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Combined lifestyle factors and the risk of LADA and type 2 diabetes – Results from a Swedish population-based case-control study

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ABSTRACT

Aims: We investigated the risk of latent autoimmune diabetes in adults (LADA) and type 2 diabetes in relation to a healthy lifestyle, the proportion of patients attributable to an unhealthy lifestyle, and the influence of family history of diabetes (FHD) and genetic susceptibility.

Methods: The population-based study included incident LADA (n = 571), type 2 diabetes (n = 1962), and matched controls (n = 2217). A healthy lifestyle was defined by BMI < 25 kg/m^2, moderate-to-high physical activity, a healthy diet, no smoking, and moderate alcohol consumption. We estimated odds ratios (OR) with 95% confidence intervals (CIs) adjusted for age, sex, education, and FHD.

Results: Compared to a poor/moderate lifestyle, a healthy lifestyle was associated with a reduced risk of LADA (OR 0.51, CI 0.34–0.77) and type 2 diabetes (OR 0.09, CI 0.05–0.15). A healthy lifestyle conferred a reduced risk irrespective of FHD and high-risk HLA genotypes. Having a BMI < 25 kg/m^2 conferred the largest risk reduction for both LADA (OR 0.54, CI 0.43–0.66) and type 2 diabetes (OR 0.12, CI 0.10–0.15) out of the individual items.

Conclusion: People with a healthy lifestyle, especially a healthy body weight, have a reduced risk of LADA including those with genetic susceptibility to diabetes.

Abbreviations: ANDIS, All New Diabetics in Scania; ANDiU, All New Diabetics in Uppsala County; CI, 95% confidence intervals; EIRA, Epidemiological Investigation of Rheumatoid Arthritis; ESTRID, Epidemiological Study of Risk Factors for LADA and Type 2 Diabetes; FFQ, Food Frequency Questionnaire; FHD, Family history of diabetes; GADA, glutamic acid decarboxylase antibodies; LADA, Latent autoimmune diabetes in adults; OR, odds ratio; PAR%, Population-attributable risk percentage

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1. Introduction

Observational studies indicate that adherence to a healthy lifestyle, including normal body weight [1] physical activity [2] a healthy diet [3,4] non-smoking [5] and moderate alcohol consumption [6] may prevent 75–91% of all cases of type 2 diabetes [4,7]. In support hereof, randomised clinical trials found that lifestyle intervention including moderate weight loss and increased physical activity reduced the risk of type 2 diabetes by 40–50% in individuals with high risk to develop the disease [8]. To what extent other forms of diabetes may be prevented by a healthy lifestyle is, however, unclear.

Latent autoimmune diabetes in adults (LADA) is a hybrid from of diabetes [9]; similar to type 1 diabetes it is characterised by autoimmune reactivity, HLA-DQB1 genotypes associated with autoimmunity [10,11] and excess risk conferred by family history of type 1 diabetes [12]. LADA also shares features with type 2 diabetes, including adult onset, insulin resistance [9,13] and, according to some studies [14,15] increased frequency of *TCF7L2* risk genotypes, which is primarily seen for less autoimmune LADA [16]. Lifestyle factors linked to type 2 diabetes have been associated with LADA [17] including overweight [18] physical inactivity [19,20] diet [21,22] smoking [23] and alcohol consumption [24]. However, to what extent the combination of healthy lifestyle factors may reduce the risk of LADA is not clear; to date only one study, based on few cases and a limited number of lifestyle factors have addressed this issue [25]. Therefore, our aim was to investigate the risk of LADA in relation to the combination of healthy lifestyle factors, and to estimate the proportion of cases attributable to an unhealthy lifestyle. We also investigated whether a healthy lifestyle may reduce the risk of LADA in individuals with family history of diabetes (FHD) or genetic susceptibility as indicated by high-risk genotypes of HLA or *TC7FL2*.

