

Characteristic	Total valid N	Statistic
Gender, women, n (%)	594	347 (58.4)
Age, years, mean (SD)	594	50.6 (13.3)
Weight, kg, mean (SD)	574	73.1 (14.5)
Waist, cm, mean (SD)	573	94.2 (12.5)
Body mass index, kg/m^2, mean (SD)	573	26.3 (4.7)
<25.0, n (%)		236 (41.2)
25.0–29.9, n (%)		235 (41.0)
≥30.0, n (%)		102 (17.8)
Education level, n (%)	575	
Not even primary school		24 (4.2)
Completed primary school		122 (21.2)
Secondary/high school		366 (63.7)
Graduate or higher		63 (11)
Work activity, n (%)	572	
Employed		393 (68.7)
Unemployed		65 (11.4)
Disability		12 (2.1)
Retired		102 (17.8)
Hypertension, n (%)	571	102 (17.9)
Dyslipidaemia, n (%)	571	131 (22.9)
Hypertriglyceridaemia, n (%)	554	22 (4.0)
Smokers, n (%)	594	
Current		152 (25.6)
Former		148 (24.9)
Alcohol consumption, n (%)	573	286 (49.9)

rate in a cohort study.[26] Twelve months after the baseline screening, we obtained a second blood draw from 89.6% of subjects found to have altered glycaemia levels at baseline (n=193 excluding undiagnosed diabetes), a retention rate also indicating acceptable validity of the results (table 3; figure 1).

Table 3 Summary of missing data for variables recorded during the first and second phase of the Mollerussa cohort

Variable of interest	Missing data, n (%)
First phase (baseline screening); n=594	
No clinical interview	17 (2.9)
No nutritional survey	28 (4.7)
No carotid echography	17 (2.9)
No laboratory results	0 (0.0)
No HbA1c measurement	10 (1.7)
No sample for biobank	22 (3.7)
Second phase (12 months follow-up); n=193	
No laboratory results	20 (10.4)

HbA1c, glycated haemoglobin.

We are currently in the phase of longitudinal follow-up of all subjects with subjects identified during the first and second phase (figure 1), and developing protocols for the analyses to explore hypotheses on different features of the epidemiology and natural history of pre-diabetes in our primary care setting. The first paper from the Mollerussa project (now in preparation) will describe results derived from the short-term follow-up of the cohort, namely the prevalence of pre-diabetes and undiagnosed diabetes, the clinical and demographic profile of patients with pre-diabetes versus those with normal glycaemic levels, a first estimation of the annual incidence of overt diabetes among subjects with pre-diabetes and the metabolic, CV and lifestyle disease-associated conditions.

STRENGTHS AND LIMITATIONS

The main strength of the Mollerussa cohort is that it includes adult patients from primary care health centres prospectively collected, and the opportunity to follow participants in the long term through healthcare electronic registries, which ensures that it will be of use for multiple future research projects. The combined short-term prospective and long-term longitudinal design has both advantages and limitations.

The prospective phase (baseline screening and 12-month short-term follow-up) prevents recall bias because the risk for diabetes was assessed before the onset of the disease, and the measurement of events in a temporal sequence allows for causes to be distinguished from effects. However, we must acknowledge a potential selection bias, since we had higher rates of women and middle-aged subjects among enlisted people than among eligible but not enlisted subjects. The influence of this potential bias will be minimised through a weighting process on the prevalence estimates, although how this original unbalance may impact the results is not clear, because IFG and HbA1c detect different categories of individuals as being at risk: IFG is substantially more common among men, and its prevalence tends to plateau in middle age, while the prevalence of pre-diabetes using HbA1c increases with age (maximum peak in those aged 60–74 years) but does not differ by gender.[27 28] Finally, we did not perform an OGTT among enrolled individuals. Although IGT is more common than IFG in most populations, it is more sensitive but slightly less specific for identifying people who will develop diabetes.[29] Additionally, the OGTT has low reproducibility and it is inconvenient in terms of costs and time consumption.[30] Conversely, HbA1c measurement is cost-effective and improves the sensitivity of FPG in the detection of early T2DM in high-risk individuals.[28 31]

On the other hand, the longitudinal, long-term phase has the advantage that cohort membership is not dependent on continuing to visit the practice from which the members were recruited. While the main strength is that

this will minimise losses to follow-up, the primary limitation is that it will rely on data that may be incomplete or inconsistently measured between subjects. An additional advantage of this design is that, since the latency from pre-diabetes to overt diabetes may be longer than the initial 12 months follow-up,[25] the long-term follow-up will allow a more accurate estimation of the time trends (cumulative incidences) and clinical features associated with progression to diabetes.

COLLABORATION

The Mollerussa study is open to future joint studies with external study groups. Investigators with an interest in hypotheses related to pre-diabetes are welcome to contact a member of the Institut Universitari d'Investigació en Atenció Primària Jordi Gol (IDIAP Jordi Gol) to submit a joint study proposal to the Scientific Committee of the institution. The group will consider these proposals if they are in accordance with the study objectives, and do not overlap with other studies already under way. If accepted, a formal written agreement will be established with the collaborative group.

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Correction notice This paper has been amended since it was published Online First. Owing to a scripting error, some of the publisher names in the references were replaced with 'BMJ Publishing Group'. This only affected the full text version, not the PDF. We have since corrected theseerrors and the correct publishers have been inserted into the references.

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Contributors MF, JRM and DM conceived and designed the study; MBV, JF-N, and AM participated in the study design; MBV, MF, ER, NA, MG-C, NM and AM collected the data; EC built and managed the database; JRM managed the database, contributed to data cleaning, performed the statistical analyses and contributed to interpretation of the data; MBV, DM, JF-N and MM-C wrote the manuscript. All authors critically reviewed the manuscript and approved the final version to be published.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethics Committee of the Primary Health Care University Research Institute (IDIAP) Jordi Gol (P12/043).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement This article describes the establishment of a longitudinal cohort and early findings, and full results will be submitted for peer-reviewed publication in due course. The authors are willing to share unpublished data with interested parties upon request because they contain identifying human information and are unsuitable for public deposition. Requests may be made to the corresponding author (didacmauricio@gmail.com).

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Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	#3	State specific objectives, including any prespecified hypotheses	4
Methods			

Study design	#4	Present key elements of study design early in the paper	4
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	4-5
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	4-5
Bias	#9	Describe any efforts to address potential sources of bias	6
Study size	#10	Explain how the study size was arrived at	6
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	6
Statistical methods	#12c	Explain how missing data were addressed	5
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	n/a
Statistical methods	#12e	Describe any sensitivity analyses	6

Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	6-7
Participants	#13b	Give reasons for non-participation at each stage	n/a
Participants	#13c	Consider use of a flow diagram	7
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	7-8
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	5
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	5
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	6-7
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6-7
Main results	#16b	Report category boundaries when continuous variables were categorized	7
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	7, 9,10
Discussion			
Key results	#18	Summarise key results with reference to study objectives	10-11

Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	13
Generalisability	#21	Discuss the generalisability (external validity) of the study results	n/a
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

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Prevalence of prediabetes and undiagnosed diabetes in the Mollerussa prospective observational cohort study in a semi-rural area of Catalonia

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ABSTRACT

Objectives: To assess the prevalence of undiagnosed diabetes and prediabetes in the healthy population in the Mollerussa cohort. As a secondary objective, to identify the variables associated with these conditions and to describe the changes in glycaemic status after one year of follow-up in subjects with prediabetes.

Design: Prospective observational cohort study.

Setting: General population from a semi-rural area.

Participants: The study included 583 participants without a diagnosis of diabetes recruited between March 2011 and July 2014.

Results: The prevalence of undiagnosed diabetes was 20, 3.4% (95% confidence interval 2.6 - 4.2) and that of prediabetes was 229, 39.3% (37.3 - 41.3). Among those with prediabetes, 18.3% had

isolated impaired fasting plasma glucose (FPG) (FPG: 100 to <126 mg/dL), 58.1% had isolated impaired HbA1c (HbA1c 5.7 - <6.5), and 23.6% fulfilled both criteria. Follow-up data were available for 166 subjects; 41.6% (37.8 - 45.4) returned to normoglycaemia, 57.6% (57.8 - 61.4) persisted in prediabetes, and 0.6% (0 -1.2) progressed to diabetes. Individuals with prediabetes had worse cardiometabolic risk profiles and sociodemographic features than normoglycaemic subjects. In the logistic regression model, variables significantly associated with prediabetes were older age (odds ratio; 95% confidence interval) (1.033; 1.011-1.056), higher physical activity (0.546; 0.360 - 0.827), body mass index (1.121; 1.029 - 1.222), and a family history of diabetes (1.543; 1.025 - 2.323). The variables significantly associated with glycaemic normalization were older age (0.948; 0.916 - 0.982) and body mass index (0.779; 0.651 - 0.931).

Conclusions: Among adults in our region, the estimated prevalence of undiagnosed diabetes was 3.4% and that of prediabetes was 39.3%. After a one-year follow-up, a small proportion of subjects (0.6%) with prediabetes progressed to diabetes, while a high proportion (41.6%) returned to normoglycaemia. Individuals with prediabetes who returned to normoglycaemia were younger and had a lower body mass index.

KEYWORDS

Prediabetes, undiagnosed diabetes, prediabetes prevalence

ARTICLE SUMMARY

Strengths and Limitations

- This was a population-based study of a small cohort that included a representative sample of a non-previously studied population of a semi-rural area in Catalonia.
- We did not perform an oral glucose tolerance test, which is a common test in most studies but is a time-consuming and expensive procedure.
- The percentage of glucose normalization among prediabetic subjects was higher than expected compared to the percentages described in previous studies.
- The small number of cases of undiagnosed diabetes precluded further statistical analyses on this topic.

BACKGROUND

Diabetes mellitus, a public health concern with an increasing incidence worldwide, is a great threat to general health and is leading to increased morbidity and mortality. These effects are mainly occurring because diabetes is a disorder of glucose metabolism that affects multiple organ systems and is associated with various micro- and macro-vascular complications and several nonvascular complications. Additionally, a large group of subjects do not fulfil the diabetes criteria but have intermediate glycaemic variables, between normal and diabetes, and are thus classified as having prediabetes. One of the most commonly used definitions of prediabetes is that of the 2010 American Diabetes Association (ADA) criteria[1, 2]: (a) impaired fasting plasma glucose (IFG), defined as fasting plasma glucose (FPG) between 100 and <126 mg/dL (5.6–5.9 mmol/L); (b) impaired glucose tolerance (IGT), defined as a 2-hour plasma glucose value after a 75 g oral glucose tolerance test (OGTT) between 140 and <200 mg/dL (7.8–11.0 mmol/L); or (c) glycated haemoglobin (HbA1c) levels between 5.7% and < 6.5% (39–46 mmol/mol).

Prediabetes is becoming increasingly important as it represents a high risk of developing type 2 diabetes (T2D) and cardiovascular diseases.[2, 3] Moreover, individuals with prediabetes are phenotypically quite similar to patients with T2D. That is, they tend to be older, with a higher body mass index (BMI) and higher blood pressure than people with normal glucose tolerance; in addition, they tend to have insulin resistance and dyslipidaemia.[4] Additionally, multiple risk factors, such as family history, gestational diabetes, and certain ethnicities as well as combined risk factors such as metabolic syndrome, are known to predispose subjects to a higher risk for prediabetes and its progression to T2D.[5] Based only on impaired glucose tolerance (IGT), the worldwide prevalence of prediabetes among adults has been estimated by the International Diabetes Federation to be 7.3% in 2017, with half of these individuals (49%) being younger than 50 years.[6] The National Diabetes Statistics Report in the United States reported that the total crude prevalence of diabetes was 9.4% (30.3 million, 2017 US population), with 23.8% undiagnosed and an additional 33.9% with prediabetes.[7]

In Spain, according to data from the Di@bet.es study, based on OGTT, FPG and HbA1c, 13.8% of the adult population, adjusted for age and sex, had diabetes, and of these individuals up to 6% had undiagnosed diabetes. Furthermore, an additional 14.8% of individuals presented with some type of prediabetic state, 3.4% based on IFG, 9.2% based on IGT and 2.2% with disturbances in both, after adjusting for age and sex.[8, 9] According to the ADA, up to 70% of people with prediabetes will develop overt diabetes throughout their lives.[10, 11] Moreover, each year, 5-10% of subjects

with prediabetes will eventually develop overt diabetes, and according to some studies, this percentage can reach up to 18% per year; however, this rate may vary with the definition of prediabetes and population characteristics.[12-15] It has been shown that over 3-5 years, approximately 25% of subjects progress to T2D, 25% return to a normal state of glucose tolerance and 50% remain in the prediabetic state.[16] Thus, the early diagnosis and screening of prediabetes are essential steps towards the prevention of its progression or at least the delay of the onset of T2D.

The primary aim of this study was to assess the prevalence of undiagnosed diabetes and prediabetes in the healthy population in the Mollerussa cohort. As a secondary objective, we aimed to assess the variables associated with these conditions and to describe the changes in glycaemic status after one year of follow-up in subjects with prediabetes.

