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Effects of Isoproterenol, a Non-Selective β -Agonist, on Lipid Metabolism of Rats: *In Vitro* and *In Vivo* Study

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It is believed that β -adrenergic receptors are involved in fat metabolism through the induction of lipolysis. On the other hand, different dietary fats show different pattern of fat accumulation in animal body. In this study, first, *in vitro* lipolytic effect of isoproterenol in rat adipocyte has been studied, then its interaction with different dietary fats on fat accumulation in animal body (*in vivo*) has been studied. For *in vitro* study, adipocytes isolated from epididymal fat were incubated with 10^{-5} to 10^{-9} M isoproterenol and freed glycerol content was measured as an index of lipolysis. For animal study, forty-eight male SD rats were divided into agonist-intaked and control groups and both of them were fed diets containing 12% of either beef tallow (BT), canola oil (CO), olive oil (OO) or safflower oil (SO) as fat source. After 4 weeks, the rats were killed and their abdominal fat mass and liver were dissected and weighed. The results of *in vitro* studies showed a potent lipolytic effect of isoproterenol on rat adipocytes. The pD_2 values for isoproterenol was 7.345 ± 0.018 . Amount of accumulated abdominal fat was not different between agonist-intaked and control. It was concluded that, although isoproterenol had a potent lipolytic effect on rat adipocytes *in vitro*, its *in vivo* effect was not obvious, possibly due to less bioavailability of the agonist at adipocytes level *in situ*.

INTRODUCTION

There are several systems involved in energy metabolism of animal body. When the energy is needed in the body, systems such as lipolysis and glycolysis stimulate energy production through catabolic processes of energy stores, mainly of lipids and saccharides. Fats are one of the main energy stores of animal body. In lipolysis, fats, mainly triglycerides, were degraded into glycerol and fatty acids to produce a considerable amount of energy. There are some control systems regulating this process. Adrenergic system is considered as one of them. Two groups of adrenoceptors of this system, *i.e.*, α and β have almost opposite effects on fat metabolism, especially in white adipose tissue (WAT). The α -adrenergic system especially α_2 -adrenoceptors show obvious anti-lipolytic activity and β -adrenergic receptors are generally considered as lipolysis-inducer of most animal species (Lafontan and Berlan, 1993). In the original classification of β -adrenergic receptors, there were only two types, *i.e.*, β_1 and β_2 -adrenoceptors (Lands *et al.*, 1967).

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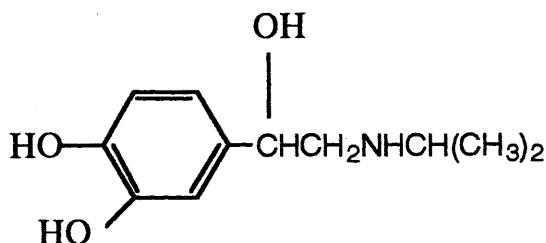


Fig. 1. Chemical structure of Isoproterenol

However, from early 80s, an “atypical” β -adrenoceptor (neither $\beta 1$ nor $\beta 2$), so-called $\beta 3$, was also found in rat adipocyte (Tan *et al.*, 1983) and its gene was cloned in many other species (Emorine *et al.*, 1989; Granneman *et al.*, 1991 and Granneman *et al.*, 1993). The patterns of distribution of different types of adrenoceptors and their functional significance in BAT and WAT are different among different species. The lipolytic activities of adrenergic system is achieved through adenylyl cyclase and resultant cAMP production in the adipocytes. cAMP acts as a “second messenger” and promotes the activation of cAMP-dependent protein kinase A which phosphorylates serine residues of hormone-sensitive lipase (HSL) and promotes its activation and its translocation towards lipid droplets (Egan *et al.*, 1992).

Since a few decades ago, obesity has been considered as a major risk factor for some cardiovascular, metabolic and endocrine diseases. Therefore, many researchers have tried to manipulate fat metabolism for preventing excess fat accumulation in the body. Lipolysis was considered as one of the potential ways for removing the excess body fat. Additionally, there have been some studies indicating the possible interactions between dietary lipids and β -adrenergic lipolytic activities. It has been suggested that dietary lipids change the cell membrane composition and then affect adrenoceptor activity (For more details refer to Fotovati and Ito, 1998).