2. Subjects, materials and methods

2.1. Study population

Analyses were based on data from a Swedish population-based case-control study with incident cases of LADA and type 2 diabetes and matched controls. The "All New Diabetics In Scania" (ANDIS) study is an extensive diabetes registry and biobank aimed to characterise all incident diabetes cases in Scania county, Sweden [26]. Nested in the ANDIS Study, the "Epidemiological Study of Risk Factors for LADA and Type 2 Diabetes" (ESTRID) is an ongoing population-based case–control study established in 2010 (described elsewhere in detail [21]). In brief, ESTRID recruits all incident cases of LADA and a random sample of patients with type 2 diabetes from ANDIS (four cases per LADA case). Since 2012, individuals from the "All New Diabetics in Uppsala County" (ANDiU) study are additionally included. Non-diabetic control individuals with age $\geq$ 35 years are randomly selected from the national population register and matched to the cases through incidence density sampling (six controls per LADA case) [27]. Analyses were based on all cases and controls collected September 2010 through October 2019 with complete information on all exposures and confounders variables (94.1% of sample); 571 LADA, 1962 type 2 diabetes, and 2217 controls. Since controls in ESTRID lack genetic data, we used population-based controls from the "Epidemiological Investigation of Rheumatoid Arthritis" (EIRA) study for stratification based on genetic risk [28]. These controls, hereafter referred to as genetic controls, were recruited 1996–2014, free of diabetes, had complete information on lifestyle except for diet, at least one of the genetic variants of interest, and were matched to the diabetes cases by sex and birth year (n = 1634). All participants gave written informed consent and the Regional Ethical Review Board in Stockholm approved the study.

2.2. Diabetes classification and laboratory analyses

Cases of LADA and type 2 diabetes were diagnosed within the health care system and blood samples were collected. GADA was determined using ELISA (RSR, Cardiff, UK); values > 250 U/mL were censored at 250 U/mL, and the cut-off level for positivity was 10 U/mL (estimated with 84% sensitivity and 98% specificity). Fasting plasma C-peptide was determined using IMMULITE 2000 (Siemens Healthcare Diagnostics, Llanberis, UK) or Cobas e 601 (Roche Diagnostics, Mannheim, Germany). LADA was defined as age $\geq$ 35 at diagnosis, GADA positivity, and C-peptide levels above the lower limit for the normal range ($\geq$0.2 nmol/L (IMMULITE) or $\geq$ 0.3 nmol/L (Cobas)). Type 2 diabetes was defined as age $\geq$ 35 at diagnosis, GADA negativity, and C-peptide levels > 0.6 nmol/L (IMMULITE) or $\geq$ 0.72 nmol/L (Cobas). Homeostatic Model Assessment (HOMA) [29] estimated insulin resistance (HOMA-IR) and beta cell function (HOMA-B) based on fasting plasma glucose and C-peptide levels. This method correlates well with the hyperinsulinaemic-euglycaemic clamp method [29]. No clinical information was available for controls.

DNA of the cases was analysed using iPLEX Gold technology (Sequenom Laboratories, San Diego, CA, USA). Imputation of missing genotypes was performed on an subset using Infinium CoreExome v1.1 (Illumina, San Diego, CA, USA) based on the Haplotype Reference Consortium (http://www.haplotype-reference-consortium. org/; version r1.1 2016) panel. Genetic controls were genotyped using GWAS data from Illumina Global Screening array or Infinium Illumina 300 K immunochip custom array (Illumina, USA). We used the single nucleotide polymorphism (SNP) most strongly associated with type 2 diabetes, *TCF7L2* rs7903146, for stratified analysis across the genetic variants (TT/CT vs. CC). Furthermore, we used tree SNPs in the HLA complex (rs3104413, rs2854275, rs9273363), shown to predict high-risk HLA DR/DQ genotypes associated with autoimmunity, with an overall accuracy of 99.3% [30]. The three SNPs were combined to identify high-risk (DR4-DQ8, DR4/3-DQ8, DR3/4, DR3/3, DR4/4, DRB1*0301-DQA1*0501-DQB1*0201) and other HLA genotypes (DR3/X, DR4/X, DR4-DQ7, DRX/X [31]; X denotes any other allele than DR3, DR4, or DR7).

2.3. Healthy lifestyle components and covariates

At the time of recruitment, cases and controls answered a similar, extensive questionnaire on lifestyle habits; cases received the questionnaire close to time of diagnosis (median time: 5 months). All participants were instructed to report

their lifestyle habits during the preceding year; cases were specifically instructed to report conditions prior to diagnosis. Based on questions on leisure-time physical activity (described in detail previously [20]), participants were categorised into four levels: sedentary, low, moderate, and high physical activity and further into low-risk (moderate and high activity) and other (sedentary and low activity) lifestyle category. Self-reported body-mass index (BMI) was calculated as kg/m^2; for cases, this information was highly correlated with clinical measurements taken at diagnosis ($r = 0.92$). BMI < 25 was defined as the low-risk lifestyle category. Based on smoking history, participants were categorized into current, former, and never smokers; the latter category was defined as the low-risk lifestyle category.

