Annals of Agricultural and Environmental Medicine 2023, Vol 30, No 3, 513–521
www.aaem.pl

Effects of Autoimmune Protocol (AIP) diet on changes in thyroid parameters in Hashimoto's disease

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Ihnatowicz P, Gębski J, Drywień ME. Effects of Autoimmune Protocol (AIP) diet on changes in thyroid parameters in Hashimoto's disease. Ann Agric Environ Med. 2023; 30(3): 513–521. doi: 10.26444/aaem/166263

Abstract

Introduction and Objective. In the scientific world, the nutritional needs of persons with Hashimoto's autoimmune thyroid disease are discussed, and there is a lot of interest in the autoimmune protocol (AIP). The aim of the study was to check the effects of AIP on thyroid parameters in euthyroid patients with Hashimoto's disease.
Materials and method. Among 28 people with Hashimoto's (including 1 male) the consumption of nutrients, anthropometrics, symptoms of the disease, values of thyroid parameters: FT3, FT4, TSH, thyroid ultrasound and autoimmune aTPO, aTG were analyzed before and after 12 weeks of using the AIP diet. The impact of changes in the consumption of selected nutrients on changes in thyroid biochemical parameters were analyzed using multiple regression models, where the dependent variables of the created models were changes in thyroid biochemical parameters.
Results. After applying the AIP diet, the number of people reporting symptoms of malaise decreased, the levels of FT3 and FT4 as well as TSH decreased, remaining within the reference concentration range. aTG decreased slightly, but aTPO increased significantly. Ultrasound examination also showed a decrease in the volume of the thyroid gland. The body weight of the subjects decreased, which indicates a caloric deficit.
Conclusions. Given the numerous advantages of the AIP diet, extending the observation time of the diet, along with its personalization in terms of food selection, energy and nutritional value, could show changes in both well-being and biochemical test results to a greater extent. The use of a personalized AIP protocol can improve the quality of life, a positive change in mental state, reduction of stress, and above all, the improvement of adverse ailments associated with Hashimoto's disease.

Key words
Hashimoto's disease, nutrients intake, Autoimmune Protocol, thyroid parameters

INTRODUCTION

In the scientific world, the nutritional needs of persons with Hashimoto's autoimmune thyroid disease are discussed [1]. There are no uniform and specific recommendations according to which doctors and dieticians could treat patients, although they could also be lacking due to individual approaches in a group of patients internally differentiated in terms of the nutritional model [2]. In 2019, Wojtas et al. (2019) proposed the Diet4Hashi protocol, but in this regard, no further consideration has been reported in publications to date [3]. Currently, the official method of treating the disease is hormone therapy extended with supplements or drugs containing selenium and vitamin D. Dietary recommendations mainly include the elimination of lactose and gluten, even for patients who do not require such interventions for medical reasons. Even if the patient does not receive such a recommendation from a specialist, he or she decides on such nutritional models, relying on information from the media [4]. In fact, there are some indications regarding gluten that its elimination may be helpful against autoimmunity [5]. In 2018, Polish scientists from POLSPEN issued a statement that there is no scientific evidence supporting the elimination of gluten or lactose in the treatment of Hashimoto's disease [6].

Although the beneficial health effects of anti-inflammatory, antioxidant, high-nutrient diets, as well as Mediterranean, DASH, Paleolithic and other diets are known, they have not been tested on Hashimoto's patients. However, they would most likely bring beneficial results against autoimmune processes, especially in Western countries [7, 8, 9, 10]. Among specialists, there is a lot of interest in the autoimmune protocol (AIP), which is not precisely defined and does not have an exact template; it is a collection of subjective dietary choices of a specialist based on incomplete scientific literature. Therefore, the AIP versions may differ more or less depending on the protocol creator. The autoimmune protocol is based on food products with appropriate nutritional but not caloric density, and the elimination of gluten is often observed [11]. Much attention is paid to the selection of food products based on their impact on the immune system, intestinal microbiota, functions of the intestinal wall and intestinal mucosa. Individual food products are therefore eliminated or included in a given protocol. It is of great importance to pay attention to potential nutritional deficiencies in patients, as well as nutrition with those ingredients that are necessary for the function of the diseased tissue or organ. In Hashimoto's disease, these include vitamin D, selenium, zinc, copper,

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Received: 29.03.2023; accepted: 16.05.2023; first published: 01.06.2023

iron, vitamin b12, iodine and fat-soluble vitamins [12]. The first application of AIP was tested in a clinical trial in people with inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis, with positive intervention results. Most of the subjects obtained remission of the disease in the 6th week of introducing the diet and maintained it in the 10th week from the start of the study [13]. This has encouraged scientists to further explore the potential of AIP in autoimmune diseases, including Hashimoto's [12]. Abbott et al. (2019), using AIP (version of the modified paleolithic diet) in 17 non-obese women with HT (Hashimoto's Thyroiditis), without other autoimmune diseases, improved their quality of life. Beneficial changes were observed in disease symptoms, number of white blood cells, level of highly sensitive reactive protein C (hs-CRP) and, statistically insignificant, improved thyroid functions, including autoimmune processes [12].

The scientific literature lacks comprehensive specific data on the effects of AIP on Hashimoto's disease, except for the description of a few individual cases [11]; hence, the presented study is an important element enriching scientific knowledge in this field. The aim of the study was to check whether and how thyroid function and quality of life would changes as a result of using AIP in Poles with Hashimoto's disease. Attention was focused on the effects of selected nutrients.