Our previous studies have shown some patterns of distribution and metabolism of intaked different dietary fats rich in either saturated fatty acids (SFAs), polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) in the rat body. In the present study, effect of isoproterenol (**Fig. 1**), as a non-selective β -adrenoceptor agonist, in inducing lipolysis in rat adipocyte (*in vitro*) has been investigated. Then, an animal study investigating whether there are any interactions between the β -adrenergic activity and the type of intaked dietary fats or not has been performed.

MATERIALS AND METHODS

In Vitro study:

Isolation of adipocytes:

About 1 mg of epididymal fat was dissected out from a 20 weeks old male SD rat and then adipocytes were isolated by collagenase digestion as previously described by Rodbell (1964). Briefly, adipose fragments were incubated for 60 min in 5 ml Krebs-Ringer

bicarbonate buffer containing albumin (3.5 g/100 ml) (KRBA), glucose (6 mM) and collagenase IV (3.5 mg/ml) at pH 7.4 and 37.5 °C in a water bath under gentle shaking at 60–70 cycles/min. After digestion, adipocyte suspension was centrifuged at 3000 g for 5 min and the supernatant was filtered through a 150 mesh stainless steel filter. Adipocytes were washed three times with KRBA buffer to eliminate collagenase. After washing, 4 to 5 samples of the final cell suspension were counted in Neubauer's hemacytometer after being diluted with trypan blue stain.

Lipolysis:

For lipolysis study, isolated adipocytes ($1\text{--}1.5 \times 10^4$ cells) were incubated in polyethylene tubes with 1 ml KRBA (pH 7.4) containing glucose (6 mM) at 37 °C with gentle shaking (60–70 cycles/min) in a water bath. Isoproterenol hydrochloride was added to the medium to reach the final concentration of agonist from 10^{-5} M to 10^{-9} M. After 90 min of incubation, the reaction was stopped by placing the tubes in ice bath and 100 μ l of aliquots of infranatant were taken for enzymatic determination of glycerol (glycerol kit, Boehringer Mannheim GmbH) by using spectrophotometer (DU-62 Spectrophotometer, BECKMAN). Measured glycerol concentration was used as an index of lipolysis. Total lipid was evaluated gravimetrically after extraction with 5 vol. of chloroform-methanol (2 to 1 in v/v) mixture (Folch *et al.*, 1957). Different concentrations of agonists and their lipolytic effects were expressed as percentage compared to maximal isoproterenol effect and dose-response curve was made accordingly. Since lipolytic effect of both agonists reached a plateau at a given concentration, the concentration of isoproterenol that produced maximum effect was determined and used as a basic value. Then, a half-maximum effect on glycerol release, *i.e.*, EC_{50} (as log mol/l) and pD2-values ($-\log EC_{50}$) was determined in relation to isoproterenol maximal effect (Van Rossum, 1963).

Animal Study:

Animals and diets:

Forty-eight 7-weeks old male Sprague-Dawley rats (purchased from Seac Co. Ltd., Japan) initially weighing 250–290 g were housed in individual stainless-steel wire-mesh cages with a free access to water and under a controlled condition, 12-h reverse light: dark cycle (08:00–20:00), 20 °C in temperature and 60% relative humidity, in animal raising facility of Biotron Institute of Kyushu University. Commercial diet (Charles River CRF-1 purchased from the same company) was used for adaptation. Four experimental diets were contained 12% of either beef tallow (BT), canola oil (CO), olive oil (OO) or safflower oil (SO) as lipid sources. Other ingredients were the same for all groups as indicated in **Table 1**. In beef tallow diet, 0.03% alpha-tocopherol (wt/wt) was added to the beef tallow itself before mixing with other ingredients, as an antioxidant.