Information on diet for ESTRID participants was obtained through a validated [32] 132 item, semi-quantitative Food Frequency Questionnaire (FFQ). Based on FFQ data, the nutrient intake of each food item and total energy intake (kcal/day) were estimated by multiplying frequency of consumption by nutrient content according to the Swedish National Food Agency Database considering age- and sex-specific portion sizes [33]. We excluded participants with implausible total energy intake, i.e. deviating > 2 standard deviations (SD) from the $\log_e$-transformed mean intake of all participants (women, <257.8 or > 6475.2 kcal/d; men, <401.1 or > 7537.7 kcal/d). Estimation of a healthy diet was based on the "Life's Simple 7" dietary goals [3] including intake of vegetables and fruit (low-risk cut-off: >400 g/day), fish (>2 servings/week), sugar-sweetened beverages (<150 g/d, equals 64 kcal), whole-grain (>48 g/d), and sodium (<1.5 g/d). We additionally included consumption of processed red meat (<1 serving/d), because it has recently been associated with increased risk of LADA and type 2 diabetes [22]. Participants were classified into three categories based on the healthy diet score, i.e. poor (0–2 dietary components), intermediate (3–4 dietary components), and healthy (≥5 dietary components achieved); the latter was defined as the low-risk lifestyle category. Participants were also asked to report how often during the previous year, they consumed alcoholic beverages by indicating one of nine predefined frequency categories, ranging from 'never' to '≥3 times/day'. Based on information about amount and frequency, the intake of alcohol as g/day was estimated [24]. Since moderate alcohol intake, defined as 5–30 g/d [34] has been shown to be associated with a reduced risk of LADA [24,34] alcohol consumption > 5 g/d was defined as the low-risk lifestyle category. Furthermore, FHD was assessed based on information on diabetes in first-degree relatives. No information on FHD was available for genetic controls.

The combined lifestyle variable was created based on accomplishments of the low-risk category of the individual lifestyle components, including moderate-to-high physical activity, a healthy diet, BMI < 25, non-smoking, and moderate alcohol consumption. The combined lifestyle was divided into three categories, similar to previous classifications in relation to type 2 diabetes [3]: poor lifestyle (≤1 low-risk components), moderate lifestyle (2–3 low-risk components), and healthy lifestyle (≥4 low-risk components). Due to missing FFQ data for genetic controls, the combined lifestyle variable for analyses stratified by HLA and *TCF7L2* was based on accomplishments of the low-risk categories of the individual lifestyle components physical activity, BMI, smoking, and alcohol consumption.

2.4. Statistical analyses

Differences in normally distributed characteristics were determined using two-sided Student's *t*-test (presented as means and SD) and Wilcoxon rank sum tests for non-normally distributed characteristics (presented as median and interquartile range, IQR). Differences in proportions were determined using Chi-squared tests.

We used conditional logistic regression to calculate odds ratios (ORs) with 95% confidence intervals (CIs). ORs for LADA and type 2 diabetes were calculated for individual lifestyle components (low-risk vs. other) as well as the combined lifestyle variable (healthy, moderate vs. poor, and healthy vs. moderate/poor). ESTRID controls were used in all analyses except for stratifications by genetic risk, where genetic controls were used. All models were adjusted for age, sex, educational level (primary school, upper secondary school, or university) and FHD, unless stated otherwise. Analyses were stratified by FHD (first degree relative, yes or no), HLA (high or low/moderate risk), and *TCF7L2* rs7903146 (TT/CT or CC). Population-attributable risk percentage (PAR%) was calculated to estimate the proportion of cases in the population that would have been prevented if all participants would have adopted the healthy lifestyle. PAR% was calculated as p*(1–1/OR), where p is prevalence (in %) of the individual (other) or combined (poor) lifestyle factor among cases and OR is the adjusted odds ratio.

In sensitivity analyses, the ORs of LADA and type 2 diabetes in relation to individual lifestyle factors were assessed after additional adjustment for BMI. Furthermore, we re-run the main analyses (Table 2) using the genetic controls to assess their validity (Table S8). We also performed separate analyses of LADA cases stratified by GADA levels (Table S4).

3. Results

3.1. Characteristics

LADA patients were younger, leaner, had lower insulin secretion, less insulin resistance and were more likely to be treated with insulin than type 2 diabetes patients (Table 1). The genetic controls were younger and more likely to be female than the ESTRID controls (Table S1); this was by matching on birthyear and sex. Overall, a moderate lifestyle (2–3 low-risk components) was achieved by 47.6% of participants and a healthy lifestyle (≥4 low-risk components) by 6.1%. Those with a moderate or healthy lifestyle were younger, less likely to have FHD, had higher education, and were slightly more likely of Swedish origin compared to those with a poor lifestyle (Table S2).

3.2. Lifestyle and LADA

Of the low-risk components, BMI < 25 was associated with the largest risk reduction for LADA (OR 0.54, 95% CI 0.43–0.66) (Table 2). Furthermore, moderate-to-high physical activity (OR 0.73, 95% CI 0.60–0.90) and moderate alcohol consump-

Table 1 – Characteristics of individuals.

