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Chapter 13

New Potential Beta-3 Adrenergic Agonists with Beta-Phenylethylamine Structure, Synthesized for the Treatment of Dyslipidemia and Obesity

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Additional information is available at the end of the chapter

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Abstract

Beta-3 adrenergic receptors have important physiological implications, being expressed in many places in the body, including brown adipose tissue. Of the effects studied in preclinical research on lipid metabolism attributable to stimulation of these receptors, we can mention the increased thermogenesis and metabolic rate in the brown adipose tissue, reduction of body weight in obese diabetic rats, lowering of intra-abdominal and subepithelial fat in nonobese and nondiabetic rats, decrease of triglyceride, and increase of HDL cholesterol levels. Carbohydrate metabolism is also changed by beta-3 adrenergic agonists, the most prevalent effects being blood glucose lowering in diabetic rats, increasing insulin secretion of the pancreas, or increasing glucose tolerance. Metabolic effects of 13 newly synthesized compounds of beta-phenylethylamine structure and reference BRL 37344 were investigated in order to identify a potential affinity for beta-3 adrenergic receptors. The antidiabetic and hypolipemiant effects were investigated on a rat model of alloxan-induced diabetes. The results demonstrated that new beta-phenylethylamine derivatives produced marked biological activity over lipid profile. All compounds have markedly decreased the values of total cholesterol, LDL cholesterol, and triglycerides and also have increased the values of antiatherogenic HDL cholesterol. The effects were significantly more intense than the reference substance BRL 37344.

Keywords: beta-3 adrenergic agonists, antidiabetic, hypolipemiant, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, hexokinase, beta-phenylethylamine

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1. Introduction

The sympathetic nervous system is part of the autonomic nervous system and innervates tissues in almost every organ system. Adrenergic system is important for maintaining the organism homeostasis and mediates the neuronal and hormonal stress response commonly known as the fight-or-flight response.

Central and peripheral adrenergic neurotransmitters are epinephrine and norepinephrine, which act on their specific adrenergic receptors.

Adrenergic receptors types and subtypes		Tissue localization	Dominant effects
α-Adrenergic receptors	α_{1A}	Heart, blood vessels, smooth muscle, liver, lung, vas deferens, prostate, cerebellum, cortex, hippocampus	Contraction of vascular smooth muscle; vasoconstriction of large resistant arterioles in skeletal muscle
	α_{1B}	Heart, kidney, spleen, lung, blood vessels, cortex, brainstem	Promotes cardiac growth and structure
	α_{1D}	Aorta, coronary artery, platelets, prostate, cortex, hippocampus	Vasoconstriction in aorta and coronary artery
	α_{2A}	Sympathetic neurons, platelets, pancreas, locus coeruleus, brainstem, spinal cord	Main inhibitory receptor on sympathetic neurons
	α_{2B}	Liver, kidney, pancreas, blood vessels	Mediates α_2 vasoconstriction
	α_{2c}	Basal ganglia, cortex, cerebellum, hippocampus	Modulates dopamine neurotransmission Inhibits hormone release from adrenal medulla
β-Adrenergic receptors	β_1	Heart, kidney, skeletal muscle, cortex, olfactory nucleus, brain stem	Positive inotropic and chronotropic effects
	β_2	Bronchial and gastrointestinal smooth muscle, blood vessels, heart, lung, skeletal muscle, cortex	Smooth muscle relaxation, skeletal muscle hypertrophy
	β_3	Adipose tissue, gastrointestinal tract, gallbladder, urinary bladder	Lipolysis, thermogenesis, relaxation of the bladder

Tissue localization and dominant effects (after Goodman & Gillman's 2011, modified).

Table 1. Types and subtypes of adrenergic receptors.

Adrenergic receptors were described for the first time by Ahlquist in 1948, who hypothesized the existence of two different types of receptors, α and β, based on the consideration that adrenaline, noradrenaline, and other pharmacological agonists regulate various physiological functions [1]. This differentiation of receptors was confirmed by the finding that there are antagonists, which selectively block α receptors (e.g., phenoxybenzamine or phentolamine) or

β receptors (e.g., propranolol). Every type of adrenergic receptors has different subtypes, which are mentioned in **Table 1** [2–4].

1.1. Beta-3 adrenergic receptor discovery and structure

In the early 1980s, Tan S and Curtis-Prior PB proposed the term of beta-3 or beta-hybrid receptor for a new type of beta-adrenergic receptor, based on some studies of four beta-adrenergic agonists on isolated rat adipose cells. They observed that lipolytic potency decreased in the order: isoprenaline (beta-1 and beta-2 agonist) > noradrenaline (beta-1 >>> beta-2 agonist) > salbutamol (beta-2 agonist) > prenalterol (beta-1 agonist). They also studied the effects of some beta-antagonists on lipolysis induced by various agonists. Propranolol (nonselective beta-antagonist) was more potent than betaxolol (selective beta-1 antagonist) or ICI 118551 (selective beta-2 antagonist). All results conducted to the idea that lipolysis in adipose tissue is regulated by other adrenergic receptor than the classical ones, beta-1 and beta-2 [5].

In 1989, Emorine et al. first characterized beta-3 receptor by discovering the gene that encodes it [6]. Before that Arch et al. observed that some nonspecific classical beta-receptor agonists, named BRL 26830A, BRL 33725A, and BRL 35135A, had antiobesity actions on obese and diabetic mice [7].

Further studies have shown that beta-3 receptor is different than beta-1 and beta-2 by some important issues:

– the specific agonists CL 316243 and BRL37344 stimulate only beta-3 receptor [8, 9];
– lack of beta-3 receptor desensitization after agonists activation [10]; and
– the need to use larger quantities of catecholamines to stimulate beta-3 receptors [11].

The structures of beta-1, beta-2, and beta-3 receptors are similar, being all members of G protein-coupled receptors. Beta-3 receptor is a protein which contains 396 amino acids, found in seven transmembrane segments, with three intracellular and three extracellular loops. The amino-terminal region is extracellular, glycosylated, and with variable length. The carboxyl-terminal region is intracellular and it does not possess phosphorylation sites, which are present at beta-1 and beta-2 receptors. Essential for interaction with the ligands are the disulfide bond between the second and the third extracellular loops and also four of the seven transmembrane segments. Other two segments are implicated in G-protein stimulation, with adenylate cyclase and second messenger activation [12].

