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Oral nicotinic acid administration effect on lipids, thyroid hormones, and oxidative stress in intact adult dogs

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Abstract

Background Nicotinic acid (niacin, Vitamin B3) is one of the most effective medicines for improving high-density lipoprotein concentrations. Obesity and related diseases are life-threatening to dogs. This study investigated the niacin effect on triglyceride, cholesterol, lipoproteins, thyroid hormones, oxidative stress, and lipid peroxidation in intact adult dogs. Blood samples were taken from seven healthy, intact adult dogs as a control group (day 0). Then, the animals received 1000 mg/dog of oral nicotinic acid tab daily for 42 days, and blood sampling was performed on days 14, 28, 42, and 56.

Result The results showed an increasing trend in high-density lipoprotein (HDL) concentration. The highest HDL concentration (138.85 ± 43.72 mg/dl) was related to day 56; the HDL level followed a statistically significant increase between day 14 and 56. Unlike HDL, there was a decreasing trend in low-density lipoprotein (LDL) concentration. The lowest LDL concentration (21.85 ± 18.60 mg/dl) was related to day 56. The concentration of apolipoprotein A-I (apoA1) was significantly increased during the study. The highest concentration of apoA1 (1.66 ± 0.06 g/l) was on day 42. There was a significant increase in apoA1 concentrations between days 0 and 14, 42, and 56. The apoA1 was significantly increased between days 14 and 42 and 56. The apoA1 followed a statistically significant increase between days 28 and 42. Changes in thyroid hormone levels did not show any constant increasing or decreasing trend. On day 14, a decreasing trend in the concentrations of TT4, FT4, and T3 was observed. However, an increasing trend was detected in the concentrations of TT4, FT4, and T3 on days 28 and 42. However, the increase in the concentrations of TT4 and FT4 was less than that on day 0. After treatment (day 56), a decreasing trend was observed in thyroid hormone concentrations. The negative correlation was detected between apoA1 and triiodothyronine (T3), total thyroxine T4 (TT4)), and free T4 (FT4) concentrations on day 42. Furthermore, a significant negative relationship was observed between HDL and T4 on day 42. However, the relationship between triglyceride and T3 was statistically positive on day 14. There was an increasing trend in serum total antioxidant capacity (TAC). The highest TAC concentration (3.83 ± 0.62 µmol /l) was on day 56; however, the malondialdehyde (MDA) concentration was decreased during the study. The total antioxidant level followed a statistically significant increase between days 0 and 56 compared to days 14 and 42.

Conclusion The study demonstrated the efficacy of nicotinic acid in improving serum HDL, apoA1, and TAC, as well as decreasing serum MDA and LDL concentrations.

Keywords Apolipoprotein A-1, Cholesterol, Dog, Lipoproteins, Niacin, Thyroid hormones

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Background

Lipid-modifying and antioxidant vitamins have a considerable impact on health. More recently, there has been an emphasis on the provision of nicotinic acid, or Vitamin B3, which can affect glycolysis, gluconeogenesis, oxidative stress, fat metabolism, inflammation, and ischemia [1]. Nicotinic acid and niacinamide are similarly effective as vitamins because they can be converted into each other within the organism. For this reason, the term Vitamin B_3 is used for both. In the last few decades, scientists have been interested in niacin's biological role and its vitamin effect. One potential advantage of nicotinic acid is reducing the risk of cardiovascular events, especially atherosclerosis [2]. This reduction can be attributed to an increase in apolipoprotein A-1 (apoA1) containing high-density lipoprotein (HDL) particles and a decline in very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and triglyceride [3]. These effects may be explained by the fact that niacin activates hydroxycarboxylic acid receptor 2 (a G-protein-coupled receptor in adipocytes) associated with hormone-sensitive lipase activity inhibition and lipolysis reduction. Another possible explanation for increased apoA1 and HDL is that niacin inhibits the surface-expressed ATP-synthase b-chain receptors leading to the HDL reuptake [4]. Furthermore, niacin enhances ATP binding cassette subfamily A member 1 (ABCA1), with a fundamental role in HDL formation and Reverse Cholesterol Transport (RCT) expression from peripheral tissues and cells to the liver [5]. Nicotinic acid may also cause a reduction in thyroxine and triiodothyronine. This effect stems from a reduction in the release of free fatty acids and interferes with the thyroid hormones binding to thyroxine-binding globulin, consequently inhibiting lipolysis by binding to GPR109A. Inhibition of lipolysis can lead to an increase in spontaneous GH secretion. GPR109A has also been shown to play a role in insulin secretion from pancreatic islet cells. Insulin is known to be a critical suppressor of GH release; thus, it is possible that this elevation in GH by niacin is due to its inhibition of insulin. Previous studies have shown that GH administration causes a significant decrease in T4 and a significant increase in T3, along with a marked unequivocal decrease in serum TSH. It is believed that GH-induced enhancement of extrathyroidal T4 to T3 conversion is responsible for this results. Additionally, nicotinic acid may affect serum thyroid hormone levels by mobilizing fatty acids from the periphery, which may interfere with the binding of thyroid hormones to thyroxine-binding globulin [6].