	Controls	LADA	Type 2 diabetes	P
No. of individuals	2217	571	1962	–
Men (%)	47.6	52.9	60.3	0.0017
Age at diagnosis/inclusion (Years, mean +/− SD)	58.9+/−13.7	59+/−12.3	63.3+/−10.4	<0.0001
BMI (kg/m2, mean +/− SD)	26+/−4.2	28.5+/−5.7	31.2+/−5.4	<0.0001
Insulin treatment (%)	–	39.1	5.6	<0.0001
GADA (IU/mL, median, IQR)	–	250 (218.5)	–	–
C-peptide (nM, median, IQR)	–	0.7 (0.7)	1.2 (0.6)	<0.0001
HOMA-B (median, IQR)	–	40.5 (53.9)	71.3 (51.1)	<0.0001
HOMA-IR (median, IQR)	–	2.8 (2.7)	3.6 (2.0)	<0.0001
Family history of diabetes (%)	25.6	45.4	51.1	0.0175
Low level of education (primary school, %)	21.9	25.9	34.9	<0.0001
Country of birth (SE, %)	88.3	89.5	87.6	0.2128

P, p-value estimating the difference between individuals with Type 2 Diabetes and LADA. Differences between levels of education were determined for the whole variable (not separated by category).

Table 2 – Odds ratios and PAR% for LADA and type 2 diabetes by individual and combined lifestyle factors.

	Individuals per group			LADA		Type 2 diabetes	
	LADA	T2D	Control	OR (95% CI)	PAR% (95% CI)	OR (95% CI)	PAR% (95% CI)
Physical activity							
Other	377	1462	1280	1	1	1	1
Low-risk	194	500	937	0.73 (0.60, 0.90)	17.6 (6.8, 26.3)	0.48 (0.41, 0.55)	39.0 (33.4, 43.9)
Diet							
Other	506	1785	1921	1	1	1	1
Low-risk	65	177	296	0.89 (0.66, 1.20)	9.5 (−17.9, 29.8)	0.75 (0.61, 0.94)	22.3 (5.5, 35.8)
BMI							
Other	410	1815	1203	1	1	1	1
Low-risk	161	147	1014	0.54 (0.43, 0.66)	33.4 (24.4, 40.7)	0.12 (0.10, 0.15)	81.1 (79.1, 83.5)
Smoking							
Other	313	1222	1100	1	1	1	1
Low-risk	258	740	1117	0.89 (0.73, 1.08)	6.1 (−4.3, 14.6)	0.73 (0.63, 0.83)	17.0 (10.3, 22.8)
Alcohol							
Other	273	1011	950	1	1	1	1
Low-risk	298	951	1267	0.75 (0.62, 0.92)	11.8 (3.8, 18.3)	0.60 (0.52, 0.69)	20.6 (15.9, 24.8)
Combined lifestyle							
Poor	262	1215	727	1	1	1	1
Moderate	280	732	1246	0.66 (0.54, 0.81)	15.5 (8.8, 21.0)	0.37 (0.32, 0.43)	38.8 (35.2, 41.9)
Healthy	29	15	244	0.40 (0.26, 0.61)	27.6 (17.9, 33.9)	0.05 (0.03, 0.09)	58.7 (56.3, 60.1)
Combined lifestyle (dichotomised)							
Poor/moderate	542	1947	1973	1	1	1	1
Healthy	29	15	244	0.51 (0.34, 0.77)	46.2 (21.8, 62.5)	0.09 (0.05, 0.15)	90.5 (84.2, 94.1)

T2D, Type 2 diabetes; OR, odds ratio; CI, confidence interval; PAR%, population attributable risk percentage. Model was adjusted for age, sex, family history of diabetes and level of education. Combined lifestyle: Poor lifestyle, 0–1 low-risk component; Moderate lifestyle, 2–3 low-risk components; Healthy lifestyle, 4 + low-risk components.

tion (OR 0.75, 95% CI 0.62–0.92) were associated with a lower risk of LADA, whereas the associations with non-smoking and a healthy diet were weaker (Table 2).