METHODS

Subjects

This was a prospective population-based cohort study from the semi-rural area of Mollerussa in Catalonia (northeast Spain) selected between March 2011 and July 2014. The description of the cohort and the procedures performed were initially published as a cohort profile.[17] Briefly, the database of the Catalan Health Institute (ICS) through its Primary Care Electronic Clinical Station (Estació Clínica Electronica d'Atenció Primaria –eCAP) was used to select the population sample. All population is passively included in the Primary Care Electronic Clinical record according to the Spanish health system, which is based on the principles of universality, free access, equity and fairness of financing.[18] Then, from a total population of 24,666 potentially eligible individuals in the health-care area (subjects older than 25 years and attending any Primary Healthcare Centre in the same health area), 2,226 subjects were randomly selected using a randomiser programme (SPSS software V.16.0 for Windows; SPSS), following the principles of simple random sampling, and were then invited to participate by telephone contact. Based on their willingness to join the study, exclusion criteria, consent and baseline laboratory data, 594 subjects aged ≥ 25 years were finally included.[17] The exclusion criteria included a previous diagnosis of diabetes (type 1 diabetes (T1D), T2D or any specific subtype of diabetes), treatment with oral antidiabetic drugs or the use of metformin for other conditions. In addition, subjects with cardiovascular disease (heart disease, heart failure, aortic stenosis), cancer, kidney disease, anaemia, hepatitis, gastrointestinal diseases, recent abdominal surgery, chronic pulmonary obstructive disease, chronic infectious

diseases, use of systemic glucocorticoids or beta blockers or major psychiatric disorders with psychotic symptoms were excluded from the study. Subjects were considered to have hypertension or dyslipidaemia if they were using anti-hypertensive or lipid-lowering agents. Prediabetes was defined as any of the following abnormal glycaemic variables: FPG 100 to <126 mg/dL or HbA1c 5.7 to <6.5%; diabetes was defined as FPG >125 mg/dL or HbA1c ≥6.5%. Normal glycaemic status was defined by FPG <100 mg/dL and HbA1c <5.7 according to the 2010 ADA criteria.[1] Eleven subjects without baseline HbA1c or FPG measurements were excluded. Subjects with prediabetes at baseline (n=229) underwent a second visit 12 months after the baseline visit, and 166 (72.5%) of them had relevant information at follow up.

A fasting blood sample was taken to determine glucose, HbA1c, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, renal function, and other parameters following standard protocols.[17] The fatty liver index (FLI) was calculated with the equation developed by Bedogni *et al*. [19] Insulin resistance was calculated by the homeostatic model assessment (HOMA2-IR); beta cell function (HOMA2-ß) and insulin sensitivity (HOMA2-S) data were calculated with a HOMA2 calculator released by the Diabetes Trials Unit, University of Oxford: HOMA Calculator. This calculator is available at: http://www.dtu.ox.ac.uk/homacalculator/ (updated October 11, 2017). [20] The estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration equation.[21]

Sociodemographic variables were recorded by researchers following a protocol for the inclusion of patients using a standardized baseline questionnaire during the clinical interview. In all cases a physical examination (including weight, height, blood pressure and waist circumference) was carried out by trained research staff. Education level and physical activity were assessed according to the International Standard Classification of Education[22] and the Spanish-validated International Physical Activity Questionnaire,[23] respectively. We classified the education level as low level (studied until primary school) and high level (secondary high school education or higher). Physical activity was classified as sedentary or active (not regularly versus regularly active).

Ethical approval

The study protocol was approved by the Ethics Committee of the Primary Health Care University Research Institute (IDIAP) Jordi Gol (P12/043) and was conducted following the Declaration of Helsinki. All study participants signed an informed consent form.

Sample size

The sample size was determined based on an estimated prediabetes prevalence of 35.5% and 38% using HbA1c levels and the 2010 ADA criteria, respectively.[1, 24, 25] It was estimated that a

random sample of 505 subjects was sufficient to assess an estimated prevalence of approximately 30% with a 95% CI and an error of ±4%.[17]

Statistical methods

Descriptive statistics of the mean (standard deviation) or median [interquartile range] were estimated for quantitative variables with a normal or non-normal distribution, respectively. Qualitative variables were assessed using absolute and relative frequencies. Normally distributed data were analysed using the Shapiro-Wilk test. Comparisons between groups of all variables were performed to evaluate the differences. Student's t-test, ANOVA, the Mann-Whitney test, or the Kruskal-Wallis test were used to assess the differences between groups. The chi-squared test or Fisher's exact test were used to determine differences in qualitative variables. Tukey's correction was applied to account for multiple tests. Multivariate logistic regression models were used to determine the association of variables with prediabetes, isolated FPG, isolated HbA1c and both FPG and HbA1c at baseline and were performed using the enter method with covariables that were clinically or statistically associated. In the prediabetes model, the variables used were age, sex, education level, physical activity, DLP, HT, family history of diabetes, BMI, waist, glomerular filtration rate and fatty liver index. A backward conditional logistic regression model was used to predict the normalization of the glycaemic state; in all models, the goodness-of-fit assumption was tested by the Hosmer-Lemeshow test. The predictive accuracy of the logistic regression model for normalization was checked by receiver-operating characteristic (ROC) curves and the area under the ROC curve (AUC_{ROC}). Odds ratios with corresponding 95% confidence intervals are shown, and statistical significance was established as a p-value <0.05. Data management and all analyses were performed using R statistical software, version 3.3.1, and SPSS software (version 22, IBM, SPSS, Chicago, Illinois, USA).

Patient and Public Involvement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

RESULTS

Out of the 594 individuals recruited, complete data on FPG and HbA1c were available from 583 (98.1%). The prevalence of undiagnosed diabetes was 20 subjects, 3.4% (95% confidence interval: 2.7-4.2), and the prevalence of prediabetes was 229 subjects, 39.3% (37.3-41.3). Furthermore, the

prevalence based on isolated FPG was 7.2%, and that based on isolated HbA1c was 22.8%, while based on the criteria of both FPG and HbA1c, the prevalence was 9.3% (Figure 1).

The differences of clinical and sociodemographic characteristics between normoglycaemic with prediabetic and diabetic groups are shown in Table 1. Except for sex, family history of diabetes, current smoking status, alcohol consumption status, triglycerides and high density lipoprotein (HDL)-cholesterol levels, there were significant differences in the majority of parameters, including age and BMI, between the three groups.

We observed an association in age, BMI, waist circumference, systolic and diastolic blood pressure (SBP and DBP), alcohol consumption status, hypertension, dyslipidaemia, triglycerides, total cholesterol, low density lipoprotein (LDL)-cholesterol, insulin test, FLI, and HOMA2-IR, which were higher in individuals with prediabetes than in individuals with normoglycaemia and were higher in the diabetic group than in the prediabetic group. On the other hand, physical activity, education level, eGFR, HOMA2-ß and HOMA2-S exhibited a negative trend between the same groups. In the prediabetic group, 41.9% had impaired FPG and 81.7% had impaired HbA1c. On the other hand, among the newly identified diabetic subjects, up to 80% met the FPG criteria and 85% met the HbA1c criteria. The prevalence of prediabetes increased with increasing age, with percentages of 17.4%, 28.6%, 46.4%, 50 and 52.9% in participants aged <35 years, 36-45 years, 46-55 years, 56-65 years and >65 years, respectively. Regarding BMI categories of normal weight (BMI <25 kg/m^2), overweight (BMI 25-29.9 kg/m^2), and obese (BMI>30 kg/m^2), the prevalence of prediabetes was 29%, 45.9%, and 49%, respectively (Supplementary file 1 Figure 1).

Table 1. Clinical and sociodemographic differences among glycaemic status groups of the Mollerussa cohort.

	Normoglycaemia FPG <100 mg/dL and HbA1c <5.7%	**Prediabetes** FPG 100 to 125 mg/dL, or HbA1c 5.7 to 6.4%	**Diabetes** FPG >125 mg/dL or HbA1c ≥6.5%	**Difference normoglycaemia vs. prediabetes**	**Difference normoglycaemia vs. diabetes**
N	334	229	20	-	-
Sex, women	193 (57.8%)	135 (59.0%)	13 (65.0%)	0.6 [-7.7- 8.8]	6.6 (-14.9-28.2)
Age, years	45.0 [37.0;55.8]	54.0 [46.0;62.0]	62.0 [53.2;69.5]	7.5 [5.4 - 9.6]	14.2 (8.4- 19.9)
BMI, Kg/m^2	25.0 [22.5;27.3]	26.4 [24.8;29.7]	30.9 [26.4;35.6]	2.1 [1.4- 2.9]	4.9 (2.9- 6.9]
BMI categories					
Normal weight	160 (50.0%)	67 (30.2%)	4 (20.0%)	-18.6 [-26.6- -10.7]	-27.9 (-46.2- -9.6]
Overweight	120 (37.5%)	106 (47.7%)	5 (25.0%)	10.4 [2.1-18.6]	10.9[-30.6-8.7]
Obesity	40 (12.5%)	49 (22.1%)	11 (55.0%)	9.4 [3.1- 15.8]	43.0 (20.9-65.1]
Waist, cm	93.0 [84.0;100]	97.0 [89.0;104]	100 [91.0;108]	5.1 [2.9- 7.2]	9.3 [3.8- 14.9]
SBP, mm Hg	119 [109;128]	125 [116;136]	132 [114;144]	6.7 [3.9- 9.5]	10.9 [3.5- 18.5]

DBP, mm Hg	75.0 [69.0;82.0]	78.0 [71.0;85.0]	79.0 [72.5;86.0]	2.5 [0.8- 4.2]	2.3 [-2.2- 6.8]
Hypertension	37 (11.1%)	49 (21.4%)	9 (45.0%)	10.3 [4.0- 16.6]	33.9 [11.9- 55.9]
Dyslipidaemia	27 (8.08%)	39 (17.0%)	5 (25.0%)	8.9 [3.2- 14.6]	16.9 [-2.3- 36.1]
Family history DM	94 (29.6%)	78 (37.0%)	8 (42.1%)	5.9 [-1.9- 13.7]	11.8 [-10.1- 33.9]
Education, high level	265 (82.6%)	145 (65.0%)	11 (55.0%)	-16.0 [-23.6- -8.4]	-24.3 [-46.6- -2.1]
Physical activity	243 (75.9%)	141 (63.2%)	10 (50.0%)	-11.2 [-19.1- -3.3]	-22.7 [-45.2- 0.3]
Current smoker	82 (24.6%)	63 (27.5%)	3 (15.0%)	3.0 [-4.4- 10.4]	-9.5 [-25.9- 6.8]
Alcohol, g/day	2.84 [0.00;10.6]	3.42 [0.04;15.9]	7.04 [1.42;11.5]	4.0 [0.9- 6.9]	2.2 [-5.8- 10.3]
FPG, mg/dL	87.0 [82.0;92.0]	97.0 [89.0;106]	126 [110;131]	10.4 [8.8- 11.9]	32.6 [28.4- 36.8]
HbA1c, %	5.30 [5.10;5.50]	5.80 [5.70;6.00]	6.50 [6.07;6.62]	0.6 [0.5- 0.6]	1.0 [0.9- 1.1]
HbA1c, mmol/mol	34.4 [32.2;36.6]	39.9 [38.8;42.1]	47.5 [42.9;48.9]	6.1 [5.6- 6.6]	11.1 [9.7- 12.5]
eGFR mL/min/1.73m^2	97.5 [87.7;106]	91.5 [78.6;102]	89.0 [68.0;101]	-6.2 [-8.5- -3.7]	-11.2 [-17.9- -4.4]
Triglycerides, mg/dL	86.0 [65.0;119]	89.0 [72.0;132]	112 [70.8;161]	6.6 [-6.8- 19.9]	11.2 [-24.7- 47.1]
T-cholesterol, g/dL	194 [169;225]	202 [184;226]	216 [187;244]	7.9 [1.9- 14.0]	16.9 [0.7- 33.1]
HDL, mg/dL	57.0 [48.0;68.0]	57.0 [50.0;68.0]	64.0 [49.8;78.0]	0.1 [-2.4- 2.6]	5.9 [-0.8- 12.6]
LDL, mg/dL	116 [95.8;140]	125 [106;146]	125 [111;150]	6.3 [1.1- 11.4]	7.5 [-6.3- 21.4]
Insulin, µU/mL	7.10 [5.30;9.70]	8.40 [6.60;11.8]	12.6 [9.25;15.6]	2.1 [1.2- 3.0]	8.2 [5.8- 10.7]
Fatty Liver Index	26.4 [10.9;53.5]	40.4 [18.9;68.2]	61.5 [37.9;92.0]	10.3 [5.5- 15.0]	25.4 [12.8- 38.0]
HOMA2-ß	100 [82.0;124]	93.3 [73.0;115]	80.2 [61.1;97.9]	-6.3 [-11.8- -0.8]	-14.9 [-29.7- -0.2]
HOMA2-S	110 [80.1;145]	87.7 [64.4;112]	59.1 [46.0;80.9]	-23.8 [-32.0 - -15.6]	-54.4 [-76.3- -32.6]
HOMA2-IR	0.90 [0.70;1.20]	1.10 [0.90;1.60]	1.70 [1.28;2.20]	0.3 [0.2 -0.4]	1.1 [0.8 – 1.4]

Median [interquartile range] and n (%). NG, normoglycaemia; PD, prediabetes; DM, diabetes; Ed level, education level; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; T-cholesterol, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA2-IR, Homeostatic Model Assessment-2_Insulin Resistance; HOMA2-ß, Homeostatic Model Assessment-2 Beta cell function; HOMA2-S, Homeostatic Model Assessment-2 Insulin Sensitivity.

