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Lipid profiles of rats fed with diets supplemented with vitamins niacin and pyridoxine

Magna da Glória Lameiro[1]; Elizabete Helbig[2,*]; Elessandra da Rosa Zavareze[1]; Fernanda Aline de Moura[1]; Rafael Aldrighi Tavares[3]; Carolina Galarza Vargas[2]; Lúcia Rota Borges[1]; Moacir Cardoso Elias[1]; Alvaro Renato Guerra Dias[1]

[1]Department of Agroindustrial Science and Technology, Federal University of Pelotas, 96010-900, Pelotas, Brazil.
[2]Department of Nutrition, Federal University of Pelotas, 96010-900, Pelotas, Brazil.
[3]Faculty of Veterinary Medicine, Federal University of Pelotas, 96010-900, Pelotas, Brazil.

ABSTRACT

Hypercholesterolemia is a major risk factor for cardiovascular disease. Supplements containing the vitamins niacin (B_3) and pyridoxine (B_6) can promote the reduction of total cholesterol and an increase in HDL cholesterol. In this study, the effects of diets supplemented with niacin (B_3) and pyridoxine (B_6) on the hepatic and serum lipid profiles of Wistar rats were assessed. The diets were prepared with combinations of three concentrations of niacin (3, 4 and 5 g/kg) and pyridoxine (6, 12 and 18 mg/kg) and one with neither vitamin. The animals were divided into eleven experimental groups of six animals per group, and nine groups were fed on a standard diet with 7.5% fat and vitamin supplementation. Another group was fed with 7.5% fat without vitamin supplements. A control group received the standard diet (AIN-93M) without modifications (4% fat). The weight gain, food intake, serum and hepatic total cholesterol, serum cholesterol fractions (HDL, LDL, and VLDL), serum and hepatic triacylglycerols and hepatic and fecal lipid contents were measured after 30 days. The diet with the highest concentration of niacin and lowest concentration of pyridoxine had the lowest level of total hepatic cholesterol. Hepatic triacylglycerols were reduced by the highest concentration of niacin (5 g/kg), and this reduction was enhanced by increasing the pyridoxine concentration. The diets supplemented with niacin and pyridoxine reduced the levels of serum total cholesterol, LDL, VLDL, triacylglycerols and hepatic lipids. These effects on the lipid profile varied with the concentrations of the two vitamins and the interactions between them.
Keywords: Cholesterol. HDL. LDL. Niacin. Pyridoxine. Vitamins.

INTRODUCTION

Increased amounts of fat in human and rat diets have been associated with the risk of obesity and hyperlipidemia and with corresponding changes in cholesterol and triacylglycerol levels (Woo *et al.,* 2009). Lipids, such as triacylglycerol, cholesterol and their esters and phospholipids, are hydrophobic molecules insoluble in water. Such molecules cannot move freely in the bloodstream and thus are carried in particles called lipoproteins (Burnett & Hooper, 2008), in which lipid molecules are combined with proteins. Lipoproteins are divided into three classes and differentiated by size, density and the chemical composition of the lipids and apoproteins. The three classes of lipoproteins are the following: very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). LDL is the main carrier of cholesterol to the cells and is associated with the initiation and acceleration of atherosclerosis (Ganji *et al.,* 2003). HDL cholesterol is important because it participates in reverse cholesterol transport and is considered antiatherogenic. Serum lipid levels have been correlated with cardiovascular diseases. Raised plasma levels of LDL cholesterol and triglycerides are risk factors for the onset of cardiovascular diseases. Additionally, high HDL cholesterol levels are considered protective factors (Friedman & Brandon, 2001).