Experimental design:

At first, all rats were raised on the commercial diet (Charles River CRF-1) for 7 days for adaptation. Then, they were assigned to two main agonist-intaked and control groups. Each group was further divided into four dietary sub-groups. Feeding diets contained 12% of either beef tallow, canola oil, olive oil or safflower oil as lipid sources. Rats were distributed by controlled randomization such that mean body weights were not

Table 1. Composition of experimental diets (g/100g of diet)

Component	Dietary sub-groups			
	Beef tallow	Canola oil	Olive oil	Safflower oil
Beef powder	20	20	20	20
Beef tallow	12	–	–	–
Canola oil	–	12	–	–
Olive oil	–	–	12	–
Safflower oil	–	–	–	12
AIN-76 Vitamin mix	1	1	1	1
AIN-76 Mineral mix	3.5	3.5	3.5	3.5
DL-Methionine	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2
Cellulose	5	5	5	5
Corn starch	27.9	27.9	27.9	27.9
Sucrose	30	30	30	30
Cholesterol	0.1	0.1	0.1	0.1

different among groups and dietary sub-groups. All groups were fed *ad libitum*. In agonist-intaked group, isoproterenol was dosed at 0.5 mg/kg BW/day. Body weight and feed consumption were measured every other day and weight gain and feed intake were calculated. After six weeks, the rats after anesthetization by ether were killed by decapitation (carried out under the control of guidelines for Animal Experiment in Faculty of Agriculture and the Graduate Course, Kyushu University and the Law [No. 105] and Notification [No. 6] of the Government). Blood was collected and serum was obtained by centrifugation (1500 g for 15 min) and stored at -80°C until use. Abdominal adipose tissues (mainly peri-renal and mesenteric adipose tissues) and liver were excised, weighed and immediately frozen in liquid nitrogen and stored at -35°C until analysis of fatty acid composition.

Lipid extraction:

Fat content of the diets and abdominal fat, liver, muscle and feces, was extracted by Folch's method (Folch *et al.*, 1957). Briefly, about 0.6 g of tissue (1 g in the case of feces and 1 ml for serum) were weighed and suspended in 5 vol. of chloroform-methanol mixture (2 to 1 in v/v). After homogenization, the suspension was filtered through a filter paper (Advantec 5B, 125 mm) and the solvent was evaporated by freeze-drying and fat content was quantified gravimetrically.

Preparation of sample for determination of fatty acid composition by gas chromatography:

Fatty acid composition of extracted lipid was analyzed by gas chromatography. For preparation of fatty acid sample, extracted lipid was hydrolyzed and methylated as previously described (Kawahara *et al.*, 1997). Briefly, about 1 mg of extracted fat was dissolved in 1 ml of acetyl chloride and methanol dehydrate mixture (1 to 10 v/v) and 100 μl benzene and hydrolyzed by incubating in hot water bath (80°C) for 120 min. Fatty acids were extracted by hexane, and after dehydration, the solvent was evaporated by

freeze-drying. Sample was dissolved in benzene as carrier, and injected to gas chromatograph (GC-14B, Gas Chromatograph, Shimadzu Co. Ltd.) equipped with a temperature programmable injector and capillary column [(30 m long with an internal diameter of 0.250 mm), Ulborn]. Column temperature was programmed from 150 to 220 °C at rate of 4 °C/min. Detection was made with a FID detector.

Chemicals:

Krebs-Ringer's and BSA were purchased from Sigma Co. (St. Louis, MO., USA). Collagenase Type IV was provided from Worthington Biochemical Co. (USA). Glycerol kits were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Filter 5B, 125 mm was obtained from Advantec (Japan). DL- α -tocopherol was purchased from Nacalai Tesque (Japan). Isoproterenol hydrochloride was purchased from Sigma Co. Ltd.. DL-Methionine, cholesterol (cholesterin), sucrose and choline bitartrate were purchased from Nacalai Tesque. Cellulose was purchased from Advantec. All other organic and inorganic chemicals and reagents were of analytical grade and were purchased from Merck (Darmstadt, Germany), Sigma and Nacalai Tesque.

Statistical analysis:

Results are shown as means \pm SEM. The differences among groups were assessed by ANOVA and was followed by Fischer PLSD for assessing the difference between all pairs of control and isoproterenol-intaked rats. *P*-value less than 0.05 is considered as statistically significant. For statistical analysis, Power Pc Version of StatView software (Abacus Concepts Inc) was used.