Table 2 shows the characteristics of prediabetic individuals by glycaemic state: isolated FPG, isolated HbA1c and both altered FPG and HbA1c. Thus, among the 229 subjects with prediabetes, 42 (18.3%) had abnormal isolated FPG, 133 (58.1%) had abnormal isolated HbA1c, and 54 (23.6%) had both abnormal FPG and HbA1c. Patients with both abnormal FPG and HbA1c were older, had larger waist circumferences, had increased FLI and HOMA2-IR, were more likely to be overweight or obese and have hypertension, and had lower HOMA2-S. The isolated FPG group had a higher proportion of subjects with a family history of diabetes, higher alcohol consumption, higher levels of total cholesterol and LDL-cholesterol and lower levels of HDL-cholesterol, although none of these differences were statistically significant. Finally, the isolated HbA1c group had an elevated HOMA2-ß. Although there were no statistically significant differences, the proportion of men was higher in the isolated FPG group, whereas the proportion of women was higher in the isolated HbA1c and both FPG and HbA1c groups. Among the three groups, no statistically significant differences were found regarding the following variables: sex, dyslipidaemia, family history of

diabetes, education level, physical activity, current smoking status, alcohol consumption, triglycerides, total cholesterol, HDL-cholesterol or LDL-cholesterol.

Table 2. Clinical and sociodemographic characteristics by glycaemic status of the individuals with prediabetes.

	Impaired HbA1c 5.7%-6.4%	Impaired FPG 100-125 mg/dL	HbA1c 5.7%-6.4% and FPG 100-125 mg/dL	*p* overall	*p* HbA1c vs. FPG	*p* HbA1c vs. Both	*p*. FPG vs. Both
N	133	42	54	-	-	-	-
Sex, Women	84 (63.2%)	19 (45.2%)	32 (59.3%)	0.12	0.181	0.74	0.369
Age, years	53.4 (12.4)	50.6 (11.8)	60.6 (10.5)	**<0.001**	0.388	**0.001**	**<0.001**
BMI, Kg/m2	25.8 [24.5;28.9]	27.8 [24.5;30.6]	27.5 [25.6;30.5]	0.056	0.534	**0.036**	0.534
BMI categories				**0.018**	0.107	**0.05**	**0.032**
Normal weight	43 (33.1%)	16 (41.0%)	8 (15.1%)				
Overweight	64 (49.2%)	12 (30.8%)	30 (56.6%)				
Obesity	23 (17.7%)	11 (28.2%)	15 (28.3%)				
Waist, cm	95.0 [88.0;102]	98.0 [90.0;106]	101 [95.0;107]	**0.008**	0.232	**0.006**	0.333
SBP, mm Hg	124 (16.1)	129 (15.5)	128 (18.4)	0.169	0.296	0.29	0.991
DBP, mm Hg	78.0 (9.44)	79.5 (12.0)	77.9 (9.39)	0.674	0.675	0.999	0.723
Hypertension	21 (15.8%)	9 (21.4%)	19 (35.2%)	**0.014**	0.542	**0.019**	0.32
Dyslipidaemia	25 (18.8%)	4 (9.52%)	10 (18.5%)	0.358	0.515	1	0.515
Family history DM	43 (34.1%)	18 (48.6%)	17 (35.4%)	0.265	0.471	1	0.471
Education, high level	91 (69.5%)	23 (59.0%)	31 (58.5%)	0.252	0.455	0.455	1
Physical activity	88 (67.2%)	21 (53.8%)	32 (60.4%)	0.281	0.547	0.68	0.68
Current smoker	38 (28.6%)	14 (33.3%)	11 (20.4%)	0.338	0.693	0.496	0.496
Alcohol, g/day	2.92 [0.00;15.2]	7.42 [0.90;16.3]	1.53 [0.00;17.9]	0.369	0.336	0.735	0.336
FPG, mg/dL	89.2 (6.89)	106 (4.97)	109 (5.96)	**<0.001**	**<0.001**	**<0.001**	0.14
HbA1c, %	5.80 [5.70;6.00]	5.40 [5.40;5.57]	5.95 [5.80;6.10]	**<0.001**	**<0.001**	**<0.001**	**<0.001**
HbA1c, mmol/mol	39.9 [38.8;42.1]	35.5 [34.7;37.4]	41.5 [39.9;43.2]	**<0.001**	**<0.001**	**<0.001**	**<0.001**
eGFR, mL/min/1.73m^2	93.6 [79.6;103]	93.2 [79.7;107]	89.3 [73.1;97.2]	0.076	0.556	0.073	0.073
Triglycerides, mg/dL	88.0 [72.0;134]	86.5 [67.0;130]	106 [74.5;132]	0.332	0.729	0.304	0.304
Total cholesterol, mg/dL	205 (34.5)	209 (28.6)	203 (29.8)	0.689	0.767	0.947	0.677
HDL-cholesterol, mg/dL	58.0 [51.0;69.0]	52.0 [45.0;65.8]	57.0 [51.0;66.0]	0.128	0.141	0.755	0.18
LDL-cholesterol, mg/dL	125 (32.2)	133 (25.5)	120 (23.5)	0.114	0.278	0.593	0.096
Insulin, µU/mL	8.00 [6.10;10.0]	9.90 [6.90;15.9]	10.9 [7.90;15.6]	**<0.001**	**0.01**	**<0.001**	0.577
Fatty Liver Index	34.4 [16.9;59.2]	42.2 [17.7;73.6]	53.8 [32.2;73.0]	**0.016**	0.373	**0.011**	0.378
HOMA2-ß	96.6 [81.5;122]	81.7 [64.5;118]	82.8 [63.0;108]	**0.001**	**0.034**	**0.002**	0.693
HOMA2-S	98.0 [77.2;127]	75.0 [47.2;107]	67.5 [47.5;91.3]	**<0.001**	**0.003**	**<0.001**	0.564
HOMA2-IR	1.00 [0.80;1.30]	1.30 [0.90;2.15]	1.50 [1.10;2.10]	**<0.001**	**0.005**	**<0.001**	0.545

Significant values are shown in bold. Mean (SD), median [interquartile range] and n (%). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA2-IR, Homeostatic Model Assessment-2_Insulin Resistance; HOMA2-ß, Homeostatic Model Assessment-2 Beta cell function; HOMA2-S, Homeostatic Model Assessment-2 Insulin Sensitivity.

Prediabetes follow-up

Of the 229 individuals with prediabetes at baseline, 166 (72.5%) had clinical and laboratory data after 12 months of follow-up. Of them, 52 (41.6%) returned to a normal glycaemic status, 112 (57.6%) persisted in their state of prediabetes, and only 2 (0.6%) progressed to diabetes. Table 3 shows the outcome of the follow-up of the isolated FPG, HbA1c and both FPG and HbA1c groups.

Table 3. Outcomes at follow-up of patients with different altered glucose metabolism statuses at baseline.

Variables	Baseline	N with follow-up	Follow up		
			Normalized	Persisted	Progressed
Prediabetes	229 (39.3%)	166 (90.7%)	52 (41.6%)	112 (57.8%)	2 (0.6%)
Isolated FPG	42 (7.2%)	3 (1.8%)	1 (33.3%)	1 (33.3%)	1 (33.3%)
Isolated HbA1c	133 (22.8%)	114 (68.7%)	47 (41.3%)	67 (58.7%)	0 (0%)
Both altered	54 (9.3%)	49 (29.5%)	4 (8.2%)	44 (89.8%)	1 (2%)

FPG, fasting plasma glucose

Association of prediabetes with glycaemic status

The multivariate logistic regression model of prediabetes *versus* normoglycaemia showed that the variables associated with prediabetes were older age (odds ratio; 95% confidence interval) (1.033; 1.011-1.056), higher physical activity levels (0.546; 0.360-0.827), higher BMI (1.121; 1.029-1.222), and a family history of diabetes (1.543; 1.025-2.323) (Figure 2a). The models for isolated FPG alterations, isolated HbA1c alterations and both FPG and HbA1c alterations are shown in Supplementary file 2 Tables 1, 2 and 3, respectively. The variables associated with isolated FPG were older age (1.032; 1.008-1.057), higher physical activity levels (0.535; 0.318-0.899), and a family history of diabetes (1.798; 1.067-3.028). On the other hand, the only variable associated with impaired HbA1c was older age (1.048; 1.029-1.067). Finally, in the model for altered FPG and HbA1c, the variables associated were older age (1.056; 1.026-1.086) and high FLI (1.031; 1.002-1.061).

Prediction of normalization

Backward conditional logistic regression, as described in the methods section, starting with the variables age, sex, waist circumference, BMI, hypertension, physical activity, family history of diabetes, education level, total cholesterol, HDL-cholesterol, FLI and HOMA2-IR, was performed to identify factors independently associated with the prediction of glycaemic status normalization (Supplementary file 2 Table 4). The variables that predicted glycaemic normalization were older

age (0.948; 0.916-0.982) and BMI (0.779; 0.651-0.931) (Figure 2b); this model had a good predictive ability (AUC_{ROC} 0.77; p<0.001) (Supplementary file 3 Figure 2).

DISCUSSION

We found that the prevalence of undiagnosed diabetes was 3.4%, and the prevalence of prediabetes was 39.3% in this semi-rural population in Catalonia (northeast Spain). The prevalence of prediabetes was three-fold higher based on HbA1c than that based on FPG. Subjects with prediabetes defined by both HbA1c and FPG criteria had unfavourable clinical and sociodemographic profiles related to increased cardiovascular risk. These factors were older age; abdominal obesity; higher triglycerides; increased FLI; and a higher proportion of overweight, obesity and hypertension. In our population, age was the variable most strongly associated with prediabetes based on all specific glycaemic status variables: isolated impaired FPG, isolated impaired HbA1c or both impaired FPG and HbA1c. Other variables associated with prediabetes were lower physical activity levels, a family history of diabetes, and obesity. Finally, the characteristics related to normalization at follow-up were younger age and lower BMI.

The prevalence of prediabetes and undiagnosed diabetes in our healthy population were within the ranges found in other population studies defining prediabetes based on the 2010 ADA criteria, using FPG and/or HbA1c. Among these studies, a large national Chinese study (with 170,287 subjects) showed a prevalence of prediabetes of 35.7% and a prevalence of undiagnosed diabetes of 6.9%.[26] In a study of the Caribbean population, the corresponding figures were 44.1% for prediabetes and 7.3% for undiagnosed diabetes.[27] In England, based on HbA1c levels, the prediabetes prevalence was 35.5% in the adult population in 2011.[24] In these studies, the prevalence of prediabetes was higher in older, overweight and obese participants.[24, 26, 27] Many other studies found this relationship of age and obesity with the risk and incidence of diabetes.[28-31]

In the 1999-2002 National Health and Nutrition Examination Survey (NHANES), the prevalence of undiagnosed diabetes was 2.8%, and up to 26% of the participants had IFG.[32] However, the age-standardized prevalence of prediabetes based on HbA1c and FPG combined was similar in the periods between 1999 and 2002 and 2003 and 2006 at 29.2% and 29.3%, respectively, but increased significantly to 36.2% in the period between 2007 and 2010.[33] This prevalence continued to increase to as high as 38% in 2012 among adults from the USA.[25] The change in

the prevalence of prediabetes over time occurred because of a significant change in elevated HbA1c, whereas the prevalence based on elevated FPG was similar over this period.[33] Thus, in our population, as in the NHANES study, HbA1c was the most significant contributor to prediabetes prevalence, followed by FPG, which is in concordance with the findings in the Caribbean population[27] and discordant with the reports from the NHANES study between 2011 and 2014 in which they reported that FPG was the most significant contributor to prediabetes prevalence followed by HbA1c.[34] Our results show that individuals with isolated impaired HbA1c when diagnosed with prediabetes might have a slightly better cardiometabolic risk profile than those with isolated FPG, while those individuals with both impaired FPG and HbA1c had the worst CV risk. These results are in line with the findings of the prospective observational study in the primary care setting of a Spanish cohort with prediabetes (PREDAPS) of our group.[35, 36]

Additionally, two meta-analyses found that among individuals with prediabetes based on the ADA criteria, all-cause and CVD mortality were increased[37] and that the risk of cardiovascular disease increased independently of the glucose assessment in comparison to the risk of normoglycaemic subjects.[38] Moreover, a recent study concluded that those who returned to normoglycaemia from FPG- or HbA1c-defined prediabetes were not at reduced risk of future CVD or death.[39] Studies of shorter duration, over 3-5 years, have shown that approximately 25% of subjects progress to diabetes, 25% return to a normal state of glucose tolerance and 50% remain in the prediabetic state;[16] after 1 year, 18.8% of subjects with prediabetes returned to normoglycaemia and approximately 30% with abnormal FPG, 29.1% with abnormal HbA1c and 7.6% with abnormalities in both FPG and HbA1c returned to a normal state of glucose tolerance.[40] In our findings from a one-year follow-up, the rate of reversion from prediabetes to normoglycaemia was approximately 40%, and approximately 60% of participants remained in the prediabetic state. On the other hand, lifestyle modifications, such as weight loss and increased physical activity, among other factors associated with prediabetes, reduced the risk of diabetes among these subjects.[13, 41] According to these reports, in our study, lower BMI was a factor that was independently associated with the normalization of the glycaemic state, and an active lifestyle decreased the risk of having prediabetes.