Moreover, niacin has essential impacts on metabolism, of which the most crucial for cell viability is the inhibition of oxidation, especially lipid peroxidation, by acting as a precursor to NAD + and NADP +. The main limitation of oxidative stress control is the decrease in NAD + and NADP + production. NADP + is a vital coenzyme for the glutathione peroxidase enzyme that acts as an antioxidant in various cells [6]. Malondialdehyde (MDA), a genotoxic product produced by lipid peroxidation autolysis, is detrimental to cell viability [7]. Some researchers have established the impact of niacin on MDA levels that contribute to lipid peroxidation and cell integrity [8, 9]. Furthermre, some clinical observations support the evidence that nicotinic acid plays a role in oxidative stress and lipid peroxidation limitations [10–12].

To the authors' knowledge, the effect of nicotinic acid on lipid profiles and oxidative stress has not yet been investigated in dogs. This study assesses the effects of nicotinic acid on triglyceride, cholesterol, lipoproteins, thyroid hormone serum levels, oxidative stress, lipid peroxidation, and biochemical and hematological factors in intact adult dogs.

Results

Serum high-density lipoprotein and apolipoprotein A-I concentration

Figure 1 presents the plasma lipid data at baseline and after using nicotinic acid for 42 days. What stands out in this table is the increase in apoA1 and HDL concentrations. HDL and apoA1 mean levels increased from 57.28 ± 27.84 mg/dl and 1.30 ± 0.05 g/l at baseline to 138.85 ± 43.72 mg/dl and 1.62 ± 0.05 g/l after 56 days of niacin treatment, respectively. Thus, the HDL and apoA1 concentrations increased by 142.40% and 24.61%, respectively during the study,. The increase in HDL concentrations only between days 14 and 56 was significant ($p < 0.05$). Furthermore, the differences between the control (day 0) apoA1 concentration and post-treatment apoA1 concentrations (day 14 ($p = 0.004$), 28 ($p = 0.009$), and 42 ($p = 0$)) were also significant. Moreover, the apoA1 was significantly increased between days 14 vs. 42 ($p = 0.01$) and 56 ($p = 0.03$). The apoA1 level followed a statistically significant increase between days 28 and 42 ($p < 0.001$) (Fig. 1; Table 1).

Serum low-density lipoprotein, triglyceride, and cholesterol

Niacin induced a significant reduction in LDL concentration. LDL levels decreased from 80.28 ± 47.39 mg/dl at baseline to 21.85 ± 18.60 mg/dl after treatment (the concentration decreased by 72.78%). A significant difference was found between LDL concentration on days 0 and 56 ($p < 0.05$). No evidence was found for changes in triglyceride levels; however, data revealed increased cholesterol serum concentrations during the study. There was no significant difference in triglyceride and cholesterol concentrations between days 0, 14, 28, 42 and 56 ($p > 0.05$; see Fig. 1; Table 1).