Nicotinic acid or niacin is a water-soluble vitamin (B_3) for which the targets are not fully known. There is evidence that this vitamin acts on specific receptors and decreases the release of fatty acids from adipose tissue (Karpe & Frayn, 2004), thus reducing the esterification of triglycerides in the liver and increasing the activity of lipoprotein lipase. Niacin has been used for the treatment of lipid disorders and cardiovascular diseases (Kamanna & Kashyap, 2008). In pharmacological doses (1-3 g/day), niacin increases levels of HDL and reduces the concentrations of total plasma cholesterol, triglycerides, VLDL, LDL and lipoprotein(a). Niacin has been noted as an excellent lipid-regulating agent that increases HDL levels (Ganji *et al.,* 2003; McKenney, 2004; Tuohimaa & Järvilehto, 2010). The main limitation of niacin is its potential for release, which can cause adverse hepatotoxicity; intermediate-release forms of niacin may have minor effects, but the rapid release of niacin can have serious effects, such as flushing. According to McKenney (2004), the use of niacin has been limited mainly by dose-dependent adverse effects, including flushing, pruritus, rashes, nausea, dyspepsia, abdominal pain and diarrhea. According to Dunbar & Gelfand (2010), niacin can cause

Autor correspondente: Elizabete Helbig - Department of Nutrition - Federal University of Pelotas - P.O. Box 96010-900 - Pelotas - Brazil - tel.: +55-53-32757258 - fax: +55-53-32757258 - E-mail address: helbignt@gmail.com

vasodilation, which is manifested as redness on the head and neck, providing a visible signal associated with other skin disorders. However, these effects can be significantly reduced by using a limited amount of this vitamin.

Pyridoxine is a water-soluble vitamin (B_6) and is essential for the formation and metabolism of tryptophan and its transformation into nicotinic acid. A deficiency of vitamin B_6 reduces the production and activity of neurotransmitters (especially acetylcholine and noradrenaline) and may cause effects such as depression, insomnia, irritability and anxiety. The total bioavailability of vitamin B_6 is lower in plant foods than in animal foods and fortified foods, which may be due to the presence of considerable amounts of β-glycosidic forms of pyridoxine in most plant products (Kall, 2003).

There has been extensive research in the field of nutrition science on the consumption of functional foods that reduce the risk of cardiovascular disease without the use of drugs. Hypercholesterolemia is a major risk factor for cardiovascular disease. Studies have shown that supplements of niacin and pyridoxine can promote the reduction of serum total cholesterol (TC), while increasing HDL cholesterol (Yang *et al.,* 2008; Ganji *et al.,* 2003; Ashen & Blumenthal, 2005). However, the action of these vitamins has not been extensively studied, and their effects on the lipid profile of rats can vary, depending on their concentration and their interactions when used in combination. The aim of this study was to investigate the effects on the hepatic and serum lipid profiles (serum TC, HDL, LDL, VLDL, triacylglycerols, and hepatic and fecal lipids) of Wistar rats fed on a high-fat diet supplemented with wheat flour and the vitamins niacin and pyridoxine.

MATERIALS AND METHODS

Materials

Wheat flour lacking folic acid and iron was supplied directly by the millers, Moinhos do Sul (Rio Grande, Brazil), who produced it specially for this experiment. The vitamins niacin (B_3) (C: N3376) and pyridoxine (B_6) (C: P5669) that were used to supplement the wheat flour were obtained from Sigma.

Experimental diets

The experimental diets were prepared as determined for the standard diet AIN-93M, described by Reeves *et al*. (1993), and supplemented with the vitamins niacin (at 3, 4 or 5 g/kg) and pyridoxine (at 6, 12 or 18 mg/kg). These concentrations of niacin and pyridoxine were based on the amounts tested in other studies (Basu & Mann, 1997) and on preliminary tests. The sources and quantity of lipids and protein source were modified from 50% wheat flour and 50% casein. The following components were added to each diet: 35 g soybean oil, 15 g hydrogenated fat and 20 g pork fat per kg diet (Table 1). The prepared diets were packed and kept frozen (-20ºC); 2 kg of each diet was prepared every week for each group.

Table 1. Experimental diets used in the biological assay with adult male Wistar rats.