The results of this study need to be interpreted in light of its strengths and weaknesses. First, the number of participants in our study is smaller in comparison to other studies. In addition, the study may not be representative of urban areas in our region. Thus, the results may not be

generalizable to other territories with different population characteristics in our country. However, the Mollerussa cohort is a representative sample of the region, which is a specific semi-rural area that has never been specifically investigated. Second, our study sample is probably healthier than the general population, as we excluded subjects with already known diabetes and other comorbidities, a lower number of subjects were counted in the denominator, thus resulting in a higher prevalence of this condition. Third, we did not assess glucose tolerance through an oral glucose tolerance test, which is common in most population studies. Although this assay is sensitive, it is also less specific for identifying subjects who could develop diabetes.[42] Furthermore, the oral glucose tolerance test has a low reproducibility and is a rather time-consuming and expensive procedure.[9, 43] Conversely, HbA1c and FPG are cost-effective and more convenient for patients. Currently, FPG is an accepted screening method to detect diabetes and prediabetes. HbA1c improves the sensitivity of FPG in the detection of early T2D in high-risk subjects[32, 44] and is a better predictor of CV events than FPG.[45] Fourth, we only followed up those participants with prediabetes. Thus, we could not analyse the probability of changing from normoglycaemia to prediabetes or diabetes in this study. Finally, it is probable that the use of the World Health Organization prediabetes criteria in our study would have resulted in a smaller proportion of subjects who returned to a normal glycaemic state. The World Health Organization established a normal concentration of FPG between 110 and <126 mg/dl.[46]

Conclusions

For the first time, our study provides information on the prevalence of diabetes and prediabetes in the Mollerussa health care area, a Mediterranean semi-rural area in northeast Spain. Individuals with prediabetes had a more unfavourable cardiometabolic risk profile than normoglycaemic subjects. Moreover, individuals with abnormalities in both criteria used to diagnose prediabetes had the worst risk profile. Finally, after one year of follow-up, few people progressed to diabetes, while more than 40% returned to a normal glycaemic state, and nearly 60% persisted in the prediabetic state. These results suggest that the use of both FPG and HbA1c criteria in clinical practice could help identify people with high diabetes and cardiovascular risk. Moreover, the identification of individuals with prediabetes provides an opportunity for intervention through lifestyle modification and pharmacological treatments not only to reduce the development of diabetes.

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Author Contributions MF, EC and DM conceived and designed the study; MBV, JFN, and MMC participated in the study design; MBV, MF, NA, MGC, NM, AM and CC collected the data; EC and JRM performed the statistical analyses; MF, EC, MMC and DM wrote the manuscript. All authors critically reviewed the manuscript and approved the final version to be published.

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Data sharing statement Readers may contact Dr Didac Mauricio (didacmauricio@gmail.com) regarding the data.

Patient consent for publication Not required.

The original protocol for the study Readers may found the Cohort description in *BMJ Open* 2017:1-8. doi:10.1136/bmjopen-2016-015158.

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FIGURE LEGENDS

Figure 1. Flow diagram of subjects at baseline and after follow-up.

Figure 2. Multivariate logistic regression models **a)** model of prediabetes *versus* normoglycaemic state in the Mollerussa cohort at baseline. Significant p values are shown in bold. eGFR, estimated

glomerular filtration rate. Hosmer and Lemeshow Test p=0.295. **b)** model of normalized *versus* persisted in subjects with follow-up data. Significant p values are shown in bold. Hosmer and Lemeshow Test p= 0.931.

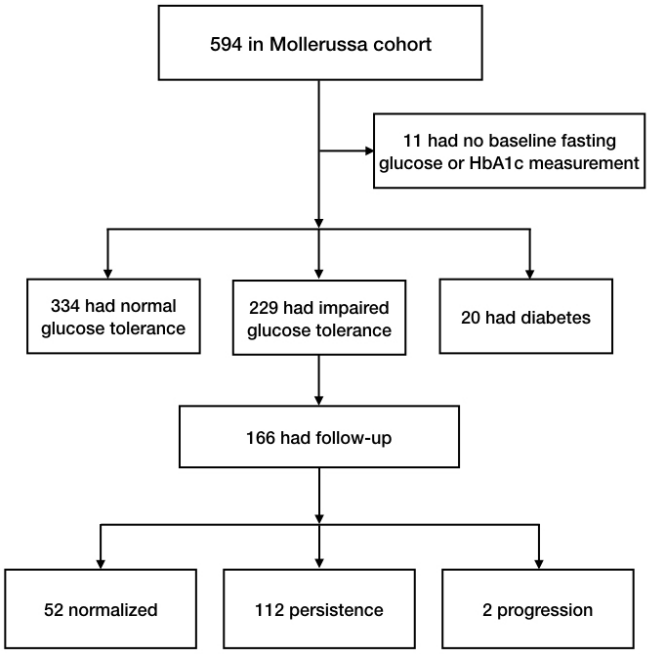

Figure 1. Flow diagram of subjects at baseline and after follow-up.

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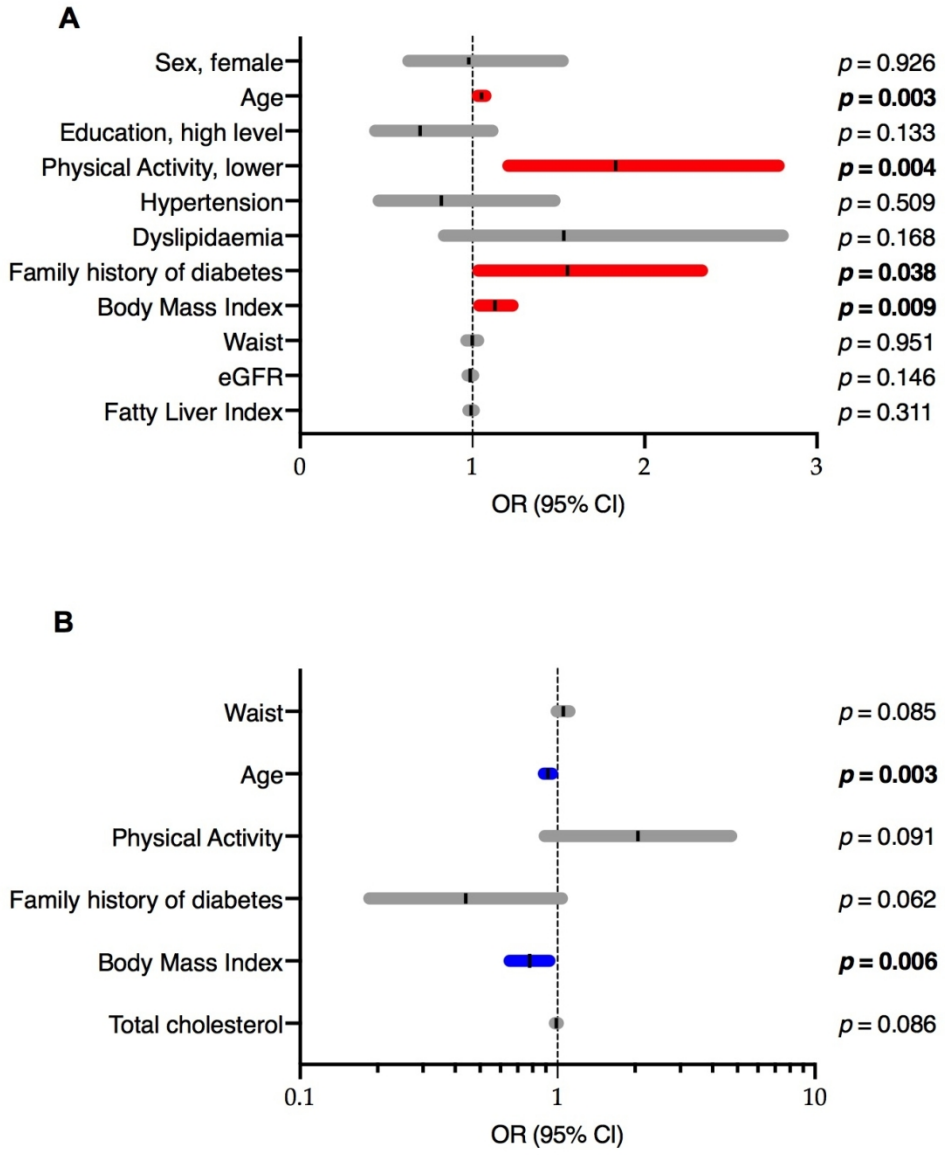
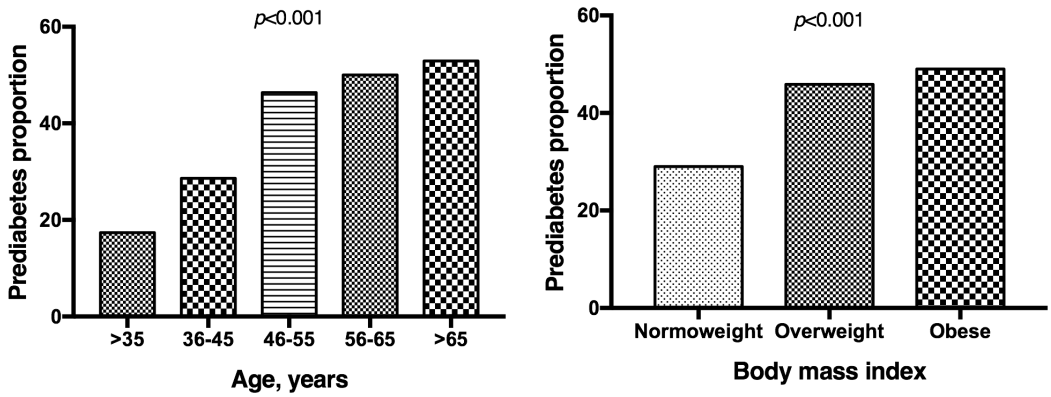


Figure 2. Multivariate logistic regression models a) model of prediabetes versus normoglycaemic state in the Mollerussa cohort at baseline. Significant p values are shown in bold. eGFR, estimated glomerular filtration rate. Hosmer and Lemeshow Test p=0.295. b) model of normalized versus persisted in subjects with follow-up data. Significant p values are shown in bold. Hosmer and Lemeshow Test p= 0.931.

116x142mm (300 x 300 DPI)



Supplementary Figure 1. Proportion of patients with prediabetes. A) Stratified by age. B) Stratified by body mass index.

Supplementary Table 1. Logistic regression model of isolated fasting plasma glucose

	OR	95% CI	p value
Sex	0.683	0.372 - 1.254	0.219
Age	1.032	1.008 - 1.057	**0.009**
Education	0.625	0.351 - 1.111	0.109
Physical activity	0.535	0.318 - 0.899	**0.018**
Family history of diabetes	1.798	1.067 - 3.028	**0.028**
Hypertension	1.423	0.722 - 2.805	0.309
Body mass index	0.994	0.890 - 1.110	0.914
Waist circumference	1.006	0.962 - 1.053	0.789
Fatty liver index	1.014	0.990 - 1.038	0.268
Total cholesterol	1.012	0.993 - 1.032	0.227
Triglycerides	0.998	0.993 - 1.003	0.402
LDL-cholesterol	0.986	0.965 - 1.008	0.212

Supplementary Table 2. Logistic regression model of isolated HbA1c

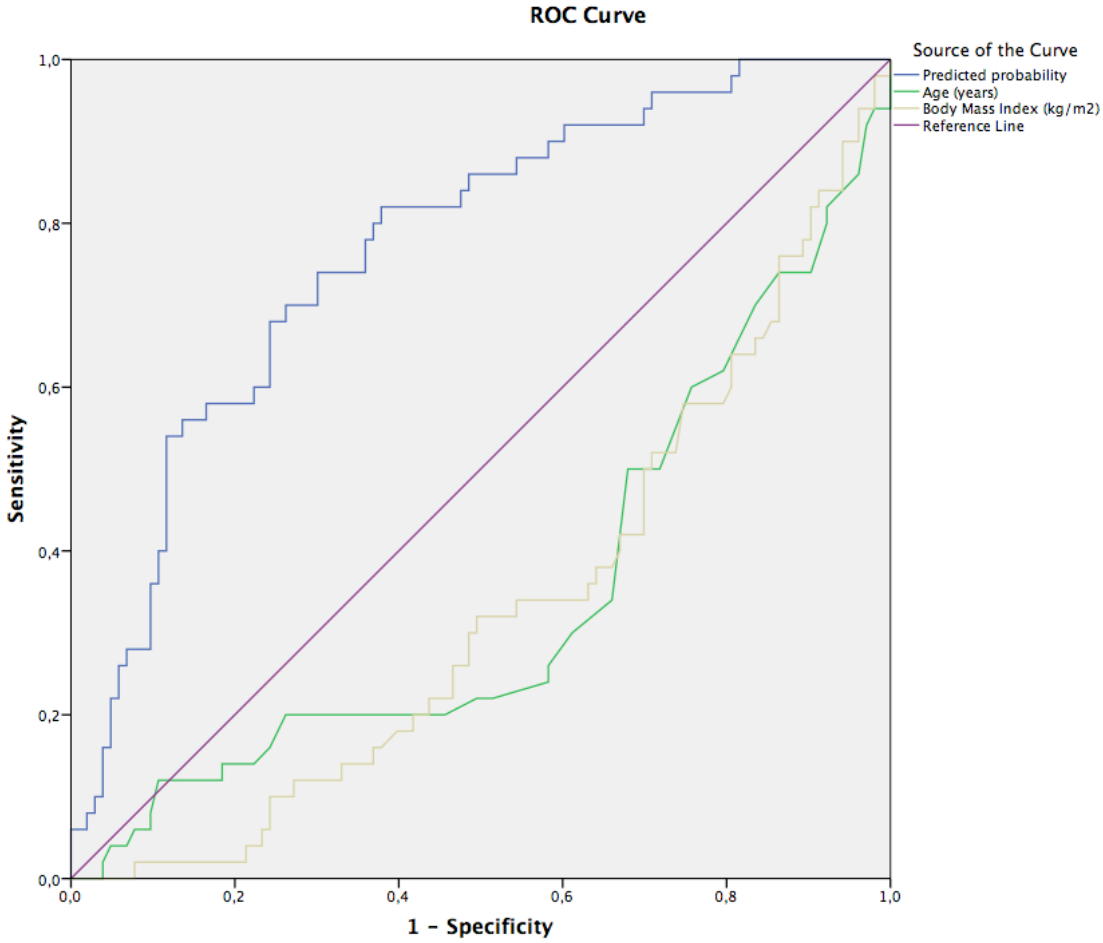
	OR	95% CI	p value
Sex	1.304	0.830 - 2.050	0.249
Age	1.048	1.029 - 1.067	**<0.001**
Education	0.917	0.575 - 1.461	0.715
Physical activity	0.668	0.440 - 1.013	0.058
Hypertension	0.848	0.481 - 1.497	0.570
Body mass index	1.080	0.990 - 1.179	0.083
Waist circumference	0.996	0.963 - 1.030	0.822
Fatty liver index	1.000	0.982 - 1.018	0.960
Total cholesterol	1.000	0.994 - 1.006	0.996
Triglycerides	0.999	0.996 - 1.002	0.724

Supplementary Table 3. Logistic regression model of fasting plasma glucose and HbA1c

	OR	95% CI	p value
Sex, female	1.559	0.753 – 3.225	0.232
Age	1.056	1.026 – 1.086	**<0.001**
Education level	0.914	0.457 – 1.828	0.799
Physical activity	0.610	0.322 – 1.157	0.130
Hypertension	1.665	0.782 – 3.545	0.186
Dyslipidaemia	0.818	0.357 – 1.870	0.633
Body mass index	0.901	0.788 – 1.030	0.128
Waist circumference	1.012	0.960 – 1.066	0.665
Triglycerides	0.999	0.993 – 1.004	0.594
Fatty liver index	1.031	1.002 – 1.061	**0.037**

Supplementary Table 4. Logistic regression model of normalized *versus* persisted in subjects with follow-up data.