1.2. Metabolic effects of beta-3 adrenergic receptor stimulation

Some of the most important effects of beta-3 receptor activation are the metabolic ones, especially in the brown adipose tissue. This thermogenic tissue has the role of keeping constant body core temperature of small animals at cold ambient temperatures. Stimulation of beta-3 adrenergic receptors not only activates brown adipose tissue thermogenesis in the short term, but also increases mitochondrial biogenesis and the expression of thermogenin

(UCP1) [13–15]. This protein mediates transport across the internal mitochondrial membrane and interrupts oxidative phosphorylation of the beta oxidation of fatty acids, increasing the use of energy [16]. There have been investigations about the effects of beta-3 adrenergic agonists on thermogenin. The results have shown that beta-3 agonists activated thermogenin, and also other uncoupling proteins, as follows:

- UCP2 that is found in many tissues.
- UCP3 that is found in skeletal muscle and has an important role in basal thermogenesis [17].

The stimulation of thermogenesis by beta-3 adrenergic agonists resulted in a number of experimental studies, which have shown that in animals, these substances lead to weight loss, a selective fat decrease, but without reducing food intake [18].

An *in vitro* study was performed on cells with high levels of beta-3 adrenoreceptors such as the adipocytes of the murine cell line 3T3-F442A. The study demonstrated that insulin and glucocorticoids downregulate beta-3 adrenoreceptor expression through a transcriptional effect. The impairment of beta-3 adrenoceptor gene expression in adipocytes of congenitally obese ob/ob mice could be related to the higher glucocorticoid plasma levels when compared to lean mice [19].

The main studied metabolic actions in preclinical research of beta-3 adrenergic agonists were the reduction of plasma insulin levels, increase glucose tolerance, and reducing body weight in obese diabetic rats. The major implication of beta-3 adrenergic receptor in glucose metabolism and hence in the release of insulin and in obesity has been demonstrated [20, 21].

The development of beta-3 adrenergic agonists was a step forward for the treatment of metabolic diseases by sympathetic activation, because norepinephrine and other relative nonselective derivatives have cardiovascular side effects which limit their use.

1.3. Preclinical studies of beta-3 agonists on carbohydrate and lipid metabolism

Numerous nonclinical studies have shown that administration of beta-3 agonists decreased glucose and lipids plasma concentrations in diabetic mice derived from genetically modified strains (kk, C57BL/KsJ- db/db) or in rats with experimentally induced diabetes [22, 23].

Several mechanisms of action were highlighted and reported:

– improving insulin resistance and increased tissue response to insulin [24],

– increasing insulin secretion in beta cells of the pancreas [25],

– decrease in glucose release from the liver, increase noninsulin-dependent uptake of glucose from white and brown adipose tissue and skeletal muscles [26], and

– increase of glucose tolerance at doses lower than those that stimulate lipolysis in adipose tissue, without affecting the amount of food intake or body weight [27].

The nonclinical research for proving the effects of beta-3 agonists on obesity were conducted on rodent species [28–30] from various strains, both normal and genetically modified to generate predisposition to obesity.

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Studies in obese rats treated with the selective beta-3 adrenergic agonists have shown a significant reduction in body weight and reduction of adipose tissue, without food intake being affected by them. It was also demonstrated that an increase of two to three times of the mRNA level and of UCP-type protein, as well as of the guanidine 5'-diphosphate coupled, a relevant index of thermogenesis, in brown and white adipose tissues for the tested rats. In addition, it has been found, after the administration of beta-3 adrenergic agonists, an improvement of glucose tolerance and a decrease of hyperinsulinemia. The researchers suggested two possible mechanisms for defining this aspect, increase of the number of insulin receptors or decrease of glucose transporters in brown and white adipose tissues, which implies an increase in glucose uptake into muscle tissue [31].

Lorente Ferrer et al. investigated the effect of beta-3 receptors agonists on thermogenesis in deep adipose tissue. In general, these agonists increase energy consumption but their effects are quickly counteracted by glucocorticoids. Thus, their potential for long-term treatment of obesity is reduced. Since the metabolic effects of beta-3 receptor agonists (β_3A) overlap only partially with those of oleoyl-estrone (OE) (loss of appetite, weight change, loss of body fat), the possibility of combining them in the energy balance in order to accelerate the decrease of fat deposits was studied. Rats receiving OE or OE + β_3A significantly reduced weight compared with the control group, the maximum reduction corresponding to the group which received the combination [32].

The effect of beta-3 adrenergic receptor agonists was investigated on two strains of rats with different genetic predisposition to obesity: male rats aged 8 weeks Osborne Mendel (OM) strain and S5B/P1 (S5B) strain. Animals were treated with beta-3 adrenergic agonist CL316243 after they have been adapted to either a high fat diet (56% fat-based energy) or low fat (10% fat-based energy), but both equivalent diets in terms of protein content (24% based on protein energy). The animals were fed *ad libitum* and were injected with CL316243 in three doses: 0.1; 0.3; and 3 mg/kg at the beginning of the night. Food intake was measured at 1, 3, and 24 hours after injection. The results showed that CL316243 significantly reduced food intake for all measurements, in both types of rats. Inhibition of food intake was still higher in S5B-type mice. CL316243 significantly decreased serum leptin and serum glucose at both types of rats, especially at S5B. In OM rats, beta-3 adrenergic agonist increased serum insulin levels, while in S5B rats fed with a low-fat diet, the level of serum insulin decreased. In another experiment, CL316243 was administered to rats kept fasting overnight. It was observed after 30 minutes a significant reduction in insulin levels in both types, more pronounced in S5B. The glucose level in OM rats decreased after 30 and 60 minutes, while in rats S5B a decrease was observed only after 30 minutes from the administration. Experiments have shown that beta-3 agonist CL316243 has a much more obvious effect on rat strain resistant to obesity induced by high-fat diet [33].

Another study used a transgenic model of mice, lacking beta-3 adrenergic receptors. CL316243 blocked the activation of adenylate cyclase and lipolysis when it was administered to these mice. A modest growth of fat tissues especially in females was observed. These mice showed an increase in the level of mRNA for beta-1 receptor, but not for receptor beta-2. This showed a functional compensation between the genes for beta-1 and beta-3 receptors. Finally, a sharp

increase of insulin levels and lipolysis after administration of CL316243 in normal mice was noted; effects were not found after administration of beta-3 adrenergic agonists in beta-3 receptor-deficient mice [34].