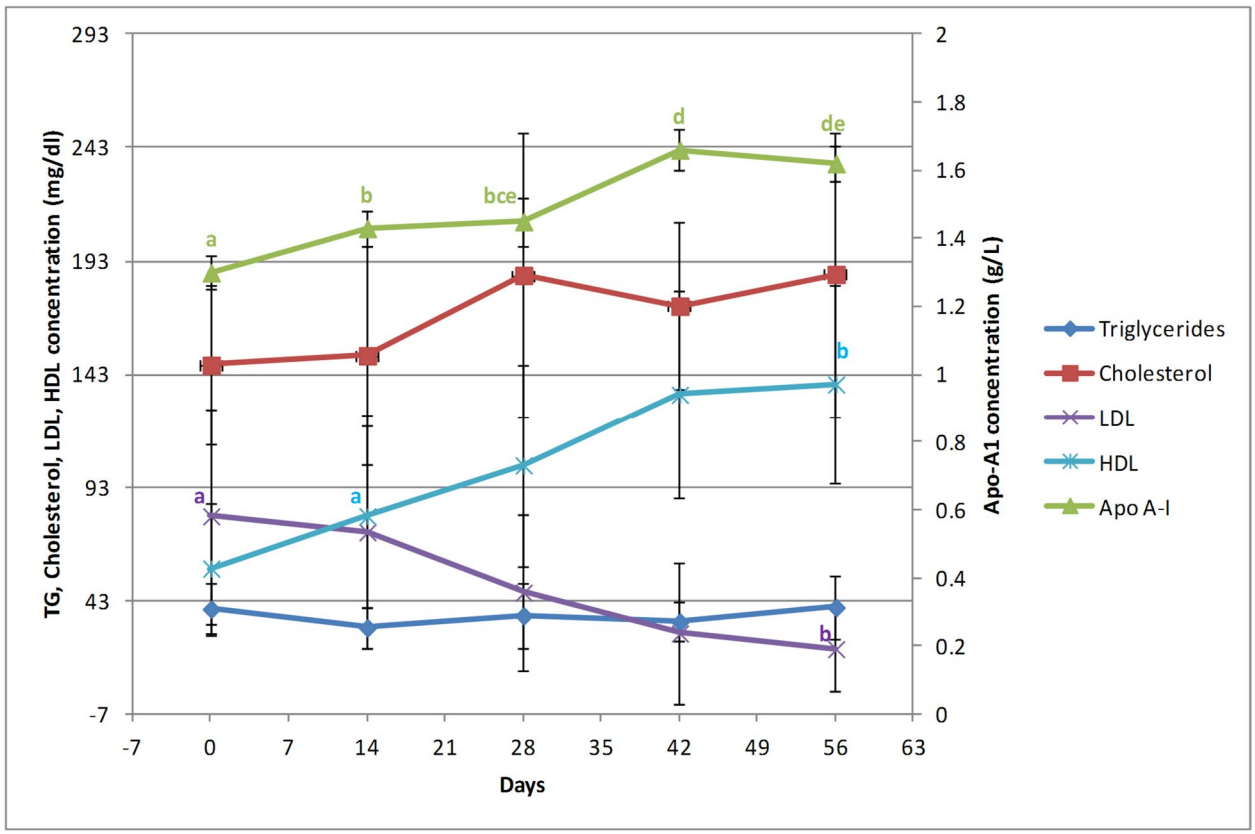

Fig. 1 Changes (mean ± SD) and comparison of serum lipid factors due to niacin administration (1000 mg/dog/day) in intact adult dogs between different days of sampling were analyzed and compared among and between groups using repeated measures ANOVA and Tukeys multiple comparison tests. Day 0 represents the day before treatment. HDL = high-density lipoprotein, LDL = low-density lipoprotein, apo A-1 = apolipoprotein A-1; [abc] Different superscript letters indicate significant differences between days of sampling

Table 1 Changes (mean ± SD) and comparison of lipid, thyroid and oxidative status factors before (Day 0), during treatment (Days 14, 28, and 42) and post treatment (Day 56) in intact male dogs ($n = 7$) treated with niacin (1000 mg/dog/day)

Days	0	14	28	42	56	Observed power[a]
Triglycerides	39.42 ± 11.51	31.21 ± 9.26	36.5 ± 14.24	33.78 ± 8.78	40.14 ± 14.14	0.927
Cholesterol	147.28 ± 35.16	151.5 ± 48.03	186.85 ± 62.78	173.28 ± 37	187.28 ± 62.58	0.275
Apo A-1	1.3 ± 0.05[a]	1.43 ± 0.05[b]	1.45 ± 0.07[bce]	1.66 ± 0.06[d]	1.62 ± 0.05[de]	1
LDL	80.28 ± 47.39[a]	73.28 ± 51.5	46.71 ± 34.34	28.71 ± 31.25	21.85 ± 18.6[b]	0.761
HDL	57.28 ± 28.74	80.28 ± 40.15[a]	102.71 ± 44.33	134.28 ± 45.79	138.85 ± 43.72[b]	0.935
FT4	1.8 ± 1.55	0.44 ± 0.63	1.54 ± 1.41	2.11 ± 1.57	1.86 ± 1.36	0.44
T3	6.23 ± 3.71	4.14 ± 1.75[a]	8.6 ± 2.57[b]	8.49 ± 4.26	4.4 ± 1.91	0.805
T4	6.56 ± 4.02	3.79 ± 2.01	6.16 ± 3.46	6.92 ± 3.05	5.23 ± 4.18	0.259
MDA	0.14 ± 0.07	0.1 ± 0.04	0.08 ± 0.03	0.08 ± 0.02	0.07 ± 0.02	0.720
TAC	2.28 ± 0.49[a]	2.24 ± 0.51[bc]	3.19 ± 0.24[abcd]	6.64 ± 1.74[b]	3.83 ± 0.62[d]	0.277

T3 Triiodothyronine, *TT4* Total Thyroxine, *FT4* Free Thyroxine, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *apoA1* apolipoprotein A-1, *MDA* malondialdehyde, *TAC* total antioxidant capacity

[a] Observed power computed using alpha = 0.05

[abcde] Different superscript letters indicated significant difference between days of the study in each row

Changes in thyroid hormones

While significant differences were noted in most lipid profiles determined at baseline and after 56 days, the treatment did not affect thyroid hormones. A significant difference was found in T3 concentration between day14 and 28 ($p < 0.05$) (Fig. 2). However, there were no significant difference in other thyroid hormones concentrations (FT4 and T4) between 0, 14, 28, and 42 days (Fig. 2). Changes in thyroid hormone levels did not show any constant increasing or decreasing trend. On day 14, a decreasing trend in the concentrations of TT4, FT4, and T3 was observed. However, an increasing trend was detected in the concentrations of TT4, FT4, and T3 on Days 28 and 42, although the increase in the concentrations of TT4 and FT4 was less than that on day 0. After treatment (day 56), a decreasing trend was observed in thyroid hormone concentrations (Table 1).

The Pearson correlation

The Pearson correlation coefficients between triglycerides, cholesterol, LDL, HDL, apolipoprotein, and thyroid hormones during the study are given in Table 2. A significant positive correlation was detected between apolipoprotein A and T3, T4, and FT4 concentrations on day 42. Additionally, a significant negative relationship was observed between HDL and T4 on day 42, and a statistically positive relationship between triglyceride and T3 was detected on day 14. No statistically significant correlations were observed between phospholipids, triglycerides, cholesterol, apolipoprotein, triiodothyronine, and total- and free-thyroxine on days 0, 28, and 56 (Table 2).

