Molecular Genetics and Metabolism xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Lifestyle factors including diet and biochemical biomarkers in acute intermittent porphyria: Results from a case-control study in northern Norway

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ARTICLE INFO

Keywords:
Diet
Alcohol
Smoking
Porphyria
Resistin
Human

ABSTRACT

Background: Lifestyle factors, including a low intake of carbohydrates, dieting, alcohol consumption, cigarette smoking and stress are some of the possible triggers of attacks in acute intermittent porphyria (AIP). The influence of lifestyle factors, including energy intake, diet and alcohol consumption on the biochemical disease activity in AIP and biochemical nutritional markers were examined.

Methods: A case-control study with 50 AIP cases and 50 controls matched for age, sex and place of residence was performed. Dietary intake was registered using a food diary in 46 matched pairs. Symptoms, alcohol intake, stress and other triggering factors of the last AIP attack were recorded on questionnaires. Porphyrin precursors, liver and kidney function markers, vitamins, diabetogenic hormones and other nutritional biomarkers were analyzed by routine methods. The Wilcoxon matched-pairs signed rank test was used to compare the cases vs. controls. The Spearman's rank correlation coefficient was used on the cases.

Results: Increasing total energy intake was negatively correlated with the biochemical disease activity. The intake of carbohydrates was lower than recommended, i.e., 40 and 39% of total energy intake in the AIP cases and controls, respectively. The plasma resistin level was significantly higher ($p = .03$) in the symptomatic than asymptomatic cases. Plasma insulin was lower in those with high porphobilinogen levels. The intake of sugar and candies were higher in the AIP cases with low U-delta aminolevulinic acid (ALA) levels ($p = .04$). Attacks were triggered by psychological stress (62%), physical strain (38%), food items (24%) and alcohol (32%) in the 34 symptomatic cases. Alcohol was used regularly by 88% of the cases (3.2 g ethanol/day) and 90% of the controls (6.3 g/day), but the intake was significantly lower in symptomatic than in asymptomatic cases ($p = .045$).

Conclusion: A high intake of energy, sugar and candies and a higher insulin level were associated with a lower biochemical disease activity. The resistin level was higher in the symptomatic than the asymptomatic cases. AIP patients drink alcohol regularly, but the intake was significantly lower in the symptomatic cases.

Trial registration: ClinicalTrials.gov Identifier: NCT01617642.

1. Introduction

Acute intermittent porphyria (AIP) is an autosomal, dominant, inherited and rare metabolic disorder. The disease is caused by a mutation in the hydroxymethyl bilan synthase (HMBS) gene, which encodes an enzyme in the heme biosynthesis [1,2]. The prevalence of heterozygous AIP mutations in the European population is between 1/75,000 to 10–20/100,000 [3,4], and about 600/100.000 in the Saltdal area in Nordland County, Norway. A recent study has shown a higher prevalence of 1/1675 of the HMBS mutation in the general population in

Abbreviations: AIP, Acute intermittent porphyria; U-ALA, urine 5-Aminolevulinic acid; ALAS1, 5-Aminolevulinic acid synthase 1; BMI, Body mass index; HCC, Hepatocellular carcinoma; U-PBG, Urine porphobilinogen; PTX3, Long-pentraxin 3

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https://doi.org/10.1016/j.ymgme.2018.12.006
Received 29 August 2018; Received in revised form 28 November 2018; Accepted 9 December 2018

Europe [5]. AIP is typically asymptomatic, and approximately 90% of those with AIP mutations never experience symptoms [2].

AIP symptoms may be triggered by mental or physical stress, alcohol, smoking, certain medications, infections or hormonal factors [1–3]. Fasting and fever can also induce heme oxygenase activity; the free heme pool is then reduced, and 5-aminolevulinic acid (ALA) synthase-1 is induced [1]. Common symptoms during the acute neurovisceral attacks include abdominal pain, nausea, vomiting, constipation, dark red urine and muscle weakness [1,6]. Bulbar or phrenic nerve paresis may occasionally lead to life-threatening respiratory failure [1,7]. A high prevalence of hypertension and renal impairment has been documented [4]. A few patients, mainly females, experience recurrent attacks. Patients who have a AIP gene mutation and have experienced symptoms of AIP are referred to as symptomatic AIP, while those who never had symptoms are referred to as asymptomatic [1]. A recent study by Lenglet et al. indicated oligogenic inheritance for AIP, and this could be the reason why only a few patients are symptomatic and why some are high and some are low excreters of ALA and PBG [8].

The first line of treatment is to remove any known triggering factor. In hospital, intravenous infusion of glucose and/or of synthetic heme can be administered [9]. The inhibiting effect of glucose on the heme synthesis is probably related to peroxisome proliferator activated cofactor 1α (PGC-1α) [10]. Liver transplantation cures AIP completely, indicating that liver metabolism plays an important part in pathogenesis [11].

The dietary advice from the American Porphyria Foundation (APF) to patients with AIP is to avoid prolonged fasting, and to have a carbohydrate intake of 55–60% of the total energy intake (E%). Further, "The carbohydrates should preferably not include large amount of refined sugars, but during less severe attacks intake of 300 g/day of dextrose or other metabolizable carbohydrate is recommended." The Nordic Nutrition recommendations for the general population recommends a carbohydrate intake of 45–60 E% [12]. A recent national diet survey (Norkost 3) showed that the average diet in Norway consisted of 43–44 E% as carbohydrates [13]. Balwani et al. stated that a "sustained adherence to a high-carbohydrate diet does not prevent attack and is not recommended" [14]. Crash dieting and bariatric surgery can precipitate porphyric attacks [15].

Ethanol may precipitate porphyric attacks in some, but not all persons with AIP mutations, and they are advised to avoid alcohol [16–18]. Smoking has been reported to be associated with a high frequency of AIP attacks [19,20]. Low levels of vitamins and micronutrients can influence overall health [12,21,22], and especially the heme biosynthesis [23]. Repeated hemin infusions may have adverse effects in AIP patients, possibly through a high iron load and inflammation [24]. Naik et al. reported that stress was a direct contributor to attacks in 56% of the examined 16 AIP cases with recurrent attacks [25].

Few studies have examined the role of the dietary intake of sugar, total carbohydrates and other dietary factors on the disease activity in AIP and simultaneously analyzing biochemical biomarkers. We hypothesized that the total energy intake and intakes of carbohydrates, sugar, alcohol, iron or diabetogenic hormones could be different in the asymptomatic vs. the symptomatic AIP cases.

2. Methods

2.1. Participants and study design

A case-control study was conducted, comparing 50 AIP cases with 50 controls matched for age, sex and place of residence. Of these cases, 15 were asymptomatic and 35 were symptomatic. Most cases had the AIP mutation W198X (n = 48), while two patients had the R167W mutation. The study was approved by the Regional Ethics Committee for Medical and Health Research. We recruited all the participants from September to November 2012, and all participants were examined once. The controls were randomly selected from the registry of residents from the Norwegian Tax Administration. The exclusion criteria for both groups were as follows: age under 18 or lacking competence to provide consent to participate in the study. An additional exclusion criterion for the AIP cases was absence of the AIP gene mutation, and one person was excluded for this reason. The exclusion criteria for the controls were also proven AIP or other porphyria, none were excluded. Four members of the control group withdrew from the study immediately before the study began and they were replaced by new matched controls, while all AIP cases agreed to participate.

2.2. Recording of symptoms, triggering factors and diet

The cases with AIP were questioned by a medical doctor regarding the presence or absence of AIP symptoms, the time of diagnosis, the number and durations of attacks, and about triggering and relieving factors during attacks. All participants were asked about their smoking and alcohol habits, physical activity, emotional stress, medications, nutritional supplements, present and chronic diseases and any surgeries prior to the interview. The dietary intake was registered using a 7-day diet logbook and was obtained from 46 cases and 46 matched controls. Of the 35 symptomatic AIP cases, 34 filled out a diet log-book. A clinical nutritionist instructed the participants on how to use the 7-day diet logbook. The logbook was scanned using the Teleform program, version 6.0 (Datascan, Oslo, Norway). Daily intakes of energy and nutrients were computed using the food database and software system (KBS, version 7) developed at the Department of Nutrition, University of Oslo. The food database (AE-10) is based mainly on the official food composition table. Energy was calculated as (g carbohydrate × 17 kJ/g) + (g protein × 17 kJ/g) + (g fat × 27 kJ/g) + (g fiber × 8 kJ/g). Resting metabolic rate (RMR) was calculated by Mifflin's formula, for women: RMR (kcal) = 10 × weight (kg) + 6.25 × height (cm) – 5 × age (years) – 161; for men: RMR (kcal) = 10 × weight (kg) + 6.25 × height (cm) – 5 × age (years). Energy requirement was calculated multiplying RMR with a physical activity factor (PAL) [12]. The PAL factors for the participants were set by individual evaluation of the description of their work and leisure physical activity. Energy requirement was converted from kcal to kJ by ×4.184. Sugar was regarded as "added sugar" and did not include naturally occurring sugars. Vitamin A was calculated as the sum of retinol and 1/12 beta-carotene. Nutritional supplements were included in the dietary calculations. Serial diastolic blood pressure was automatically measured over a 20–30 min period, every second minute using a CAS 740 monitor (CAS Medical Systems Inc., Branford, CT, USA). The body weight, length and waist/hip-ratio were measured according to the WHO recommendations.

2.3. Blood sampling

Blood samples were obtained between 8 and 9 am by venipuncture after an overnight fast. Vacuette citrate, EDTA and serum tubes were used (Greiner Bio-one Gmbh, Frickenhausen, Germany). The EDTA tubes for analyzing diabetogenic hormones in plasma were immediately placed on crushed ice, centrifuged at 1500*g* for 15 min at +4 °C, and the plasma was stored at −80 °C until analysis.

2.4. Routine biochemistry tests

Hematology parameters were analyzed on a Siemens ADVIA 2120i Hematology System (Siemens Healthcare Diagnostics Ltd., Camberly, UK). Serum (*S*)-Na^+, K^+, Mg^{2+}, creatinine, urine creatinine, S-iron and other routine clinical chemistry parameters were analyzed on a ADVIA®1800 system (Siemens Medical Solutions Diagnostics, Japan) using reagents from Siemens Healthcare Diagnostics Ltd. The relative estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) using

the creatinine equation and expressed as ml/min/1.73 m^2 [26]. Serum ferritin, folate and vitamin B12 were analyzed on a ADVIA Centaur® XP system (Siemens). A BN ProSpec® nephelometer (Siemens Healthcare Diagnostics Ltd.) was used to measure S-prealbumin and alpha-1 antitrypsin. Urine porphobilinogen (U-PBG) and U-ALA were analyzed using a kit from Bio-Rad Laboratories (Munich, Germany). Total and fractionated porphyrins were analyzed by high-performance liquid chromatography (HPLC) as previously described [27]. Epinephrine, norepinephrine and vanillylmandelic acid (VMA) in a random morning urine sample were analyzed by HPLC using kits from Chromsystems Instruments & Chemicals GmbH (Munich, Germany). Total 25-OH Vitamin D were analyzed using kits from Chromsystems and an Ultimate 3000 HPLC system (Thermo Scientific, Waltham, MA, USA) equipped with a TSQ Quantum Access MAX triple quadrupole mass spectrometry detector.

2.5. Enzyme immunoassay

A Bio-Plex pro human diabetes 10-plex kit from Bio-Rad Laboratories Inc. (Hercules, CA, USA) was used to measure plasma (P)-insulin, visfatin, resistin and C-peptide in EDTA plasma on a Bio-Plex 200 system from Bio-Rad. The Enzygnost F1 + 2 (monoclonal) kit (Dade Behring, Marburg GmbH, Germany) was used to analyze prothrombin fragment 1 + 2 (PTF1.2) in EDTA plasma. Long-pentraxin 3 (PTX3) was analyzed by an enzyme-linked immunosorbent assay (ELISA) kit from R&D Systems Inc. (Minneapolis, MN, USA). The optical density was measured on a MRX microplate reader (Dynex Technologies, Denkendorf, Germany).

2.6. Statistical analyses

The sample consisted initially of 50 cases with AIP and 50 controls matched for age, sex and residence. The dietary data arise from (n = 46) AIP-cases and their 46 matched controls. When comparing groups of AIP cases with high (> 1.5) and low (≤1.5 μmol U-PBG/mmol creatinine) U-PBG levels and high (> 3.9) and low (≤3.9 μmol U-ALA/mmol creatinine) ALA levels, we used data from all 47 AIP cases that had filled in the dietary logbook. We used the Wilcoxon matched-pairs signed rank test on the matched case-control data. The Spearman's rank correlation coefficient was calculated, giving an r and two-tailed p. The Mann-Whitney *U* test was used on non-matched data, i.e., on symptomatic vs. asymptomatic cases. The Fischer's exact test was used on categorical variables. Statistical significance was considered at $p < .05$. The statistical analysis was performed using Prism version 6.0 from GraphPad Software Inc. (CA, USA).