Regarding the combination of individual lifestyle components, a reduced risk of LADA was seen in individuals with a moderate (2–3 low-risk components) lifestyle (OR 0.66, 95% CI 0.54–0.81), which was more pronounced in individuals adhering to the healthy (≥4 low-risk components) lifestyle (OR 0.40, 95% CI 0.26–0.61) when compared to those with a poor lifestyle (Table 2). A healthy lifestyle was associated with a reduced LADA risk in both women and men (Fig. 1, Table S3). Estimation of PAR% indicated that almost half of all individuals with LADA (46.2%, 95% CI 21.8%-62.5%) could be ascribed to a lifestyle that did not conform to the low-risk pattern (≥4 low-risk components) (Table 2). When restricting the analysis to highly autoimmune LADA cases (GADA ≥ 250 U/mL), both a moderate (OR 0.74, 95% CI 0.57–0.97) and a healthy (OR 0.50, 95% CI 0.29–0.85) lifestyle were associated with a reduced risk (Table S4). The PAR% was estimated at 21.1% (95% CI 6.2–29.8) for a poor vs. healthy lifestyle. These results were more pronounced for less autoimmune LADA (GADA in the lowest tercile; < 67.46 U/mL); OR was 0.15, 95% CI 0.05–0.42) for a healthy lifestyle and PAR% was 47.4 (95% CI 32.2–52.9) for healthy vs. poor lifestyle (Table S4).

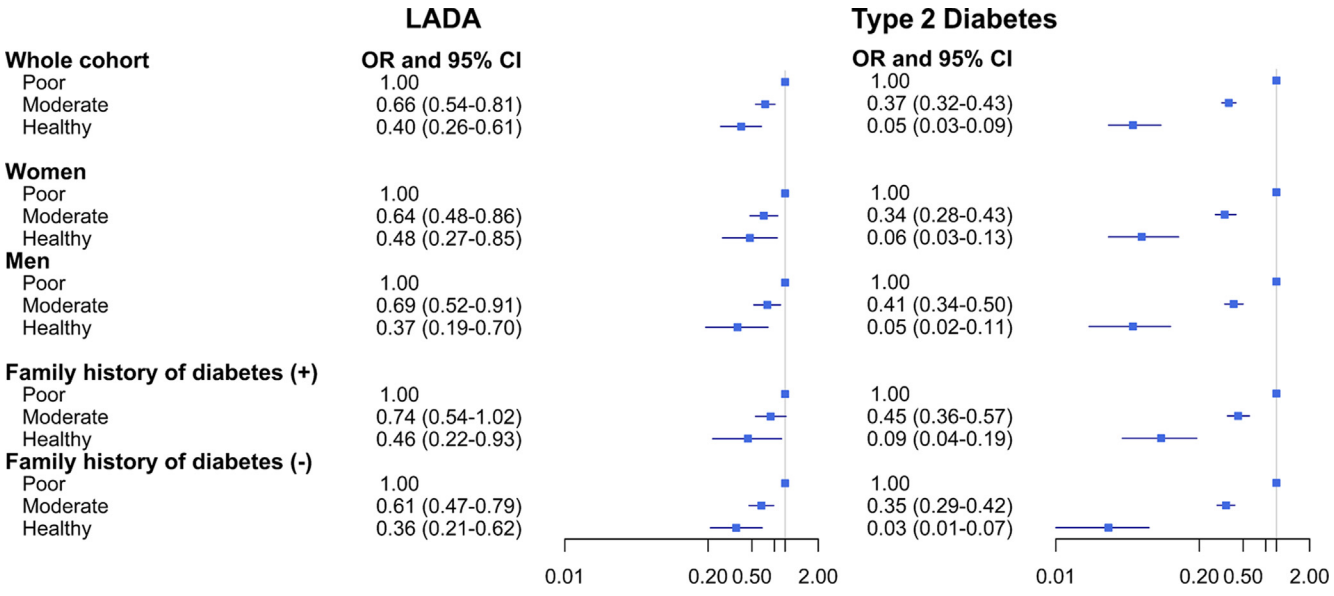

Fig. 1 – Odds ratios for LADA and type 2 diabetes by combined lifestyle components. Odds ratios (ORs) and 95% confidence intervals (CIs) for incident LADA (left panel) and type 2 diabetes (right panel) are presented for non-stratified individuals (whole cohort, upper panel), and individuals stratified by sex (middle panel), or by family history of diabetes (with (+) or without (-) known history of diabetes in first-degree family, lower panel). ORs were adjusted for age, sex, and level of education. Horizontal lines display ORs as indicated by square and 95% CIs. Poor lifestyle, 0–1 low-risk component; Moderate lifestyle, 2–3 low-risk components; Healthy lifestyle, 4 + low-risk components.

As shown previously in this population [12,18] the risk of LADA was associated with FHD, and HLA and *TCF7L2* risk genotypes (Table S5). A healthy lifestyle was associated with a reduced risk of LADA in those with (OR 0.46, 95% CI 0.22–0.93) and without (OR 0.36, 95% CI 0.21–0.62) FHD (Fig. 1, Table S3). Similarly, an inverse association was seen both in carriers of high-risk (OR 0.32, 95% CI 0.11–0.94) and in those with low/intermediate-risk (OR 0.28, 95% CI 0.08–0.93) HLA genotypes (Table 3). Furthermore, those with moderate lifestyle had a reduced risk of LADA irrespective of *TCF7L2* genotype (Table 3).