In another model of knockout mice lacking functional beta-3 adrenoreceptors, there were no responses for food intake and insulin secretion in white and brown adipocytes after administration of beta-3 adrenergic agonist CL316243, indicating the implication of beta-3 receptors in these metabolic effects [35].

An increase in insulin levels during "fasted/fed" transition in rats has been demonstrated, associated with a decrease in the mRNA level of beta-3 adrenergic receptor and a decrease of the response in brown and white adipose tissues. It was concluded that there is a close relationship between the food intake, plasma levels of insulin, and beta-3 adrenergic receptors. Downregulation of the beta-3 receptors could be a possible mechanism by which insulin determines lipid storage and prevents lipid mobilization after food intake [36].

In another study, CL316243 was administered in obese diabetic KKAy mice for 2 weeks. The results showed a decrease of serum levels of glucose, insulin, triglyceride, free fatty acid, and tumor necrosis factor-alpha (TNF-alpha) and an increase of adiponectin. The beta-3 adrenergic receptor agonist recovered the mRNA expressions of adiponectin, adiponectin receptors, and beta-3 adrenoreceptor, which were reduced in epididymal white adipose tissue in KKAy mice. Also, CL316243 suppressed the overexpressed mRNA level of TNF-alpha in both epididymal white and brown adipose tissues. It was concluded that the normalization of adiponectin, adiponectin receptors and TNF-alpha could contribute at the amelioration of obesity-induced insulin resistance [28].

In the study conducted on nonobese/nondiabetic Sprague-Dawley rats, the selective beta-3 agonist CL 312243 increased food intake, metabolic rate, and body temperature after 7 days of treatment. The author also showed a decrease in intra-abdominal and subepithelial fat, a hepatic glucose level independent of variations in body weight, an increase in interscapular fat, and in total glucose, which stimulates the production of insulin. According to the results, only white and brown adipose tissues have been affected. A more important role of adipose tissue in glucose uptake underlining the potential role of beta-3 adrenergic agonist drugs for the treatment of obesity and insulin-resistant diabetes was suggested [8].

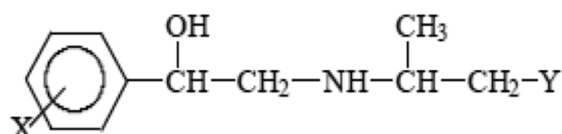
By a critical analysis of the published data in nonclinical research, it was concluded that beta-3 adrenergic receptor activation in the experiment-induced diabetes and obesity [37, 38] determines an increase of glucose tolerance and lipolysis activation in adipose tissue [22]. It seems that the effects are dose dependent and the selectivity of actions for glucose metabolism occurs at lower doses than those used for influencing lipid metabolism.

Comparing the effects of beta-3 agonists in rats and humans, Arch and Wilson stated that these compounds, with remarkable effects on rodents, have not convinced in clinical studies because of limited efficacy or serious side effects. The explanations mentioned by the authors included low pharmacokinetic properties and a low biotransformation to active compounds. A possible more important distinction between rats and humans is the different structure of beta-3 receptors, leading to lower efficacy of compounds in humans than in rats. In addition, it seems

that the number of beta-3 receptors is lower than beta-1 and beta-2 receptors in the tissues that mediate thermogenesis in humans [39]. This is one of the reasons why the clinical studies conducted on beta-3 selective agonists had some contradictory results.

Mirabegron, a currently approved drug for the treatment of overactive bladder, was recently studied in humans for its effects on brown adipose tissue. This drug has several advantages over other members of its class, including a higher bioavailability and a higher *in vitro* affinity for the human beta-3 adrenoreceptor. Mirabegron was orally administered in the dose of 200 mg to 12 healthy male subjects with detectable brown adipose tissue. The results showed that all treated subjects had a higher brown adipose tissue metabolic activity, measured with ^{18}F-fluorodeoxyglucose (^{18}F-FDG) using positron emission tomography (PET) combined with computed tomography (CT). These are promising results for a possible future use of beta3 agonists for metabolic disease [40].

Based on all these preclinical and clinical considerations new chemical entities with beta-phenylethylamine nucleus, substituted in various positions on the nucleus or side chain, with potential action on diabetes and/or obesity were synthesized [41, 42]. Chemists led synthesis in order to obtain derivatives with increased beta-3 receptor selectivity. The compounds were conventionally named A1-βPhEA–A13-βPhEA (**Figure 1**).



- X = H, alkoxy, halogen, dihalogen
- Y = 4-carbopropoxy-phenoxy, 4-carbomethoxymethylene-phenoxy, 4-carbomethoxyethylene-phenoxy

Figure 1. General structure of the newly synthesized compounds. X = H, alkoxy, halogen, dihalogen; Y = 4-carbopropoxy-phenoxy, 4-carbomethoxymethylene-phenoxy, 4-carbomethoxyethylene-phenoxy.

2. Objectives

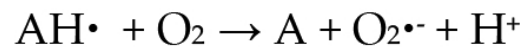
The purpose of this study was to test the effects of the newly synthesized compounds over lipid profile and body weight of rats to which alloxanic diabetes was induced, being a known fact that this metabolic disorder induces alterations in plasma lipids.

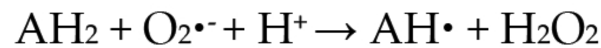
Alloxan was chosen for induction of type II diabetes mellitus because its pancreatic toxicity was demonstrated in nonclinical trials using isolated islet cell or entire perfused rat pancreas and afterwards by directly administering the substance to rodent or nonrodent animals. In the first stage, alloxan stimulates on short-term insulin secretion, which is followed by total suppression of the response of the islet cells to glucose, regardless of its concentration [43].

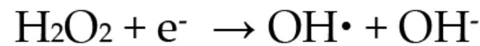
Alloxan is readily absorbed by beta pancreatic cell, a process that contributes to diabetogenic action. Its absorption also takes place in liver although hepatocytes are more resilient to its action compared to beta cells, therefore more protected against its toxicity [44].

2.1. Mechanism of alloxan toxicity

The mechanism of toxic action resides in formation of reactive species of oxygen [45], the alloxan exhibiting increased affinity to substrates containing thiolic groups (reduced glutathione, cysteine, proteins with sulfide groups, enzymes) [46]. The product of alloxan reduction, the dialuric acid, is reoxidized to alloxan, the redox cycle thus formed being responsible for releasing superoxide radicals—see **Figure 2** [47].