3. Results

3.1. Baseline characteristics of cases with acute intermittent porphyria and matched controls

The baseline characteristics of the study population, which consisted of AIP cases and controls matched for age, sex and place of residence, are shown in Table 1. Most variables were similar in the two groups (Table 1). Of the 50 controls and 50 AIP cases, 6 and 10 persons, respectively, were current smokers (p = .55) [26]. Of the 34 symptomatic AIP cases, 15% had experienced one AIP attack ever, and 85% have had more than one porphyria attack. Some of them have had more than twenty AIP attacks during their life. Of the 34 symptomatic cases 50% had been treated with glucose intravenously and 21% with Normosang intravenously, during their AIP attacks. None of the AIP cases were under an AIP attack, and none were having glucose or Normosang treatment at the time of study.

Overweight defined as BMI 25–29.9, was found in 52% of the AIP cases and 38% of the matched controls. Similarly, 24% of the cases and 30% of the controls had obesity (BMI ≥ 30). Of the 50 controls and 50 cases, 3 controls and 4 cases had diabetes mellitus. No significant differences in the frequency of inflammatory diseases were observed, except for a near-significant difference in the number of persons with gout [26]. Statins were used by 7 AIP cases and 8 of their matched controls. However, only 5 of 46 (11%) of the AIP cases had an adequate total energy intake, i.e. 89% had a too low energy intake according to the dietary logbook. Of the controls, 4 of 46 (9%) had an adequate total energy intake, while 42 (91%) had a too low energy intake.

3.2. Dietary intake of carbohydrates, sugar, protein, fat and fatty acids in cases with acute intermittent porphyria and matched controls

The dietary intake of carbohydrates as E% was similar, but lower than the Nordic 2012 recommendations in 78% of the AIP cases and 91% of the controls (Fig. 1A). The intake of added sugar was similar between the groups but was higher than the recommendations in 37% of cases and 26% of the controls (Fig. 1B). The intake of protein (Fig. 1C), fiber (Fig. 1D), total fat (Fig. 1E), saturated fatty acids (SFA, Fig. 1F) and monounsaturated fatty acids (MUFA, Fig. 1G) was similar in the cases and controls. However, the intake of fiber given as g/MJ (Fig. 1D) was lower and the intakes of total fat and SFA given as E% (Fig. 1E, F) were higher than recommended. The intake of polyunsaturated fatty acids (PUFA) was significantly lower (p = .04) in cases than in the controls, and 15% of cases and 9% of controls had a lower intake of PUFA given as E% intake than recommended (Fig. 1H).

3.3. Dietary intakes of vitamin D, calcium, vitamin B12, folate and the corresponding serum levels in cases with acute intermittent porphyria and matched controls

The intake of vitamin D was similar in the two groups, but below the recommended 10 μg/day in 63% of the AIP cases and 54% of the controls (Fig. 2A). The intake of calcium was insufficient in 65% of cases and 50% of controls. The total S-calcium levels were within the normal reference limits in 98% of cases and 96% of the controls. The vitamin B12 intake was similar and sufficient in both groups (Fig. 1E), and S-vitamin B12 levels were above the lower reference limit in both cases and controls (Fig. 1F). One case and one control used vitamin B12 injections (Fig. 1F). In comparison, the intake of folate was lower than recommended in 82% of cases and 78% of the controls, when considering men and postmenopausal women (Fig. 2G), and 88% of cases and 78% of controls, when considering premenopausal women. However, only 8% of the controls had S-folate levels below the lower reference limit (Fig. 2H).

3.4. Intake of vitamin supplements

Doctor-prescribed regular vitamin B12 injections and vitamin D medications was used by 2% of cases and 2% of controls (App Table A). Of the cases, 2% used isotretinoin (vitamin A), and 2% of controls used magnesium supplements (App. Table A). Iron medication was taken by 6% of cases and 12% of the controls. Over-the-counter vitamin supplements were taken by several cases and controls, including vitamin B, vitamin D, multi vitamins, vitamin K and herbal remedies (Appendix Table A).

3.5. Homocysteine levels, kidney function, calcium, albumin, parathyroid hormone and porphobilinogen levels in asymptomatic and symptomatic cases and matched controls

The homocysteine levels in plasma were significantly higher in the symptomatic AIP cases compared to their matched controls, but were similar in the asymptomatic cases and controls (Fig. 3A). The kidney function given as eGFR was significantly lower (p = .0008) in the symptomatic than in the asymptomatic AIP cases (Fig. 3B). Diastolic

Table 1
Baseline characteristics of the study population.

Characteristics	Controls			Cases			p	RI[a] (Norway)	RDA[b], AI (Am.)
	25%ile	Median	75%ile	25%ile	Median	75%ile			
Age, years	31.8	52	68	31.8	52	68.3	0.39		
Height, cm	163	172	179	164	171	180	0.84		
Weight, kg	71.5	80.3	91.4	69.4	81	92.3	0.6		
BMI, kg/m^2	23.8	26.8	30	25	27	29.8	0.92	20–25[c]	18.5–24.9[c]
Hip circumference, cm	93.9	98.8	106	93.9	99.4	107	0.75		
Waist circumference, cm	89	94.6	104	89.3	94.8	106	0.84	≤93/≤79[d,e]	
Waist/Hip –ratio	0.9	0.97	1.01	0.88	0.98	1.02	> 0.99		
RMR[f] W[g], kJ/day	5054	5448	6125	4971	5515	6268	0.67		
Energy req.[h], W[g] kJ/day	9117	9690	10,933	7786	9590	11,343	0.45		
Energy intake W[g], kJ/day	5532	7266	9001	5978	7141	7947	0.84		
RMR[f] M[i], kJ/day	6335	7289	7540	6519	7234	7728	0.12		
Energy req.[h], M[i] kJ/day	10,899	11,983	13,539	11,288	13,188	13,866	0.30		
Energy intake M[i], kJ/day	7735	8988	11,111	8209	9383	12,058	0.53		
RMR[f] all, kJ/day	5581	6318	7355	5535	6523	7397	0.40		
Energy req.[h], all, kJ/day	9786	11,016	12,426	9678	11,527	13,334	0.81		
Energy intake all, kJ/day	6996	8367	10,621	7099	8162	10,763	0.72		
Protein, g/day	65.6	80.6	105	68.8	77.6	98.9	0.92		56/46[e]
Fat, g/day	72	86.8	112	70	84.5	115	0.76		
Saturated fat, g/day	29	36.8	43.9	28.7	36	47.6	0.38		
C-MUFA[l], [m], g/day	23	29.1	36.9	21.9	28.4	37.1	0.58		
C-PUFA[k], g/day	12.5	16.7	20.7	10.8	13.8	20.5	0.19		
Cholesterol, mg/day	264	356	434	280	383	483	0.56		
Carbohydrates, g/day	156	190	211	166	201	248	0.12		130
Sugar, g/day	27.3	36.6	50	30.7	42.8	56	0.18		
Vitamin A, µg/day	645	929	1074	707	909	1160	0.25		
Retinol, µg/day	515	701	893	568	750	945	0.31	900/700[e]	900/700[e]
B-carotene, µg/day	1194	1875	3104	1101	1728	2368	0.69		
Tocopherol, mg/day	8.0	14.2	20.8	9.35	12.3	17.3	0.79	10/8[e]	15
Thiamin, mg/day	1.00	1.31	1.85	1.01	1.32	1.92	0.94	[l]	1.2/1.1[e]
Riboflavin, mg/day	1.27	1.55	2.42	1.26	1.66	2.52	0.77	[m]	1.3/1.1[e]
Vitamin B6, mg/day	1.03	1.44	2.13	1.12	1.49	2.32	0.67	1.5/1.2[n]	1.3[n]
Vitamin C, mg/day	44.8	63.5	145	51	73.5	134	0.72	75	90/75[e]
Fe, mg/day	7.8	9.4	14.4	7.7	9.8	14.3	0.48	9/15[e,o]	8/18[e,p]
Potassium, mg/day	2716	3537	4325	2637	3201	4108	0.49	3500/3100[k]	4700
Mg, mg/day	283	332	412	258	289	406	0.33	350/280 [e]	420/320[q,e]
Zn, mg/day	8.0	10.6	13.3	7.9	10.5	15.8	0.39	9/7[e]	11/8[e]
Se, µg/day	34.5	49.5	73.3	37.5	52.5	75.5	0.77	60/50[e]	55
Cu, mg/day	0.81	1.1	1.34	0.87	1.08	1.61	0.59	0.9	0.9
Phosphorus, mg/day	1147	1457	1733	1156	1392	1799	0.84	600[r]	700
Sugar, E%[s]	6.3	7.5	10.6	6.1	9.1	12.4	0.1	< 10	< 10
Alcohol, E%[s]	0	2.5	4.8	0	0.95	4.1	0.55	< 5	

The data represent median values and interquartile ranges; 25%ile and 75%ile. The data were analyzed using the Wilcoxon matched-pairs signed-rank test on the AIP cases (n = 46) versus matched controls on all data except age, height, weight, BMI, hip circumference, waist circumference and Waist/Hip-ratio (n = 50) AIP cases and 50 matched controls. Of the 46 matched pairs 20 are women and 26 are men. The p-values are exact, two-tailed.

[a] RI = Recommended intake, from Norwegian guidelines on diet, nutrition and physical activity, The Norwegian Directorate of Health, 2014.

[b] RDA = Recommended Dietary Allowance, AI = Adequate intake, from Dietary Guidelines for Americans. 8th Edition 2015–2020, January 2016, US Department of Health and Human Services and US Department of Agriculture.

[c] Normal body mass index, BMI. For persons above 70 years (y): 22–27.

[d] Indicates low risk level metabolic disease.

[e] Men/Women.

[f] RMR, Resting metabolic rate calculated by Mifflin's formula.

[g] W = women.

[h] Energy requirement = RMR* PAL.

[i] M = men.

[j] C-MUFA = Cis-mono unsaturated fatty acids, [k]C-PUFA = Cis-poly unsaturated fatty acids.

[l] Thiamin: M: 1.4 mg/d for 18–30 y 1.3: 31–60 y, 1.2: > 60. W: 1.1 mg/d for 18–60 y, 1.0: > 60 y.

[m] Riboflavin; M: 1.6 mg/d for 18–30 y, 1.5: 31–60 y, 1.4: 61–74, 1.3: > 75 y, W: 1.3 mg/d for 18–30 y, 1.2 > 30 y.

[n] Vitamin B6 Nordic rec.: W > 60 y: 1.2, [n] Vitamin B6 American rec.: W > 51 y: 1.5, M > 51 y: 1.7.

[o] Iron, Women: > 61 y: 9.

[p] Iron, Women, American rec. > 51 y: 8.

[q] Mg: M:19–30: 400 W:19–30 y: 310.

[r] P: 18–20 y: 700.

[s] E% = % of total energy intake.

blood pressure was significantly higher in the symptomatic than in the asymptomatic cases (Fig. 3C), while the U-epinephrine level was lower (Fig. 3D). S-calcium and albumin were significantly lower (Fig. 3E, F) and PTH levels in plasma were significantly higher (Fig. 3G) in the symptomatic cases than in the asymptomatic cases. As expected, U-PBG levels were significantly higher in the asymptomatic and symptomatic cases compared with their respective matched controls (Fig. 3H).