3.3. *Lifestyle and type 2 diabetes*

All five individual, low-risk lifestyle components were inversely associated with type 2 diabetes (Table 2), including moderate-to-high physical activity, a healthy diet, BMI < 25, non-smoking, and moderate alcohol consumption. The risk was reduced in those with a moderate (OR 0.37, 95% CI 0.32–0.43) and healthy lifestyle (OR 0.05, 95% CI 0.03–0.09); both in men and women (Fig. 1, Table S3). Estimation of PAR% indicated that 90.5% of type 2 diabetes cases (95% CI 84.2%-94.1%) were attributable to a lifestyle that did not conform to the low-risk pattern. The inverse associations between moderate and healthy lifestyles and T2D were seen irrespective of FHD (Fig. 1, Table S3) and genotypes of *TCF7L2* (Table 3).

3.4. *Clinical characteristics by lifestyle*

LADA patients with a poor lifestyle had higher HOMA-B and C-peptide level, and lower levels of GADA, but were more insulin resistant (HOMA-IR) than those with moderate or healthy lifestyle (Table S6). Similarly, in type 2 diabetes a poor lifestyle was characterised by higher HOMA-B, C-peptide, and HOMA-IR values than a moderate or healthy lifestyle (Table S6).

3.5. *Sensitivity analyses*

When we adjusted analyses of the individual lifestyle components for BMI, the association between physical activity and LADA was attenuated, whereas the association with alcohol consumption remained (Table S7). For type 2 diabetes, the associations with physical activity, smoking, and alcohol consumption remained after adjustment for BMI, but the association with diet was eliminated (Table S7). Substituting the ESTRID controls for the genetic controls yielded similar associations between the individual lifestyle factors and LADA and type 2 diabetes, respectively (Table 2, Table S8).

4. Discussion

In this large population-based study, adherence to a healthy lifestyle, including moderate-to-high physical activity, a healthy diet, BMI < 25, non-smoking, and moderate alcohol consumption, was associated with a 60% reduction in the risk of LADA. Furthermore, almost half of all patients were attributable to a lifestyle that did not conform to the low-risk pattern. These findings are in line with results based on the Norwegian HUNT study [25]. In addition, we observed, for the first time, that a healthy lifestyle was associated with a reduced risk of LADA irrespective of sex, FHD, and high-risk genotypes of HLA and *TCF7L2*. Notably, we found a markedly larger risk reduction for adopting a healthy over a moderate

Table 3 – Odds ratios and PAR% for LADA and Type 2 Diabetes by combined lifestyle factors stratified by HLA and *TCF7L2*.

			Individuals per group			LADA		Type 2 diabetes	
			LADA	T2D	Control	OR (95% CI)	PAR% (95% CI)	OR (95% CI)	PAR% (95% CI)
Whole cohort	**Combined lifestyle**	Poor	199	874	422	1	1	1	1
		Moderate	178	392	1097	0.40 (0.29, 0.55)	30.6 (23.0, 36.2)	0.23 (0.18, 0.30)	52.9 (48.1, 56.4)
		Healthy	13	5	115	0.31 (0.14, 0.67)	35.2 (16.8, 43.9)	0.03 (0.01, 0.08)	66.7 (63.3, 68.1)
	(dichotomised)	Poor/moderate	377	1266	1519	1	1	1	1
		Healthy	13	5	115	0.55 (0.26, 1.16)	43.5 (−15.5, 71.5)	0.05 (0.02, 0.18)	94.6 (81.7, 97.6)
HLA high risk	**Combined lifestyle**	Poor	119	278	133	1	1	1	1
		Moderate	109	120	353	0.36 (0.22, 0.59)	32.1 (20.6, 39.2)	0.19 (0.12, 0.30)	56.6 (48.9, 61.5)
		Healthy	9	0	41	0.32 (0.11, 0.94)	34.1 (3.0, 44.7)	NA	NA
	(dichotomised)	Poor/moderate	228	398	486	1	1	1	1
		Healthy	9	0	41	0.61 (0.22, 1.71)	37.5 (−68–3, 75.0)	NA	NA
HLA low/inter. risk	**Combined lifestyle**	Poor	80	596	289	1	1	1	1
		Moderate	69	272	744	0.39 (0.24, 0.62)	31.9 (19.9, 39.7)	0.25 (0.18, 0.34)	51.2 (45.1, 56.0)
		Healthy	4	5	74	0.28 (0.08, 0.93)	37.6 (3.7, 48.1)	0.03 (0.01, 0.11)	66.2 (60.8, 67.6)
	(dichotomised)	Poor/moderate	149	868	1033	1	1	1	1
		Healthy	4	5	74	0.50 (0.15, 1.60)	48.7 (−58.4, 82.8)	0.06 (0.02, 0.22)	93.5 (76.0, 95.4)
***TCF7L2* (TT or TC)**	**Combined lifestyle**	Poor	110	451	170	1	1	1	1
		Moderate	90	214	509	0.36 (0.23, 0.57)	34.3 (23.1, 41.3)	0.23 (0.16, 0.34)	52.0 (44.6, 56.7)
		Healthy	5	3	57	0.18 (0.05, 0.62)	44.0 (20.4, 51.0)	0.03 (0.01, 0.12)	65.5 (59.4, 66.8)
	(dichotomised)	Poor/moderate	200	665	679	1	1	1	1
		Healthy	5	3	57	0.34 (0.10, 1.13)	64.4 (−12.7, 87.8)	0.06 (0.01,0.26)	93.6 (72.2, 96.6)
***TCF7L2* (CC)**	**Combined lifestyle**	Poor	89	423	252	1	1	1	1
		Moderate	88	178	588	0.43 (0.26, 0.70)	27.4 (14.4, 35.6)	0.21 (0.14, 0.30)	55.4 (49.1, 60.3)
		Healthy	8	2	58	0.53 (0.19, 1.52)	22.6 (−25.0, 39.0)	0.03 (0.01, 0.21)	68.0 (55.4, 69.4)
	(dichotomised)	Poor/moderate	177	601	840	1	1	1	1
		Healthy	8	2	58	0.90 (0.33, 2.44)	9.6 (−137.8, 64.1)	0.06 (0.01, 0.44)	93.7 (55.8, 98.7)