Changes in total antioxidant levels

The increase in serum total antioxidant levels is presented in Fig. 3. Nicotinic acid induced a significant improvement in TAC levels compared to baseline. TAC mean levels increased from 2.28 ± 0.49 µmol/l at baseline to 6.64 ± 1.74 µmol/l after 42 days of niacin treatment, resulting in an increase of 67.98%. The results obtained from the statistical analysis of TAC concentrations revealed significant increases between day 0 vs. 14 ($p = 0.02$) and day 42 ($p = 0.04$). Moreover, the total antioxidant level followed a statistically significant increase between day 56 and both days 14 and 42 (Fig. 3; Table 1).

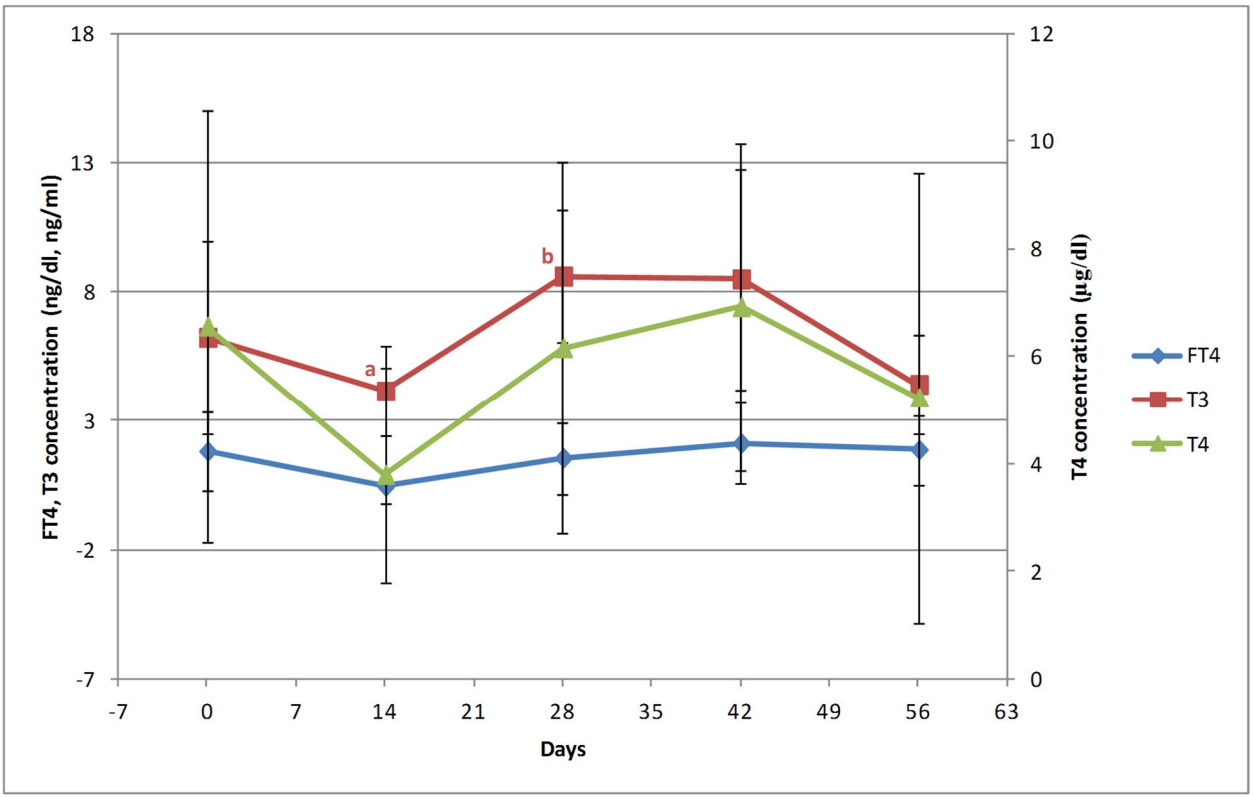

Fig. 2 Changes (mean ± SD) and comparison of thyroid hormones due to niacin administration (1000 mg/dog/day) in intact adult dogs were analyzed over 42 days. Day 0 was the day before treatment. Data were analyzed and compared among and between groups using repeated measures ANOVA and Tukeys multiple comparison tests. T3 = Triiodothyronine, TT4 = Total Thyroxine, FT4 = Free Thyroxine; [abc] Different superscript letters indicate significant differences between days of sampling

Table 2 The correlation coefficient was calculated using the Pearson correlation coefficients test between blood lipids and thyroid hormones of niacin-treated dogs on different days of sampling

Factors		Triglyceride	Cholesterol	LDL	HDL	apoA1
Day 0	**T3**	0.373	0.259	−0.247	−0.426	0.314
	T4	0.490	0.522	−0.100	−0.199	0.345
	FT4	−0.328	−0.082	−0.372	−0.376	0.193
Day 14	**T3**	0.955***	0.649	0.089	0.535	0.385
	T4	0.586	0.216	0.215	0.398	0.292
	FT4	−0.240	−0.207	0.477	−0.268	−0.00
Day 28	**T3**	0.321	0.182	−0.016	0.207	−0.346
	T4	−0.131	−0.021	−0.157	−0.221	−0.551
	FT4	−0.376	−0.137	−0.448	−0.197	−0.610
Day 42	**T3**	0.208	−0.053	−0.338	−0.451	0.857*
	T4	0.395	−0.325	−0.664	−0.818*	−0.835*
	FT4	0.502	0.070	−0.144	−0.431	−0.835*
Day 56	**T3**	−0.390	−0.346	−0.024	−0.307	−0.281
	T4	−0.163	0.199	−0.055	−0.345	0.057
	FT4	−0.164	−0.134	−0.372	−0.416	−0.00