	OR	95% CI	p value
Sex(1)	1.025	0.392 – 2.682	0.960
Age	0.956	0.917 – 0.998	**0.039**
Education(1)	1.327	0.513 – 3.431	0.560
Physical_Activity(1)	1.885	0.798 – 4.453	0.149
Treat_HT(1)	0.865	0.268 – 2.791	0.808
Family_history_DM(1)	0.428	0.176 – 1.040	0.061
BMI	0.749	0.605 – 0.926	**0.007**
Waist	1.037	0.959 – 1.121	0.365
FLI	1.022	0.982 – 1.063	0.292
HOMA2_IR	0.620	0.240 – 1.604	0.324
Total_cholesterol	0.986	0.972 – 1.000	0.052
HDL_cholesterol	1.015	0.978 – 1.052	0.435



Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Predicted probability	0.771	0.040	0.000	0.693	0.849
Age	0.347	0.049	0.002	0.251	0.442
Body Mass Index	0.332	0.045	0.001	0.244	0.421

Supplementary Figure 2. Receiver operating characteristics (ROC) curve showing the relationship between sensitivity and 1-specificity in determining the discriminatory ability of the logistic regression model and the variables age and body mass index separately.

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	#3	State specific objectives, including any prespecified hypotheses	4
Methods			

Study design	#4	Present key elements of study design early in the paper	4
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	4-5
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	4-5
Bias	#9	Describe any efforts to address potential sources of bias	6
Study size	#10	Explain how the study size was arrived at	6
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	6
Statistical methods	#12c	Explain how missing data were addressed	5
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	n/a
Statistical methods	#12e	Describe any sensitivity analyses	6

Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	6-7
Participants	#13b	Give reasons for non-participation at each stage	n/a
Participants	#13c	Consider use of a flow diagram	7
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	7-8
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	5
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	5
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	6-7
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6-7
Main results	#16b	Report category boundaries when continuous variables were categorized	7
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	7, 9,10
Discussion			
Key results	#18	Summarise key results with reference to study objectives	10-11

Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	13
Generalisability	#21	Discuss the generalisability (external validity) of the study results	n/a
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

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Prevalence of prediabetes and undiagnosed diabetes in the Mollerussa prospective observational cohort study in a semi-rural area of Catalonia

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Secondary Subject Heading:	Epidemiology
Keywords:	Prediabetes, Undiagnosed diabetes, prediabetes prevalence

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Prevalence of prediabetes and undiagnosed diabetes in the Mollerussa prospective observational cohort study in a semi-rural area of Catalonia

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ABSTRACT

Objectives: To assess the prevalence of undiagnosed diabetes and prediabetes in the healthy population in the Mollerussa cohort. As a secondary objective, to identify the variables associated with these conditions and to describe the changes in glycaemic status after one year of follow-up in subjects with prediabetes.

Design: Prospective observational cohort study.

Setting: General population from a semi-rural area.

Participants: The study included 583 participants without a diagnosis of diabetes recruited between March 2011 and July 2014.

Results: The prevalence of undiagnosed diabetes was 20, 3.4% (95% confidence interval 2.6, 4.2) and that of prediabetes was 229, 39.3% (37.3, 41.3). Among those with prediabetes, 18.3% had

isolated impaired fasting plasma glucose (FPG) (FPG: 100 to <126 mg/dL), 58.1% had isolated impaired HbA1c (HbA1c 5.7 - <6.5), and 23.6% fulfilled both criteria. Follow-up data were available for 166 subjects; 41.6% (37.8, 45.4) returned to normoglycaemia, 57.6% (57.8, 61.4) persisted in prediabetes, and 0.6% (0, 1.2) progressed to diabetes. Individuals with prediabetes had worse cardiometabolic risk profiles and sociodemographic features than normoglycaemic subjects. In the logistic regression model, variables significantly associated with prediabetes were older age (odds ratio; 95% confidence interval) (1.033; 1.011, 1.056), higher physical activity (0.546; 0.360, 0.827), body mass index (1.121; 1.029, 1.222), and a family history of diabetes (1.543; 1.025, 2.323). The variables significantly associated with glycaemic normalization were older age (0.948; 0.916, 0.982) and body mass index (0.779; 0.651, 0.931).

Conclusions: Among adults in our region, the estimated prevalence of undiagnosed diabetes was 3.4% and that of prediabetes was 39.3%. After a one-year follow-up, a small proportion of subjects (0.6%) with prediabetes progressed to diabetes, while a high proportion (41.6%) returned to normoglycaemia. Individuals with prediabetes who returned to normoglycaemia were younger and had a lower body mass index.

KEYWORDS

Prediabetes, undiagnosed diabetes, prediabetes prevalence

ARTICLE SUMMARY

Strengths and Limitations

- This was a population-based study of a small cohort that included a representative sample of a non-previously studied population of a semi-rural area in Catalonia.
- We did not perform an oral glucose tolerance test, which is a common test in most studies but is a time-consuming and expensive procedure.
- The small number of cases of undiagnosed diabetes precluded further statistical analyses on this topic.

BACKGROUND

Diabetes mellitus, a public health concern with an increasing incidence worldwide, is a great threat to general health and is leading to increased morbidity and mortality. These effects are mainly occurring because diabetes is a disorder of glucose metabolism that affects multiple organ

systems and is associated with various micro- and macro-vascular complications and several nonvascular complications. Additionally, a large group of subjects do not fulfil the diabetes criteria but have intermediate glycaemic variables, between normal and diabetes, and are thus classified as having prediabetes. One of the most commonly used definitions of prediabetes is that of the 2010 American Diabetes Association (ADA) criteria[1, 2]: (a) impaired fasting plasma glucose (IFG), defined as fasting plasma glucose (FPG) between 100 and <126 mg/dL (5.6–5.9 mmol/L); (b) impaired glucose tolerance (IGT), defined as a 2-hour plasma glucose value after a 75 g oral glucose tolerance test (OGTT) between 140 and <200 mg/dL (7.8–11.0 mmol/L); or (c) glycated haemoglobin (HbA1c) levels between 5.7% and < 6.5% (39–46 mmol/mol).

Prediabetes is becoming increasingly important as it represents a high risk of developing type 2 diabetes (T2D) and cardiovascular diseases.[2, 3] Moreover, individuals with prediabetes are phenotypically quite similar to patients with T2D. That is, they tend to be older, with a higher body mass index (BMI) and higher blood pressure than people with normal glucose tolerance; in addition, they tend to have insulin resistance and dyslipidaemia.[4] Additionally, multiple risk factors, such as family history, gestational diabetes, and certain ethnicities as well as combined risk factors such as metabolic syndrome, are known to predispose subjects to a higher risk for prediabetes and its progression to T2D.[5] Based only on impaired glucose tolerance (IGT), the worldwide prevalence of prediabetes among adults has been estimated by the International Diabetes Federation to be 7.3% in 2017, with half of these individuals (49%) being younger than 50 years.[6] The National Diabetes Statistics Report in the United States reported that the total crude prevalence of diabetes was 9.4% (30.3 million, 2017 US population), with 23.8% undiagnosed and an additional 33.9% with prediabetes.[7]

In Spain, according to data from the Di@bet.es study, based on OGTT, FPG and HbA1c, 13.8% of the adult population, adjusted for age and sex, had diabetes, and of these individuals up to 6% had undiagnosed diabetes. Furthermore, an additional 14.8% of individuals presented with some type of prediabetic state, 3.4% based on IFG, 9.2% based on IGT and 2.2% with disturbances in both, after adjusting for age and sex.[8, 9] According to the ADA, up to 70% of people with prediabetes will develop overt diabetes throughout their lives.[10, 11] Moreover, each year, 5-10% of subjects with prediabetes will eventually develop overt diabetes, and according to some studies, this percentage can reach up to 18% per year; however, this rate may vary with the definition of prediabetes and population characteristics.[12-15] It has been shown that over 3-5 years,

approximately 25% of subjects progress to T2D, 25% return to a normal state of glucose tolerance and 50% remain in the prediabetic state.[16] Thus, the early diagnosis and screening of prediabetes are essential steps towards the prevention of its progression or at least the delay of the onset of T2D.

The primary aim of this study was to assess the prevalence of undiagnosed diabetes and prediabetes in the healthy population in the Mollerussa cohort. As a secondary objective, we aimed to assess the variables associated with these conditions and to describe the changes in glycaemic status after one year of follow-up in subjects with prediabetes.

METHODS

Subjects

This was a prospective population-based cohort study from the semi-rural area of Mollerussa in Catalonia (northeast Spain) selected between March 2011 and July 2014. The description of the cohort and the procedures performed were initially published as a cohort profile.[17] Briefly, the database of the Catalan Health Institute (ICS) through its Primary Care Electronic Clinical Station (Estació Clínica Electronica d'Atenció Primaria –eCAP) was used to select the population sample. All population is passively included in the Primary Care Electronic Clinical record according to the Spanish health system, which is based on the principles of universality, free access, equity and fairness of financing.[18] Then, from a total population of 24,666 potentially eligible individuals in the health-care area (subjects older than 25 years and attending any Primary Healthcare Centre in the same health area), 2,226 subjects were randomly selected using a randomiser programme (SPSS software V.16.0 for Windows; SPSS), following the principles of simple random sampling, and were then invited to participate by telephone contact. Based on their willingness to join the study, exclusion criteria, consent and baseline laboratory data, 594 subjects aged ≥ 25 years were finally included.[17] The exclusion criteria included a previous diagnosis of diabetes (type 1 diabetes (T1D), T2D or any specific subtype of diabetes), treatment with oral antidiabetic drugs or the use of metformin for other conditions. In addition, subjects with cardiovascular disease (heart disease, heart failure, aortic stenosis), cancer, kidney disease, anaemia, hepatitis, gastrointestinal diseases, recent abdominal surgery, chronic pulmonary obstructive disease, chronic infectious diseases, use of systemic glucocorticoids or beta blockers or major psychiatric disorders with psychotic symptoms were excluded from the study. Subjects were considered to have hypertension or dyslipidaemia if they were using anti-hypertensive or lipid-lowering agents.

Prediabetes was defined as any of the following abnormal glycaemic variables: FPG 100 to <126 mg/dL or HbA1c 5.7 to <6.5%; diabetes was defined as FPG >125 mg/dL or HbA1c ≥6.5%. Normal glycaemic status was defined by FPG <100 mg/dL and HbA1c <5.7 according to the 2010 ADA criteria.[1] Eleven subjects without baseline HbA1c or FPG measurements were excluded. Subjects with prediabetes at baseline (n=229) underwent a second visit 12 months after the baseline visit, and 166 (72.5%) of them had relevant information at follow up.

A fasting blood sample was taken to determine glucose, HbA1c, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, renal function, and other parameters following standard protocols.[17] The fatty liver index (FLI) was calculated with the equation developed by Bedogni *et al*. [19] Insulin resistance was calculated by the homeostatic model assessment (HOMA2-IR); beta cell function (HOMA2-ß) and insulin sensitivity (HOMA2-S) data were calculated with a HOMA2 calculator released by the Diabetes Trials Unit, University of Oxford: HOMA Calculator. This calculator is available at: http://www.dtu.ox.ac.uk/homacalculator/ (updated October 11, 2017).[20] The estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration equation.[21]

Sociodemographic variables were recorded by researchers following a protocol for the inclusion of patients using a standardized baseline questionnaire during the clinical interview. In all cases a physical examination (including weight, height, blood pressure and waist circumference) was carried out by trained research staff. Education level and physical activity were assessed according to the International Standard Classification of Education[22] and the Spanish-validated International Physical Activity Questionnaire,[23] respectively. We classified the education level as low level (studied until primary school) and high level (secondary high school education or higher). Physical activity was classified as sedentary or active (not regularly versus regularly active).

Ethical approval

The study protocol was approved by the Ethics Committee of the Primary Health Care University Research Institute (IDIAP) Jordi Gol (P12/043) and was conducted following the Declaration of Helsinki. All study participants signed an informed consent form.