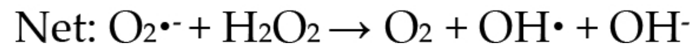


Figure 2. Mechanism of diabetes induction by administration of alloxan [47]. A, Alloxan; AH·, alloxan radical; AH_2, dialuric acid; $O_2\cdot^-$, superoxide radical; OH·, hydroxyl radical.

An optimal protection against cytotoxic action of alloxan and dialuric acid is offered by an association between superoxide-dismutase (SOD) and catalase (CAT), in order to completely prevent the redox cycle and consecutively the formation of any reactive oxygen species [48].

Glucose confers complete protection against toxic effects of alloxan both *in vivo* and *in vitro*, by blocking glucokinase inhibition by it and also contributing to maintaining the antioxidant protection mechanism of the beta cells [49].

One of the targets for the reactive oxygen species is the DNA of the pancreatic islet cells. The DNA fragmentation occurs in beta cells exposed to alloxan [49, 50].

The DNA alteration stimulates poly-ADP-ribose, enzyme which contributes to affected DNA repair. In several trials it was stated that glucose administration contributes to counteracting the alloxan cytotoxicity. Such ability is the result not only of glucokinase protection but also of interaction with glucose carrier GLUT2 resulting in reduced alloxan absorption [51].

3. Materials and methods

Diabetes was induced to white Wistar male rats by intraperitoneal administration of extemporaneously prepared alloxan (Sigma-Aldrich) solution, in the dose of 130 mg/kg weight [52]. After 48 hours, glycemia was determined using ACCU-CHEK Active device (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). The blood was harvested from tail veins by vein puncture. The determination was used for the selection of diabetic animals, thus presenting a glycemia over 200 mg/dL.

From the total rat collectivity, a percentage of 66.34% became diabetic with the remaining 33.66% becoming hyperglycemic. Fifteen groups of diabetic animals were designated for experimental research (eight animals/group), being treated as follows:

- diabetic control group (D Control)—distilled water, 1 mL/100 g weight p.o.;
- reference group—BRL 37344, 50 mg/kg weight, p.o.;
- A1-βPhEA—20 mg/kg weight, p.o.;
- A2-βPhEA—50 mg/kg weight, p.o.; and
- A3-βPhEA through A13-βPhEA, 100 mg/kg weight, p.o.

At the same time, a nondiabetic control group was designated (ND control), treated with distilled water, 1 mL/100 g weight p.o. Administration of tested and reference substances (BRL 37344) continued for 14 days, once daily.

The doses chosen for investigating the metabolic effects of the newly synthesized derivatives were established following previous determinations of acute toxicity which allowed to set the LD_{50} [53, 54].

At the end of the experiment, the animals were sacrificed and biochemical and enzymatic determinations were performed: glycemia, glucose-6-phosphate dehydrogenase (transforming glucose by pentose-phosphate pathway), glucose–6–phosphatase (catalyzes the hydrolysis of glucose–6–phosphate to glucose and inorganic phosphate), hexokinase (catalyzes the phosphorylation of glucose), total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides.

4. Results and discussion

The model of alloxan-induced diabetes has produced alterations in the activity of the three enzymes [55–57] involved in glucose metabolism homeostasis (**Figure 3**).

Moreover, the lipid profile was altered by the administration of the pancreatic toxic, registering statistically significant increases in total cholesterol, LDL cholesterol, and triglycerides, together with decreases in HDL cholesterol (**Figure 4**).

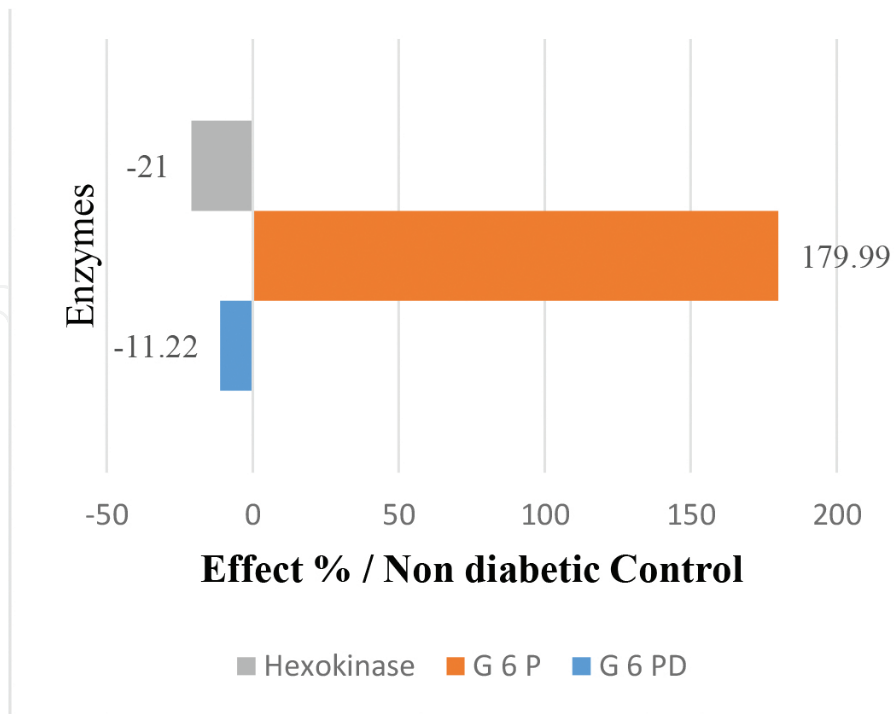


Figure 3. Variations of the activity of the enzymes involved in glucose metabolism in diabetic animals compared to nondiabetic animals.