T3 Triiodothyronine, *TT4* Total Thyroxine, *FT4* Free Thyroxine, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *apoA1* apolipoprotein A-I

* At the level of $P < 0.05$ is significant

*** At the level of $P < 0.01$ is significant

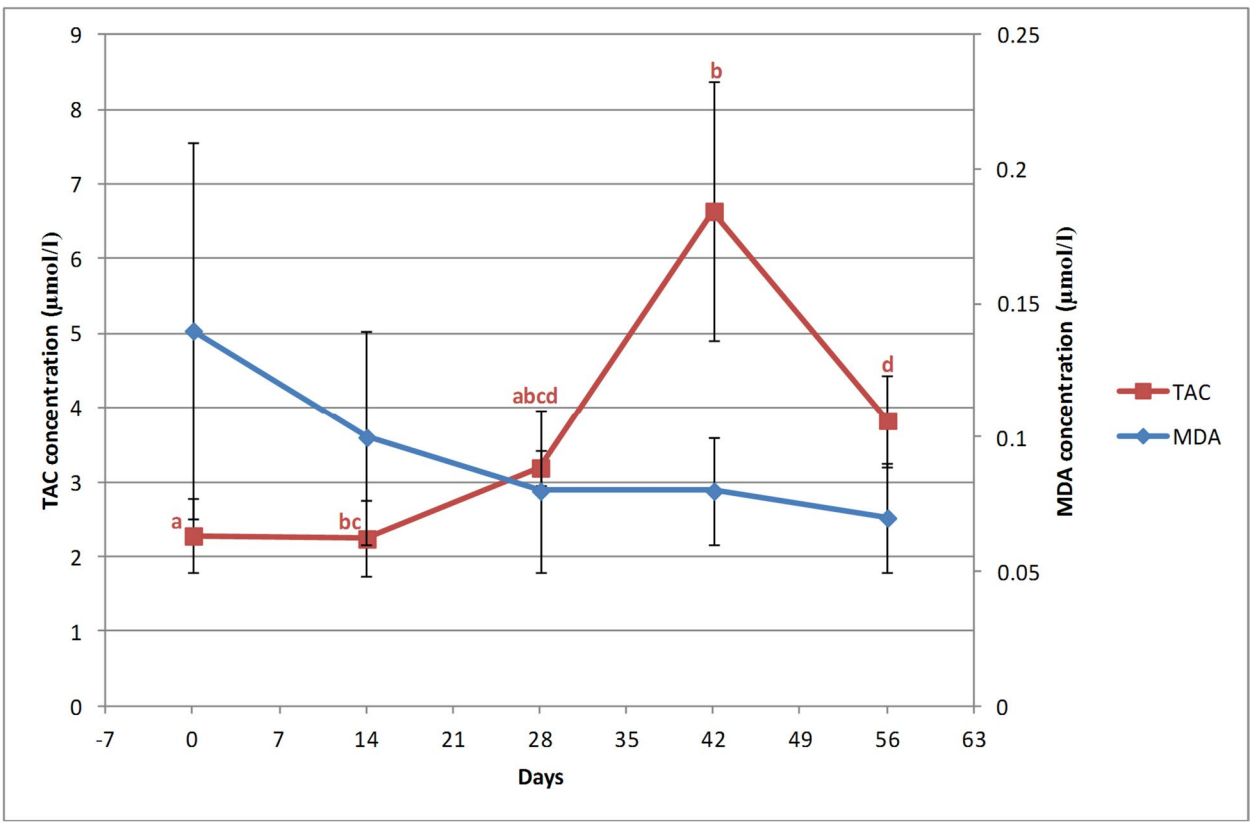

Fig. 3 Changes and comparison of serum TAC and MDA levels (mean ± SD) due to niacin consumption (42 days) in intact adult dogs before (Day 0) and after oral administration of niacin (1000 mg/dog/day) were observed. TAC: total antioxidant capacity; MDA: malondialdehyde; [abc] Different superscript letters indicate significant differences between days of sampling

Changes in malondialdehyde

The decrease in plasma MDA levels can be observed in Fig. 3. The MDA levels decreased from 0.14 ± 0.07 µmol/l at baseline to 0.07 ± 0.02 µmol/l after 42 days of nicotinic acid treatment, represnting a decrease of 50%. No significant difference was observed in MDA levels on different days of the study ($p > 0.05$) (Fig. 3; Table 1).

Biochemistry and hematology profile

No adverse effects and no significant differences in hematology factors, hepatic enzymes, BUN, creatinine, total protein, and glucose levels were detected in all dogs after niacin therapy, compared with baseline data (Table 3).