Cases and controls were stratified using three SNPs to identify high-risk and low/intermediate *HLA* genotypes (see Materials and Methods for details) and by *TCF7L2*-rs7903146 (TT/TC vs. CC). Analyses were based on cases with genetic information (LADA: n = 390, 68.3% of sample; Type 2 diabetes: n = 1271, 64.8% of sample) and genetic controls (n = 1634). OR, odds ratio; CI, confidence interval; PAR%, population attributable risk percentage. Models were adjusted for age, sex and birth year (matching variables in the genetic analyses), and level of education. Poor lifestyle, 0–1 low-risk component; Moderate lifestyle, 2–3 low-risk components; Healthy lifestyle, 4 + low-risk components. The combined lifestyle variable was based on accomplishments of the low-risk category of the individual lifestyle components physical activity, BMI, smoking, and alcohol consumption.

lifestyle in type 2 diabetes; in LADA, on the other hand, the risk reduction was more comparable between the moderate and healthy lifestyle. The influence of lifestyle on type 2 diabetes risk was more pronounced; a 91% risk reduction was seen in individuals with a healthy lifestyle, and 91% of all patients could be attributed to an unhealthy lifestyle. These findings are in accordance with a recent *meta*-analysis wherein individuals with the healthiest lifestyle had a 75% reduced risk of type 2 diabetes [7]. A moderate lifestyle was also associated with a reduced risk of LADA and type 2 diabetes, suggesting that improving at least some lifestyle factors already reduces the risk of diabetes, whereas adhering to a healthier lifestyle may decrease the risk further.

Our findings suggest that lifestyle modifications may substantially reduce the risk of LADA also in individuals with genetic susceptibility. Similar findings were seen in type 2 diabetes, confirming those of a previous study based on two prospective cohorts [35]. Our findings further confirmed those of the HUNT study, wherein 69% of individuals with LADA were estimated to be preventable by adhering to a healthy lifestyle [25]. The preventive potential was smaller in our study, i.e. 46%, which may either reflect the heterogenous nature of LADA [11,36] or differences in the definitions of a healthy lifestyle, because smoking and diet were not included in the previous study. It should be noted that the present study was based on more than three times as many LADA cases as the previous [25].

Notably, all individual lifestyle components were associated with a reduced risk of type 2 diabetes, whereas only moderate-to-high physical activity, BMI < 25, and moderate alcohol consumption were significantly associated with LADA. Among the low-risk components, BMI < 25 was associated with the largest risk reduction for both LADA and type 2 diabetes, in accordance with previous studies [4,25]. This highlights the importance of maintaining a healthy weight to reduce diabetes risk. In the present study, only 6.1% of study participants adhered to a healthy lifestyle, illustrating the tremendous potential for improvement. Notably, a poor lifestyle was more common in individuals who were older, had low education, FHD, and who were of non-Swedish origin, indicating that these groups may benefit most from preventive efforts.