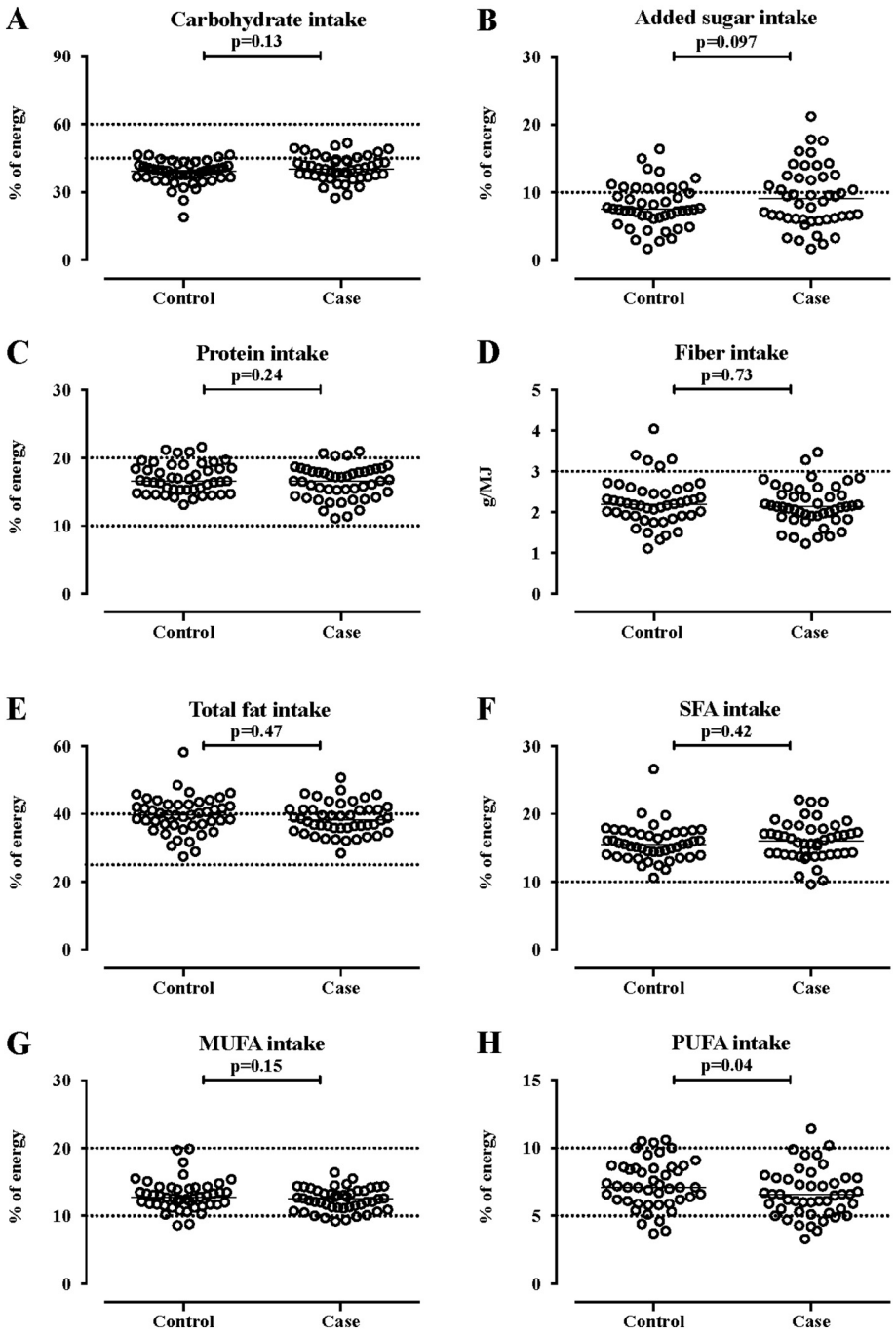


Fig. 1. The dietary intake of carbohydrates, added sugar, protein, fiber, total fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in 46 acute intermittent porphyria (AIP) cases (Case) compared with their matched controls (Control, n = 46). The dietary intake of (A) carbohydrates, (B) added sugar, (C) protein, (E) total fat, (F) saturated fatty acids (SFA), (G) MUFA and (H) PUFA are expressed as % of total energy intake, except for fiber intake (g/MJ). The dietary intake was calculated from a seven-day dietary logbook. The results are shown as scatterplots with the median. The two horizontal dotted gridlines show the recommended intake (RI)-interval for adults above 18 years for carbohydrate, protein, fiber, total fat, MUFA and PUFA. The one horizontal dotted gridline for added sugar and SFA indicates the upper recommended intake level. All dietary recommendations in the figure are from the current Norwegian guidelines on diet, nutrition and physical activity. The data were analyzed using the Wilcoxon matched-pair signed rank test. The p-values are exact, two-tailed.

3.6. Intake of sugar/candies, carbohydrates, protein, meat and alcohol and plasma resistin and alpha-1 antitrypsin levels in asymptomatic and symptomatic cases and matched controls

The intake of sugar/candies and carbohydrates was not significantly different between the symptomatic and asymptomatic AIP cases (Fig. 4A, B). The median intake of protein was close to be significantly different (p = .052) between the symptomatic and asymptomatic cases (Fig. 4C). The intake of meat given as g/day was significantly lower in the symptomatic than in the asymptomatic cases (Fig. 4D). The P-resistin level were significantly higher (p = .03) in the symptomatic than in the asymptomatic AIP cases and were significantly lower (p = .004) in the asymptomatic cases than in their matched controls (Fig. 4E). Interestingly, the intake of alcohol both given as g/day (Fig. 4G) and as E% (Fig. 4F) was significantly lower in the symptomatic than in the asymptomatic AIP cases. However, the alcohol intake was not significantly different from the matched controls (Fig. 3E, F).

3.7. Alcohol intake, cigarette smoking and intake of carbohydrates, added sugar and sugar/candies in acute intermittent porphyria cases with low and high levels of porphyrin precursors

We next examined if lifestyle factors, including alcohol, cigarette smoking and intake of carbohydrates and sugar/candies in the diet were different in the AIP cases with low and high biochemical disease activity. The intake of alcohol given as g/day (Fig. 5A) and E% (Fig. 5B) was similar in the AIP cases with low and high U-PBG levels. The number of cigarettes smoked per day (Fig. 5C), fat intake as E% (Fig. 5D), carbohydrate intake as E% (Fig. 5E) and added sugar intake as E% (Fig. 5F) were not different in the AIP cases with high and low U-

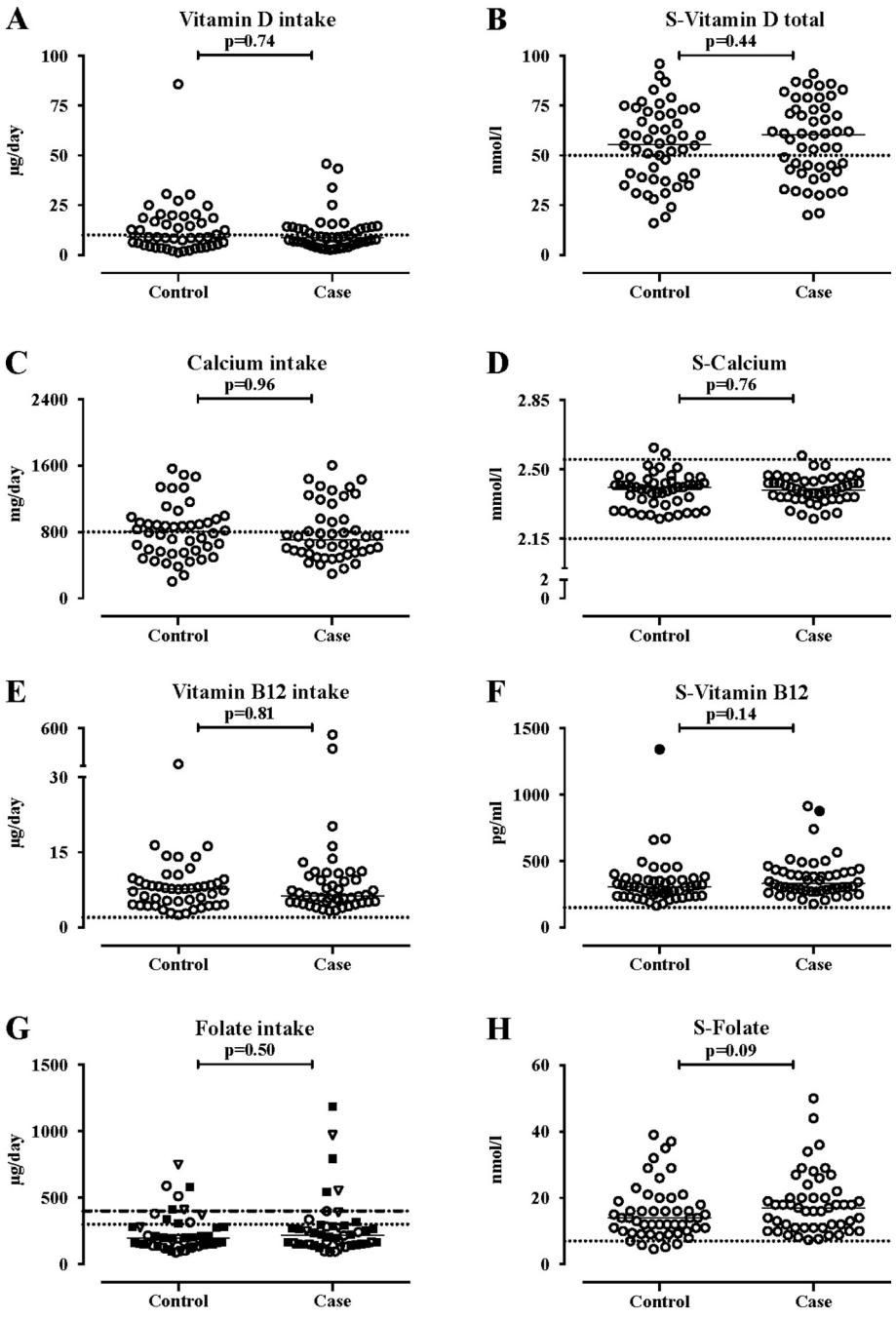


Fig. 2. Dietary intake of vitamin D, calcium, vitamin B12 and folate and the corresponding serum (S) levels in acute intermittent porphyria (AIP) cases and matched controls. The intake of (A) vitamin D, (C) calcium, (E) vitamin B12 and (G) folate in AIP cases (n = 46) was compared with their matched controls (n = 46). The dietary intakes were calculated from a seven-day dietary logbook and expressed as µg/day, except for the calcium intake (mg/day). The horizontal dotted gridline shows the recommended intake (RI) for adults above 18 years for the following: (A) vitamin D intake: 10 µg/day, but for persons > 75 years, the RI is 20 µg/day; (C) calcium intake: 800 mg/day, but for persons 18–20 years, the RI is 900 mg/day, and for postmenopausal women, a supplement of calcium of 500–1000 mg/day might reduce age-related bone loss and (E) Vitamin B12 intake: 2 µg/day, (G) The RI for folate intake is 300 µg/day for postmenopausal women (open circles) and men (black squares). The upper horizontal dot-line gridline in panel (G) shows the RI of folate 400 µg/day for women in fertile age (open triangles). All dietary recommendations are from the current Norwegian guidelines on diet, nutrition and physical activity. The serum level of (B) total 25-OH vitamin D was analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) and expressed as nmol/l. The serum levels of (D) total calcium (mmol/l), (F) S-vitamin B12 (pg/ml) and (H) S-folate (nmol/l) were analyzed using standard biochemical methods and expressed as mmol/l, pg/ml and nmol/l, respectively. "The control and the AIP case who received regular vitamin B12 injections are marked as black dots." The results are shown as scatter-plots with the median. The horizontal dotted gridlines indicate the reference limits for: (B) S-25 OH vitamin D total (50 nmol/l), (D) S-calcium (2.15–2.55 mmol/l), (F) S-vitamin B12 (150 pmol/l) and (H) S-folate (7 nmol/l). The data were analyzed using the Wilcoxon matched-pair signed rank test. The p-values are exact, two-tailed.

PBG levels. Interestingly, the intake of sugar/candies given as g/day (Fig. 5G) was significantly higher (p = .04) in the AIP cases with a low U-ALA levels (Fig. 5G). However, the sugar/candies intake was not significantly different in the AIP cases with low and high PBG levels (Fig. 5H).

3.8. Meat intake and serum iron levels, folate intake and folate levels, and creatinine and homocysteine levels in acute intermittent porphyria cases with low and high levels of porphyrin precursors

The intake of meat given as g/day (Fig. 6A) and folate given as µg/day (Fig. 6C) was similar in the AIP cases with low and high U-PBG levels. The S-iron levels (Fig. 6B) were close to be significantly different (p = .053) between the AIP cases with low and high U-PBG levels. The intake of folate (Fig. 6C) and the S-folate levels (Fig. 6D) were similar in the AIP cases with low and high PBG levels. However, the S-creatinine (Fig. 6E) and P-homocysteine levels (Fig. 6F) were significantly higher in the AIP cases with high U-PBG levels than in those with low PBG levels.

