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Thermogenic and lipolytic effect of yohimbine in the dog

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1 Lipid mobilization during a hypocaloric diet may be enhanced by a pharmacological approach using α_2-adrenoceptor antagonists since these drugs are known to increase sympathetic tone and stimulate lipolysis. Studies were undertaken in the dog in order to evaluate the effects of oral yohimbine administration (α_2-adrenoceptor antagonist) on heat production, metabolic, endocrinological and cardiovascular parameters.

2 Acute oral yohimbine (0.25 or 0.40 mg kg^{-1}) provoked an increase in plasma non-esterfied fatty acids. The drug increased sympathetic nervous system activity as indicated by the increased level of plasma noradrenaline. These effects persisted during the entire experimental period (4 h). The increase in plasma noradrenaline level was two fold higher with the higher dose of yohimbine (0.4 mg kg^{-1}). The plasma adrenaline level was increased only with the higher dose.

3 Yohimbine transiently increased plasma insulin and the effect was dose-dependent.

4 Yohimbine (0.25 mg kg^{-1}) enhanced heart rate and arterial blood pressure.

5 The effect of yohimbine on oxygen consumption, carbon dioxide and heat production was determined by indirect calorimetry. The drug (0.25 mg kg^{-1}) increased O_2 consumption and CO_2 and heat production 30 min after its administration and the effect persisted over the experimental period. The respiratory quotient, rather low in the fasting animals, remained unchanged.

6 The present work indicates that thermogenesis and lipid mobilization are enhanced during fasting in the dog by α_2-adrenoceptor blockade. Yohimbine also induced a transient increase in plasma insulin level and increased heart rate and blood pressure. The lipid mobilization plus the action on thermogenesis observed after yohimbine draw attention to the putative interest of α_2-antagonists in the pharmacological treatment of obesity during restricted calorie intake.

Keywords: Yohimbine; lipomobilization; catecholamines; thermogenesis; fasting dog

Introduction

Starvation or energy restriction induce compensatory changes in metabolic rate as reflected by the reduction in total energy expenditure. Reduction of the size of the digestive tract, reduction of the protein turnover and of ionique active transport (mainly Na^+/K^+) contribute to compensatory mechanisms (Ma & Foster, 1985; Farrel & Williams, 1989; Rumpler *et al.*, 1989). Lipid mobilization is accelerated during the early period of energy restriction or fasting in man and animals. The decrease in plasma insulin levels is a major factor involved in the induction of lipid mobilization (Cahill *et al.*, 1966; Livavivathana *et al.*, 1978; Jensen *et al.*, 1987). In man (James *et al.*, 1981; Scheen *et al.*, 1981; Dulloo & Miller, 1987) and in the rat (Young & Landsberg, 1977), starvation is followed by a lowering of the level of adrenergic activity. Thus, a probable decrease in sympathetic nervous system activity could be partly responsible for the reduction of energy expenditure and the weight-loss resistance usually observed during obesity therapy based on caloric restriction (Landsberg & Young, 1978) suggesting that enhancement of sympathetic activity could offer new perspectives (Dulloo, 1988).

A pharmacological approach aiming at an improvement in lipid mobilization and an increase in thermogenesis could be of potential interest in the treatment of obesity. The direct stimulation of β-adrenoceptors with β-agonists increases the metabolic rate and lipolysis. However, the use of classical (isoprenaline) or new (RO 16-8714) β-adrenoceptor agonists should be avoided since these agents possess powerful cardiovascular effects (Henny *et al.*, 1987) and some of them are rapidly eliminated after administration. The new atypical β-adrenoceptor agonist (BRL 26830A) which increases lipolysis and thermogenesis in animals may represent a more attractive approach since minor cardiovascular effects were observed after acute or chronic administration in man (Connacher *et al.*, 1988).

The use of α_2-adrenoceptor antagonists may also be of interest for both lipid mobilization and thermogenesis increment. These compounds have been shown to increase the sympathetic tone either through an action on the central nervous system and/or the blockade of the presynaptic α_2-adrenoceptors located on the sympathetic nerve endings. In consequence, these drugs raise plasma catecholamine and non-esterified fatty acid (NEFA) levels. The effect on NEFA levels is due both to a direct blockade of antilipolytic α_2-adrenoceptors located on the adipocytes and an indirect action through the stimulation of fat cell β-adrenoceptors by noradrenaline. These two mechanisms could exert additive effects on lipid mobilization (Zahorska-Markiewicz *et al.*, 1986; Galitzky *et al.*, 1988; Berlan *et al.*, 1991).