Table 3 Changes and comparison of biochemical and hematologic factors (mean ± SD) between the day before (Day 0) and after 56 days of treatment with niacin (1000 mg/dog/day) in intact adult dogs

Factors	Days	
	Control (day 0)	Post treatment (day 56)
Glucose (mg/dl)	91.31 ± 5	90.58 ± 4.97
Urea (mg/dl)	32.71 ± 3.77	32.14 ± 3.71
Creatinine (mg/dl)	0.76 ± 0.13	0.79 ± 0.13
AST (U/L)	22.28 ± 3.25	23.85 ± 5.36
ALT (U/L)	49.57 ± 5.88	50 ± 5.62
ALP (U/L	108.57 ± 10.75	109.14 ± 15.71
Ca (mg/dl)	10.01 ± 0.68	10.07 ± 0.84
P (mg/dl)	4.62 ± 0.40	4.71 ± 0.62
Total protein (g/dl)	5.85 ± 0.29	5.78 ± 0.49
Albumin (g/dl)	3.01 ± 0.50	3.14 ± 0.45
RBC (10^6/µl)	6.60 ± 0.555770	6.26 ± 0.727264
WBC (10^3/µl)	7415.98 ± 4161.97	7997.14 ± 2028.64
Platelet	182.07 ± 56.43	174.85 ± 24.71
Neutrophil (/µl)	6262.71 ± 2634.52	4792.14 ± 1775.22
Eosinophil (/µl)	1252.00 ± 912.39	1621.22 ± 955.12
Monocyte (/µl)	261.00 ± 180.47	522.94 ± 267.84
Lymphocyte (/µl)	1424.42 ± 898.09	788.571 ± 596.19
MCV	62.20 ± 2.23	61.95 ± 1.84
MCHC (g/dl)	33.71 ± 0.93	33.82 ± 0.87
MCH	20.90 ± 0.69	20.94 ± 0.64
HCT (%)	41.04 ± 3.51	38.75 ± 4.16
Band (/µl)	64.71 ± 94.26	272.25 ± 229.15
Hb (g/dl)	13.81 ± 0.89	13.08 ± 1.22

AST Aspatate transaminase, *ALT* Alanine transaminase, *ALP* Alkaline phosphatase, *Ca* Calcium, *P* Phosphorus, *RBC* Red blood cell, *WBC* white blood cell, *MCV* Mean corpuscular volume, *MCHC* Mean corpuscular hemoglobin concentration, *MCH* Mean corpuscular hemoglobin, *HCT* hematocrit, *Hb* hemoglobine

Discussion

Niacin's preventive effect on atherosclerosis and its progressive impact on HDL and apoA1 levels have recently received a great deal of attention [12]. Nicotinic acid has been used to improve human cardiovascular events [3, 13, 14], and it is commonly assumed that it affects cardiovascular conservation in animal species. The most prominent conclusion of our study supporting this hypothesis is that oral nicotinic acid administration (1000 mg/dog) for 42 days markedly improves HDL and apoA1 concentrations and decreases LDL levels in dogs. The second major conclusion is that niacin administration causes increased TAC concentration and decreased MDA in serum. In the present study, clinically relevant dosages of nicotinic acid were well tolerated by the animals, and no negative effects or hematologic abnormalities were observed during the niacin administration. The influential effects of niacin on lipid metabolism are attributed to the acceleration of the protein ABCA1 expression as an apoA1 regulator and stabilizer, diminishment of HDL uptake in the liver, and inhibition of triglyceride lipolysis in adipocytes, leading to a reduction of VLDL and LDL particles [13–18]. Moreover, the antioxidant effects of niacin have been attributed to its role as a precursor to NAD + and $NADP^+$ [19–21].

The infuential effect of nicotinic acid on thyroid hormones stems from the inhibition of lipolysis, which leads to increased spontaneous GH secretion. Growth Hormone plays a role in a significant decrease in T4 and a significant increase in T3 along with a marked unequivocal decrease in serum TSH [22]. Additionally, nicotinic acid may affect serum thyroid hormone levels by mobilizing fatty acids from the periphery, which may subsequently interfere with the binding of thyroid hormones to thyroxine-binding globulin. Previous studies have confirmed that niacin administration can cause hypothyroidism. A one year clinical trial showed that nicotinic acid leads to significantly reduced TT4,FT4,T3, and TBG levels [4]. The concentration of FT4 fluctuates within the normal range, as reported using a canine FT4 ELISA kit before. The fluctuation of FT4 concentration depends on the age, breed, size of animals, and time of sampling [23, 24]. In our study, the age, breed, size of animals and time of sampling (hour in a day) were matched and consistent. The observed power for T3 changes was 0.8 during the 42-day treatment period of our study.

Many previous studies in laboratory animal models broadly support the beneficial impact of niacin on lipid profiles. Yang et al. [25] confirmed that administratering of niacin (200 mg/kg/day) for six weeks could reduce serum leptin levels, an adipocyte-derived hormone, in rabbits fed with a cholesterol-rich diet. Lamon-Fava et al. [26] showed that oral nicotinic acid administration (2000 mg/day) improved HDL and decreased LDL levels in hyper dyslipidemic men. Rasti et al. [3] prescribed niacin to a patient with atherosclerosis, and found that after the niacin treatment, HDL and apolipoprotein increased

significantly, whereas LDL, VLDL, and cholesterol decreased. In contrast to Rasti et al. (2020), however, no evidence of cholesterol decrease was detected in the current research [3]. The observed power for HDL and TG changes was 0.935 and 0.927, respectively, during the 42-day treatment period of our study.