3.9. Serum glucose, plasma insulin, glucose/insulin ratio, pre-albumin and plasma C-peptide levels in acute intermittent porphyria cases with low and high levels of porphyrin precursors

The glucose levels were similar in the AIP cases with low and high U-PBG levels (Fig. 7A). The median plasma insulin level was significantly (p = .02) higher (Fig. 7B) and the glucose/insulin-ratio (Fig. 7C) was significantly lower (p = .02) in the AIP cases with low compared to those with high U-PBG levels. The pre-albumin levels (Fig. 6D) and plasma C-peptide levels (Fig. 6E) were similar in the AIP cases with low and high U-PBG levels. The C-peptide levels were not significantly different between the cases with low and high U-ALA

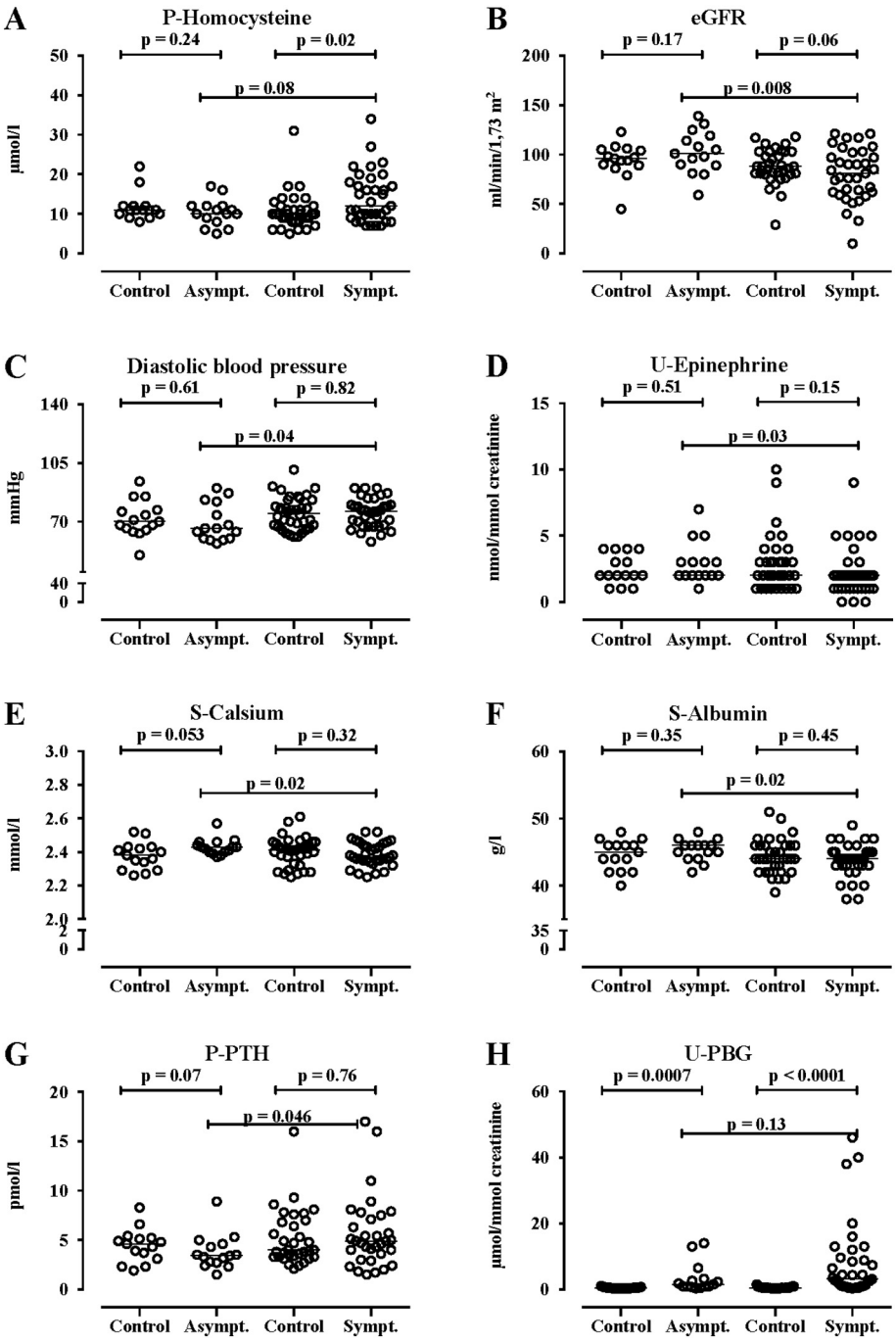


Fig. 3. Levels of homocysteine, kidney function, diastolic blood pressure, urine (U)-epinephrine, serum (S)-calcium, S-albumin, plasma (P)-PTH and U-porphobilinogen (U-PBG) levels in asymptomatic (Asympt.) and symptomatic (Sympt.) acute intermittent porphyria (AIP) cases (n = 50) and matched controls (Control, n = 50). The analytes were analyzed using standard biochemical methods. (A) P-homocysteine is expressed as µmol/l, (B) relative eGFR (estimated glomerular filtration rate) chronic kidney disease (CKD-EPI) as ml/min/1.73 m^2 with the creatinine equation, (C) diastolic blood pressure as mm Hg, (D) U-epinephrine as nmol/mmol creatinine, (E) S-calcium as mmol/l, (F) S-albumin as g/l, (G) P-PTH as pmol/l and (H) U-PBG as µmol PBG/mmol creatinine. Serial diastolic blood pressure was automatically measured over a 20–30 min period, every second minute using a CAS 740 blood pressure monitor. The paired case-control data were analyzed using the Wilcoxon matched-pair signed rank test. The asymptomatic and symptomatic AIP cases were compared using the Mann-Whitney *U* test.

levels (Fig. 7F). The AIP cases (n = 2) who received anti-diabetic medications are indicated with black dots (Fig. 7). When the p values for the comparison of biomarkers in the AIP cases with low and high U-PBG were calculated after the exclusion of those who received anti-diabetic medication, the results were; S-glucose (p = .11) as before, P-insulin (p = .04) and Glucose/Insulin-ratio (p = .04).

3.10. Porphyrin precursors, serum cholesterol, triglycerides and other biochemical biomarkers in cases with acute intermittent porphyria and controls

The levels of all porphyrins and porphyrin precursors were, as expected, significantly higher in the AIP cases than in the controls (Table 2). The S-total cholesterol, S-LDL cholesterol, and S-triglyceride levels were significantly higher in AIP cases than in the controls (Table 2). The S-phosphate levels were lower in the cases than in the controls (Table 2). The VMA levels in random urine samples were significantly lower in the AIP cases than in the controls, but the levels of U-norephinephrine and U-ephineprine were similar. All biomarkers of iron deficiency, including hemoglobin and erythrocyte numbers, were similar in the AIP cases compared to those of the controls (Table 2). The S-CDT%, S-electrolytes and cortisol levels were similar in cases and controls (Table 2).

3.11. Correlations between dietary intake, alcohol, smoking, biochemical biomarkers of kidney and liver function, and porphyrin precursors in cases with acute intermittent porphyria

The U-PBG levels correlated significantly and positively with P-homocysteine, S-creatinine, PTX3, HDL and total cholesterol levels

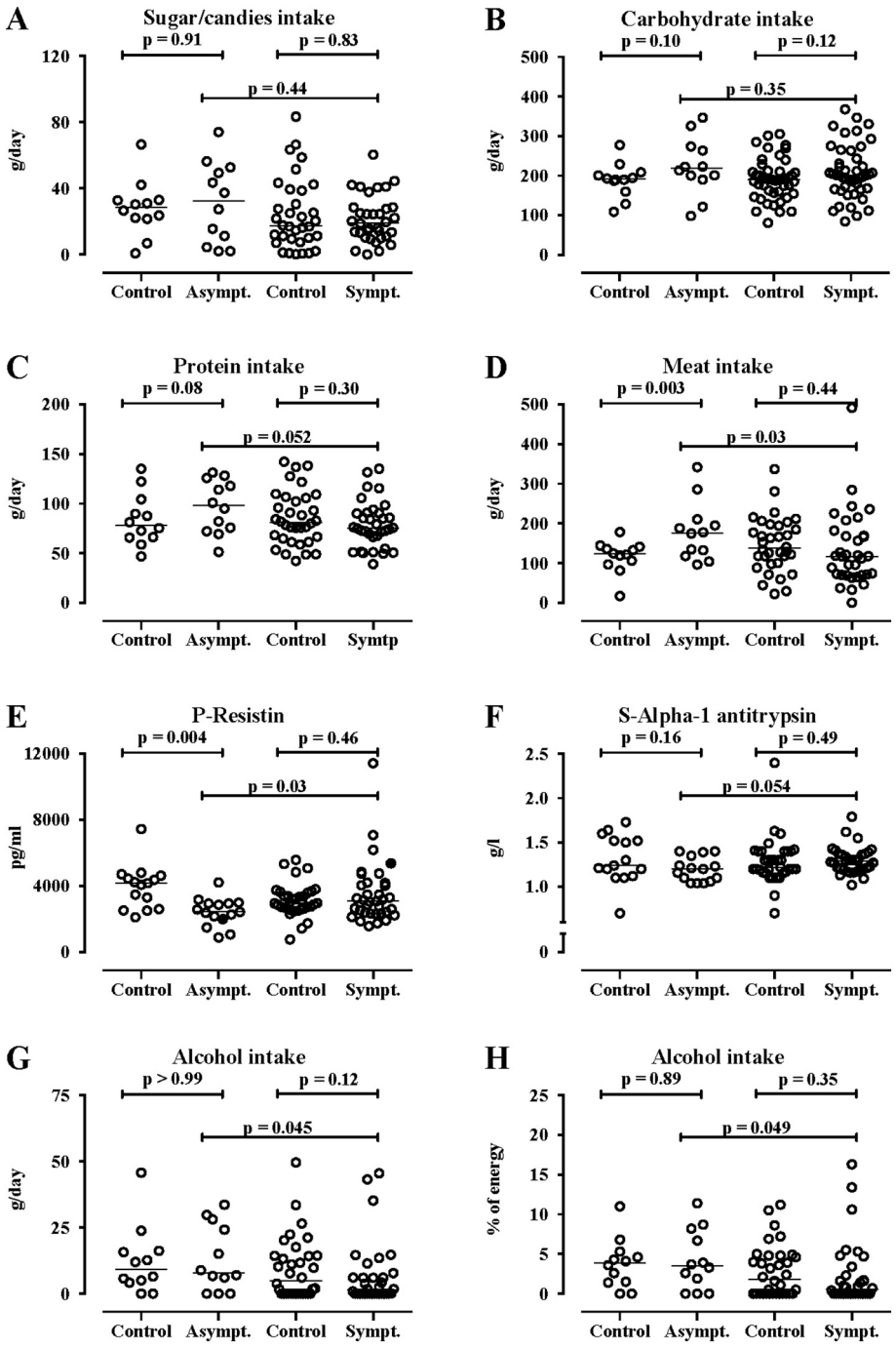


Fig. 4. Dietary intake of sugar and candies, carbohydrates, protein, meat, alcohol and urine-porphobilinogen (U-PBG) levels in asymptomatic (Asympt.) and symptomatic (Sympt.) acute intermittent porphyria (AIP) cases and matched controls (Control). (A) Intake of sugar and candies is given as g/day, (B) carbohydrate intake as g/day, (C) protein intake as g/day, (D) meat intake as g/day, (E) P-resistin as pg/ml, and the AIP cases and controls who received antidiabetic medications are indicated by black dots, (F) S-alpha-1 antitrypsin as g/l, (G) alcohol intake as g/day and (H) alcohol intake as % of total energy intake. The dietary intake was calculated from a seven-day dietary logbook from 46 matched pairs, i.e., 12 asymptomatic and 34 symptomatic cases and 12 and 34 matched controls. S-alpha-1 antitrypsin was analyzed using standard biochemical methods and P-resistin was analyzed using a Diabetes 10-plex assay in 15 asymptomatic and 35 symptomatic cases and matched controls. The paired case-control data were analyzed using the Wilcoxon matched-pair signed rank test. The asymptomatic and symptomatic AIP cases were compared using the Mann-Whitney *U* test.

(Fig. 8). U-PBG levels correlated significantly and negatively with pre-albumin, total energy intake and PUFA intake (Fig. 8). In comparison, U-ALA correlated significantly and negatively with S-pre-albumin and sugar/candies intake (Fig. 8). U-ALA correlated significantly and positively with smoking given as cigarettes smoked/day (Fig. 8). Furthermore, the inflammatory marker PTX3 correlated significantly and negatively with the carbohydrate intake given as g/day, but not with carbohydrates as E% (Fig. 8). As expected, the alcohol intake correlated positively and significantly with the CDT% (data not shown).

3.12. Correlations between biomarkers of glucose metabolism, diabetogenic hormones and biomarkers of disease activity in cases with acute intermittent porphyria

The porphyrin precursor U-PBG correlated positively and significantly with U-ALA and S-iron levels (Fig. 9). U-ALA correlated significantly and positively with S-cortisol levels (Fig. 9). Both U-PBG and U-ALA correlated with P-PTF1.2 levels. B-reticulocytes, P-leptin, P-ghrelin and P-resistin also correlated positively and significantly with PTF1.2 levels (Fig. 9). Furthermore, resistin and U-VMA showed a positive correlation. Plasma resistin levels in the cases were not correlated with BMI (data not shown). As expected, S-glucose correlated significantly and positively with HbA1c levels (Fig. 9).