Sample size

The sample size was determined based on an estimated prediabetes prevalence of 35.5% and 38% using HbA1c levels and the 2010 ADA criteria, respectively.[1, 24, 25] It was estimated that a random sample of 505 subjects was sufficient to assess an estimated prevalence of approximately 30% with a 95% CI and an error of ±4%.[17]

Statistical methods

Descriptive statistics of the mean (standard deviation) or median [interquartile range] were estimated for quantitative variables with a normal or non-normal distribution, respectively. Qualitative variables were assessed using absolute and relative frequencies. Normally distributed data were analysed using the Shapiro-Wilk test. Comparisons between groups of all variables were performed to evaluate the differences. Student's t-test, ANOVA, the Mann-Whitney test, or the Kruskal-Wallis test were used to assess the differences between groups. The chi-squared test or Fisher's exact test were used to determine differences in qualitative variables. Tukey's correction was applied to account for multiple tests. Multivariate logistic regression models were used to determine the association of variables with prediabetes, isolated FPG, isolated HbA1c and both FPG and HbA1c at baseline with covariables that were clinically or statistically associated. In the prediabetes model, the variables used were age, sex, education level, physical activity, DLP, HT, family history of diabetes, BMI, waist, glomerular filtration rate and fatty liver index. A stepwise method with selection of variables by backward elimination was used to build the final logistic regression model to predicts the normalization of the glycaemic state; in all models, the goodness-of-fit assumption was tested by the Hosmer-Lemeshow test. The predictive accuracy of the logistic regression model for normalization was checked by receiver-operating characteristic (ROC) curves and the area under the ROC curve (AUC_{ROC}). Odds ratios with corresponding 95% confidence intervals are shown, and statistical significance was established as a p-value <0.05. Data management and all analyses were performed using R statistical software, version 3.3.1, and SPSS software (version 22, IBM, SPSS, Chicago, Illinois, USA).

Patient and Public Involvement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

RESULTS

Out of the 594 individuals recruited, complete data on FPG and HbA1c were available from 583 (98.1%). The prevalence of undiagnosed diabetes was 20 subjects, 3.4% (95% confidence interval: 2.7, 4.2), and the prevalence of prediabetes was 229 subjects, 39.3% (37.3, 41.3). Furthermore, the prevalence based on isolated FPG was 7.2%, and that based on isolated HbA1c was 22.8%, while based on the criteria of both FPG and HbA1c, the prevalence was 9.3% (Figure 1).

The differences of clinical and sociodemographic characteristics between normoglycaemic with prediabetic and diabetic groups are shown in Table 1. Except for sex, family history of diabetes, current smoking status, alcohol consumption status, triglycerides and high density lipoprotein (HDL)-cholesterol levels, there were significant differences in the majority of parameters, including age and BMI, between the three groups.

We observed an association in age, BMI, waist circumference, systolic and diastolic blood pressure (SBP and DBP), alcohol consumption status, hypertension, dyslipidaemia, triglycerides, total cholesterol, low density lipoprotein (LDL)-cholesterol, insulin test, FLI, and HOMA2-IR, which were higher in individuals with prediabetes than in individuals with normoglycaemia and were higher in the diabetic group than in the prediabetic group. On the other hand, physical activity, education level, eGFR, HOMA2-β and HOMA2-S exhibited a negative trend between the same groups. In the prediabetic group, 41.9% had impaired FPG and 81.7% had impaired HbA1c. On the other hand, among the newly identified diabetic subjects, up to 80% met the FPG criteria and 85% met the HbA1c criteria. The prevalence of prediabetes increased with increasing age, with percentages of 17.4%, 28.6%, 46.4%, 50 and 52.9% in participants aged <35 years, 36-45 years, 46-55 years, 56-65 years and >65 years, respectively. Regarding BMI categories of normal weight (BMI <25 kg/m^2), overweight (BMI 25-29.9 kg/m^2), and obese (BMI>30 kg/m^2), the prevalence of prediabetes was 29%, 45.9%, and 49%, respectively (Supplementary file 1 Figure 1).

Table 1. Clinical and sociodemographic differences among glycaemic status groups of the Mollerussa cohort.

	Normoglycaemia FPG <100 mg/dL and HbA1c <5.7%	**Prediabetes** FPG 100 to 125 mg/dL, or HbA1c 5.7 to 6.4%	**Diabetes** FPG >125 mg/dL or HbA1c ≥6.5%	**Mean difference NG vs. PD (95% CI)**	**Mean difference NG vs. DM (95% CI)**
N	334	229	20	-	-
Sex, women	193 (57.8%)	135 (59.0%)	13 (65.0%)	1.2 (-7.1, 9.4)	7.2 (-14.3, 28.8)
Age, years	47.1 (12.8)	54.6 (12.3)	61.2 (13.6)	7.5 (5.4, 9.6)	14.2 (8.4, 19.9)
BMI, Kg/m^2	25.3 (4.27)	27.5 (4.75)	30.2 (5.48)	2.1 (1.4, 2.9)	4.9 (2.9, 6.9)
BMI categories					
Normal weight	160 (50.0%)	67 (30.2%)	4 (20.0%)	-18.6 (-26.6, -10.7)	-27.9 (-46.2, -9.6)
Overweight	120 (37.5%)	106 (47.7%)	5 (25.0%)	10.4 (2.1, 18.6)	-10.9 (-30.6, 8.7)
Obesity	40 (12.5%)	49 (22.1%)	11 (55.0%)	9.4 (3.1, 15.8)	43.0 (20.9, 65.1)
Waist, cm	91.9 (11.9)	97.0 (12.3)	101 (16.8)	5.1 (2.9, 7.2)	9.3 (3.8, 14.9)
SBP, mm Hg	119 (16.3)	126 (16.6)	130 (18.6)	6.7 (3.9, 9.5)	10.9 (3.5, 18.5)
DBP, mm Hg	75.7 (10.0)	78.2 (9.88)	78.0 (9.24)	2.5 (0.8, 4.2)	2.3 (-2.2, 6.8)
Hypertension	37 (11.1%)	49 (21.4%)	9 (45.0%)	10.3 (4.0, 16.6)	33.9 (11.9, 55.9)
Dyslipidaemia	27 (8.08%)	39 (17.0%)	5 (25.0%)	8.9 (3.2, 14.6)	16.9 (-2.3, 36.1)

Family history DM	94 (29.6%)	78 (37.0%)	8 (42.1%)	5.9 (-1.9, 13.7)	11.8 (-10.1, 33.9)
Education, high level	265 (82.6%)	145 (65.0%)	11 (55.0%)	-16.0 (-23.6, -8.4)	-24.3 (-46.6, -2.1)
Physical activity	243 (75.9%)	141 (63.2%)	10 (50.0%)	-11.2 (-19.1, -3.3)	-22.7 (-45.2, -0.3)
Current smoker	82 (24.6%)	63 (27.5%)	3 (15.0%)	3.0 (-4.4, 10.4)	-9.5 (-25.9, 6.8)
Alcohol, g/day	8.33 (13.9)	12.3 (21.3)	10.6 (17.2)	4.0 (0.9, 6.9)	2.2 (-5.8, 10.3)
FPG, mg/dL	86.6 (7.04)	97.0 (11.2)	119 (15.2)	10.4 (8.8, 11.9)	32.6 (28.4, 36.8)
HbA1c, %	5.25 (0.26)	5.80 (0.29)	6.26 (0.54)	0.6 (0.5, 0.6)	1.0 (0.9, 1.1)
HbA1c, mmol/mol	33.8 (2.81)	39.9 (3.12)	45.0 (5.92)	6.1 (5.6, 6.6)	11.1 (9.7, 12.5)
eGFR mL/min/1.73m^2	96.6 (14.1)	90.4 (15.9)	85.5 (18.1)	-6.2 (-8.7, -3.7)	-11.2 (-17.9, -4.4)
Triglycerides, mg/dL	104 (90.0)	111 (63.2)	116 (47.3)	6.6 (-6.8, 19.9)	11.2 (-24.7, 47.1)
T-cholesterol, g/dL	197 (38.2)	205 (32.4)	214 (31.1)	7.9 (1.9, 14.0)	16.9 (0.7, 33.1)
HDL, mg/dL	58.7 (15.0)	58.8 (14.3)	64.6 (17.6)	0.1 (-2.4, 2.6)	5.9 (-0.86, 12.6)
LDL, mg/dL	119 (31.4)	125 (29.4)	126 (27.7)	6.3 (1.1, 11.4)	7.5 (-6.3, 21.4)
Insulin, µU/mL	7.99 (3.78)	10.1 (5.46)	16.2 (17.1)	2.1 (1.2, 3.0)	8.2 (5.8, 10.7)
Fatty Liver Index	34.0 (26.9)	44.3 (28.6)	59.4 (33.0)	10.3 (5.5, 15.0)	25.4 (12.8, 38.0)
HOMA2-β	104 (31.8)	97.7 (31.9)	89.0 (48.4)	-6.3 (-11.8, -0.8)	-14.9 (-29.7, -0.2)
HOMA2-S	118 (52.1)	94.0 (43.4)	63.3 (26.7)	-23.8 (-32.0, -15.6)	-54.4 (-76.3, -32.6)
HOMA2-IR	1.03 (0.48)	1.33 (0.72)	2.16 (2.08)	0.3 (0.2, 0.4)	1.1 (0.8, 1.4)

Mean (SD) and n (%). NG, normoglycaemia; PD, prediabetes; DM, diabetes; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; T-cholesterol, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA2-IR, Homeostatic Model Assessment-2_Insulin Resistance; HOMA2-ß, Homeostatic Model Assessment-2 Beta cell function; HOMA2-S, Homeostatic Model Assessment-2 Insulin Sensitivity.

Table 2 shows the characteristics of prediabetic individuals by glycaemic state: isolated FPG, isolated HbA1c and both altered FPG and HbA1c. Thus, among the 229 subjects with prediabetes, 42 (18.3%) had abnormal isolated FPG, 133 (58.1%) had abnormal isolated HbA1c, and 54 (23.6%) had both abnormal FPG and HbA1c. Patients with both abnormal FPG and HbA1c were older, had larger waist circumferences, had increased FLI and HOMA2-IR, were more likely to be overweight or obese and have hypertension, and had lower HOMA2-S. The isolated FPG group had a higher proportion of subjects with a family history of diabetes, higher alcohol consumption, higher levels of total cholesterol and LDL-cholesterol and lower levels of HDL-cholesterol, although none of these differences were statistically significant. Finally, the isolated HbA1c group had an elevated HOMA2-β. Although there were no statistically significant differences, the proportion of men was higher in the isolated FPG group, whereas the proportion of women was higher in the isolated HbA1c and both FPG and HbA1c groups. Among the three groups, no statistically significant differences were found regarding the following variables: sex, dyslipidaemia, family history of diabetes, education level, physical activity, current smoking status, alcohol consumption, triglycerides, total cholesterol, HDL-cholesterol or LDL-cholesterol.

Table 2. Clinical and sociodemographic characteristics by glycaemic status of the individuals with prediabetes.

	Impaired HbA1c 5.7%-6.4%	Impaired FPG 100-125 mg/dL	HbA1c 5.7%-6.4% and FPG 100-125 mg/dL	*p* overall	*p* HbA1c vs. FPG	*p* HbA1c vs. Both	*p*. FPG vs. Both
N	133	42	54	-	-	-	-
Sex, Women	84 (63.2%)	19 (45.2%)	32 (59.3%)	0.12	0.181	0.74	0.369
Age, years	53.4 (12.4)	50.6 (11.8)	60.6 (10.5)	**<0.001**	0.388	**0.001**	**<0.001**
BMI, Kg/m2	25.8 [24.5;28.9]	27.8 [24.5;30.6]	27.5 [25.6;30.5]	0.056	0.534	**0.036**	0.534
BMI categories				**0.018**	0.107	**0.05**	**0.032**
Normal weight	43 (33.1%)	16 (41.0%)	8 (15.1%)				
Overweight	64 (49.2%)	12 (30.8%)	30 (56.6%)				
Obesity	23 (17.7%)	11 (28.2%)	15 (28.3%)				
Waist, cm	95.0 [88.0;102]	98.0 [90.0;106]	101 [95.0;107]	**0.008**	0.232	**0.006**	0.333
SBP, mm Hg	124 (16.1)	129 (15.5)	128 (18.4)	0.169	0.296	0.29	0.991
DBP, mm Hg	78.0 (9.44)	79.5 (12.0)	77.9 (9.39)	0.674	0.675	0.999	0.723
Hypertension	21 (15.8%)	9 (21.4%)	19 (35.2%)	**0.014**	0.542	**0.019**	0.32
Dyslipidaemia	25 (18.8%)	4 (9.52%)	10 (18.5%)	0.358	0.515	1	0.515
Family history DM	43 (34.1%)	18 (48.6%)	17 (35.4%)	0.265	0.471	1	0.471
Education, high level	91 (69.5%)	23 (59.0%)	31 (58.5%)	0.252	0.455	0.455	1
Physical activity	88 (67.2%)	21 (53.8%)	32 (60.4%)	0.281	0.547	0.68	0.68
Current smoker	38 (28.6%)	14 (33.3%)	11 (20.4%)	0.338	0.693	0.496	0.496
Alcohol, g/day	2.92 [0.00;15.2]	7.42 [0.90;16.3]	1.53 [0.00;17.9]	0.369	0.336	0.735	0.336
FPG, mg/dL	89.2 (6.89)	106 (4.97)	109 (5.96)	**<0.001**	**<0.001**	**<0.001**	0.14
HbA1c, %	5.80 [5.70;6.00]	5.40 [5.40;5.57]	5.95 [5.80;6.10]	**<0.001**	**<0.001**	**<0.001**	**<0.001**
HbA1c, mmol/mol	39.9 [38.8;42.1]	35.5 [34.7;37.4]	41.5 [39.9;43.2]	**<0.001**	**<0.001**	**<0.001**	**<0.001**
eGFR, mL/min/1.73m^2	93.6 [79.6;103]	93.2 [79.7;107]	89.3 [73.1;97.2]	0.076	0.556	0.073	0.073
Triglycerides, mg/dL	88.0 [72.0;134]	86.5 [67.0;130]	106 [74.5;132]	0.332	0.729	0.304	0.304
Total cholesterol, mg/dL	205 (34.5)	209 (28.6)	203 (29.8)	0.689	0.767	0.947	0.677
HDL-cholesterol, mg/dL	58.0 [51.0;69.0]	52.0 [45.0;65.8]	57.0 [51.0;66.0]	0.128	0.141	0.755	0.18
LDL-cholesterol, mg/dL	125 (32.2)	133 (25.5)	120 (23.5)	0.114	0.278	0.593	0.096
Insulin, µU/mL	8.00 [6.10;10.0]	9.90 [6.90;15.9]	10.9 [7.90;15.6]	**<0.001**	**0.01**	**<0.001**	0.577
Fatty Liver Index	34.4 [16.9;59.2]	42.2 [17.7;73.6]	53.8 [32.2;73.0]	**0.016**	0.373	**0.011**	0.378
HOMA2-β	96.6 [81.5;122]	81.7 [64.5;118]	82.8 [63.0;108]	**0.001**	**0.034**	**0.002**	0.693
HOMA2-S	98.0 [77.2;127]	75.0 [47.2;107]	67.5 [47.5;91.3]	**<0.001**	**0.003**	**<0.001**	0.564
HOMA2-IR	1.00 [0.80;1.30]	1.30 [0.90;2.15]	1.50 [1.10;2.10]	**<0.001**	**0.005**	**<0.001**	0.545

Significant values are shown in bold. Mean (SD), median [interquartile range] and n (%). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA2-IR, Homeostatic Model Assessment-2_Insulin Resistance; HOMA2-β, Homeostatic Model Assessment-2 Beta cell function; HOMA2-S, Homeostatic Model Assessment-2 Insulin Sensitivity.