Components (g)	Treatments									
	1	2	3	4	5	6	7	8	9	10
Casein [a]	73.8	73.8	73.8	73.8	73.8	73.8	73.8	73.8	73.8	73.8
Maltodextrin [a]	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
Sucrose [a]	93.0	90.0	90.0	90.0	89.0	89.0	89.0	88.0	88.0	88.0
Soybean oil	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Hydrogenated fat	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Pork fat	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Wheat fiber [a]	77.3	77.3	77.3	77.3	77.3	77.3	77.3	77.3	77.3	77.3
Mineral mix [a]	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Vitamin mix [a]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Niacin	0.0	3.0	3.0	3.0	4.0	4.0	4.0	5.0	5.0	5.0
Pyridoxine	0.000	0.006	0.012	0.018	0.006	0.012	0.018	0.006	0.012	0.018
L-cysteine [a]	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Choline [a]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
TBHQ [a]	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Wheat flour	631.0	631.0	631.0	631.0	631.0	631.0	631.0	631.0	631.0	631.0

[a] According to the AIN-93M formulation (g/kg). TBHQ (tetra-butyl hydroquinone), choline (choline bitartrate).

Experimental model

Male Wistar rats were housed at 21 ± 1°C with a 12-hour light/dark cycle at 50 to 60% relative humidity. The animals, of mean body weight 168 g, were randomly divided into 11 experimental groups of 6 animals per group. Nine groups received the diet with 7.5% fat and supplemented with vitamins B_3 and B_6. One group received the diet with 7.5% lipids without vitamin supplementation and another group received the standard diet (AIN-93M) without modification (4% fat) as a control for the experiment. The animals were kept in metabolic cages and had free access to food and water. After 30 days of treatment with the

above diets, the rats were anesthetized with ethyl ether and cardiac puncture was performed. Subsequently, euthanasia was performed by increasing the anesthetic to a lethal dose. The blood (1000*g*) was centrifuged for 15 minutes to obtain serum. The serum was stored at -20°C until analyzed for TC, HDL cholesterol and triglycerides (TG). The weight gain of the animals, taken from their weight at the beginning and end of the experiment, and their food intake were recorded. The experiment was approved by the Ethics Commission on Animal Experiments, Federal University of Pelotas, RS, Brazil.

Biochemical analyses

The serum and hepatic TC and TG were determined by the method proposed by Haug & Hostmark (1987), which employs an enzymatic system (cholesterol esterase, cholesterol oxidase and peroxidase; Labtest Diagnostic® cholesterol liquiform cat. 76-2/100). High-density lipoprotein cholesterol (HDL-c) was determined by the precipitation of chylomicrons. Very low-density lipoprotein cholesterol (VLDL-c) and low-density lipoprotein cholesterol (LDL-c) were determined by precipitation with phosphotungstic acid and magnesium chloride. After centrifugation (1000*g*) for 10 min, the HDL-c fraction remaining in the supernatant was determined by using the enzyme kit described previously. LDL-c was estimated by the Friedwald equation (LDL = TC - HDL - VLDL). The concentration of VLDL-c was calculated by the equation: VLDL = TG/5.

Hepatic and fecal lipid content

The hepatic and fecal lipid contents were determined by the method described by Bligh & Dyer (1959). Samples were initially macerated, homogenized and dehumidified in an oven at 50°C for 4 h.

Statistical analysis

The results were subjected to analysis of variance (ANOVA), followed by Tukey's test, differences being taken as significant when $p < 0.05$.

RESULTS

Weight gain and food intake

The weight gain and food intake results from the diets with niacin and pyridoxine are shown in Table 2. The supplementation of diets with the highest concentrations of niacin and pyridoxine (treatment 10) significantly affected weight gain in the rats: a reduction of 84.2% relative to treatment 1. The rats fed the highest concentrations of niacin and pyridoxine had the lowest food intake of allthe test groups (Table 2).

Table 2. Weight gain and food intake of rats fed for 30 days on experimental diets.