3.13. Triggering factors for attacks and symptoms in cases with acute intermittent porphyria

The most frequent AIP symptoms and signs were abdominal pain (91%), dark/red urine (80%), tiredness (71%), muscle ache (60%) and muscle weakness (60%), followed by vomiting, headaches, decreased

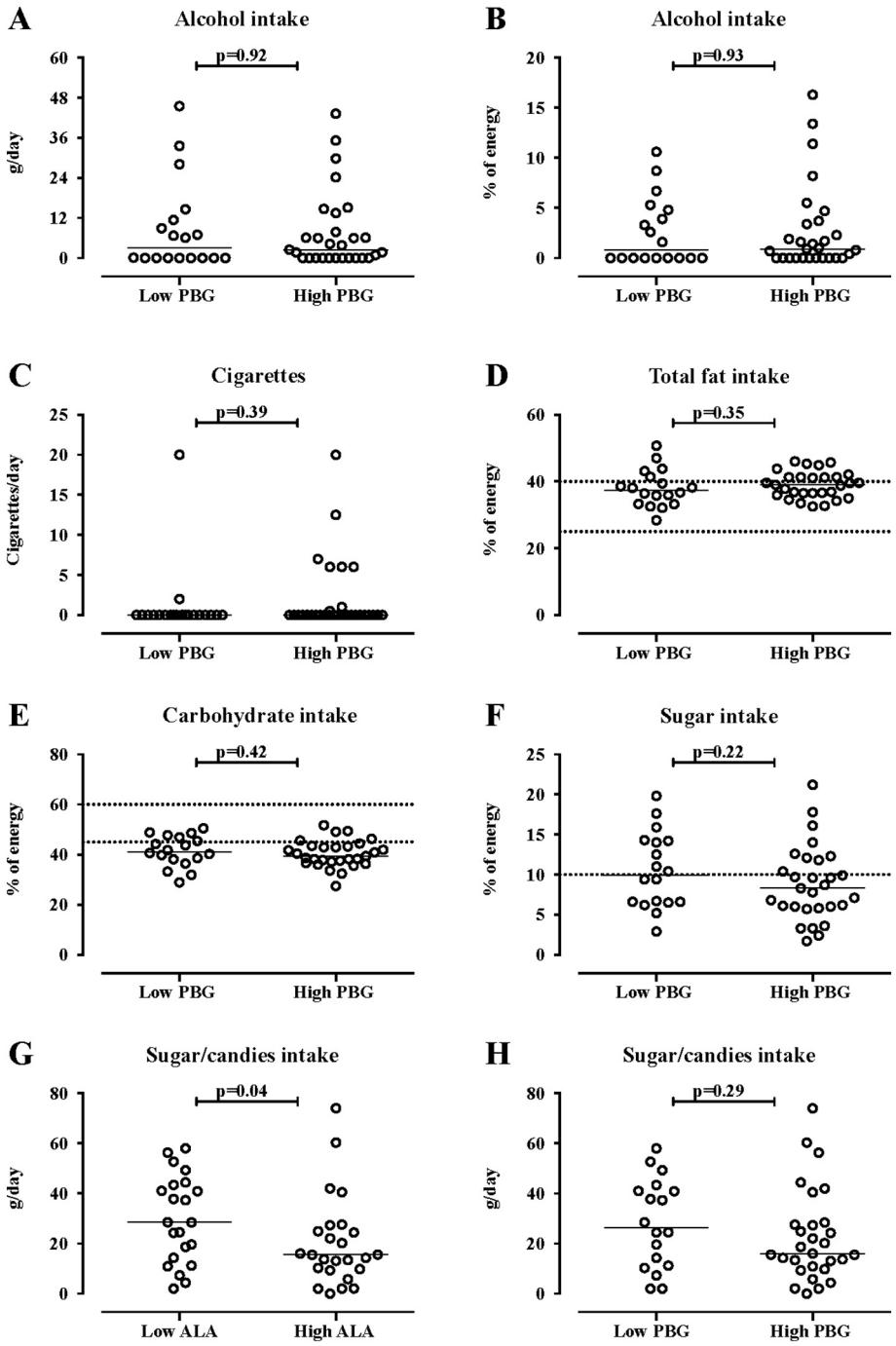


Fig. 5. Lifestyle factors, including (A, B) alcohol intake, (C) cigarette smoking, (D) total fat intake, and (E) intake of carbohydrates and (F) total sugar in acute intermittent porphyria (AIP) cases with low urine (U)-porphobilinogen (PBG) compared with AIP cases with high U-PBG levels. (G) Intake of sugar and candies in AIP cases with low (< 3.9) U-delta-aminolevulinic acid (ALA) compared with AIP cases with high U-ALA (> 3.9 µmol ALA/mmol creatinine), i.e., the reference limit for this assay. A low U-PBG level was defined as values ≤ 1.5 µmol PBG/mmol creatinine, i.e., the reference limit for this assay. The dietary components were calculated from a seven-day dietary logbook. The results are shown as scatter-plots with the median. The two horizontal dotted gridlines on total fat intake (D) and carbohydrate intake (E) indicate the recommended intake (RI) range, and the horizontal dotted gridline (F) for added sugar intake indicates the upper RI, as % of total energy for adults above 18 years. All dietary recommendations are from the current Norwegian guidelines on diet, nutrition and physical activity. The data were analyzed using the Mann-Whitney *U* test. The P-values are exact, two-tailed.

awareness, palpitations, psychological symptoms, constipation, paresis and epilepsy (Table 3). The most prevalent triggering factors for porphyric attack in the AIP cases were psychological stress (62%), followed by work environment (41%), which was described in most cases as stress at work or stressful working (Table 3). Physical strain were the third most prevalent trigger (38%), followed by other diseases including infections, sleep deprivation, food items, hunger, alcohol, dieting and medications. Cigarette smoking was a triggering factor in 6 % of the symptomatic AIP cases. The menstrual cycle was a trigger for 17% of the attacks in female AIP cases. Of the asymptomatic and symptomatic AIP males, 75% and 69%, respectively, had finished one year of military service.

4. Discussion

To our knowledge, this is the first report that both analyzes the dietary intake using a dietary logbook and simultaneously analyzes biochemical nutritional markers and diabetogenic hormones in blood samples in AIP cases and matched controls. The results indicate that the intake of slow-release carbohydrates was lower, and the intakes of sugar and saturated fatty acids was higher than recommended for the Nordic countries, both in the AIP cases and controls. Vitamin D deficiency was the most common vitamin deficiency in both the cases and controls. The intake of alcohol was unexpectedly high in the AIP cases, but lower in the symptomatic than in the asymptomatic cases. Insulin was higher in the cases with low U-PBG levels. Plasma resistin levels were higher in the symptomatic than the asymptomatic cases. These

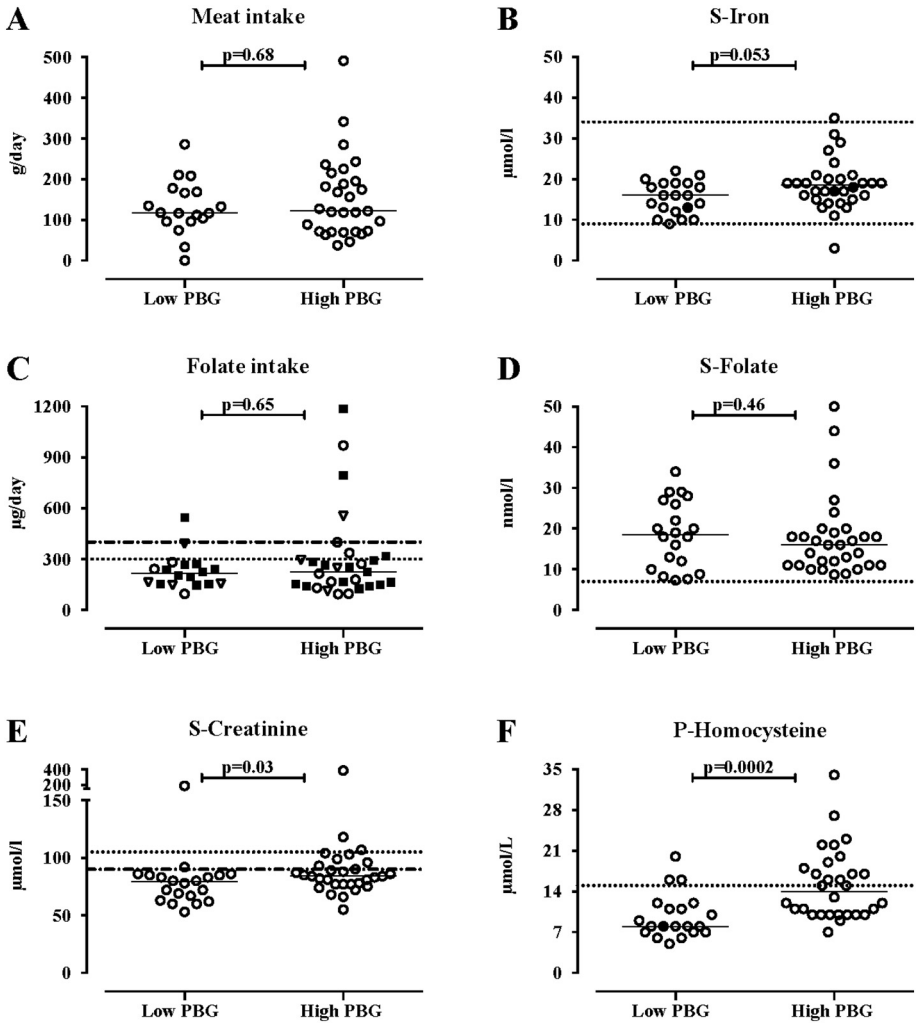


Fig. 6. Intake of meat and folate and the serum (S) levels of iron, folate, plasma (P) homocysteine and kidney function markers in acute intermittent porphyria (AIP) cases with low U-porphobilinogen (PBG) compared with AIP cases with high U-PBG. Low U-PBG was defined as ≤1.5 µmol PBG/mmol creatinine, corresponding to the upper reference limit. The dietary components were calculated from a seven-day dietary logbook including intake of (A) meat and (C) folate. (B) S-iron, (D) S-folate, (E) S-creatinine and (F) P-homocysteine levels were analyzed using standard biochemical assays and the results are shown as scatter-plots with the median. The two horizontal dotted gridlines in panel B indicates the reference values for S-iron (9–34 µmol/l) and black dots indicate AIP cases who received iron supplements. (C) The RI for folate intake is 300 µg/day for postmenopausal women (open circles) and men (black squares). The upper horizontal dot-line gridline in panel (C) shows the RI of folate 400 µg/day for women in fertile age (open triangles). All dietary recommendations in the figure are from the current Norwegian guidelines on diet, nutrition and physical activity. The horizontal dotted gridlines indicate the reference value for: (D) S-folate (> 7 nmol/l), (E) S-creatinine in women (lower line, 90 µmol/l) and for men (upper line, 105 µmol/l), and (F) P-homocysteine (> 15 µmol/l) and a black dot indicates the AIP case who received B12 injections. The data were analyzed using the Mann-Whitney U test. The P-values are exact, two-tailed.

findings may have several explanations and implications.

The energy intake was sufficient in all AIP cases and controls since the BMI results indicate that half of the AIP cases and about 4 out of 10 of the matched controls in this study were overweight. Similarly, 1 out of 4 of the cases and 3 out of 10 of the controls had obesity in line with the high and increasing frequency of obesity in Norway. However, when the energy intake was calculated from the diet logbook, most cases and controls had an insufficient energy intake. However, the dietary intake is probably slightly (17%) underreported according to unpublished data from a validation study of the 7- day diet log book among adults (personal communication from A.M. Wetting Johansen, the Department of Nutrition, Univ. of Oslo). Interestingly, an increasing energy intake was negatively correlated with the U-PBG levels in the AIP cases suggesting that the total energy intake affects the biochemical disease activity also in periods with no dieting.

The intake of carbohydrates in most AIP cases and controls was lower than the recommended 45–60 E% in Norway and other Nordic countries [12,22] and in the US [21]. Thus, the AIP cases did not have a high intake of slow-release carbohydrate foods, in line with a previous report on 16 Spanish AIP cases [28]. In addition, the intake of added sugar was higher than the recommended maximum 10% of total energy intake in many AIP cases and controls, and this may lead to obesity. The intake of sugar and candies was higher in those with low ALA levels than in the group with high ALA levels, suggesting an effect of this dietary factor on the biochemical disease activity. However, no difference in sugar and candies or carbohydrate intake was found between the asymptomatic and symptomatic AIP cases. Finally, no significant correlation was found between the intake of carbohydrates and disease activity in the AIP cases, although a near significant correlation was found between carbohydrate intake in g/day and U-PBG. This may be due to a too low consumption of carbohydrates in the AIP cases, since the carbohydrate intake in two previous studies were up to 500 g/day [29,30], compared to a median intake of 201 g carbohydrates/day in the cases in this report. However, the previous finding that the AIP cases who acquired type 2 diabetes mellitus did not have symptoms of AIP supports the protective role of elevated glucose levels [31]. Furthermore, glucose reduced the synthesis of ALA and PBG in a mouse model of AIP [10]. However, the plasma glucose level is regulated by multiple hormones, including insulin, resistin, glucagon, cortisol and catecholamines. A previous study indicated that the S-glucose level was unchanged, but the fasting levels of insulin were higher and the glucagon was lower in AIP mice than in wild-type mice [32]. We previously found that the fasting insulin levels were decreased in the symptomatic AIP cases compared to the matched controls, but no difference was found between asymptomatic and symptomatic cases [26]. This report suggests that the fasting insulin was lower and the glucose/insulin ratio was higher in the AIP cases with high compared to the group with low U-PBG level. This finding, and the report by Handschin et al. [10] indicating an inhibitory effect of insulin on porphyrin precursor levels in mice, suggests that glucose should be administered together with insulin during porphyric attacks.