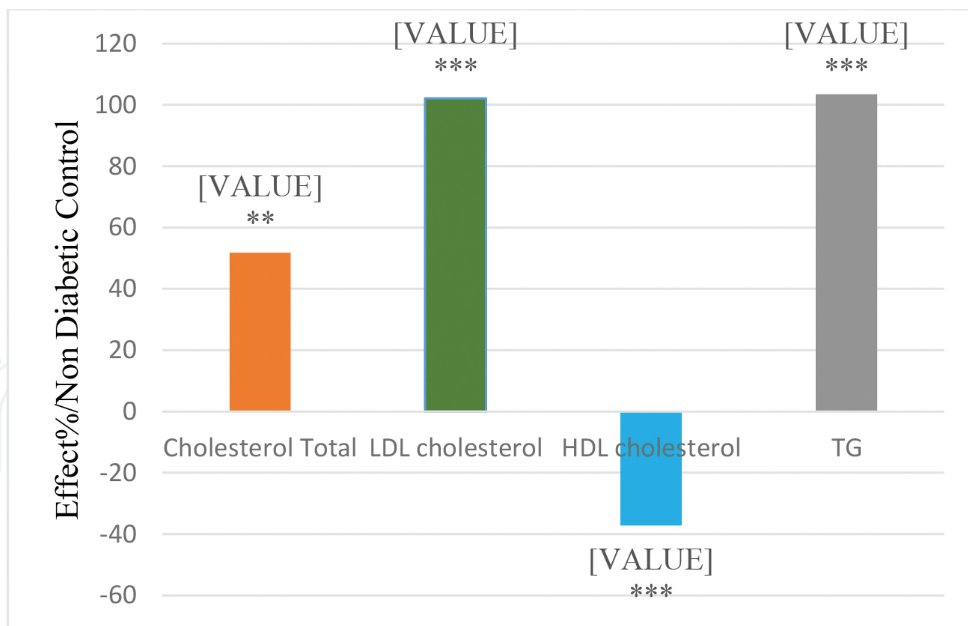


Figure 4. Variation of plasma lipids in diabetic animals compared to nondiabetic animals ** $p<0.01$; *** $p<0.001$.

To what concern the effect of new compounds with possible affinity for beta-3 adrenergic receptors, seven of those (A1-βPhEA, 20 mg/kg weight; A3-βPhEA, 100 mg/kg weight; A4-βPhEA, 100 mg/kg weight; A6-βPhEA, 100 mg/kg weight; A8-βPhEA, 100 mg/kg weight; A9-

βPhEA, 100 mg/kg weight; A13-βPhEA, 100 mg/kg weight) have markedly reduced the values of total cholesterol (**Table 2**, **Figure 5**).

Group	M ± SE	ANOVA	Dunnett posttest/ND control	ANOVA	Dunnett posttest/D control
Nondiabetic control	78.22 ± 9.553	0.0002***		<0.0001***	**
Diabetic control	119.3 ± 11.89		**		
BRL 37344 50 mg/kg	77.65 ± 2.196		ns		*
A1-βPhEA 20 mg/kg	62.03 ± 5.005		ns		***
A2-βPhEA 50 mg/kg	74.83 ± 13.10		ns		*
A3-βPhEA 100 mg/kg	70.42 ± 9.716		ns		**
A4-βPhEA 100 mg/kg	69.27 ± 6.423		ns		**
A5-βPhEA 100 mg/kg	113.0 ± 11.41		ns		ns
A6-βPhEA 100 mg/kg	61.00 ± 4.749		ns		***
A7-βPhEA 100 mg/kg	75.05 ± 5.286		ns		*
A8-βPhEA 100 mg/kg	72.31 ± 6.148		ns		**
A9-βPhEA 100 mg/kg	69.25 ± 6.542		ns		**
A10-βPhEA 100 mg/kg	77.73 ± 7.884		ns		*
A11-βPhEA 100 mg/kg	66.60 ± 3.331		ns		*
A12-βPhEA 100 mg/kg	75.32 ± 5.966		ns		*
A13-βPhEA 100 mg/kg	62.15 ± 6.673		ns		**

Table 2. The effect of new derivatives of beta-phenylethylamine on total cholesterol in rats with alloxan-induced diabetes * p<0.05; ** p<0.01; *** p<0.001.

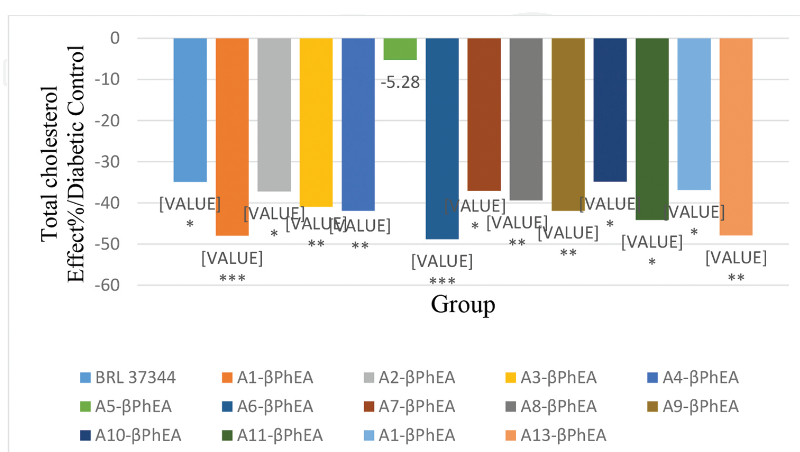


Figure 5. Alterations of total cholesterol in diabetic animals treated with reference substance (BRL 37344) or newly synthesized derivatives of beta-phenyl ethylamine compared to diabetic control group * p<0.05; ** p<0.01; *** p<0.001.

The majority of the tested compounds (**Table 3**) have markedly reduced the values of LDL cholesterol (**Figure 6**), the effect probably due to increased plasma clearance for this lipid fraction as total cholesterol serum concentration decreased. Smaller reductions, still statistically significant (**Table 3**) in values of LDL cholesterol, and comparable to those of the beta-3adrenergic agonist, BRL 37344, were produced by the compounds: A7-βPhEA, A10-βPhEA, A12-βPhEA, and A13-βPhEA (**Figure 6**).