RESULTS

In vitro lipolytic study:

Spontaneous glycerol release, *i.e.*, basal lipolysis, was $0.322 \pm 0.031 \mu\text{mol}/100 \text{ mg}/90 \text{ min}$. Isoproterenol was full agonist and induced a dose-dependent lipolysis on isolated rat epididymal adipocytes (**Fig. 2**). Lipolytic effect of agonist, determined as concentration of released glycerol, reached a plateau at about 10^{-6} M . The mean half-maximum effect (pD₂) values for isoproterenol was 7.345 ± 0.018 .

Animal study:

In all of dietary sub-groups, there was no significant difference in daily feed intake and weight gain between control and agonist treated rats (**Figs. 3a and b**). However, among control groups, daily feed intake in BT (27.615 ± 1.468) was significantly higher than SO (25.062 ± 2.163) ($P < 0.05$) (**Fig. 3a**). Weight gain was also significantly higher in control group of BT (7.320 ± 0.900) than SO (5.563 ± 1.125) ($P < 0.05$) (**Fig. 3b**). There was no significant difference in liver weight among the dietary groups and also between agonist-treated and control in each dietary group (**Fig. 4a**). However, among control groups, liver weights in BT (20.950 ± 1.973) and CO (19.862 ± 3.040) were significantly higher than SO (17.580 ± 3.235) ($P < 0.05$) (**Fig. 4a**). Amount of abdominal fat was not significantly different between agonist-intaked and control rats in all dietary groups (**Fig. 4b**).

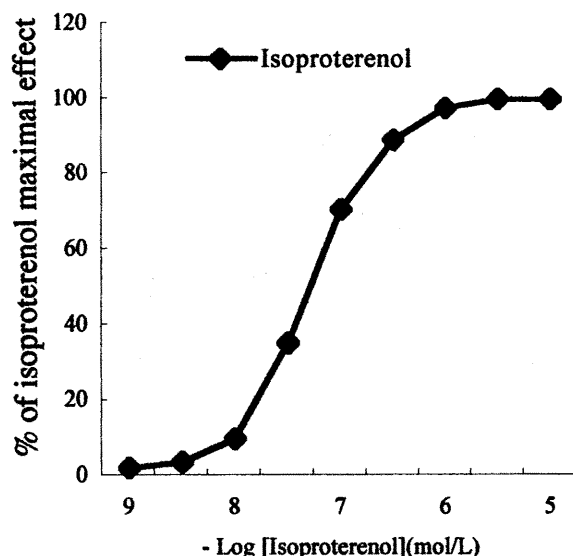


Fig. 2. Lipolysis induced by increasing the concentration of (—) isoproterenol in rat epididymal adipocytes. The effects are expressed as % relative to isoproterenol maximal effect. Values are means \pm SEM from 4–5 separate experiments.

Fatty acid composition of experimental diets is shown in **Table 2**. In every diet, MUFA was the most abundant fatty acid. BT contained a large amount of SFA, while CO and SO contained larger amount of PUFA. Fatty acids composition of abdominal fat, as a typical example, of control and isoproterenol-intaked rats of four dietary sub-groups is shown in **Fig. 5**. There was no significant difference in fatty acid composition between agonist-treated and control rats in every dietary Sub-group. The situation was similar in the case of liver, muscle and serum (Data not shown).

DISCUSSION

The results of this study showed *in vitro* lipolytic effect of isoproterenol and its failure to induce lipolysis *in vivo* in the rats fed different dietary fats.

In this study, isoproterenol showed a dose-dependent lipolytic activity in rat adipocytes (**Fig. 2**). This was in consistent with some previous studies (Hollenga *et al.*, 1991; Umekawa *et al.*, 1996; Carpéné *et al.*, 1999; Fotovati *et al.*, 2000). Isoproterenol is considered as a mixed non-selective β -agonist (Lafontan and Berlan, 1993), however, different concentration of it may play an important role in determining the type of β -receptor to be stimulated. It has been shown that low concentration of catecholamine (10 nM or lower) activates lipolysis through β_1 -adrenoceptor activation, whereas the stimulation of β_3 -adrenoceptor occurred at higher concentration (more than 1 μ M).