T [a]	Niacin (g/kg)	Pyridoxine (mg/kg)	Weight gain (g)	Food intake (g)
1	0	0	40.64 ± 5.44 [abc]	20.19 ± 0.79 [a]
2	3	6	43.28 ± 5.07 [ab]	19.27 ± 1.13 [a]
3	3	12	48.90 ± 6.73 [a]	20.15 ± 1.15 [a]
4	3	18	46.33 ± 8.65 [ab]	20.26 ± 0.73 [a]
5	4	6	35.04 ± 5.52 [bcd]	19.07 ± 0.32 [a]
6	4	12	25.62 ± 6.12 [d]	19.31 ± 1.21 [a]
7	4	18	39.57 ± 3.98 [abc]	19.62 ± 0.13 [a]
8	5	6	29.53 ± 6.54 [cd]	19.79 ± 0.59 [a]
9	5	12	40.56 ± 6.17 [abc]	19.11 ± 0.59 [a]
10	5	18	6.43 ± 1.50 [e]	17.23 ± 0.56 [b]

[a] T: treatments; Different letters in the same column differ statistically ($p<0.05$). Standard diet (AIN-93M): weight gain: 26.82 ± 3.28 g and food intake: 19.29 ± 1.42 g.

Hepatic total cholesterol, lipids and triacylglycerols and fecal lipids

The results for hepatic TC and TG and hepatic and fecal lipids in male Wistar rats that were fed for 30 days on diets supplemented with the vitamins niacin and pyridoxine are shown in Table 3. The combinations of niacin and pyridoxine did not have a uniform effect on the hepatic TC. In comparison with the rats that were fed the diet without these supplements (TC = 23.87 mg/dL), those fed on the diet containing the highest niacin concentration (5 g/kg) and lowest pyridoxine concentration (6 mg/kg) showed the lowest hepatic TC (17.07 mg/dL), representing a 28.5% reduction in total hepatic cholesterol. However, the hepatic TC resulting from the diet with 4 g/kg niacin and 18 mg/kg pyridoxine (33.22 mg/dL) was higher than that for the diet without addition of these vitamins (Table 3). There was a notable variation in the effects of these vitamins, depending on the combination of their concentrations in the diet.

Supplementation with niacin and pyridoxine in the diet reduced the hepatic TG of Wistar rats. The lowest level of triacylglycerols (126.41 mg/dL) resulted from the highest niacin and pyridoxine concentrations; this TG level was 37.6% lower than that for the treatment 1 diet (202.64 mg/dL). The highest niacin concentration (5 g/kg) resulted in the lowest hepatic TG and, at this level of niacin, there was a reduction in hepatic TG with rising pyridoxine concentration. However, at lower niacin concentrations (3 and 4 g/kg), the pyridoxine concentrations of 6 and 18 mg/kg resulted in raised hepatic triacylglycerol contents, compared to the diet used in treatment 1 (Table 3). Therefore, raising the niacin concentration promotes a reduction in TG levels and, at the highest concentration of niacin, an increase in the pyridoxine concentration reduces hepatic triacylglycerols.

The hepatic lipid content of the rats was reduced by the niacin and pyridoxine supplements in the diet.

Fecal lipids were reduced, relative to the supplement-free control (treatment 1), only in the presence of the lowest niacin concentration (3 g/kg). There was an increase in hepatic lipids, in the presence of a high niacin concentration (5 g/kg) and low pyridoxine concentration (6 mg/kg), of 21.5% relative to treatment 1 (Table 3).

Table 3. Hepatic total cholesterol and triacylglycerols and hepatic and fecal lipids of male Wistar rats fed for 30 days on experimental diets supplemented with niacin and pyridoxine.