MATERIALS AND METHODS

Ethical Approval. The study was approved by the Ethics Committee of the Faculty of Human Nutrition and Consumer Science, Warsaw University of Life Sciences, Poland (Resolution No. 40_1/2017 of 28.02.2018). The study was conducted according to the guidelines laid down in the Declaration of Helsinki. Before starting the study, the researchers explained the purpose of the study and asked the respondents for consent to participate.

Study design and sample collection. A group of 28 people were examined, including 27 women (96.43%) and 1 male (3.57%), the average age was 35 years (range 23–55 years). In the study group, 15 people (53.57%) lived in cities with more than 100,000 inhabitants, 7 people (25%) in cities with less than 100,000 inhabitants, and 4 people (14.29%) lived in a village. 2 people did not provide information. Most of the respondents (23 people; 82.14%) worked professionally, 3 (10.71%) did not work, and 2 people did not provide information. Thus, 28 people started the study and 20 completed it, dropout rate – 28.6%.

The following inclusion criteria were used in the selection of people for the study: Hashimoto's disease diagnosed by a doctor in respondents aged between 19–50 years, BMI > 19, and written consent to participate in the study. The exclusion criteria were pregnancy and breastfeeding, BMI<19, diagnosed celiac disease, renal failure, liver failure, advanced atherosclerotic disease, malnutrition, and eating disorders. The exclusion criteria also included people who had used an elimination diet during the year preceding the study, and people with implanted electrostimulators.

Anthropometric research. Body height was measured using a stadiometer (Seca), weight and body composition (BIA method) using a Tanita MC-580 body composition analyzer with medical certificates.

Biochemical tests carried out in DIAGNOSTYKA Laboratories Medical (Poland) in accordance with the according methodology, and included determination of the following concentrations: thyroid stimulating hormone (TSH) (to assess the metabolic state of the thyroid gland); total triiodothyronine (T3); serum circulating thyroxine (T4); antibodies (anti-Tg); anti-peroxidase antibodies (anti-TPO); free fraction of triiodothyronine (fT3); free fraction of thyroxine (fT4). Blood for testing was obtained at the collection point of the Student Health Centre at the Warsaw University of Life Sciences,.

Imaging examinations of the thyroid gland were performed using a medically certified ultrasound machine (VINNO G60). On the basis of the examination, the size and structure of the right and left thyroid lobes were assessed.

Biochemical tests and ultrasound examinations of the thyroid gland were performed under medical supervision in the Student Health Centre of the Warsaw University of Life Sciences (SGGW).

Information on respondents' previous diet, and the amount of food and liquids consumed was collected using the 3-day food records method. To determine the daily intake of energy and nutrients, their content in the consumed products was calculated using the computer programme Dieta 5.0. Then, in order to plan an individual AIP diet, energy needs were determined for each person (Harris-Benedict formula) taking into account the physical activity coefficient (PAL). The level of recommended nutrient intake was determined based on standards for the Polish population (Jarosz et al., 2020). On the basis of the collected data, individual menus were developed for each of the subjects, in accordance with the AIP protocol.

During the study, the respondents' intake of supplements and medications was not interfered with, which means that they continued their current supplementation and medication. For each of the subjects, however, the value of the recommended intake of the most important nutrients was determined individually, which had a positive effect on the functioning of the body and constituted a necessary supplement to the developed menu, e.g. magnesium, potassium, Vitamin B6, B12, D, iron (Tab. 2A). This intervention made it possible to supplement nutritional deficiencies by adjusting the AIP, without having to change other factors. Anthropometric parameters (weight, height, fat and muscle mass) of the body, biochemical indices (TSH, fT3, fT4, aTPO, aTG) and ultrasound images of the thyroid gland were measured in the subjects before the introduction of the AIP diet. BMI and thyroid function parameters (TSH, fT3, fT4) were normal, but the titer of anti-thyroid antibodies aTPO and aTG was elevated in all patients. All subjects were then given individual AIP diets for 12 weeks, and the previously mentioned measurements were repeated.

The newly implemented menu was analyzed in terms of energy and nutritional value, to determine to what extent the change in nutrient intake before and after the AIP protocol affected thyroid parameters. All nutrients in the diet were balanced, therefore additional supplementation was not recommended, although the subjects continued their previous supplementation. The intervention diet for the single man differed from the women's diet in terms of the amount of energy, micro- and macroelements, which resulted from individual needs. However, the principles of constructing the menu were the same for men and women. In addition, the subjects assessed their well-being through the subjectively perceived symptoms characteristic of Hashimoto's disease, before and after applying the AIP diet. Compliance with the intervention programme was monitored on an ongoing basis via remote contact, and consultations were held at monthly online meetings.

Characteristics of the AIP protocol. The principles of the AIP protocol were proposed by Dr. Sarah Ballantyne, promoter of the Paleolithic diet,. The assumptions of the protocol focus on the nutritional density of the diet, which is based in particular on whole grains or unprocessed products, excluding products potentially associated with dysregulation of the immune system. In addition, within the scope of modification, the impact of food on the intestinal microbiota and the intestinal mucosa is taken into account. Based on the above, individual products, as well as entire groups of food products, are recommended or excluded from the protocol [14].