Hamoud et al. [27] investigated the impact of oral nicotinic acid (niacin) on hypercholesterolemia patients for three months. They received oral niacin (1000 mg/day, 2000 mg/day, and 3000 mg/day) in the first, second, and third months, respectively. Niacin could increase HDL-C and apoA1 levels significantly and reduce oxidative stress [27]. Another research on niacin in alcoholic pellagra patients for 27 days showed this drug to increase serum glutathione peroxidase concentrations and decrease serum levels of MDA; this finding broadly supports the present research results [11]. On the other hand, other studies have demonstrated the cholesterol-lowering, anti-inflammatory, and antioxidant effects of statins on patients. The most obvious difference between statins and niacin is the latter's impact on boosting HDL and apoA1 concentrations. However, concomitant use of simvastatin with niacin in people with cardiovascular disease and low HDL levels has been shown to have major clinical benefits [28, 29].

The direct effects of niacin and the mechanisms affecting lipid, thyroid and oxidative stress status following nicotinic acid administration were summarized in Fig. 4. The present study is an empirical investigation into the impact of nicotinic acid on canine lipid profiles, oxidative stress, thyroid hormone, and lipid peroxidation in serum. However, the results and conclusions of this experimental study are limited by the small number of dogs and the short period devoted to monitoring. It is suggested to perform the study as a case–control experimental study.

Conclusion

The present study demonstrated the efficacy of oral administration of nicotinic acid for 42 days in improving serum HDL, apoA1, and TAC levels. Furthermore, nicotinic acid was found to effectively decrease serum MDA and LDL concentrations in adult intact dogs.

Material & methods

Animal rights statement

The present study was performed under the supervision of the Iranian Society for the Prevention of Cruelty to Animals and the Research Council of Shiraz University (IACUC No. 6387/63). The experimental protocols were conducted in accordance with the Iranian Animal Ethics Framework.

Animals

Seven adult intact mixed breed dogs weighing 20 ± 2 kg and 2 ± 1 years old were selected, and kept by the Veterinary Medicine School at Shiraz University. The dogs were fed 300 g of commercial dog food per day

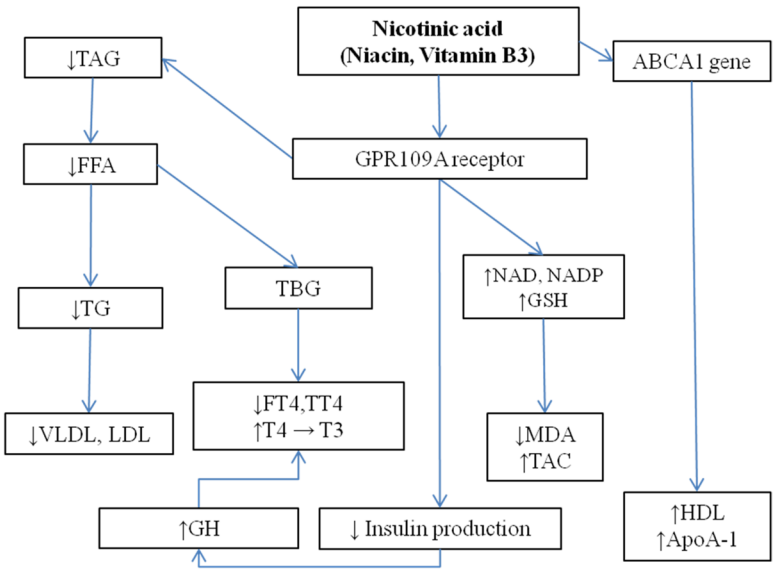

Fig. 4 A schematic diagram indicated the direct effects of niacin and the mechanisms affecting lipid, thyroid and oxidative stress status following nicotinic acid administration. Low-density lipoprotein (LDL); High-density lipoprotein (HDL); apolipoprotein A-1 (apoA1); Triiodothyronine (T3); Free Thyroxine (FT4); Total Thyroxine (TT4); Growth hormone(GH); total antioxidant capacity (TAC); malondialdehyde (MDA); ATP Binding Cassette Subfamily A Member 1 (ABCA1); G Protein-Coupled Receptor 109A (GPR109A receptor); Thyroxine-binding globulin (TBG); Triacyl glycerol (TAG); Free fatty acid (FFA); Triglycerides (TG); Growth Hormone (GH)

(Nutri® dry dog food; Behintash Co. Iran), with free access to water. The food analysis revealed at least 21% crude protein, at least 9% crude fat, a maximum of 3% crude fiber, a maximum of 0.8% salt and 3000 kcal/kg. The dogs were adapted to the new conditions for two weeks. Antiparasitic treatment was carried out for the dogs using mebendazole (22mg/kg; Parazol®, ZagrosPharmed, Iran) and praziquantel (10 mg/kg; Lorensit®, ZagrosPharmed, Iran). All the dogs were neutered at the end of the study and kept in a non-governmental organization shelter for adoption.