T *	Niacin (g/kg)	Pyridoxine (mg/kg)	TC* (mg/dL)	TG* (mg/dL)	Hepatic lipids (g/100 g)	Fecal lipids (g/100 g)
1	0	0	23.87 ± 2.44 ^{b}	202.64 ± 4.00 ^{d}	5.05 ± 0.01 ^{a}	7.45 ± 0.24 ^{bc}
2	3	6	30.79 ± 2.44 ^{a}	234.16 ± 2.10 ^{b}	4.63 ± 0.01 ^{ab}	5.80 ± 0.24 ^{d}
3	3	12	19.37 ± 2.93 ^{bc}	168.98 ± 3.47 ^{f}	4.36 ± 0.01 ^{bc}	6.70 ± 0.33 ^{d}
4	3	18	30.11 ± 3.43 ^{a}	251.48 ± 1.40 ^{a}	5.10 ± 0.02 ^{a}	6.97 ± 0.35 ^{cd}
5	4	6	23.52 ± 2.93 ^{b}	204.62 ± 4.98 ^{d}	3.78 ± 0.01 ^{d}	7.57 ± 0.30 ^{bc}
6	4	12	29.06 ± 0.97 ^{a}	178.55 ± 4.46 ^{e}	4.18 ± 0.01 ^{bcd}	7.07 ± 0.27 ^{bcd}
7	4	18	33.22 ± 1.95 ^{a}	224.42 ± 3.18 ^{c}	4.11 ± 0.01 ^{cd}	8.41 ± 0.32 ^{ab}
8	5	6	17.07 ± 1.71 ^{c}	159.41 ± 3.96 ^{g}	4.65 ± 0.02 ^{ab}	9.05 ± 0.24 ^{a}
9	5	12	23.53 ± 0.12 ^{b}	149.01 ± 3.02 ^{h}	4.42 ± 0.01 ^{bc}	7.37 ± 0.31 ^{bc}
10	5	18	20.41 ± 2.45 ^{bc}	126.41 ± 3.48 ^{i}	4.06 ± 0.02 ^{cd}	7.60 ± 0.35 ^{bc}

Different letters in the same column differ statistically ($p<0.05$). Standard diet (AIN-93M): TC: 21.11 ± 1.46 mg/dL; TG: 113.20 ± 4.46 mg/dL; hepatic lipids: 3.24 ± 0.01 g/100 g and fecal lipids: 7.86 ± 0.10 g/100 g. * T: treatments; TC: total cholesterol; TG: triacylglycerols.

Serum cholesterol and triacylglycerols

The assay results for serum TC, HDL-c, LDL-c, VLDL-c and TG of the male Wistar rats that were fed experimental diets for 30 days are shown in Table 4. Diets including niacin and pyridoxine had effects on the total serum cholesterol of the experimental animals. Treatments 3, 4, 7, 9 and 10 led to a lower serum TC than treatment 1; lower values were observed when the dosage of pyridoxine was equal to or higher than 12 mg/kg (Table 4). Supplementation with niacin and pyridoxine at intermediate levels decreased the HDL cholesterol. However, in the presence of the maximum levels of niacin and pyridoxine, there was an increase in HDL-c compared to other treatments performed with various niacin and pyridoxine contents. The LDL cholesterol level of rats was reduced in the diets with 4 or 5 g/kg of niacin and 18 mg/kg of pyridoxine, compared to the diet of treatment 1 (Table 4).

At high pyridoxine levels, niacin and pyridoxine in the diets decreased the total serum cholesterol and LDL cholesterol. The maximum concentration of pyridoxine enhanced the effects on serum triacylglycerol, both in the reduction of triacylglycerol at a niacin concentration of 5 g/kg and in the raising of this lipid profile at niacin concentrations of 3 and 4 g/kg. The diet with 5 g/kg of niacin and 18 mg/kg of pyridoxine resulted in the lowest serum triacylglycerol level. Supplementation of the diet with the highest levels of niacin and pyridoxine reduced the VLDL-c of animals by 9.6%, relative to the diet of the treatment 1 (Table 4).

Table 4. Total serum cholesterol, fractions of serum cholesterol and serum triacylglycerols in rats fed on diets supplemented with niacin and pyridoxine for 30 days.