In the current study, products supporting the work of the intestines and intestinal microbiota were included in the developed individual protocols. Food additives and food products that disturb or potentially disturb the functioning of the immune system were eliminated. Excluded from AIP were: chicken eggs, dairy products and cereals. Patients could use gluten-free flours, such as coconut, chestnut and arrowroot, among others. It was noted that the protocol should consist of 'live' food, i.e. fermenting or maturing food, pickled, free from heat treatment, or heavy technological processing. Despite the lack of decisive data, it was decided to relinquish potentially harmful lectin products, such as nightshades and legumes.

The specification of food products or their groups for the protocol and an exemplary 3-day menu can be found in the supplementary diet (in turn, SF AIP List of products excluded and allowed and SF Sample 3-day AIP) (Tab. 1A).

The results of the research were subjected to a preliminary statistical analysis.

Changes in the value of energy intake from individual sources. The results of the anthropometric tests, and the results of thyroid function, the autoimmunity and thyroid volume doubled in measurements, before and after the therapy, were statistically assessed using the t-test for paired samples. Frequency of symptoms characteristic of Hashimoto's disease before and after the AIP diet were compared using the Chi^2 independence test.

The impact of changes in the consumption of selected nutrients on changes in thyroid biochemical parameters were analyzed using multiple regression models, where the dependent variables of the created models were changes in thyroid biochemical parameters. The same set of independent (explanatory) variables in the form of changes in nutrient intake were used in all models. Only those variables that were statistically significant at the level of $p<0.05$ were used in the models. The selection of variables for the models was carried out by automatic selection of model variables by stepwise elimination. All statistical analysis were performed using the SAS 9.4 statistics package at the level of significance $\alpha=0.05$.

RESULTS

Changes in the energy and nutritional value of the diet. On analyzing changes in energy intake with diet before and after AIP, no significant differences were observed. However, the volume of energy obtained from protein increased and from fat decreased, statistically significantly (Tab. 1).

Table 1. Change in average energy intake before and after AIP

	Before AIP	After AIP	Change [%]	p-value
Protein energy (%)	16.77	24.91	48.5*	<.0001
Carbohydrate energy (%)	45.63	44.44	-2.6	0.256
Fat energy (%)	33.80	30.65	-9.3*	<.0001
Energy intake (kcal)	2 067	1 997	-3.4	0.872
Energy intake (kJ)	8 665	8 360	-3.5	0.893

* statistically significant difference at the significance level α=0.05 (paired T-test)

Table 1A. Sample 3-day AIP menu used in the study

Day 1	Day 2	Day 3
Meal 1		
Mackerel with vegetables	Turkey with vegetables	Pumpkin bread Avocado butter Carrot bread spread
Meal 2		
Sweet baked potatoes	Sweet potato and carrot fries	Sweet potatoes with dill
Meal 3		
Beef stew with vegetables	Clear broth	Baked chicken leg
Meal 4		
Sweet potato fries Clear broth	Sweet potato pudding	Spicy puree Smoked cod
Energy and macronutrients content		
E: 2007 P: 113 F: 61 CA: 213 DF: 51	E: 2006 P: 105 F: 68 CA: 206 DF: 50	E: 2006 P: 117 F: 74 CA: 185 DF: 48

E - energy [kcal]; P - protein [g]; F - fat [g]; CA - assimilable carbohydrates [g]; DF - dietary fibre [g]

The use of AIP resulted in an increase in the nutritional density of the diet in relation to the previously used diet, and an increase in the intake of beta-carotene (5-fold), an increase in the consumption of dietary fibre by more than half, as well as an increase in the consumption of minerals and vitamins. The largest increase was observed in the consumption of both vitamin A and vitamin C, respectively a 3- and 9-fold increase, compared to the intake before the start of AIP. The values of changes in the consumption of individual ingredients are presented in the Table in the supplementary materials (Tab. 2A). Reduction in intake was noted for: sucrose (-15%), lactose (-72%), cholesterol, saturated fatty acids, caffeine (-100%), starch (-1%), sodium (-1%), zinc (-12%) and calcium (-27%).

Changes in the value of anthropometric and biochemical indicators. As a result of the applied AIP nutrition scenarios, body weight, BMI, percentage of muscle and fat tissue in the subjects changed. A statistically significant decrease in individual values was noted (Tab. 2).

Table 2. Mean values of anthropometric indicators before and after AIP

	Before AIP (SD)	After AIP (SD)	Difference [%]	p-value
Body mass [kg]	69.0 (12.12)	65.5 (10.81)	-4.83*	<.0001
BMI [kg/m^2]	25.6 (3.59)	24.25 (3.06)	-5.15*	<.0001
Body fat [%]	33.01 (7.60)	29.42 (7.96)	-9.94*	<.0001
Body muscle [%]	22.96 (7.95)	21.04 (5.03)	-4.37*	0.032

*-statistically significant difference at the significance level α=0.05 (paired T-test)

The main parameters of thyroid function TSH, fT3, fT4 were within the generally accepted norm, although the AIP diet significantly lowered their values. In addition, an increase in aTPO by as much as 40%, and a decrease in thyroid volume by 5.3–6.2%, was observed (Tab. 3).

Changes in subjects well-being. The subjects reported many symptoms of malaise before applying the AIP diet. The most frequently reported symptoms were fatigue, drowsiness, poor concentration and dry skin. As a result of applying the new AIP menu, the number of people suffering from these ailments has decreased (Tab. 4).