The higher resistin level in the symptomatic than the asymptomatic cases, and the positive correlation with leptin levels may indicate that inflammation [26], adipokines and hormones affecting insulin

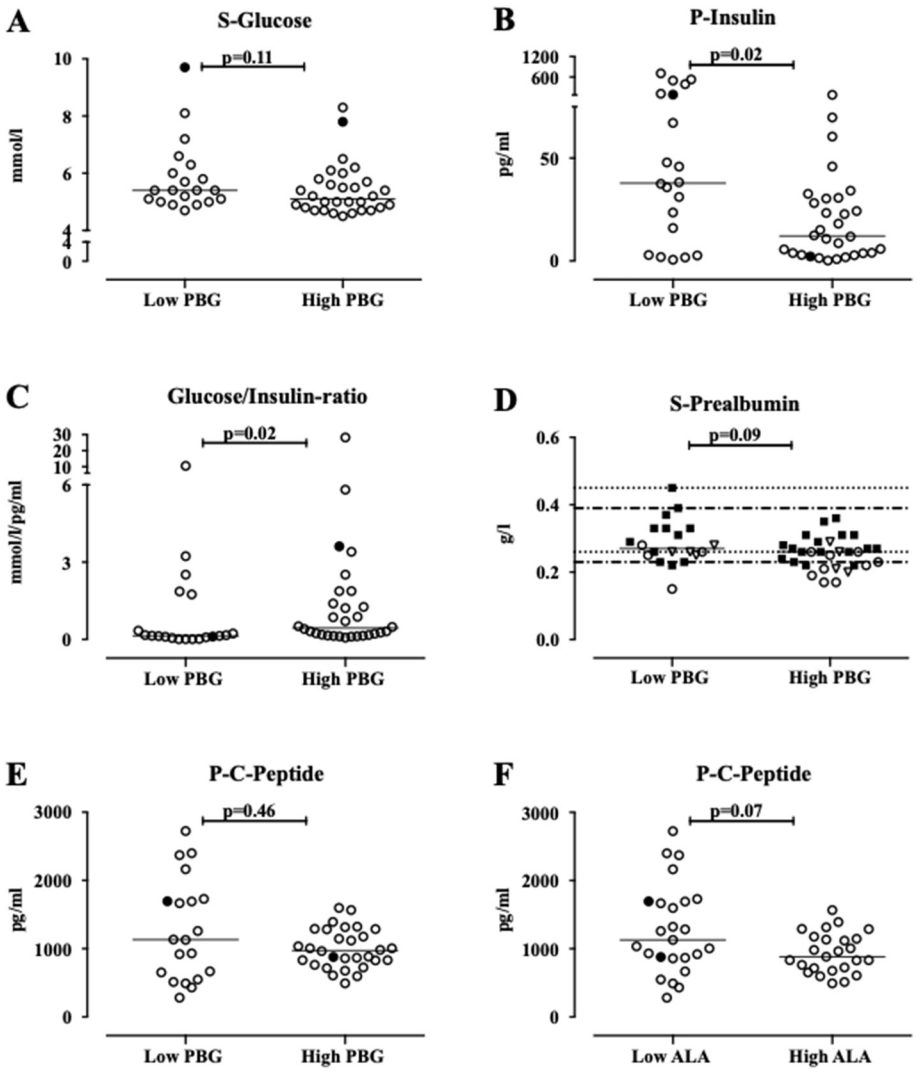


Fig. 7. Glucose, insulin, glucose/insulin ratio, prealbumin and C-peptide levels in acute intermittent porphyria (AIP) cases with low U-porphobilinogen (PBG) compared with acute intermittent porphyria (AIP) cases with high U-PBG. (A) serum glucose levels (mmol/l), (B) insulin (pg/ml), (C) glucose/insulin ratio (mmol/l/pg/ml), (D) S-pre-albumin (g/l), (E) C-peptide levels (pg/ml) in AIP cases with low (n = 20) and high (n = 30) levels of porphyrin precursors in urine. The black dots in panels A, B, C, E and F, indicate AIP cases who received anti-diabetic medications. A low U-porphobilinogen (PBG) level was defined as ≤1.5 μmol PBG/mmol creatinine, i.e., the reference value in urine. (F) C-peptide levels in AIP cases with low U-delta aminolevulinic acid (ALA) defined as < 3.9 μmol ALA/mmol creatinine (n = 25). S-glucose and pre-albumin were analyzed using standard biochemical assays, while the other markers were analyzed using a Bio-PlexPro Human Diabetes 10-plex immunoassay from Bio-Rad. The results are shown as scatter-plots with the median. The two horizontal dotted gridlines in panel (D) prealbumin are the normal reference values for men (black squares) and for women > 50 years (open circles). For women < 50 years (open inversed triangles) the reference values are 0.23–0.39 g/l (dotline gridline). The data were analyzed using the Mann-Whitney U test. The P-values are exact, two-tailed.

resistance may possibly be involved in a high disease activity [33].

However, we have no explanation for these differences, but this indicate that the role of hormones regulating glucose uptake such requires further investigation. Acute inflammation can trigger AIP attacks [1], but the inflammation observed as increased resistin levels found in the AIP cases could be an effect of the AIP disease itself, since it was observed in periods between attacks and outside acute inflammation. Resistin activates TLR4 on macrophages and increases the release of cytokines [34]. Furthermore, increased resistin levels is probably not an effect of other inflammatory diseases, as we did not find enhanced frequency in inflammatory diseases that could explain the difference in cytokines [26]. On the other hand, the slightly higher frequency of smoking, higher LDL levels and lower prealbumin levels indicating liver inflammation in the AIP cases, may partly explain the enhanced inflammation [26]. Another possible pathway from AIP with increased ALA, PBG and porphyrins to inflammation could be that ALA, PBG and porphyrins cause tissue damage that creates elevated cytokines [26]. Additionally, damage associated patterns (DAMPS) such as elevated uric acid due to reduced kidney function in some AIP cases could lead to cytokine production [26].

The intake of fat, and especially saturated fatty acids, was higher than recommended in both the AIP cases and controls and is associated with enhanced risk of cardiovascular disease [35]. The higher cholesterol levels in the cases can not be explained by a higher intake of SFA in the AIP cases, since the intake of SFA was elevated in both groups. Replacement of dietary saturated fats with PUFA normally reduces cholesterol levels and the CVD risk [35], and the lower PUFA intake in the AIP cases, may partly explain the higher total cholesterol and triglyceride levels in the AIP cases than in the controls. The intake of protein was within the national recommendations both in the AIP cases and the controls. The fiber intake was similar but was lower than recommended in both AIP cases and controls, in line with a previous report [28]. The intake of meat was lower in the AIP cases than in the controls. This may have several reasons, but we speculate that this may be due to the enhanced prevalence of reduced kidney function and maybe gout [26] in the AIP cases since patients with gout are advised to reduce the intake of purine-rich foods [36].

The intake of some vitamins, including vitamin D, was insufficient in many AIP cases and controls, and vitamin D deficiency was common in both groups. This may partly explain the enhanced PTH levels in some AIP cases, although reduced kidney function may also increase PTH levels [37]. Vitamin D deficiency is very common in Norway due to insufficient dietary sources and a lack of sunlight in northern Norway [38]. Vitamin D deficiency may have several negative effects, including increased risk of osteoporosis [37]. S-iron was close to be significantly higher in the AIP cases with high PBG levels, but iron deficiency was rare in the AIP cases. The homocysteine levels were increased in the symptomatic AIP cases compared to their matched controls, but this was probably not due to a severe deficiency of folate or vitamin B12. The increased homocysteine levels in the symptomatic cases was most likely due to reduced kidney function since homocysteine and creatinine levels were highly and positively correlated. However, a deficiency

Table 2
Biomarker levels in acute intermittent porphyria cases and controls.

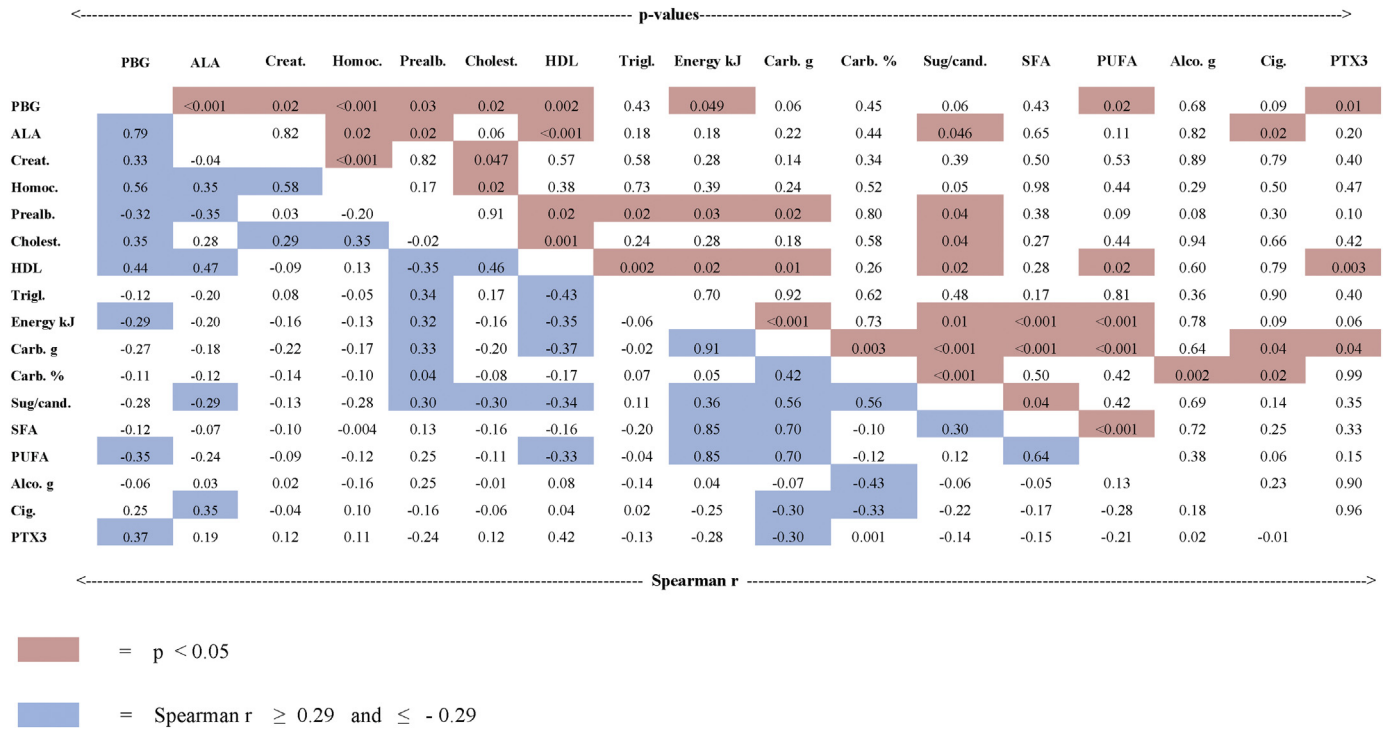
	Controls			Cases			p
	25%ile	Median	75%ile	25%ile	Median	75%ile	
U-ALA[a], µmol/mmol creatinine	1.6	1.9	2.3	2.4	3.9	7.0	< 0.0001
U-PBG[b], µmol/mmol creatinine	0.3	0.4	0.5	0.9	2.6	8.5	< 0.0001
U-Total porphyrins, nmol/mmol creatinine	4.4	6.8	11	8.5	26	83	< 0.0001
U-Uroporphyrins, nmol/mmol creatinine	1	1.25	1.7	1.3	2.8	19.7	< 0.0001
U-Heptaporphyrins, nmol/mmol creatinine	0.2	0.3	0.5	0.3	0.7	1.3	< 0.0001
U-Hexaporphyrins, nmol/mmol creatinine	0.1	0.1	0.3	0.0	0.2	0.7	0.045
U-Pentaporphyrins, nmol/mmol creatinine	0.0	0.1	0.2	0.1	0.4	1.4	< 0.0001
U-Coproporphyrins, nmol/mmol creatinine	3.0	4.5	8.8	6.3	14	44	< 0.0001
S-Total Cholesterol, mmol/l	4.6	5.1	5.7	5.1	5.7	6.4	0.002
S-LDL[c]-Cholesterol, mmol/l	2.3	3.1	3.7	3.0	3.5	3.9	0.02
S-HDL[d]-Cholesterol, mmol/l	1.3	1.5	1.8	1.1	1.4	1.8	0.50
S-Triglycerides, mmol/l	0.7	1.0	1.4	1.0	1.2	1.85	0.01
S-CDT[e], %	1.2	1.4	1.7	1.3	1.5	1.6	0.49
B-Hemoglobin, g/dl	13.7	14.4	15.3	13.6	15.0	15.8	0.19
B-EVF[f]	0.40	0.42	0.45	0.41	0.43	0.47	0.08
Erc-MCV[g], fl	85	87	91	86	89	93	0.18
Erc-MCH[h], pg	29	30	31	29	30	32	0.57
B-EPK[i], $\times 10^{12}$/l	4.6	4.9	5.1	4.7	5	5.3	0.25
Erc-Reticulocytes, %	0.8	0.9	1.2	0.8	1	1.2	0.47
Rtkc-CHr[j], pg	32.6	33.7	34.7	32.7	33.9	35	0.71
Erc-Hypo[k], %	0.1	0.2	0.3	0.1	0.2	0.4	0.59
B-Platelets, $\times 10^{9}$/l	224	266	299	194	243	287	0.05
S-Ferritin, µg/l	49	120	186	57	111	222	0.76
S-Iron, µmol/l	13	19	22	14	17	19	0.81
S-TIBC[l], µmol/l	59	62	70	63	67	75	0.06
S-Soluble transferrin receptor, mg/l	1.0	1.2	1.5	1.0	1.23	1.5	0.70
S-Sodium, mmol/l	140	142	143	140	141	142	0.20
S-Potassium, mmol/l	4.1	4.2	4.4	4.2	4.3	4.5	0.11
S-Magnesium, mmol/l	0.85	0.90	0.93	0.88	0.91	0.96	0.31
S-Phosphate, mmol/l	0.99	1.13	1.25	0.87	1.06	1.21	0.03
S-Cortisol, nmol/l	385	460	587	406	549	584	0.45
U-Norepinephrine, nmol/mmol creatinine	12	16	23	9	14	20	0.09
U-Epinephrine, nmol/mmol creatinine	1.75	2.0	3.25	1.0	2.0	3.0	0.37
U-VMA[m], µmol/mmol creatinine	1.1	1.6	2.1	0.8	1.3	1.9	0.01

The data represent the median values and interquartile range; 25 percentile (25%-ile) and 75 percentile (75%-ile).
Wilcoxon's matched-pairs signed-rank test was used on all case-control data in this table. N = 50 matched pairs.