T	Niacin (g/kg)	Pyridoxine (mg/kg)	TC (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)	TG (mg/dL)
1	0	0	125.21 ± 0.37ab	56.88 ± 1.24a	59.15 ± 1.46cd	9.18 ± 0.15f	45.91 ± 0.77f
2	3	6	126.92 ± 1.79^{a}	52.29 ± 0.54c	63.01 ± 0.88bc	11.62 ± 0.32cd	58.11 ± 1.62cd
3	3	12	119.23 ± 1.70de	46.86 ± 1.66de	60.29 ± 3.10cd	12.09 ± 0.24c	60.44 ± 1.19c
4	3	18	118.59 ± 1.28de	44.11 ± 0.99e	58.50 ± 0.52 cd	15.98 ± 0.24a	79.90 ± 1.19a
5	4	6	122.65±1.96bcd	52.86 ± 0.67bc	58.84 ± 1.26cd	10.95 ± 0.24d	54.73 ± 1.19 d
6	4	12	123.93±1.61abc	43.88 ± 1.66e	69.99 ± 0.22a	10.06 ± 0.32e	50.32 ± 1.62e
7	4	18	117.95 ± 1.70e	47.09 ± 0.93de	55.97 ± 0.98d	14.89 ± 0.39b	74.45 ± 1.96b
8	5	6	124.79±0.98abc	46.38 ± 1.07de	66.83 ± 1.40ab	11.57 ± 0.32cd	57.85 ± 1.62cd
9	5	12	116.88 ± 0.94e	47.75 ± 1.03d	60.05 ± 1.73cd	9.08 ± 0.39fg	45.40 ± 1.96fg
10	5	18	120.94±0.91cde	56.17 ± 1.37 ab	56.47 ± 2.11d	8.30 ± 0.24g	41.50 ± 1.19g

Different letters in the same column differ statistically ($p<0.05$). Standard diet (AIN-93M): TC: 133.54 ± 1.95 mg/dL; TG: 106.09 ± 0.90 mg/dL; HDL-c: 45.69 ± 2.09 mg/dL; LDL-c: 66.63 ± 2.96 mg/dL; VLDL-c: 21.21 ± 0.17 mg/dL. T: treatments; TC: total cholesterol; TG: triacylglycerols.

DISCUSSION

The water-soluble vitamins, the B complex group and vitamin C, are stored in limited quantities; therefore, they must form part of the daily diet. However, the amounts ingested must be controlled, without excess or deficiency, because otherwise metabolic processes could be affected. According to Flanagan *et al.* (2010), niacin and pyridoxine act as cofactors in the formation of carnitine, a non-essential amino acid that plays a vital role in energy production and the metabolism of fatty acids. The decreased weight gain and food intake of rats fed with niacin and pyridoxine may be explained by the highest concentrations of these vitamins having caused a slight acceleration in metabolism, highlighting their role as coenzymes in metabolic routes. Basu & Mann (1997) also observed a reduced weight gain in rats that were fed a diet supplemented with 400 or 4000 mg niacin/kg. These authors also reported that a combination of these two vitamins may be a better choice of therapy to lower the lipid status than niacin alone. A plausible explanation for this effect is the hypothesis that there is a synergy between the two vitamins, increasing their effects on weight reduction.

Although the use of niacin has been associated in the past with adverse effects such as hepatic toxicity, more recent studies, using new formulations of niacin, have not shown hepatic toxicity but have shown similar effects on the plasma lipid profile. These new formulations have stimulated interest in the use of niacin as a lipid regulating agent and its role in increasing HDL cholesterol levels (Ganji *et al.,* 2003). Niacin reduces the mobilization of fatty acids from adipose tissue by inhibiting triacylglycerol lipolysis. According to Ganji *et al.* (2003), one mechanism for the reduction of triacylglycerol levels in the blood is the inhibition of lipolysis. Fat cells are specialized for the synthesis and storage of triacylglycerols, in the form of free fatty acids and glycerol, and for their mobilization to the liver. According to Yang *et al.* (2008), treating rabbits with niacin for 6 weeks resulted in a reduction in the levels of total cholesterol and LDL cholesterol and an increase in HDL cholesterol in the plasma. Ashen & Blumenthal (2005) reported that therapy with niacin leads to a reduction of free fatty acids (20-40%), triacylglycerols (20-40%), lipoprotein (a) (30-40%) and LDL cholesterol (20-40%) and a significant rise in HDL cholesterol (20-35%). Furthermore, Ganji *et al.* (2009) reported that niacin has antioxidant potential.