Table 2A. Mean intake of selected nutrients before and after the AIP diet

Nutrients	Before AIP	After AIP	Difference	Difference [%]
Alanine [mg]	4 055.00	6 808.70	2753.7?	167.91
Arginine [mg]	4 451.62	6 555.95	2104.33	147.27
Beta-carotene [µg]	4 896.40	26 960.25	22063.85	550.61
Total protein [g]	82.39	122.55	40.16	148.74
Vegetable protein [g]	27.09	32.15	5.06	118.68
Animal protein [g]	54.52	88.63	34.11	162.56
Dietary fiber [g]	23.49	38.04	14.55	161.94
Cholesterol [mg]	369.28	326.42	-42.86	88.39
Zinc [mg]	9.58	13.13	3.55	137.06
Cystine [mg]	1 174.01	1 268.85	94.84	108.08
Long-chain polyunsaturated fatty acids [g]	0.68	1.78	1.10	261.76
Energy in kcal [kcal]	2 067.95	1 997.28	-70.67	96.58
Energy in kJ [kJ]	8 665.71	8 360.21	-305.50	96.47
Phenylalanine [mg]	3 485.64	4 627.90	1 142.26	132.77
Folates (dietary equivalents) [µg]	319.46	631.87	312.41	197.79
Phosphorus [mg]	1 429.15	1 844.80	415.65	129.08
Glycine [mg]	3 397.84	5 931.74	2 533.90	174.57
Histidine [mg]	2 443.25	3 557.88	1 114.63	145.62
Isoleucine [mg]	3 833.56	5 884.45	2 050.90	153.50
Iodine [µg]	117.92	188.16	70.25	159.57
Caffeine [mg]	229.97	0.00	-229.97	0.00
Aspartic acid [mg]	6 970.35	12 986.37	6 016.03	186.31
Glutamic acid [mg]	15 062.64	17 888.08	2 825.43	118.76
Fatty acids: total MUFA [g]	31.69	32.78	1.09	103.44
Fatty acids: total SFA [g]	27.89	16.77	-11.12	60.14
Fatty acids: total PUFA + intake from supplements [g]	13.58	13.73	0.15	101.07
Lactose [g]	4.47	1.25	-3.22	28.03
Leucine [mg]	6 097.56	8 845.15	2 747.59	145.06
Lysine [mg]	5 374.11	9 833.18	4 459.06	182.97
Magnesium + intake from supplements [mg]	426.85	640.99	214.14	150.17
Manganese [mg]	4.37	4.51	0.14	103.10
Methionine [mg]	1 964.06	2 941.72	977.66	149.78
Copper [mg]	1.45	2.60	1.15	179.12

Nutrients	Before AIP	After AIP	Difference	Difference [%]
Niacin [mg]	23.62	40.98	17.36	173.50
Ash [g]	16.45	24.29	7.84	147.65
Potassium + intake from supplements [mg]	3 880.33	7 620.28	3 739.95	196.38
Percentage of energy from protein [%]	16.77	24.91	8.14	148.55
Percentage of energy from fat [%]	33.80	30.65	-3.15	90.68
Percentage of energy from carbohydrates [%]	45.63	44.44	-1.20	97.38
Proline [mg]	5 031.52	5 146.88	115.36	102.29
Retinol [µg]	653.63	830.16	176.54	127.01
Riboflavin [mg]	1.64	2.35	0.70	142.78
Saccharose [g]	42.93	36.49	-6.43	85.01
Serine [mg]	3 760.60	4 905.34	1 144.73	130.44
Starch [g]	107.47	106.35	-1.12	98.96
Sodium [mg]	3 031.98	2 988.75	-43.23	98.57
Thiamine [mg]	1.31	1.80	0.49	137.55
Fat [g]	80.26	69.41	-10.85	86.48
Threonine [mg]	3 326.08	5 245.79	1 919.71	157.72
Tryptophan [mg]	1 021.60	1 606.10	584.50	157.21
Tyrosine + intake from supplements [mg]	2 813.62	3 676.73	863.10	130.68
Valine [mg]	4 476.70	6 665.87	2 189.16	148.90
Calcium [mg]	654.10	476.65	-177.45	72.87
Total carbohydrates [g]	258.97	256.03	-2.94	98.86
Vitamin A (retinol equivalent) [µg]	1 528.58	5 220.45	3 691.86	341.52
Vitamin B12+ intake from supplements [µg]	16.43	26.54	10.11	161.55
Vitamin B6 + intake from supplements [mg]	4.43	8.07	3.64	182.14
Vitamin C + intake from supplements [mg]	164.96	1 462.66	1 297.69	886.65
Vitamin D + intake from supplements [µg]	53.04	55.91	2.87	105.41
Vitamin E (alpha-tocopherol equivalent) [mg]	11.80	15.54	3.74	131.68
Water [g]	1 407.86	1 868.15	460.29	132.69
Iron + intake from supplements [mg]	14.90	20.22	5.32	135.74

Table 3. Mean values of biochemical indicators and thyroid volume before and after AIP

Indicator (Reference Value)	Before AIP (SD)	After AIP (SD)	Unit difference	Difference [%]	p-value
TSH (0.27-4.2) [mU/l]	3.72 (4.14)	2.69 (21.55)	-1.03	-27.68	0.416
FT3 (2-4.4) [pmol/l]	3.31 (0.40)	2.88 (0.78)	-0.43	-12.99*	0.009
FT4 (0.93-1.7) [ng/dl]	1.36 (0.28)	1.20 (0.29)	-0.16	-11.76*	0.044
aTPO (<34) [IU/ml]	210.19 (204.12)	293.73 (384.06)	83.54	39.74	0.152
aTG (<115) [IU/ml]	317.11 (272.42)	300.89 (219.77)	-16.22	-5.11	0.634
pp-usg [ml]	7.17 (3.85)	6.79 (3.21)	-0.38	-5.30*	0.020
pl-usg [ml]	5.31 (2.71)	4.98 (2.32)	-0.33	-6.21*	0.014