Group	M ± SE	ANOVA	Dunnett posttest/ND control	ANOVA	Dunnett posttest/D control
Nondiabetic control	51.07 ± 3.610	<0.0001***		<0.0001***	***
Diabetic Control	103.2 ± 7.899		***		
BRL 37344 50 mg/kg	78.71 ± 4.940		ns		**
A1-βPhEA 20 mg/kg	56.87 ± 1.887		ns		***
A2-βPhEA 50 mg/kg	57.47 ± 2.831		ns		***
A3-βPhEA 100 mg/kg	50.91 ± 1.205		ns		***
A4-βPhEA 100 mg/kg	57.08 ± 2.302		ns		***
A5-βPhEA 100 mg/kg	56.26 ± 2.134		ns		***
A6-βPhEA 100 mg/kg	56.88 ± 2.496		ns		***
A7-βPhEA 100 mg/kg	75.91 ± 6.447		**		***
A8-βPhEA 100 mg/kg	54.62 ± 2.458		ns		***
A9-βPhEA 100 mg/kg	53.70 ± 2.128		ns		***
A10-βPhEA 100 mg/kg	76.00 ± 7.567		**		***
A11-βPhEA 100 mg/kg	57.52 ± 3.112		ns		***
A12-βPhEA 100 mg/kg	66.77 ± 7.334		ns		***
A13-βPhEA 100 mg/kg	50.21 ± 1.188		ns		***

Table 3. The effect of new derivatives of beta-phenylethylamine on LDL cholesterol values in alloxan-induced diabetic rats ** p<0.01; *** p<0.001.

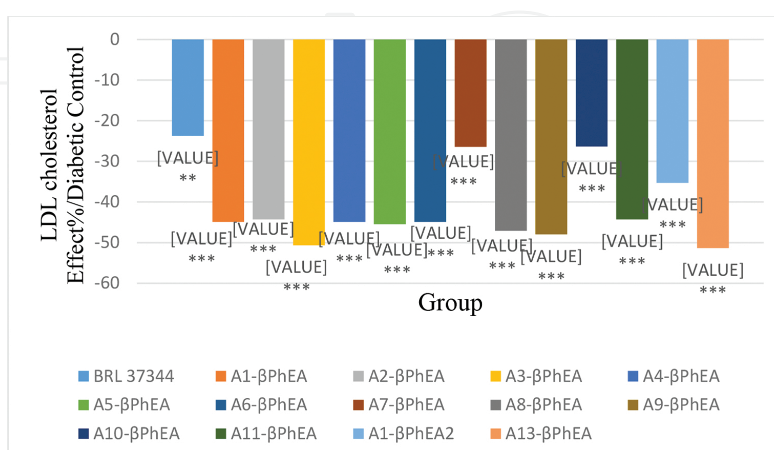


Figure 6. Alterations of LDL cholesterol values in diabetic animals treated with reference substance (BRL 37344) or new derivatives of beta-phenylethylamine compared to diabetic control group ** p<0.01; *** p<0.001.

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The results for lowered total cholesterol and LDL cholesterol are in line with other literature data showing that several beta-3adrenergic agonists have induced similar effects in mice with apolipoprotein E deficiency and in wild C57BL/6J strain animals. For such substances an increase in apolipoprotein A1 and PPARα and PPARγ receptors (peroxisome proliferator-activated receptor) expression in liver was demonstrated [58].

Other trials showed that, due to effects on lipid metabolism but also to glycemia reduction, the β_3 adrenergic agonist BRL 37344 has induced a reduction in the process of formation of atherosclerotic plaque in ApoE(-/-) mice [59].

A pivotal role in slowing down the process of atherosclerosis stands with HDL cholesterol, which is a small size alpha-lipoprotein, formed in liver or shed from chylomicrons dismemberment. These lipoproteins have a cholesterol-rich core with type 1 and 2 apolipoproteins at the surface, ensuring the reverse transport of cholesterol, from tissues to liver. The trial conducted by Shi et al. demonstrated the increases in mARN and apoA1 expression.

Group	M ± SE	ANOVA	Dunnett posttest/ND control	ANOVA	Dunnett posttest/D control
Nondiabetic control	46.36 ± 2.930	<0.0001***		<0.0001***	***
Diabetic control	29.34 ± 3.478		***		
BRL 37344 50 mg/kg	34.82 ± 3.216		ns		ns
A1-βPhEA 20 mg/kg	48.09 ± 3.621		ns		***
A2-βPhEA 50 mg/kg	42.96 ± 2.102		ns		*
A3-βPhEA 100 mg/kg	46.24 ± 4.104		ns		***
A4-βPhEA 100 mg/kg	53.35 ± 4.875		ns		***
A5-βPhEA 100 mg/kg	49.79 ± 2.926		ns		***
A6-βPhEA 100 mg/kg	46.07 ± 3.679		ns		***
A7-βPhEA 100 mg/kg	52.18 ± 3.653		ns		***
A8-βPhEA 100 mg/kg	51.04 ± 1.662		ns		***
A9-βPhEA 100 mg/kg	47.81 ± 4.664		ns		***
A10-βPhEA 100 mg/kg	45.66 ± 4.880		ns		***
A11-βPhEA 100 mg/kg	38.04 ± 2.596		ns		ns
A12-βPhEA 100 mg/kg	38.46 ± 2.095		ns		ns
A13-βPhEA 100 mg/kg	41.94 ± 1.330		ns		*

Table 4. The effect of new derivatives of beta-phenyl ethylamine on HDL cholesterol in rats with alloxan-induced diabetes * $p<0.05$; *** $p<0.001$.

The results of the experimental research have shown that all tested compounds have induced statistically significant increases in HDL cholesterol, compared to diabetic control group (**Table 4**). A smaller effect of increase of the values for this lipid fraction was produced by the compounds A11-βPhEA and A12-βPhEA, still being similar to the one for beta$_3$ adrenergic agonist BRL 37344 (**Figure 7**).

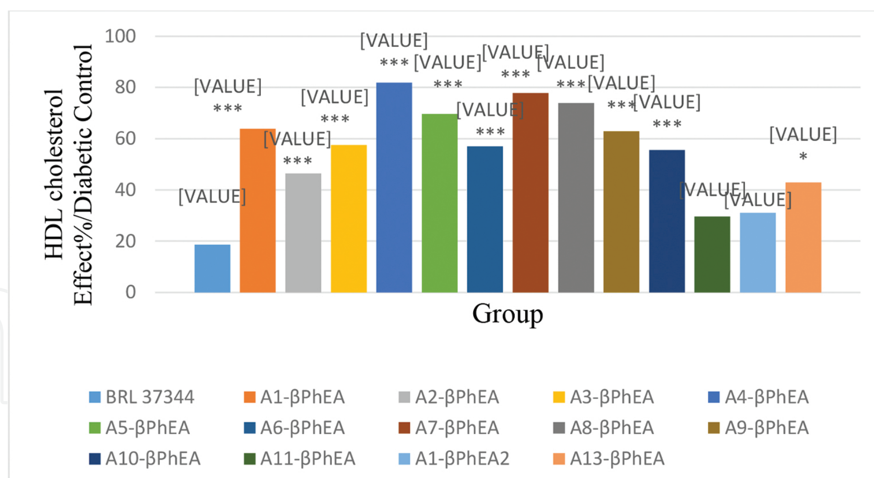

Figure 7. Alteration of HDL cholesterol in diabetic animals treated with reference substance (BRL 37344) or with derivatives of beta-phenylethylamine compared to diabetic control group * p<0.05; *** p<0.001.