Study design

This study was designed as an experimental cohort study to reduce the impact of individual variability on the treatment results. Day 0 was considered as a control group before oral administration of nicotinic acid for 42 days. Blood samples were collected on day 0 (as a control group), 14, 28, 42 (treatment), and 56 (post-treatment). Blood sampling was performed at 10 a.m. prior to feeding and oral administration of niacin every day. They were collected from the jugular vein into simple glasses and EDTA tubes and centrifuged at 3000 rpm for 10 min; then, the serum samples were kept at −20°C. Dogs received 50 mg/kg (1000 mg/dog) of an oral nicotinic acid tab (Letrofem®, Iran Hormone, Iran) daily for 42 days. The canine dose of the nicotinic acid was calculated according to the human dose and the equation below: [Canine allometric coefficient (1.8) × human dose (mg/kg)] [15].

Laboratory measurements

All laboratory measurement methods and commercial kits were validated for serum samples of dogs in the special clinical pathology laboratory of the School of Veterinary Medicine at Shiraz University. Validity and repeatability of measurements had been confirmed in previous published studies.

Assay for cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL)

A commercial kit (Pars Azmoon Co., Tehran, Iran) was used to measure total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Further, a biochemical autoanalyzer (Alpha Classic AT + +, Sanjesh Company, Iran) was used to analyze them.

Assay for apolipoprotein A-1 (ApoA1)

A quantitative sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure serum apolipoprotein A-I (apoA1) using a commercial dog-specific competitive ELISA kit (CUSABIO, Shanghai, China; Code CSB-EL001913DO). The kit sensitivity was 7 ng/ml, with a detection limit of 28.12–1800 ng/ml. The intra- and inter-assay coefficient of variation (CVs) of the apolipoprotein A-1 kit were < 8% and < 10%, respectively.

Assay for thyroid hormones

A solid-phase sandwich enzyme-linked immunosorbent assay (ELISA kit; Dia Zist, Tehran, Iran) was used to measure serum T3 and T4. The sensitivities of the T3 and T4 kits were 0.1 ng/ml and 0.3 µg/dl, respectively. The intra- and inter-assay CVs of the T3 and T4 kits were < 5% and < 10%, respectively. Additionally, a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA kit; Monobind Inc., USA) was used to measure serum fT4. The sensitivity of the fT4 kit was 0.314 ng/dl. The intra- and inter-assay CVs of the fT4 kit were < 6% and < 15%, respectively [30].

Assay for total antioxidant capacity (TAC) and malondialdehyde (MDA)

In order to determine the TAC level, a commercial kit (ZellBio GmbH, Germany) was utilized. At the end of the process, the chromogenic substrate color product (tetramethyl benzidine) developed. The color difference was measured calorimetrically using a spectrophotometer (Jenway 6300 Spectrophotometer, UK) at 450 nm as mmol/L. With a sensitivity of 0.1 mM (100 µmol/L), TAC can be determined by this method. The intra- and inter-assay CVs were lower than 3.4% and 4.2%, respectively. A kit provided by ZellBio GmbH (Germany) was employed to measure MDA (µmol/L; Cat. no. ZB-MDA96A) according to its reaction with thiobarbituric acid at high temperature in an acidic condition. The color complex was calorimetrically measured at 535 nm. The sensitivity of the assay kit was 0.1 µM (inter-assay CV: 5.8%) for MDA [31].

A veterinary hematology counter (Nihon Kohden, MEK-6450 Celltac Alpha, Tokyo, Japan) was used to determine hematological parameters, while a commercial kit (Pars Azmoon Co., Tehran, Iran) was used to measure serum biochemical parameters, which were analyzed using a biochemical autoanalyzer (Alpha Classic AT + +, Sanjesh Company, Iran) [32].

Statistical analysis

The data were entered into SPSS version 26, and the mean and standard deviation (SD) of the quantitative variables were reported. Normal distribution of

the data was assessed and confirmed using the Shapiro-Wilk test. A one-way repeated measures ANOVA test was used to compare data among different sampling days, and Tukey's multiple comparison tests was employed to compare the data between sampling days. Observed power was calculated for a 42-day treatment period during the study. A two-tailed P value < 0.05 was considered significant.

Abbreviations

ELISA	Enzyme-linked immunosorbent assay
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
apoA1	Apolipoprotein A-I
T3	Triiodothyronine
FT4	Free Thyroxine
TT4	Total Thyroxine
GH	Growth hormone
TAC	Total antioxidant capacity
MDA	Malondialdehyde
RM ANOVA	Repeated measure analysis of variance

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Not applicable.

Authors' contributions
All authors have seen and approved the final version of the manuscript being submitted. All authors contributed in all parts of study from designing study to writing and preparing manuscript. AM, SN, ND, MRD, ZD, MSK, and MZA contributed in study design, performing study, sampling, laboratory metabolites analysis, data collection and analysis and preparing manuscript. All authors reviewed the manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was reported in accordance with ARRIVE guidelines, and approved by the Iranian Laboratory Animal Ethics Framework under the supervision of the Iranian Society for the Prevention of Cruelty to Animals and Shiraz University Research Council (IACUC no. 4687/63). A written informed consent was obtained from the shelter, and the recommendations of the European Council Directive (2010/63/EU) of September 22, 2010, regarding the standards in the protection of animals used for experimental purposes, were also followed.

Consent for publication
NA.

Competing interests
The authors declare no competing interests.

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