*-statistically significant difference at the significance level α=0.05 (paired T-test)

Table 4. Frequency of symptoms characteristic of Hashimoto's disease before and after AIP diet

Symptoms	Before AIP [% of subjects]	After AIP [% of subjects]	Difference [% of subjects]
heartburn	14	11	3
constipation	36	11	25*
flatulence	61	29	32*
diarrhea	29	7	22*
intestinal gases	61	21	40*
intestinal colic / abdominal pain	29	14	15
Nausea / vomiting	7	0	7**
taste disturbances	7	7	0
reflux	18	11	7
peptic ulcer disease	7	4	3**
tiredness	82	29	53*
increased drowsiness	64	21	43*
headaches	39	11	28*
impaired concentration	71	18	53*
fatigue	50	11	39*
depression	29	4	25*
muscle cramps	43	7	36*
arthralgia	57	21	36*
hair falling out	64	32	32*
weak nails	39	25	14
skin problems	57	7	50*
dry skin	71	32	39*
edema/swelling	39	11	28*
weight gain	57	11	46*

* statistically significant difference at the significance level of α=0.05 (Chi-Square Test of Independence)
** no possibility to perform the test (unfulfilled assumptions)

Among the subjects, a decrease in body weight was also noted (Tab. 1) caused by a decrease in glucose and insulin levels, which subsequently resulted in a decrease in the level of hormones – primarily estrogens.

Effect of changes in nutrient intake and on changes in biochemical indicators. Regression models were used to determine the effect of changes in nutrient intake and on changes in the biochemical parameters of the thyroid gland after applying the AIP diet, where $\Delta = Value_{after} - Value_{before}$. The dependent variables of the created models there were changes in the following values: TSH, fT3, fT4, aTPO, aTG, volume of the right and left thyroid lobes. In the independent variables, there were changes in energy and nutrient intakes: protein (total, animal, vegetable), fat, total carbohydrates, digestible carbohydrates, dietary fibre, percentage of energy from protein, fat and carbohydrates, amino acids, isoleucine, leucine, lysine, methionine, tryptophan, valine; fatty acids: total, polyunsaturated, monounsaturated and saturated, total polyunsaturated fatty acids with intake from supplements, individual fatty acids with different chain lengths and unsaturation; minerals: ash, zinc, phosphorus, magnesium, magnesium + intake from supplements, manganese, copper, potassium, potassium + intake from supplements, sodium, calcium, iron, iron + intake from supplements, iodine; vitamins: folates; niacin; retinol and vit. A as a retinol equivalent; riboflavin; thiamine; vitamin B_{12} and vit. B_{12} + intake from supplements; vitamin B_6, vit. B_6 + intake from supplements; vitamin C and vit. C + intake from supplements; vitamin D and vit. D + intake from supplements and vitamin E, other ingredients: beta-carotene, caffeine, sucrose, starch, lactose, cholesterol.

The performed regression analysis showed that the change in the amount of manganese (Δ Mn) taken in with the AIP diet had a statistically significant effect on the change in the TSH value in the blood according to the equation:

$$\Delta\ TSH = 6.22 - 6.28 * \Delta\ Mn.$$

The AIP diet had a significant effect on the change in the fT3 value. Significant factors were changes in intake (in order of importance): vitamin D along with intake from supplements (Δ Vit D), caffeine (Δ Caf), iodine (Δ I), vitamin C along with intake from dietary supplements (Δ Vit C), % energy from carbohydrates (% Carb) and folates equivalents (Δ Fol). Nutrients are presented in the order of statistically significant change in the fT3 value (Tab. 5 and 6), according to the equation:

$$\Delta\ fT3 = 0.50 - 0.06 * \Delta\ D + 0.01 * \Delta\ Caf - 0.01 * \Delta\ I + 0.0004 * \Delta\ Vit\ C + 0.04 * \Delta\ \%\ Carb - 0.001 * \Delta\ Fol$$

The AIP diet changed the value of fT4, mainly by changing the intake of long-chain polyunsaturated fatty acids (Δ LcPUFA), iodine and vitamin C along with intake from supplements. The components are presented according to the statistical significance of their effect on fT4 concentration (Tab. 5 and 6). According to the equation:

$$\Delta\ fT4 = -0.17 - 0.25 * \Delta\ LcPUFA - 0.002 * \Delta\ I + 0.0002 * \Delta\ C$$

The change in the characteristics of the diet resulted in changes in the value of aTPO, which were responsible in the order of importance of the effect: retinol (Δ Ret) and isoleucine (Δ Ileu) (Tables 5 and 6), according to the equation:

$$\Delta\ aTPO = -61.48 - 0.06 * \Delta\ Ret + 0.06 * \Delta\ Ileu$$

Changes in the intake of certain types of fatty acids had a statistically significant effect on the change in the aTG value. The most significant effect was the change in the intake of total monounsaturated fatty acids (Δ MUFA), followed by