However, the main effect of isoproterenol on lipolysis was attributable to the activation of the atypical β_3 -adrenoceptor, whereas the typical β_1 -adrenoceptor play a small, subordinate role (Hollenga and Zaagsma, 1989). The results of our other study and results of other authors have shown that, although isoproterenol strongly stimulates lipolysis in rat adipocytes, its lipolytic effect is about 10 times weaker than selective β_3 -agonists such as BRL 35135 (Fotovati *et al.*, 2000; Hollenga *et al.*, 1991; Umekawa *et al.*, 1996). The order of the potency of lipolytic effect of isoproterenol compared to selective β_1 , β_2 and β_3 -agonists in rat was suggested as follows: β_3 -agonists (CL316243 and BRL37344) > isoproterenol as mixed agonist > β_1 -agonist (dobutamine) > β_2 -agonist (procaterol) (Atgie *et al.*, 1997). Lipolytic activity of isoproterenol on other animal and human adipocytes has been studied *in vitro* (Milavec-Krizman and Wagner, 1978; Langin *et al.*, 1991; Carpené *et al.*, 1999). Large species-specific differences exist in fat cell adrenoceptor distribution and in their relative importance in the control of the fat cell function. Although functional β_3 -adrenoceptors coexist with β_1 - and β_2 -adrenoceptors in a

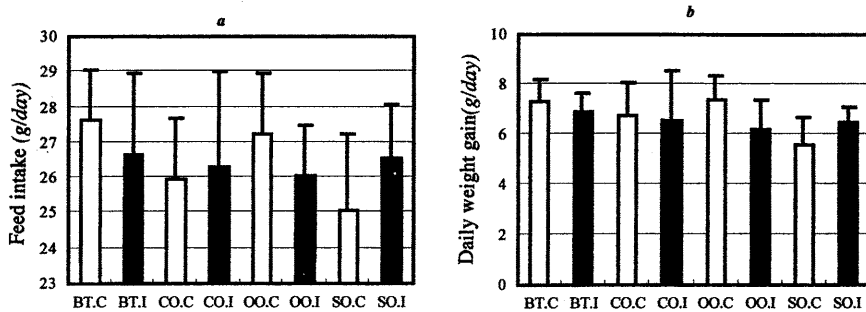


Fig. 3. Daily feed intake (a) and weight gain (b) in control (C) and isoproterenol-treated (I) rats. BT=Beef tallow, CO=Canola oil, OO=Olive oil, SO=Safflower oil. Values are means \pm SEM.

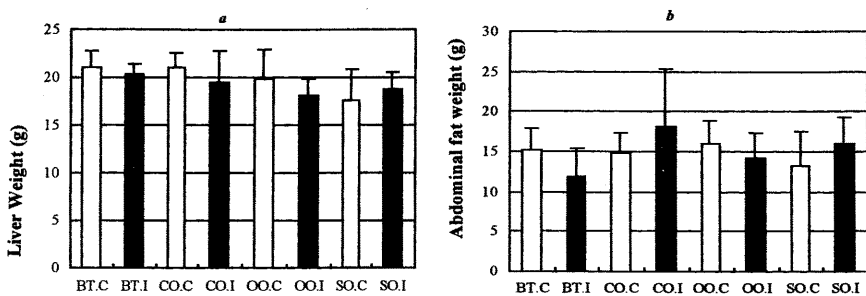
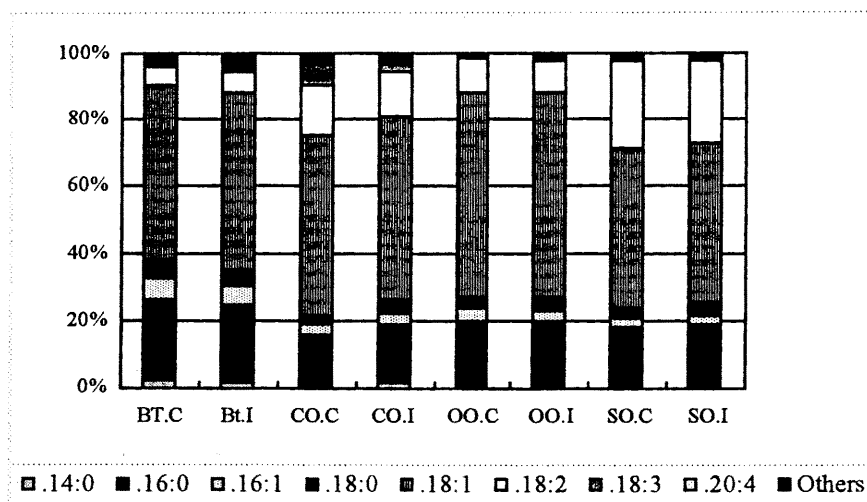


Fig. 4. Liver weight (a) and abdominal fat weight (b) in control (C) and isoproterenol-treated (I) rats. BT=Beef tallow, CO=Canola oil, OO=Olive oil, SO=Safflower oil. Values are means \pm SEM.