Prediabetes follow-up

Of the 229 individuals with prediabetes at baseline, 166 (72.5%) had clinical and laboratory data after 12 months of follow-up. Of them, 52 (41.6%) returned to a normal glycaemic status, 112

(57.6%) persisted in their state of prediabetes, and only 2 (0.6%) progressed to diabetes. Table 3 shows the outcome of the follow-up of the isolated FPG, HbA1c and both FPG and HbA1c groups.

Table 3. Outcomes at follow-up of patients with different altered glucose metabolism statuses at baseline.

Variables	Baseline	N with follow-up	Follow up		
			Normalized	Persisted	Progressed
Prediabetes	229 (39.3%)	166 (90.7%)	52 (41.6%)	112 (57.8%)	2 (0.6%)
Isolated FPG	42 (7.2%)	3 (1.8%)	1 (33.3%)	1 (33.3%)	1 (33.3%)
Isolated HbA1c	133 (22.8%)	114 (68.7%)	47 (41.3%)	67 (58.7%)	0 (0%)
Both altered	54 (9.3%)	49 (29.5%)	4 (8.2%)	44 (89.8%)	1 (2%)

FPG, fasting plasma glucose

Association of prediabetes with glycaemic status

The multivariate logistic regression model of prediabetes *versus* normoglycaemia showed that the variables associated with prediabetes were older age (odds ratio; 95% confidence interval) (1.033; 1.011, 1.056), higher physical activity levels (0.546; 0.360, 0.827), higher BMI (1.121; 1.029, 1.222), and a family history of diabetes (1.543; 1.025, 2.323) (Figure 2a). The models for isolated FPG alterations, isolated HbA1c alterations and both FPG and HbA1c alterations are shown in Supplementary file 2 Tables 1, 2 and 3, respectively. The variables associated with isolated FPG were older age (1.032; 1.008, 1.057), higher physical activity levels (0.535; 0.318, 0.899), and a family history of diabetes (1.798; 1.067, 3.028). On the other hand, the only variable associated with impaired HbA1c was older age (1.048; 1.029, 1.067). Finally, in the model for altered FPG and HbA1c, the variables associated were older age (1.056; 1.026, 1.086) and high FLI (1.031; 1.002, 1.061).

Prediction of normalization

Logistic regression model, as described in the methods section, starting with the variables age, sex, waist circumference, BMI, hypertension, physical activity, family history of diabetes, education level, total cholesterol, HDL-cholesterol, FLI and HOMA2-IR, was performed to identify factors independently associated with the prediction of glycaemic status normalization (Supplementary file 2 Table 4). The variables that predicted glycaemic normalization were older age (0.948; 0.916, 0.982) and BMI (0.779; 0.651, 0.931) (Figure 2b); this model had a good predictive ability (AUC_{ROC} 0.77; $p<0.001$) (Supplementary file 3 Figure 2).

DISCUSSION

We found that the prevalence of undiagnosed diabetes was 3.4%, and the prevalence of prediabetes was 39.3% in this semi-rural population in Catalonia (northeast Spain). The prevalence of prediabetes was three-fold higher based on HbA1c than that based on FPG. Subjects with prediabetes defined by both HbA1c and FPG criteria had unfavourable clinical and sociodemographic profiles related to increased cardiovascular risk. These factors were older age; abdominal obesity; higher triglycerides; increased FLI; and a higher proportion of overweight, obesity and hypertension. In our population, age was the variable most strongly associated with prediabetes based on all specific glycaemic status variables: isolated impaired FPG, isolated impaired HbA1c or both impaired FPG and HbA1c. Other variables associated with prediabetes were lower physical activity levels, a family history of diabetes, and obesity. Finally, the characteristics related to normalization at follow-up were younger age and lower BMI.

The prevalence of prediabetes and undiagnosed diabetes in our healthy population were within the ranges found in other population studies defining prediabetes based on the 2010 ADA criteria, using FPG and/or HbA1c. Among these studies, a large national Chinese study (with 170,287 subjects) showed a prevalence of prediabetes of 35.7% and a prevalence of undiagnosed diabetes of 6.9%.[26] In a study of the Caribbean population, the corresponding figures were 44.1% for prediabetes and 7.3% for undiagnosed diabetes.[27] In England, based on HbA1c levels, the prediabetes prevalence was 35.5% in the adult population in 2011.[24] In these studies, the prevalence of prediabetes was higher in older, overweight and obese participants.[24, 26, 27] Many other studies found this relationship of age and obesity with the risk and incidence of diabetes.[28-31]

In the 1999-2002 National Health and Nutrition Examination Survey (NHANES), the prevalence of undiagnosed diabetes was 2.8%, and up to 26% of the participants had IFG.[32] However, the age-standardized prevalence of prediabetes based on HbA1c and FPG combined was similar in the periods between 1999 and 2002 and 2003 and 2006 at 29.2% and 29.3%, respectively, but increased significantly to 36.2% in the period between 2007 and 2010.[33] This prevalence continued to increase to as high as 38% in 2012 among adults from the USA.[25] The change in the prevalence of prediabetes over time occurred because of a significant change in elevated HbA1c, whereas the prevalence based on elevated FPG was similar over this period.[33] Thus, in

our population, as in the NHANES study, HbA1c was the most significant contributor to prediabetes prevalence, followed by FPG, which is in concordance with the findings in the Caribbean population[27] and discordant with the reports from the NHANES study between 2011 and 2014 in which they reported that FPG was the most significant contributor to prediabetes prevalence followed by HbA1c.[34] Our results show that individuals with isolated impaired HbA1c when diagnosed with prediabetes might have a slightly better cardiometabolic risk profile than those with isolated FPG, while those individuals with both impaired FPG and HbA1c had the worst CV risk. These results are in line with the findings of the prospective observational study in the primary care setting of a Spanish cohort with prediabetes (PREDAPS) of our group.[35, 36]

Additionally, two meta-analyses found that among individuals with prediabetes based on the ADA criteria, all-cause and CVD mortality were increased[37] and that the risk of cardiovascular disease increased independently of the glucose assessment in comparison to the risk of normoglycaemic subjects.[38] Moreover, a recent study concluded that those who returned to normoglycaemia from FPG- or HbA1c-defined prediabetes were not at reduced risk of future CVD or death.[39] Studies of shorter duration, over 3-5 years, have shown that approximately 25% of subjects progress to diabetes, 25% return to a normal state of glucose tolerance and 50% remain in the prediabetic state;[16] after 1 year, 18.8% of subjects with prediabetes returned to normoglycaemia and approximately 30% with abnormal FPG, 29.1% with abnormal HbA1c and 7.6% with abnormalities in both FPG and HbA1c returned to a normal state of glucose tolerance.[40] In our findings from a one-year follow-up, the rate of reversion from prediabetes to normoglycaemia was approximately 40%, and approximately 60% of participants remained in the prediabetic state. On the other hand, lifestyle modifications, such as weight loss and increased physical activity, among other factors associated with prediabetes, reduced the risk of diabetes among these subjects.[13, 41] According to these reports, in our study, lower BMI was a factor that was independently associated with the normalization of the glycaemic state, and an active lifestyle decreased the risk of having prediabetes.

The results of this study need to be interpreted in light of its strengths and weaknesses. First, the number of participants in our study is smaller in comparison to other studies. In addition, the study may not be representative of urban areas in our region. Thus, the results may not be generalizable to other territories with different population characteristics in our country. However, the Mollerussa cohort is a representative sample of the region, which is a specific semi-

rural area that has never been specifically investigated. Second, our study sample is probably healthier than the general population, as we excluded subjects with already known diabetes and other comorbidities, a lower number of subjects were counted in the denominator, thus resulting in a higher prevalence of this condition. Third, we did not assess glucose tolerance through an oral glucose tolerance test, which is common in most population studies. Although this assay is sensitive, it is also less specific for identifying subjects who could develop diabetes.[42] Furthermore, the oral glucose tolerance test has a low reproducibility and is a rather time-consuming and expensive procedure.[9, 43] Conversely, HbA1c and FPG are cost-effective and more convenient for patients. Currently, FPG is an accepted screening method to detect diabetes and prediabetes. HbA1c improves the sensitivity of FPG in the detection of early T2D in high-risk subjects[32, 44] and is a better predictor of CV events than FPG.[45] Fourth, we only followed up those participants with prediabetes. Thus, we could not analyse the probability of changing from normoglycaemia to prediabetes or diabetes in this study. Finally, it is probable that the use of the World Health Organization prediabetes criteria in our study would have resulted in a smaller proportion of subjects who returned to a normal glycaemic state. The World Health Organization established a normal concentration of FPG between 110 and <126 mg/dl.[46]

Conclusions

For the first time, our study provides information on the prevalence of diabetes and prediabetes in the Mollerussa health care area, a Mediterranean semi-rural area in northeast Spain. Individuals with prediabetes had a more unfavourable cardiometabolic risk profile than normoglycaemic subjects. Moreover, individuals with abnormalities in both criteria used to diagnose prediabetes had the worst risk profile. Finally, after one year of follow-up, few people progressed to diabetes, while more than 40% returned to a normal glycaemic state, and nearly 60% persisted in the prediabetic state. These results suggest that the use of both FPG and HbA1c criteria in clinical practice could help identify people with high diabetes and cardiovascular risk. Moreover, the identification of individuals with prediabetes provides an opportunity for intervention through lifestyle modification and pharmacological treatments not only to reduce the development of diabetes.

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Author Contributions MF, EC and DM conceived and designed the study; MBV, JFN, and MMC participated in the study design; MBV, MF, NA, MGC, NM, AM and CC collected the data; EC and JRM performed the statistical analyses; MF, EC, MMC and DM wrote the manuscript. All authors critically reviewed the manuscript and approved the final version to be published.

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Competing interests None declared.

Ethics approval The project was approved by the Ethics Committee of the Primary Health Care University Research Institute (IDIAP) Jordi Gol (PI12/043) Barcelona, Spain.

Data sharing statement Readers may contact Dr Didac Mauricio (didacmauricio@gmail.com) regarding the data.

Patient consent for publication Not required.

The original protocol for the study Readers may found the Cohort description in *BMJ Open* 2017:1-8. doi:10.1136/bmjopen-2016-015158.

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FIGURE LEGENDS

Figure 1. Flow diagram of subjects at baseline and after follow-up.

Figure 2. Multivariate logistic regression models **a)** model of prediabetes *versus* normoglycaemic state in the Mollerussa cohort at baseline. Significant p values are shown in bold. eGFR, estimated glomerular filtration rate. Hosmer and Lemeshow Test p=0.295. **b)** model of normalized *versus* persisted in subjects with follow-up data. Significant p values are shown in bold. Hosmer and Lemeshow Test p= 0.931.

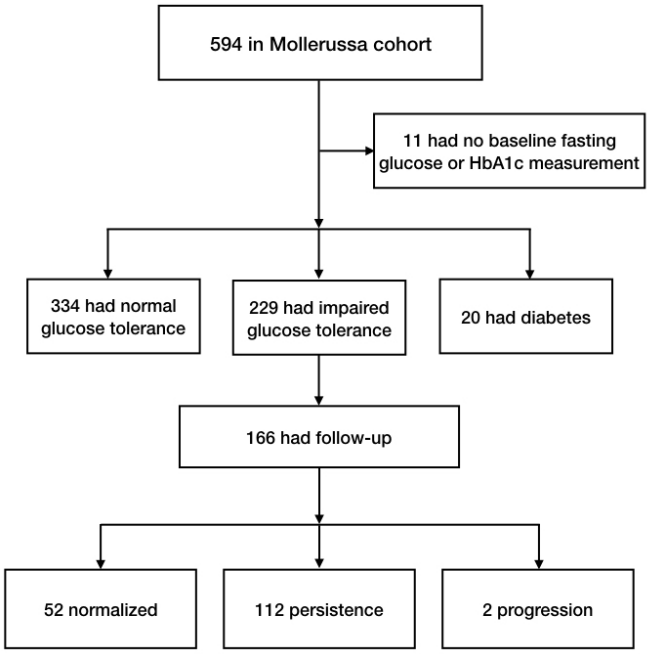

Figure 1. Flow diagram of subjects at baseline and after follow-up.