Table 5. Effect of nutrients intake with the AIP diet - regression coefficients

	Intercept	ΔPP	ΔLcPUFA	ΔFol	ΔIleu	ΔI	ΔCaf
Model 1 (ΔTsh)	**6.216**	ns	ns	ns	ns	ns	ns
Model 2 (ΔFt3)	**0.593**	ns	ns	**-0.002**	ns	**-0.007**	**0.004**
Model 3 (ΔFt4)	**-0.167**	ns	**-0.252**	ns	ns	**-0.002**	ns
Model 4 (ΔaTPO)	**-61.475**	ns	ns	ns	**0.057**	ns	ns
Model 5 (ΔaTG)	**5.711**	ns	ns	ns	ns	ns	ns
Model 6 (Δpp-Vol)	**0.484**	ns	ns	ns	ns	ns	ns
Model 7 (Δpl-Vol)	**0.344**	**0.057**	ns	ns	ns	ns	ns

ΔPP – Plant Protein; ΔLcPUFA – Long chain Polyunsaturated Fatty Acids; ΔFol – Folate; ΔIleu – Isoleucine; ΔI – Iodine; ΔCaf – Caffeine

Table 6. Effect of nutrients intake with the AIP diet - regression coefficients

	Δ C16:0	Δ C20:0	Δ C20:4	ΔMUFA	ΔMn	Δ %Carb	Δ Ret	ΔVit C	ΔVit D
Model 1 (ΔTsh)	ns	ns	ns	ns	**6.216**	ns	ns	ns	ns
Model 2 (ΔFt3)	ns	ns	ns	ns	ns	**0.043**	ns	**0.491×10^{-3}**	**-0.058**
Model 3 (ΔFt4)	ns	ns	ns	ns	ns	ns	ns	**0.226×10^{-3}**	ns
Model 4 (ΔaTPO)	ns	ns	ns	ns	ns	ns	**-0.060**	ns	ns
Model 5 (ΔaTG)	**12.019**	**1145.08**	ns	**-13.796**	ns	ns	ns	ns	ns
Model 6 (Δpp-Vol)	ns	ns	**-7.943**	ns	ns	ns	ns	ns	ns
Model 7 (Δpl-Vol)	ns	ns	**-7.486**	ns	ns	ns	ns	ns	ns

Δ C16:0 – Palmitic acid ; Δ C20:0 – Oleic acid; Δ C20:4 – Arachidonic acid ; ΔMUFA – Monounsaturated fatty acids; ΔMn – Manganese; Δ % Carb – percentage of energy from carbohydrates; Δ Ret – Retinol; ΔVit C – Antiscorbutic vitamin ; ΔVit D – Antirachitic vitamin

arachidic acid (Δ 20:0) and palmitic acid (Δ 16:0) (Tab. 5 and 6). The model is expressed by the equation:

$$aTG = 5.71 + 1145.08 * \Delta\ 20{:}0 - 13.8 * \Delta\ MUFA + 12.02 * \Delta\ 16{:}0$$

The change in arachidonic acid intake (Δ 20:4) significantly differentiated the change in the volume of the right lobe (pp) examined during thyroid ultrasound (Tab. 5 and 6). Descriptive model of the analysis:

$$\Delta\ pp\text{-}Vol = 0.48 - 7.94 * \Delta\ 20{:}4$$

The change in the intake of fatty acids 20:4, together with the change in the intake of vegetable protein (Δ PP), also contributed to the change in the volume of the left thyroid lobe (pl) (Tab. 5 and 6), according to the equation:

$$\Delta\ pl\text{-}Vol = 0.34 - 7.49 * \Delta\ 20{:}4 + 0.06 * \Delta\ PP$$

DISCUSSION

The lack of knowledge of the specific mechanisms of the pathogenesis of Hashimoto's disease means that there is a need to study the relationship and cause-and-effect relationships between the consumption of individual ingredients with the diet, and the function of the thyroid gland or the immune system. The current analysis broadens the spectrum, and the reported relationships indicate that thyroid function or autoimmunity is affected by many factors whose mechanism of action has not yet been explained [15]. The study shows that there may be relationships between the characteristics of the AIP diet and the function or autoimmunity of the thyroid gland.

After 12 weeks of AIP, the participants' weight and BMI decreased by an average of 4.8% and 5.2%, respectively. In the vast majority, the more than twice as much loss of body weight resulted from the loss of adipose tissue (-9.9%) rather than muscle tissue, which is important for metabolism (4.37%). Similar effects were also observed in people with excessive initial body weight [12], which indicates that beneficial changes in body composition can be expected in both overweight and normal weight people, although this is not always guaranteed by using the AIP [16].

The observed decrease in body weight, despite the theoretical lack of a caloric deficit, allows the conclusion that the participants were in an a state of energy deficiency. Along with a significant increase in protein intake, the AIP intervention alone could suppress the activity of the HPT axis, leading to a decrease in TSH and free thyroid hormones [17]. Subsequently, along with the loss of body fat, the participants probably experienced changes in the concentration of adipokines regulating the HPT axis [18]. This complicates the ability to assess the impact of the nutrients themselves on the parameters under study.

The use of AIP in the subjects reduced the concentrations of TSH, fT3 and fT4 by 28, 13 and 12%, respectively; however, they were within valid ranges of values, which makes it difficult to explain this effect. A far-reaching hypothesis is the possibility that the improved nutritional status of the body increased the sensitivity of tissues to the action of these hormones. Hence, in principle, the feedback mechanism could have muted the pituitary-hypothalamic-thyroid axis. However, it is closer to the truth that it was the energy deficit that acted as a stressor. An interesting observation is the increase in the level of aTPO antibodies by as much as 40%. The concentration of these antibodies is related to the intensity of Hashimoto's disease symptoms [19], as well as lymphocytic infiltration of the thyroid gland even in euthyroidism [20]. According to this, the disease symptoms should have worsened, which was not observed. On the contrary, the number of people with diagnosed symptoms decreased except for one. It is possible that aTPO levels increased as a result of natural fluctuations in autoimmune processes seen in autoimmune diseases.