Table 2. Fatty acid composition of diets.

Diet	Fatty acids								
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	others
Beef tallow	2.13	26.5	4.45	10.04	48.52	1.79	0.11	0.26	6.2
Canola oil	0.0	9.51	1.21	4.42	52.53	17.86	8.59	1.14	4.74
Olive oil	0.5	15.2	1.72	2.63	68.73	6.95	0.84	1.06	2.37
Safflower oil	0.09	10.7	0.09	2.23	41.21	39.62	0.76	1.24	4.06

**Fig. 5.** Pattern of distribution of fatty acids in abdominal fat of control (C) and isoproterenol-treated (I) rats raised on different dietary fats. BT: Beef tallow, CO: Canola oil, OO: Olive oil, SO: Safflower oil.

number of fat cells; they are weakly active in guinea pig, primate, and human fat cells (Lafontan and Berlan, 1993).

In vivo lipolytic effects of isoproterenol has been shown in human and animals. It is believed that β_1 and β_2 -adrenoceptors could play a role in triglyceride breakdown *in vivo* (Harms *et al.*, 1982). Intravenous infusion of isoproterenol in fasted rabbits significantly elevated plasma non-esterified fatty acids, indicating increased lipolysis (Reverte and Rivascabanero, 1996). Injection of 0.05–0.15 mg/kg of isoproterenol to mice induced a dose-dependant lipolysis (Shih *et al.*, 1995). However, in the present animal study, inclusion of isoproterenol failed to show lipolytic activity in the rat body. There was no significant difference in the amount of abdominal fat between agonist-intaked and control rat in all dietary groups (**Fig. 4b**). This might be due to weaker lipolytic activity of isoproterenol as a non-selective β -agonist, compared to selective β_3 -agonists such as BRL35135. The other possibility is desensitization of β -adrenoceptors due to long term dosage of isoproterenol as indicated by the results of one study (Green *et al.*, 1996). Low bio-availability of isoproterenol due to so-called “*first-pass loss*” by the liver is an

another potential cause of failure of its activation in the present animal study (Pond and Tozer, 1984; Roland and Tozer, 1995). It is believed that large parts of isoproterenol will be eliminated by the liver during passing the gastrointestinal tract. Failure of lipolytic activity of isoproterenol was observed in all dietary groups. It was indicated that lipolytic activity of adrenoceptors might be reduced by feeding rats with SFA-rich diets such as beef tallow (Matsuo *et al.*, 1997) and similar results have been observed in our other study using selective β 3-adrenoceptors (Fotovati *et al.*, 2000). However, this phenomenon could not be observed in this study because of the basic failure of activation of the receptor by this agonist.

Future studies by using alternative administration techniques, *i.e.*, parenteral, which provides higher concentration of agonist at the adipocyte level may provide more information regarding non-selective adrenergic-induced lipolysis in rats fed different dietary fats. Such studies also provide more detailed information regarding the effect of diet itself on obesity and other fat-related metabolic disorders.

CONCLUSION

Although isoproterenol as one of the non-selective β -adrenergic agonist activated lipolysis in adipocytes *in vitro*, its lipolytic effect was not significant in animal body. This might be due to following reasons: 1) Non-selective agonist activities of isoproterenol, 2) lesser lipolytic activity of β 1-adrenoceptor, compared to β 3-adrenoceptor, 3) desensitization of β -adrenoceptors due to the long term dosage of isoproterenol and finally 4) lower concentration of the agonist bound to the adrenoceptors located on the surface of the adipocytes due to the reduction in the level of biologically available agonist after first-pass loss in the liver. Results of the present study could not show any interaction between isoproterenol and the type of dietary lipids.

ACKNOWLEDGEMENT

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