The investigated lifestyle factors have primarily been linked type 2 diabetes through effects on insulin resistance [7]. In support hereof, we found that both in LADA and type 2 diabetes, a poor lifestyle was associated with higher HOMA-IR. Considering that the primary defect of LADA is insulin deficiency caused by autoimmunity [9], it is not surprising that the risk reduction for both the combined and individual lifestyle factors were less pronounced for LADA than type 2 diabetes, where insulin resistance plays a key role in pathogenesis. Moreover, a healthy lifestyle was associated with a larger risk reduction for less autoimmune than for more autoimmune LADA, in whom autoimmunity and subsequent insulin deficiency is likely to be the main driver of disease onset. We noted that LADA patients adhering to a healthy lifestyle tended to have higher GADA levels than those with a poor lifestyle, worse beta cell function (HOMA-B), but less insulin resistance (HOMA-IR). One explanation could be that people with an unhealthy lifestyle develop insulin resistance, which will increase the demand on the beta cells; this could hypothetically unmask an underlying autoimmune process at an earlier stage, i.e. when there is more remaining beta cell function than in people with a healthy lifestyle who are not as insulin resistant. The idea that the degree of insulin resistance may determine at what stage in the autoimmune process LADA will become manifest has been proposed previously by Naik et al [37].

The strengths of this study include the population-based design and large number of incident LADA patients. In addition, detailed information on lifestyle and potential confounders were available, including a validated FFQ [32]. We used a case-control design with incident cases, which is the most efficient design for rare outcomes like LADA [26]. A limitation is that information on lifestyle is collected at time of diagnosis; recall bias is introduced if cases have modified their lifestyle following diagnosis and report accordingly. Cases may also overestimate how unhealthy their previous lifestyle was, which would lead to overestimation of associations. The validity of the study design is supported by the fact that our type 2 diabetes results were in strong agreement with findings from previous prospective studies where lifestyle information was obtained several years prior to diagnosis [7]. Even though the specificity of the GADA assay was high, misclassification of type 2 diabetes cases into the LADA group could have contributed to an association between a healthy lifestyle and LADA. On the other hand, there was an inverse association between a healthy lifestyle and highly autoimmune LADA, where misclassification of type 2 diabetes cases is unlikely. Some control individuals may have undiagnosed diabetes at inclusion; this would lead to underestimation of the associations. Another limitation is the use of external controls for stratification analyses based on genetic risk. Importantly, we observed the similar results when we repeated the main analysis using the genetic controls (Table S8), which supports their validity. The estimated potential for prevention is determined by our definition of low-risk components and a healthy lifestyle, which was determined *a priori* and similar to definitions used in previous type 2 diabetes studies [4,7,25,38]. We used the same definition for both outcomes to allow for comparisons. In addition, we defined a healthy diet based on the Life's Simple 7 recommendations [3] whereas other dietary factors may also play a role. We could adjust for several potential confounders; still, there may be residual confounding due to unmeasured or inaccurately determined factors. PAR% should be interpreted cautiously, because it was, by definition, based both on the assumption of causality and absence of measurement errors and bias. Finally, the study was conducted in Sweden, and adherence to different lifestyle patterns may not be representative for other populations. Still, previous studies from other countries found similar proportions of participants adhering to low-risk behaviours [3,7]. Incidence of autoimmune diabetes is relatively high in Scandinavia and the results may not be generalisable to low incidence regions. The prevalence of LADA varies across populations; this could reflect that the proportion carrying high-risk HLA genotypes varies [39]. In addition, prevalence of lifestyle risk factors, especially obesity, may contribute to these differences. In general, research focusing on the risk of LADA in relation to lifestyle factors is limited

and primarily based on Scandinavian studies [17,21,36]. Confirmation of our findings in other populations is clearly needed.

In conclusion, we find a reduced risk of LADA as well as type 2 diabetes in people with a healthy lifestyle, including BMI < 25, no smoking, a healthy diet, moderate alcohol consumption, and physical activity. Out of these components, maintaining normal weight was associated with the largest risk reduction, but the combined healthy lifestyle resulted in a lower risk for LADA than any lifestyle component alone. Intervention studies have shown that it is possible to prevent type 2 diabetes through lifestyle modification. Such studies are lacking in LADA but clearly needed to elucidate whether the observed associations are causal and the disease in part preventable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Data collection was a collaborative effort by SC, RH, and JEL (ESTRID), LG, EA, and TT (ANDIS), and LA (EIRA). SC and TT conceptualised the research objectives, designed the study, and thoroughly revised the manuscript. KH analysed the data, developed the study objectives, and drafted the manuscript. All authors contributed to the interpretation of the results and critically revised and approved the final version of the manuscript. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2021.108760.

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