In the current study, similar to that by Abbot [12], no significant changes were observed in the levels of TSH and free thyroid hormones, which were within the normal reference range from the beginning. It is possible that as a result of the change in diet, there was a reduction in inflammatory processes and lower levels of hs-CRP. However, measuring this factor was not within the scope of the current study. Therefore, it can be assumed that the AIP diet, regardless of the modification method, does not cause any negative effects on thyroid function.

Improvement of health and basic anthropometric parameters are important as a result of diet therapy, but it is equally important to improve the well-being and quality of life of the subjects. This can be felt already during the first few weeks [16] and not only as a result of weight reduction [13]. Similar to Abott et al. (2019), in the current study it was observed that many complaints reported before the use of the AIP diet resolved in a significant number of people after 12 weeks of its use. This can be considered as an argument for the positive effect of the 'tailor-made' AIP diet.

The resolution of the subjectively reported complaints by the participants was surprising in the light of the changes in biochemical parameters. This mainly concerns the aTPO titer and volume of the thyroid gland. Free thyroid hormones also decreased along with TSH, although all remained within normal limits. In addition, the volume of the left and right thyroid lobes decreased. Despite this, participants felt better at the end of the study than before it began. This is consistent with many observations, where obtaining euthyroidism did not improve the quality of life, or symptoms in patients until proper nutrition was taken care of.

The presented analysis shows that only manganese intake affected the reduction of TSH concentration. This may be related to the role of manganese in the functions of the cellular mitochondrion necessary to generate energy useful in the production of thyroid hormones. Manganese is a component of superoxide dismutase that protects the mitochondria against oxidative stress and cell death [21]. It is possible that this mitochondrial support resulted in the better function of thyroid cells, which did not require initial TSH values for their work. It is known from other studies that nutritional support of cellular mitochondria is conducive to Hashimoto's therapy [22].

After AIP diet therapy, the level of fT3 decreased by 13%, still remaining within the normal range. It is difficult to explain these relationships with some nutrients because there is no comparison in the literature, it can only be commented on in the context of iodine intake. Negligible amounts of fT3 are secreted from the thyroid gland, and its blood titre is mainly derived from conversion from T4 in peripheral tissues. It is possible that increased iodine intake affected deiodinase 1 and/or 2 converting T4 to T3. The latter hormone is therefore also an indicator of the activity of deiodinase from which it is formed [23].

It is possible that the above nutrients, directly or indirectly, influenced the activity of deiodinase. However, the decrease in fT3 levels may be related to the concomitant decrease in fT4, as it has been shown to decrease due to a decrease in T4 secretion by the thyroid gland, independently of environmental, dietary factors and physical activity [24]. It seems that the decrease in fT3 is more related to the decrease in the synthesis and secretion of T4 by the thyroid gland than to the limitation of the work of deiodinase 1 and 2. TSH stimulation of the thyroid gland decreased because the level of this hormone itself decreased by as much as 28% from 3.72 to 2.69 mU/L. This probably also resulted in a decrease in the synthesis and secretion of T4, and consequently a decrease in the level of fT4 and fT3 in the blood of the subjects.

Dietary iodine may have played a significant role in limiting thyroid function among the nutrients listed above. Excessive iodine intake can lead to oxidative damage to the thyroid gland, which could explain the observed 40% increase in aTPO titre. Its importance is the greater, the more it is not compensated by selenium intake. Thyroid damage is more influenced by the ratio of iodine to selenium than iodine intake *per se* [25]. Selenium deficiency limits the metabolism of iodine and its effectiveness in Hashimoto's disease [1]. Selenium affects the activity of suppressor lymphocytes by stimulating them, hence limiting the activity of autoreactive T and B lymphocytes. Thus, it reduces the production of antibodies, damage to the thyroid gland itself, and the reduction of thyroid hormone levels [25]. On the other hand, if the selenium intake was excessive, this could also cause a decrease in fT3 [15]. The increase in the consumption of other nutrients suggests that the consumption of selenium had also increased. In addition, iodine may reduce the sensitivity of the thyroid itself to TSH, as well as limit its production capacity [26]. In the current analysis, iodine was found to be associated with fT3 and fT4 levels. Before starting AIP, participants consumed a sufficient amount of iodine per day (118 μg/d), which increased significantly (by 60% to 188 μg/d), and may have been responsible for the reduction in thyroid function and size. One of the limitations of our study is the lack of analysis of selenium intake by the subjects before and after AIP.

The fT4 level was affected by changes in the consumption of long-chain polyunsaturated fatty acids, vitamin C and iodine. No direct explanation was found for this relationship, but it is possible that the fT3, fT4 levels decreased due to the reduction in thyroid volume. It is possible that these effects were indirectly related to a 49% increase in protein energy intake, which affected thyroid function [27]. A relationship was observed between plant protein intake and left thyroid lobe volume which, at this stage of knowledge, cannot be explained.