[a] U-ALA = Urine-delta aminolevulinic acid.
[b] U-PBG = Urine-porphobilinogen.
[c] S-LDL = Serum–low-density lipoproteins.
[d] S-HDL = Serum–high-density lipoproteins.
[e] S-CDT% = percentage of carbohydrate-deficient transferrin.
[f] B-EVF = Whole blood erythrocyte volume fraction.
[g] Erc-MCV = Erythrocyte mean corpuscular volume.
[h] Erc-MCH = Erythrocyte mean corpuscular hemoglobin.
[i] B-EPK = Whole blood erythrocyte-particle concentration.
[j] Rtkc-Chr = Reticulocyte hemoglobin content.
[k] Erc-Hypo = Percentage of hypochromic red cells.
[l] S-TIBC = Serum-total iron-binding capacity.
[m] U-VMA = Urine vanillylmandelic acid.

of vitamin B1 and B6 cannot be excluded since they were not measured. The diastolic blood pressure and PTH was higher and S-albumin and calcium were slightly lower in the symptomatic compared to the asymptomatic AIP cases, probably also due to a reduced kidney function in the symptomatic AIP cases.

The use of alcohol in the AIP cases was much higher than expected, and was higher than the generally recommended upper limit of 1 alcohol unit (12 g) per day in a few cases. However, the alcohol intake was significantly lower in the symptomatic AIP cases than in the asymptomatic cases. Since alcohol had triggered attacks in 32% of the AIP cases in line with a previous study [18], we speculate that the symptomatic cases reduced the intake to avoid attacks. No correlation was found between the alcohol intake and disease activity. The finding that most AIP cases in this study used moderate amounts of alcohol regularly may indicate that a regular, but moderate intake of alcohol is less porphyrinogenic than an acute intake of alcohol [17,39]. However, the alcohol intake observed in both the AIP cases and controls, may partially hide the real impact of alcohol in the AIP group. AIP cases are advised to avoid alcohol. According to a WHO report from 2016, the mean total alcohol intake per capita in Norwegian adults above 15 years age was 7.5 l pure alcohol per year, compared to 9.8, 9.8, 6.3 and 7.3 in the whole European region, the US, the African and the Western Pacific Regions, respectively [40]. The mean total alcohol intake was 5.7 and 4.3 l per year in the controls for the asymptomatic and symptomatic AIP cases, respectively, and 6.1 and 3.1 l in the asymptomatic and symptomatic AIP cases, respectively. This indicates that the alcohol intake in the cases was not high compared to the rest of Norway and other countries. Furthermore, the alcohol habits vary between different countries, and different individuals, and this should be kept in mind when our results from Norway are being evaluated. It is well known that alcohol is toxic to the liver and is associated with enhanced risk of hepatocellular carcinoma (HCC) and liver cirrhosis [41].



	PBG	ALA	Creat.	Homoc.	Prealb.	Cholest.	HDL	Trigl.	Energy kJ	Carb. g	Carb. %	Sug/cand.	SFA	PUFA	Alco. g	Cig.	PTX3
PBG		<0.001	0.02	<0.001	0.03	0.02	0.002	0.43	0.049	0.06	0.45	0.06	0.43	0.02	0.68	0.09	0.01
ALA	0.79		0.82	0.02	0.02	0.06	<0.001	0.18	0.18	0.22	0.44	0.046	0.65	0.11	0.82	0.02	0.20
Creat.	0.33	-0.04		<0.001	0.82	0.047	0.57	0.58	0.28	0.14	0.34	0.39	0.50	0.53	0.89	0.79	0.40
Homoc.	0.56	0.35	0.58		0.17	0.02	0.38	0.73	0.39	0.24	0.52	0.05	0.98	0.44	0.29	0.50	0.47
Prealb.	-0.32	-0.35	0.03	-0.20		0.91	0.02	0.02	0.03	0.02	0.80	0.04	0.38	0.09	0.08	0.30	0.10
Cholest.	0.35	0.28	0.29	0.35	-0.02		0.001	0.24	0.28	0.18	0.58	0.04	0.27	0.44	0.94	0.66	0.42
HDL	0.44	0.47	-0.09	0.13	-0.35	0.46		0.002	0.02	0.01	0.26	0.02	0.28	0.02	0.60	0.79	0.003
Trigl.	-0.12	-0.20	0.08	-0.05	0.34	0.17	-0.43		0.70	0.92	0.62	0.48	0.17	0.81	0.36	0.90	0.40
Energy kJ	-0.29	-0.20	-0.16	-0.13	0.32	-0.16	-0.35	-0.06		<0.001	0.73	0.01	<0.001	<0.001	0.78	0.09	0.06
Carb. g	-0.27	-0.18	-0.22	-0.17	0.33	-0.20	-0.37	-0.02	0.91		0.003	<0.001	<0.001	<0.001	0.64	0.04	0.04
Carb. %	-0.11	-0.12	-0.14	-0.10	0.04	-0.08	-0.17	0.07	0.05	0.42		<0.001	0.50	0.42	0.002	0.02	0.99
Sug/cand.	-0.28	-0.29	-0.13	-0.28	0.30	-0.30	-0.34	0.11	0.36	0.56	0.56		0.04	0.42	0.69	0.14	0.35
SFA	-0.12	-0.07	-0.10	-0.004	0.13	-0.16	-0.16	-0.20	0.85	0.70	-0.10	0.30		<0.001	0.72	0.25	0.33
PUFA	-0.35	-0.24	-0.09	-0.12	0.25	-0.11	-0.33	-0.04	0.85	0.70	-0.12	0.12	0.64		0.38	0.06	0.15
Alco. g	-0.06	0.03	0.02	-0.16	0.25	-0.01	0.08	-0.14	0.04	-0.07	-0.43	-0.06	-0.05	0.13		0.23	0.90
Cig.	0.25	0.35	-0.04	0.10	-0.16	-0.06	0.04	0.02	-0.25	-0.30	-0.33	-0.22	-0.17	-0.28	0.18		0.96
PTX3	0.37	0.19	0.12	0.11	-0.24	0.12	0.42	-0.13	-0.28	-0.30	0.001	-0.14	-0.15	-0.21	0.02	-0.01	

= $p < 0.05$

= Spearman r ≥ 0.29 and ≤ -0.29

Fig. 8. Correlation matrix of porphyrin precursors, diet, alcohol, cigarette smoking, diet-related biomarkers and PTX3 in the acute intermittent porphyria (AIP) cases (n = 47). The data in the left lower part are Spearman's correlation coefficients, in the AIP cases (n = 47), r ≥ 0.29 and r ≤ −0.29 (blue color). The correlation matrix shows pairwise correlations of the different variables. In the upper right part the corresponding significant p values ($p < .05$) are indicated by red color. The variables included are: urine porphobilinogen ratio (U-PBG); urine 5-aminolevulinic acid ratio (ALA); S-creatinine (Creat.); P-homocysteine (Homoc.); S-pre-albumin (Prealb.); S-total cholesterol (Cholest.); S-high-density lipoprotein (HDL); S-triglycerides (Trigl.); intake of energy kJ/day (Energy KJ); carbohydrates g/day (Carb. g); Carbohydrates % of total energy (Carb. %); sugar/candy intake g/day (Sug/Cand.); saturated fatty acids (SFA) (g/day); polyunsaturated fatty acids (PUFA) g/day; alcohol g/day (Alco. g); current smoking of cigarettes per day (Cig.); and pentraxin 3 (PTX3), ng/ml. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

However, in the study by Innala et al., none of the AIP cases developing HCC were abusing alcohol [42]. This indicates that the general recommendation of no or a very low alcohol intake in AIP cases should be continued.

Psychological stress and physical strain was reported as triggers of attacks in 62% and 38%, respectively, of the symptomatic AIP cases. This result is in line with the report by Naik et al. who reported that 56% of patients with acute hepatic porphyrias said that stress, mainly emotional stress or caused by work, was a direct contributing factor to their acute attacks [25]. Several stress hormones were analyzed in this study, outside of AIP attacks, but only the levels of VMA were decreased in the AIP cases compared to the controls. In comparison, the levels of epinephrine and norepinephrine were similar in the cases and controls. We have no explanation for these findings, but speculate that the catecholamine release or VMA formation might be slightly decreased in the AIP cases in periods outside attacks. Stress may also affect the levels of stress hormones, which in turn affect the metabolism of glucose and fat. A previous report by Pozo et al. showed reduced levels of urine adrenal steroid hormones, including cortisol metabolites, in AIP cases compared to controls [43]. In this report, S-cortisol levels were similar in the AIP cases and controls, but urine metabolites of cortisol were not examined.

This report indicates that 2%, 6% and 20% of the AIP cases used porphyrinogenic, probably porphyrinogenic or possibly porphyrinogenic medications, respectively. Furthermore, 29% of the symptomatic cases reported that medications had triggered attacks. This is an unexpected finding since it is well known that correct medication is important in AIP cases and that many medications are porphyrinogenic [44]. The most likely explanation for this is that some medical doctors still have limited knowledge about AIP despite repeated information given regarding correct medication.

Current smoking was slightly more common among the AIP cases than among the controls. However, smoking had triggered attacks in only 6 % of the symptomatic AIP cases in this report. Among the asymptomatic and symptomatic cases, one out of five were smoking tobacco. Furthermore, the number of cigarettes smoked per day was correlated with the biochemical disease activity of ALA, but not of PBG, in the AIP cases. This is partially in agreement with Bylesjo et al. who reported that smoking was associated with a high AIP attack frequency [20]. In addition, even low cigarette usage is associated with an enhanced risk of several diseases, including lung cancer, coronary heart disease and stroke and should be avoided [45].

The kidney function was lower in the symptomatic AIP cases, which is in agreement with several previous reports indicating that the porphyrin precursors are toxic to the kidney tubuli and endothelial cells causing a chronic tubulointerstitial nephropathy [5,6]. In line with this, the S-albumin and P-PTH levels were slightly lower and higher in the cases than controls, respectively. Furthermore, the pre-albumin levels were reduced in the AIP cases, indicating reduced liver function, malnutrition or inflammation of the liver [26,46].