Group	M ± SE	ANOVA	Dunnett posttest/ND control	ANOVA	Dunnett posttest/D control
Nondiabetic control	81.74 ± 2.261	0.0002***		<0.0001***	***
Diabetic control	166.3 ± 6.793		***		
BRL 37344 50 mg/kg	77.13 ± 3.639		ns		***
A1-βPhEA 20 mg/kg	91.25 ± 4.573		ns		***
A2-βPhEA 50 mg/kg	88.01 ± 4.158		ns		***
A3-βPhEA 100 mg/kg	90.23 ± 4.643		ns		***
A4-βPhEA 100 mg/kg	67.38 ± 8.630		ns		***
A5-βPhEA 100 mg/kg	66.32 ± 5.682		ns		***
A6-βPhEA 100 mg/kg	66.58 ± 9.860		ns		***
A7-βPhEA 100 mg/kg	80.60 ± 3.113		ns		***
A8-βPhEA 100 mg/kg	84.24 ± 2.831		ns		***
A9-βPhEA 100 mg/kg	87.80 ± 4.384		ns		***
A10-βPhEA 100 mg/kg	86.21 ± 2.935		ns		***
A11-βPhEA 100 mg/kg	87.86 ± 5.032		ns		***
A12-βPhEA 100 mg/kg	84.35 ± 3.656		ns		***
A13-βPhEA 100 mg/kg	91.25 ± 6.193		ns		***

Table 5. The effect of new derivatives of beta-phenyl ethylamine on serum TG in rats with alloxan-induced diabetes *** p<0.001.

In Wistar rats alloxan has induced a high increase, statistically significant, of serum triglycerides (TG) (**Table 5**). Compared to diabetic control group, all the tested compounds have reduced the values of serum triglycerides with high statistical significance. These effects could

be due to increased expression [58] of PPARα (liver, kidney, muscle, adipose tissue) and PPAR γ receptors (subtypes 1, 2, 3 in adipose tissue) resulting in increased expression of the gene for lipoprotein lipase.

4.1. Effects on body weight

During the research, animals had free access to standard food and water. The body weight was determined initially, at 48 hours after alloxan administration and then in day 5, 10, and 14 of the experiment. For nondiabetic control group the same determination were performed as in the case of diabetic groups. The food was dispensed daily in same amounts and body weight was determined before the next feeding.

Throughout this determination it was apparent that alloxan-induced diabetes produces, in 48 hours from administration, a statistically nonsignificant reduction (**Figure 8**) of body weight in treated animals (203.4 ± 0.7004 vs. 205.9 ± 0.7078).

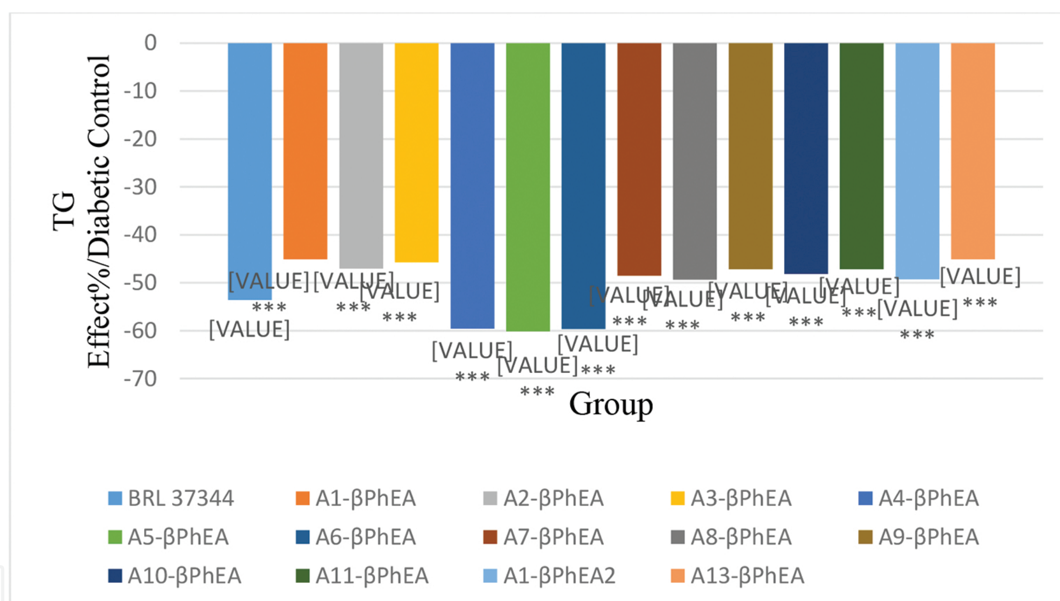

Figure 8. Body weight of animals initially and at 48 hours after alloxan administration *** p<0.001.

The research results showed that, for diabetic control group, animal body weight increases after alloxan administration, reaching a significant higher value in day 14 of the experiment (p = 0.0097**). For the groups treated with reference substance, the body weight varied statistically nonsignificantly in all moments of determination (**Table 6**). The amount of consumed food increased for the diabetic control group, while for the treated groups, it remained constant. In the determinations of day 14, for nondiabetic control group it was registered an increase of 1.07% in body weight against the initial measurement, while for the diabetic control group the increase reached 5.65%. The variation in body weight at the end of the experiment against initial and compared to diabetic control group was calculated using the formulas:

Group	Parameter	Body weight basal	Body weight 48 h after alloxan	Body weight Day 5	Body weight Day 10	Body weight Day 14
ND control	M ± SE	203.8 ± 3.301	200.2 ± 4.176	204.3 ± 2.894	205.5 ± 2.766	206.0 ± 2.733
	ANOVA/48 h	0.7341 ns				
D control	M ± SE	203.2 ± 3.429	197.0 ± 3.967	206.5 ± 2.363	209.7 ± 2.305	214.7 ± 1.382**
	ANOVA/48 h after A	0.0097**				
BRL 37344	M ± SE	204.3 ± 2.963	198.7 ± 3.920	203.8 ± 2.868	202.7 ± 2.246	201.5 ± 2.513
	ANOVA/48 h after A	0.6778 ns				
A1-βPhEA	M ± SE	202.5 ± 2.872	197.2 ± 3.640	201.7 ± 2.836	200.8 ± 3.114	199.8 ± 3.092
	ANOVA/48 h after A	0.7823 ns				
A2-βPhEA	M ± SE	200.2 ± 3.781	195.5 ± 4.123	198.5 ± 3.667	198.7 ± 3.556	199.0 ± 3.540
	ANOVA/48 h after A	0.9238 ns				
A3-βPhEA	M ± SE	208.0 ± 2.206	201.8 ± 3.936	206.0 ± 2.145	205.5 ± 2.247	205.0 ± 2.556
	ANOVA/48 h after A	0.6202 ns				
A4-βPhEA	M ± SE	204.8 ± 2.701	199.5 ± 3.805	202.8 ± 2.651	203.2 ± 2.151	202.0 ± 3.088
	ANOVA/48 h after A	0.4047 ns				
A5-βPhEA	M ± SE	207.2 ± 2.868	201.1 ± 4.083	206.0 ± 3.044	204.7 ± 2.552	206.7 ± 3.232
	ANOVA/48 h after A	0.6717 ns				
A6-βPhEA	M ± SE	205.0 ± 2.966	199.1 ± 3.973	203.2 ± 2.868	202.5 ± 2.754	202.0 ± 2.781
	ANOVA/48 h after A	0.7505 ns				
A7 -βPhEA	M ± SE	206.7 ± 2.552	201.1 ± 3.989	204.8 ± 2.358	204.2 ± 2.167	203.7 ± 2.290
	ANOVA/48 h after A	0.7213 ns				
A8-βPhEA	M ± SE	204.8 ± 1.990	201.7 ± 3.802	203.5 ± 1.586	202.7 ± 1.926	203.7 ± 2.290
	ANOVA/48 h after A	0.9261 ns				
A9 -βPhEA	M ± SE	208.2 ± 1.249	202.1 ± 3.667	205.5 ± 0.7638	204.7 ± 1.022	203.8 ± 1.558
	ANOVA/48 h after A	0.3638 ns				
A10-βPhEA	M ± SE	206.3 ± 2.741	200.8 ± 4.036	205.0 ± 2.477	204.8 ± 2.587	204.0 ± 2.671
	ANOVA/48 h after A	0.7431 ns				
A11-βPhEA	M ± SE	208.0 ± 3.066	202.2 ± 4.401	206.2 ± 2.738	205.8 ± 2.713	205.7 ± 2.813
	ANOVA/48 h after A	0.7922 ns				
A12-βPhEA	M ± SE	207.3 ± 2.894	201.7 ± 4.234	205.7 ± 2.565	205.3 ± 2.603	204.5 ± 2.320
	ANOVA/48 h after A	0.7561 ns				
A13-βPhEA	M ± SE	209.3 ± 2.591	203.0 ± 4.244	206.7 ± 2.028	206.7 ± 2.390	205.0 ± 2.266
	ANOVA/48 h after A	0.6257 ns				

ns, nonsignificant; A, alloxan.

Table 6. The effect of new derivatives of beta-phenylethylamine on body weight in rats with alloxan-induced diabetes ** $p<0.01$.

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$$\text{Effect } \%\frac{\text{BW}}{\text{basal}} = \frac{\text{BW basal}(g) - \text{BW day14}(g)}{\text{BW basal}(g)} \times 100 \quad (1)$$

$$\begin{aligned}&\textit{Effect } \% \textit{ BWSubstance / Diabetic control } = \textit{Effect } \% \textit{ BW} \\ &\textit{Diabetic control } + \textit{Effect } \% \textit{ BW Substance}\end{aligned} \quad (2)$$

Taking into account that body weight increased for the animals in diabetic control group and that for all tested substances it has decreased against initial, the effect of the tested substances was determined compared to diabetic control group (**Figure 9**). It was therefore noted that newly synthesized derivatives of beta-phenyl ethylamine and the reference substance BRL 37344 had produced decreases in body weight between 5.89 and 7.76%, compared to diabetic control animals after 14 days of treatment.

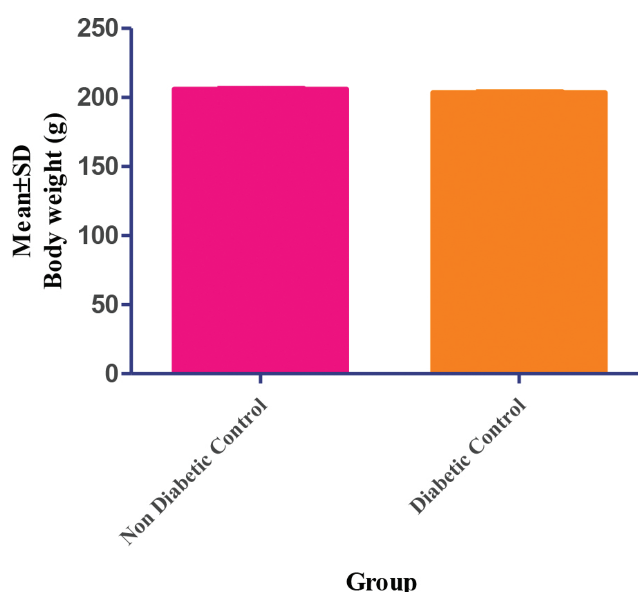

Figure 9. Variation of body weight for animals treated with reference substance and tested substances compared to diabetic control group at day 14 determination.

5. Conclusions

The results of this experimental research have demonstrated that newly synthesized derivatives of beta-phenylethylamine produce marked biological activity over lipid profile which is altered in diabetes induced by alloxan administration in rats.

All tested compounds have markedly decreased the values of total cholesterol, LDL cholesterol, and triglycerides, the effect being more intense than with reference substance BRL 37344. They also have increased the values of antiatherogenic HDL cholesterol, significantly more

than the reference substance. Overall, the activity on body weight was of reduction even if the food consumption of the animals was not altered. These experimental data suggest that the tested new chemical entities have high therapeutical potential in the treatment of dislipidemias and/or obesity.

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