As a result of the AIP diet, an increase was observed in the value of aTPO, which may result from an increase in iodine intake, which in the case of selenium deficiency, may lead to thyroid damage and an increase in the presentation of its antigens. It is also possible that it was the increased intake of iodine, or perhaps more the insufficient intake of selenium or selenium saturation of the thyroid gland, that caused damage. They are associated with a decrease in the potential of the thyroid gland to produce hormones, lowering their concentration in the blood, an increase in cellular damage patterns, e.g. HMGB1 protein, and an increase in thyroid antigens in the bloodstream and also in the membranes of the thyroid cells themselves. All this stimulates autoimmune and inflammatory processes, which could result in the observed increase in aTPO.

The volume of the right and left thyroid lobes in the subjects decreased by 5 and 6%, respectively, during the study, which suggests that the decrease in secretion of thyroid hormones due to the destruction of the organ may actually be justified.

Intracellular components appearing in the blood that are molecular patterns for pattern recognition receptors (e.g. TLR-4) derived from thyroid cells, may be responsible for the increase in aTPO at the end of the study. A decrease in thyroid volume with an increase in aTPO titre suggests an increase in the titre of inflammatory markers. The increase in aTPO level may have resulted from the disintegration of thyroid cells, which may be suggested by its reduced volume at the 12th week of the study. This suggests guidelines for further research that would allow us to check the possible relationship between the intensity of inflammatory processes, and the activity of deiodinases.

In the current analysis, the concentration of thyroid antibodies aTPO and aTG was related to the intake of retinol and isoleucine, as well as the total monounsaturated fatty acids, arachidic and palmitic acids, respectively. The titer of these immunoglobulins increased by 40 and 5%, respectively, for which there is no explanation. It is assumed that the above-mentioned ingredients had a modulating effect on the cells of the immune system by indirect ways, e.g. on the basis of the intestinal microbiota-immune system axis, or by directly changing the functions of these cells.

The presented analysis not only raises many questions, but also provides important data for further research. For example, if individual amino acids or peptides affect the HPT axis, do fatty acids also modulate it? What is responsible for the observed effect of 20:4 fatty acid intake on the parameters of the thyroid lobes in the ultrasound image?

According to the TSH index, thyroid function seems to have improved, but this is contradicted by the decrease in the level of fT3 and fT4. However, it is known that TSH alone is not an ideal indicator for assessing thyroid function. Therefore, since the patients took significantly more nutrients, are the positive changes in the symptoms felt due to improved nutrition or dietary elimination on which AIP is based? Rationalization of nutrition would probably be enough for many Hashimoto's patients. In a number of studies, beneficial relationships have been observed between nutrition with nutrients and the health of patients. The studies focused on improving nutrition, which gave positive results. Eliminating potentially harmful foods may help with autoimmunity, but it is not possible to assess this based on the current study.

The results of the study indicate that AIP may be effective in reducing disease symptoms in euthyroid patients. Changes in thyroid parameters were within physiological limits, although an increase in the level of aTPO was observed. These parameters are affected by the percentage of individual nutrients in the diet, the ratio of one to another, as well as the amount of energy consumed. Therefore, when analyzing the effects of the AIP diet or comparing them, differences between individual protocols and participants should be taken into account. The impact of the consumption of the analyzed nutrients in the AIP in this study could be different in people with hypothyroidism or obesity. Hashimoto's patients themselves differ in terms of nutrition, hence the effect of AIP may be different in individual clusters. The demonstrated effect of changes in the intake of individual nutrients on the examined parameters, indicate the importance of individual components on the function and volume of the thyroid gland.

Study limitations. The limitations include the small number of participants (n=28), lack of blinding, subjective assessment of disease symptoms, potential bias of researchers when selecting participants, and the bias of participants when answering questions, e.g. about body weight. Future studies should be longer and include more participants. Information on stress management, light management, sleep hygiene, susceptibility to environmental stress, and physical activity would be helpful. To the best knowledge of the authors of this study, with the exception of the work of Krishnamurthy et al. (2021), this is the first to examine in detail the impact of multinutrient intake on thyroid parameters and autoimmunity. It is also one of the few studies where the effect of AIP on Hashimoto's disease was checked. The results of the tested parameters may be different in subsequent studies, taking into account the genotypic variability of humans. Hashimoto's patients differ significantly in terms of nutrition [11]. It would be valuable to know the antioxidant potential of the plasma of the subjects, glutathione levels or oxidative stress indicators. Hashimoto's disease is associated with oxidative stress, hence one of the directions of diet therapy is its reduction.

CONCLUSIONS

This study is the first to explore in detail the effects of the AIP diet on well-being and biochemical indices. The AIP diet during the 12 weeks did not radically reduce the value of key indicators and thyroid volume reduction. However, the AIP diet has many advantages, but it should not be used alone by people who do not have the appropriate knowledge – it is necessary to consult a dietitian and a doctor in order to determine the appropriate composition of the diet and monitor its physiological effects. In most cases it is not harmful, and worth testing and adapting to personal requirements. Given the numerous advantages of the AIP diet, extending the observation time of the diet, along with its personalization in terms of food selection, energy and nutritional value, it could show changes in both well-being and biochemical test results to a greater extent. In addition, the use of a personalized AIP protocol can lead to an improvement in the quality of life, a positive change in mental state, reduction of stress, greater ease in dealing with the daily effects of the diet, and above all, to the improvement of adverse ailments associated with Hashimoto's disease.

Acknowledgements
The study was financially supported by from the resources of the Polish Ministry of Education and Sciences within funds of the Institute of Human Nutrition Sciences, University of Life Sciences (WULS) in Warsaw, for scientific research.

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