This study has several limitations that might have affected the results. First, most of the AIP cases had the same AIP mutation. Second, many different parameters were analyzed, implying that the risk of false positive results in the statistical analysis is high. Third, the number of AIP cases was limited to 50, mainly due to the rarity of the disease. However, to our knowledge this is the first report on the role of dietary factors in AIP cases using a dietary logbook and biochemical analysis in blood samples for many of the same nutrients and vitamins. The registration of food intake, however, may have been underreported since the reported energy intake was lower than the calculated energy

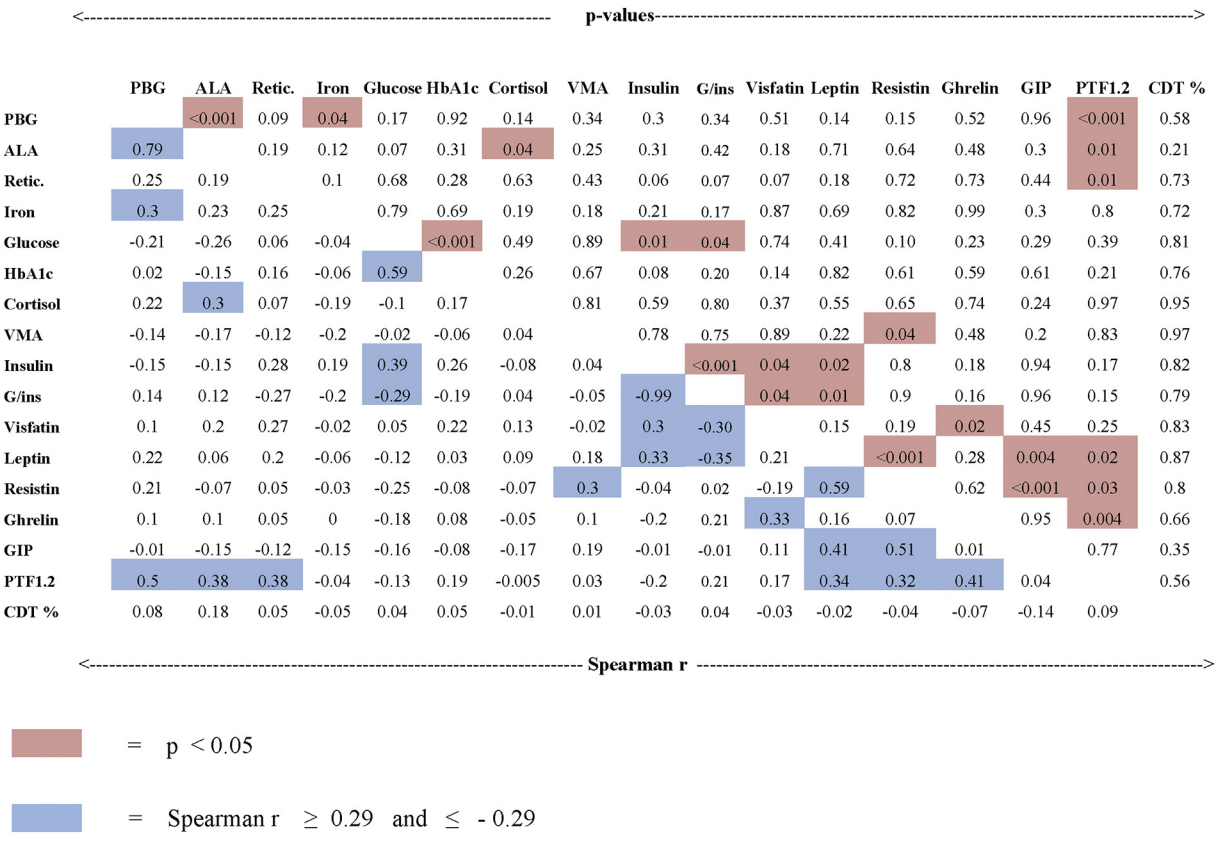


Fig. 9. Correlation matrix of porphyrin precursor and biomarkers of glucose metabolism- and diet-related biomarkers, prothrombin factor 1 + 2 (PTF1.2) and CDT%. In the lower left part are Spearman correlation coefficients, r, in the acute intermittent porphyria group (n = 47), r ≥ 0.29 and r ≤ −0.29 (blue color). The correlation matrix shows pairwise correlations of the different markers. In the upper right part are the corresponding significant p values (p < .05), which are indicated by red color. The variables included are the following: urine porphobilinogen ratio (PBG); urine 5-aminolevulinic acid ratio (ALA); Erc-Reticulocytes % (Retic.); S-iron (Iron); S-glucose (glucose); B-HbA1c (HbA1c); S-cortisol (cortisol); urine vanillylmandelic acid (VMA); P-insulin (Insulin); S-glucose/P-insulin ratio (G/ins-r); P-visfatin (Visfatin); P-leptin (Leptin); P-resistin (Resistin); P-ghrelin (Ghrelin); P-gastrointestinal peptide (GIP); P-prothrombin F1 + 2 (PTF1.2) and percentage of carbohydrate deficient transferrin (CDT%). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Symptoms and triggering factors of attacks in acute intermittent porphyria cases.

Symptoms during AIP attacks[a]	%	Triggering factors of attacks[b]	%	Triggering factors of attacks,women[c]	%	Military service accomplished[d]	%
Abdominal pain	91	Psychological stress	62	Birth control pills	0	Asymptomatic men	75
Dark/red urine	80	Work environment (mainly stress)	41	Menstrual cycle	17	Symptomatic men	69
Tiredness	71	Physical strain	38	Menstrual cycle, first day	17		
Muscle ache	60	Other disease	32	Menstrual cycle, ovulation	0		
Muscle weakness	60	Sleep deprivation	32	Menstrual cycle, other	0		
Other[e]	43	Alcohol	32				
Vomiting	43	Medication	29				
Headaches	43	Food items	24				
Decreased sensibility	40	Hunger	24				
Palpitations	40	Dieting	9				
Psychological symptoms	26	Other (alternative medications)	6				
Constipation	23	Smoking	6				
Paresis	17						
Epilepsy	6						

The data were obtained from interviews done by medical doctors and questionnaires.
[a] % of AIP symptomatic cases, (n = 35), 18 women, 17 men.
[b] % of AIP symptomatic cases, (n = 34), one data missing.
[c] % of (n = 18) symptomatic women, regarding menstrual cycle.
[d] % of 12 asymptomatic and % of 16 symptomatic men regarding military service (one of 17 symptomatic men had missing data on question on military service).
[e] e.g: Diarrhea, visual disturbances, fecal incontinence, impaired memory.

requirement, and the RMR-factor (energy intake divided by RMR) was lower than expected from their activity level in some of the cases and controls [47]. However, the underreporting seems to be similar in both cases and controls, indicating that the intake in the cases and controls can still be compared. Another limitation is that the comparison against the reference energy and nutritional recommendations are for Norway, Nordic Countries and Americans, and it may, thus, be questioned with respect to the extent that this issue may limit the generalization of

results to other countries, for instance, in Asia and Southern Europe, etc. However, the dietary recommendations do not vary much between the Nordic countries, European countries and the US [48], indicating that the comparison of intakes in the AIP case and control group with the recommended intake is valid for many countries. Furthermore, the comparison of the intake in the AIP case and control groups is also valid, although the actual dietary habits may vary in different countries. The differences in dietary habits between the Nordic area [12], the Mediterranean area [49], and other parts of the world should be kept in mind when our results are generalized to other AIP patients. In general, the individual dietary pattern should be assessed when individualized dietary advice are given to AIP patients. Furthermore, the biomarkers on glucose metabolism were possibly affected by the antidiabetic medication used by two AIP cases..

In summary, several lifestyle factors, including total energy intake, alcohol, stress and medications may affect the disease activity of AIP cases. The distinction between slow-release carbohydrates and added sugar is not always precisely explained to the AIP patients, leaving some of them on a diet high in added sugar which may lead to obesity. Resistin levels were higher in the symptomatic than asymptomatic AIP cases, and insulin was higher in the cases with low U-PBG levels suggesting that hormones regulating glucose uptake may be disturbed in AIP. Several of these lifestyle factors, including diet, alcohol intake and stress, are known to affect the expression of genes through epigenetic changes. A recent study suggested oligogenic inheritance in AIP, pointing to several factors that dictate who manifests symptoms [8]. Future studies should therefore examine the role of other related mutations and other epigenetic changes that could explain why only a few of those with an AIP mutation have symptoms of the disease, while most cases are asymptomatic.

Acknowledgements

This work was supported by grants from Northern, Regional Health Authorities, and from the Somatic research fund at the Nordland Hospital Trust.

Disclosure

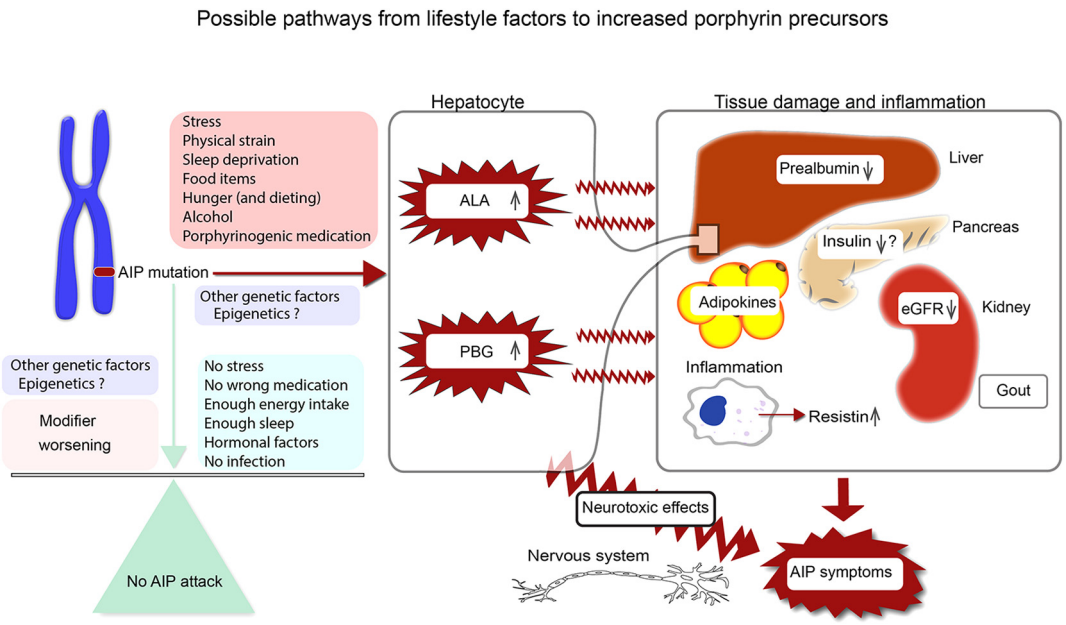
Dr. E. Storjord has received research grants from the Somatic research fund at the Nordland Hospital Trust and has received grants from the Northern Regional Health Authorities in Norway. Dr. Ole-L. Brekke has received grants from the Northern Regional Health Authorities in Norway.

Appendix A. Appendix Table A Porphyrinogenic medications, vitamin supplements, dietary supplements and herbal remedies in acute intermittent porphyria cases and controls

	Controls n (%)	Cases n (%)	p
Medications			
PSP[a] medications	4 (8)	10 (20)	0.15
PRP[b] medications	12 (24)	3 (6)	0.02
P[c] medications	1 (2)	1 (2)	1.00
Medications containing vitamins, Mg or iron			
Vitamin B12 injection	1 (2)	1 (2)	1.00
Etalpha (1α(OH)-vitamin D_3)	1 (2)	1 (2)	1.00
Isotretinoin (Vitamin A)	0 (0)	1 (2)	1.00
Magnesium	4 (8)	0 (0)	0.12
Iron	6 (12)	3 (6)	0.27
Supplements			
Vitamin B	6 (12)	4 (8)	0.74
Vitamin C	5 (10)	2 (4)	0.44
Vitamin D	4 (8)	5 (10)	1.00
Multi-vitamins	4 (8)	5 (10)	1.00
Calcium	3 (6)	2 (4)	1.00
Vitamin K	0 (0)	1 (2)	1.00
Fish oil	18 (36)	15 (30)	0.67
Persons using herbal remedies	8 (16)	7 (14)	1.00

The data were obtained from questionnaires from all participants, and from the 7-day diet logbook from n = 47 cases and 48 controls, and are given as n and % of AIP cases (n = 50) and of their age-, sex- and place of residence-matched controls (n = 50).
The herbal remedies are not counted in the PSP, PRP and P-data. Herbal remedies containing vitamins, calcium, magnesium or iron are also counted in supplements where applicable. Two-tailed P values were calculated using Fischers exact test.

[a] PSP = Possibly porphyrinogenic medication.
[b] PRP = Probably porphyrinogenic medication.
[c] P = Porphyrinogenic medication.



Appendix Fig. A. Possible pathways from lifestyle factors to increased porphyrin precursor levels and symptoms in acute intermittent porphyria. The possible role of lifestyle factors in the biochemical disease activity and symptoms in AIP cases are summarized. An AIP mutation is the prerequisite for AIP disease. To acquire elevated porphyrin precursor levels often requires several triggering factors at the same time such as stress, physical strain, dieting, alcohol and/or porphyrinogenic medications (red box), which may partly explain the low manifestation rate of symptoms in the AIP cases. Many factors may contribute to the worsening or improvement of porphyrin precursor levels and symptoms, such as genetic and epigenetic modifiers (blue box), the intensity of triggers as the level of protective factors such as a higher sugar intake and relaxation. Other diseases, such as diabetes mellitus, may also protect those with an AIP mutation. High levels of ALA and PBG affects the nervous system, the liver and kidney and result in abdominal pain, and vomiting etc. during the acute nevrovisceral attacks. Enhanced U-PBG levels are possibly associated with lower insulin levels. Resistin levels are higher in the symptomatic than the asymptomatic cases, and are associated with inflammation. As illustrated, most AIP cases tolerate different amounts of single triggering factors and probably have sufficient protective factors (marked in green) to avoid an increase in the porphyrin precursors and attacks. This conforms to what we have found, for example, with alcohol intake, since there was no difference in the PBG levels in those who drank more or less alcohol.

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