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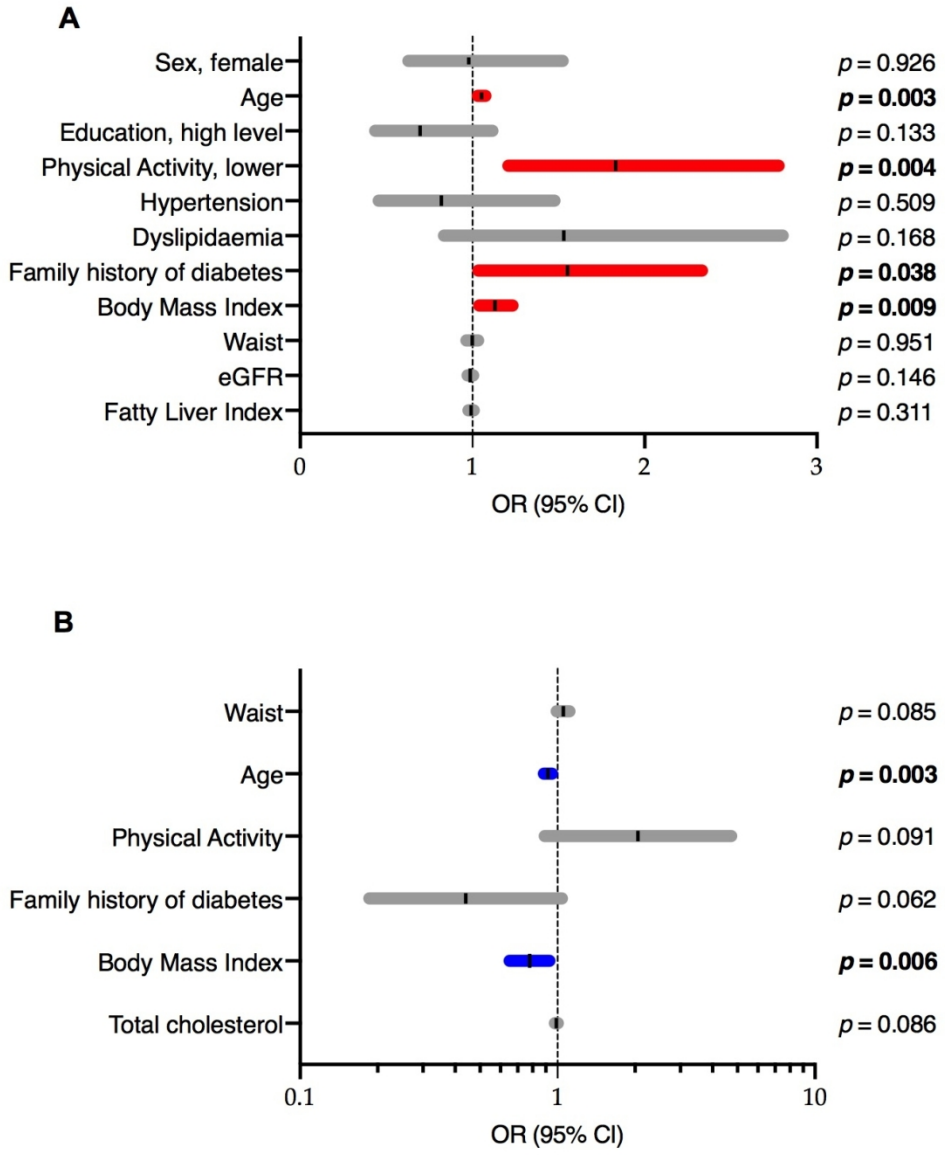

Figure 2. Multivariate logistic regression models a) model of prediabetes versus normoglycaemic state in the Mollerussa cohort at baseline. Significant p values are shown in bold. eGFR, estimated glomerular filtration rate. Hosmer and Lemeshow Test p=0.295. b) model of normalized versus persisted in subjects with follow-up data. Significant p values are shown in bold. Hosmer and Lemeshow Test p= 0.931.

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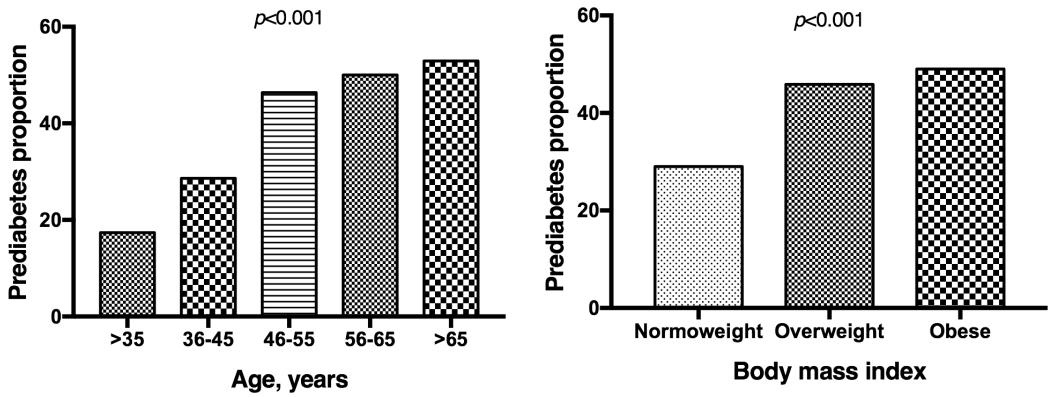


Supplementary Figure 1. Proportion of patients with prediabetes. A) Stratified by age. B) Stratified by body mass index.

Supplementary Table 1. Logistic regression model of isolated fasting plasma glucose

	OR	95% CI	p value
Sex	0.683	0.372 - 1.254	0.219
Age	1.032	1.008 - 1.057	**0.009**
Education	0.625	0.351 - 1.111	0.109
Physical activity	0.535	0.318 - 0.899	**0.018**
Family history of diabetes	1.798	1.067 - 3.028	**0.028**
Hypertension	1.423	0.722 - 2.805	0.309
Body mass index	0.994	0.890 - 1.110	0.914
Waist circumference	1.006	0.962 - 1.053	0.789
Fatty liver index	1.014	0.990 - 1.038	0.268
Total cholesterol	1.012	0.993 - 1.032	0.227
Triglycerides	0.998	0.993 - 1.003	0.402
LDL-cholesterol	0.986	0.965 - 1.008	0.212

Supplementary Table 2. Logistic regression model of isolated HbA1c

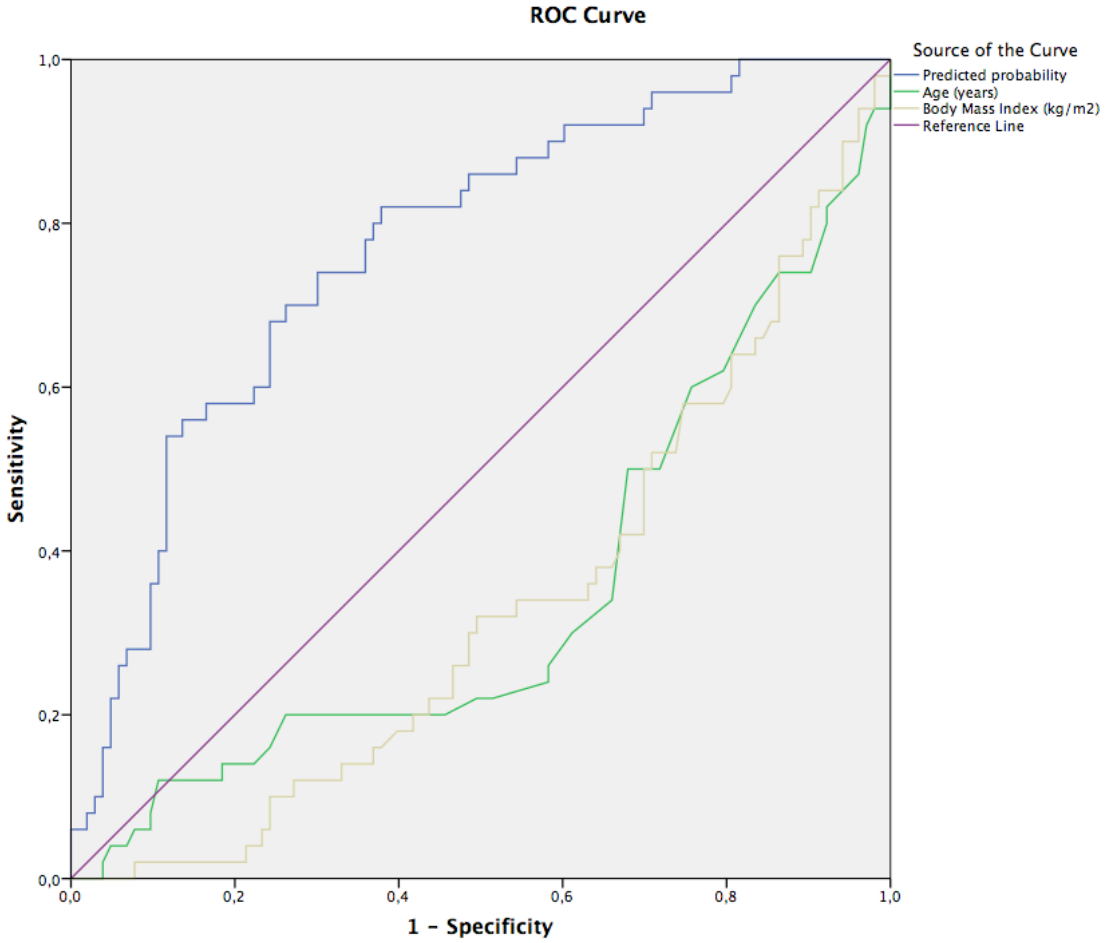
	OR	95% CI	p value
Sex	1.304	0.830 - 2.050	0.249
Age	1.048	1.029 - 1.067	**<0.001**
Education	0.917	0.575 - 1.461	0.715
Physical activity	0.668	0.440 - 1.013	0.058
Hypertension	0.848	0.481 - 1.497	0.570
Body mass index	1.080	0.990 - 1.179	0.083
Waist circumference	0.996	0.963 - 1.030	0.822
Fatty liver index	1.000	0.982 - 1.018	0.960
Total cholesterol	1.000	0.994 - 1.006	0.996
Triglycerides	0.999	0.996 - 1.002	0.724

Supplementary Table 3. Logistic regression model of fasting plasma glucose and HbA1c

	OR	95% CI	p value
Sex, female	1.559	0.753 – 3.225	0.232
Age	1.056	1.026 – 1.086	**<0.001**
Education level	0.914	0.457 – 1.828	0.799
Physical activity	0.610	0.322 – 1.157	0.130
Hypertension	1.665	0.782 – 3.545	0.186
Dyslipidaemia	0.818	0.357 – 1.870	0.633
Body mass index	0.901	0.788 – 1.030	0.128
Waist circumference	1.012	0.960 – 1.066	0.665
Triglycerides	0.999	0.993 – 1.004	0.594
Fatty liver index	1.031	1.002 – 1.061	**0.037**

Supplementary Table 4. Logistic regression model of normalized *versus* persisted in subjects with follow-up data.

	OR	95% CI	p value
Sex(1)	1.025	0.392 – 2.682	0.960
Age	0.956	0.917 – 0.998	**0.039**
Education(1)	1.327	0.513 – 3.431	0.560
Physical_Activity(1)	1.885	0.798 – 4.453	0.149
Treat_HT(1)	0.865	0.268 – 2.791	0.808
Family_history_DM(1)	0.428	0.176 – 1.040	0.061
BMI	0.749	0.605 – 0.926	**0.007**
Waist	1.037	0.959 – 1.121	0.365
FLI	1.022	0.982 – 1.063	0.292
HOMA2_IR	0.620	0.240 – 1.604	0.324
Total_cholesterol	0.986	0.972 – 1.000	0.052
HDL_cholesterol	1.015	0.978 – 1.052	0.435



Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Predicted probability	0.771	0.040	0.000	0.693	0.849
Age	0.347	0.049	0.002	0.251	0.442
Body Mass Index	0.332	0.045	0.001	0.244	0.421

Supplementary Figure 2. Receiver operating characteristics (ROC) curve showing the relationship between sensitivity and 1-specificity in determining the discriminatory ability of the logistic regression model and the variables age and body mass index separately.

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	#3	State specific objectives, including any prespecified hypotheses	4
Methods			

Study design	#4	Present key elements of study design early in the paper	4
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	4-5
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	4-5
Bias	#9	Describe any efforts to address potential sources of bias	6
Study size	#10	Explain how the study size was arrived at	6
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	6
Statistical methods	#12c	Explain how missing data were addressed	5
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	n/a
Statistical methods	#12e	Describe any sensitivity analyses	6

Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	6-7
Participants	#13b	Give reasons for non-participation at each stage	n/a
Participants	#13c	Consider use of a flow diagram	7
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	7-8
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	5
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	5
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	6-7
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6-7
Main results	#16b	Report category boundaries when continuous variables were categorized	7
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	7, 9,10
Discussion			
Key results	#18	Summarise key results with reference to study objectives	10-11

Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	13
Generalisability	#21	Discuss the generalisability (external validity) of the study results	n/a
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13