Brattström *et al.,* (1990) reported a reduction of 10% and 17% in total cholesterol and LDL cholesterol, respectively, in elderly men who ate a diet supplemented with 120 mg of pyridoxine daily for 8 weeks; however, there was no effect on HDL cholesterol. According to Hardman & Limbird (1996), nicotinic acid is able to inhibit the hormone-sensitive lipase, an enzyme responsible for lipolysis of adipose tissue, and increase the activity of lipoprotein lipase. In lipid metabolism, nicotinic acid increases cholesterol catabolism, decreases the synthesis of LDL cholesterol and probably VLDL cholesterol, increases the formation of HDL and inhibits the release of free fatty acids from adipose tissue.

According to Brown *et al.* (2001), nicotinic acid acts at various steps of lipid metabolism by inhibiting the mobilization of fatty acids from adipose tissue, reducing the intake of these fatty acids into the liver and, consequently, decreasing the production of VLDL cholesterol and of LDL cholesterol. Niacin also inhibits the degradation of HDL cholesterol particles, by mechanisms that are not well understood, and can be used in the treatment of dyslipidemia. Bloc'h *et al.* (2010) also reported that the use of niacin reduces plasma triacylglycerols, total cholesterol, VLDL cholesterol and LDL cholesterol. Assmann & Gotto (2004) reported that nicotinic acid has been used to increase the HDL cholesterol in humans and also contributes to the reduction of LDL cholesterol and triacylglycerols. In the present study, joint supplementation with niacin and pyridoxine had different effects, depending on the dose of each vitamin. Robinson *et al.* (1998) suggested that a low level of vitamin B_6 (pyridoxine) in the blood is a risk factor for developing cardiovascular disease. Basu & Mann (1997) reported that large doses of niacin have a negative effect on serum cholesterol fractions. It is possible that niacin induces the catabolism and loss of vitamin B_6 or introduces bias into vitamin B_6 metabolic routes. This study demonstrates that hypercholesterolemia can be treated successfully by taking modest amounts of vitamin B_6.

The relevance of the fecal excretion of lipids and cholesterol is not only in the regulation of total serum cholesterol and its fractions, but also in preventing certain diseases. Two of the most important dietary factors that might predispose people to a high incidence of colorectal cancer are low intake of non-absorbable dietary fibers and high intake of fat (Forman *et al.,* 2004). Diets rich in lipids increase cholesterol synthesis in the intestine, where it is required for the absorption and transport of high levels of circulating lipids in the diet. There is also a reduction in the fecal excretion of bile acids.

Supplementation with niacin and pyridoxine in the diets of rats resulted in a reduction in the levels of total serum cholesterol, LDL, VLDL, triacylglycerols and hepatic lipids. This supplementation had varying effects on the lipid profiles, which depended on both the vitamin concentrations and the interaction between the two vitamins. The combination of these two vitamins intensified the effects on the lipid profiles of Wistar rats, and the highest vitamin contents reduced the weight gain in the animals. The diet with the highest concentration of niacin and lowest concentration of pyridoxine led to the lowest level of total hepatic cholesterol. A diet supplemented with niacin and pyridoxine reduced the hepatic triacylglycerol levels and the lowest values were achieved at the highest niacin concentration. When the concentration of niacin remained the same, there was a reduction in hepatic triacylglycerol with increasing pyridoxine concentration. It is essential to perform clinical trials to determine whether these experimental results can be extrapolated to humans. The use of niacin and pyridoxine is a viable alternative therapy in the treatment of high cholesterol; however, human studies that assess adverse reactions and toxicity are needed to conduct safe and effective treatment.

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