The present study was undertaken to evaluate lipomobilization together with sympathetic nervous system activation and thermogenesis. This comparative approach was carried out using the dog as a model. The properties of dog fat cell adrenoceptors have previously been defined and are very similar to those reported in human adipocytes (Milavec-Krisman & Wagner, 1978; Berlan *et al.*, 1982; Taouis *et al.*, 1987).

Methods

Endocrino-metabolic and cardiovascular measurements

Six normal female mongrel dogs (9–19 kg) were used for the studies. Several days before each experiment, the dogs were trained to stand still for 3 to 4 h on a Pavlov table, and accustomed to immobilization and blood sampling. At 09 h 00 min., after 18–20 h fasting, the dogs were placed on the Pavlov table. The systolic and diastolic blood pressures were recorded by means of a catheter introduced into the abdominal aorta

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Table 1 Effect of oral yohimbine administration (0.25 or 0.40 mg kg^{-1}) on plasma glucose, insulin, noradrenaline and adrenaline levels and on cardiovascular parameters (heart rate, systolic and diastolic blood pressure) in 6 conscious dogs

Time (min)	0		60		240	
Dosage (mg kg^{-1})	0.25	0.40	0.25	0.40	0.25	0.40
Glucose (mmol l^{-1})	5.2 ± 0.1	5.2 ± 0.1	4.5* ± 0.2	4.5* ± 0.1	4.7 ± 0.1	4.5 ± 0.4
IRI (μu ml^{-1})	19.6 ± 2.8	22.9 ± 6.7	41.0* ± 7.3	64.9* ± 23	29.1 ± 8.5	25.0 ± 7.8
Noradrenaline (pg ml^{-1})	242 ± 35	404 ± 92	1031* ± 189	2006* ± 505	979* ± 194	1403* ± 335
Adrenaline (pg ml^{-1})	180 ± 31	146 ± 22	294 ± 52	384* ± 116	269 ± 93	253 ± 47
Heart rate (beats min^{-1})	72 ± 7	—	101* ± 12	—	89 ± 17	—
SBP (mmHg)	161 ± 12	—	198* ± 17	—	185 ± 7	—
DBP (mmHg)	98 ± 8	—	127* ± 9	—	101 ± 7	—

IRI: immuno-reactive insulin; SBP: systolic blood pressure; DBP: diastolic blood pressure.
Values are mean ± s.e.mean.
* $P < 0.05$ when compared to corresponding values measured at time 0 min.

via the left fermoral artery under local anaesthesia (xylocaine 5%) and connected to a Gould P23ID transducer on one channel of a Honeywell recorder. Mean blood pressure was measured by electrical integration of systolic and diastolic blood pressure. Heart rate was obtained with a heart period meter triggered by blood pressure. Another catheter was placed in the jugular vein for blood sampling. The dog remained quiet until the beginning of the experiment. One blood sample was taken before drug administration. Then, yohimbine (pellets from Houde Laboratory, Paris) was administered orally. The dog received placebo or various doses of yohimbine (0.25 or 0.40 mg kg^{-1} orally). The different administrations were separated by at least one week. Other blood samples were taken 15, 30, 60, 120, 180, 240 min after yohimbine administration.

The blood samples were collected in heparinized tubes and immediately centrifuged; plasma was collected and frozen at −20°C until used. Blood glucose was determined with a glucose-oxidase technique (Biotrol kit), and insulin (IRI) by radio-immunoassay (commercial radiommunoassay kit, Institut Pasteur, Paris) with human insulin used as a standard. Non-esterified fatty acids (NEFA) were determined by an enzymatic procedure by the Wako method (commercial kit, Biolyon, Lyon) with oleic acid (1 mmol l^{-1}) in 0.2% Triton X-100 used as a standard.

For catecholamine determination, blood was collected on lithium heparin with 10 mM sodium metabisulphite and centrifuged at 5000 g at 0°C; the plasma was stored at −80°C. Catecholamines were selectively isolated from the plasma by adsorption on activated alumina, then eluted with 0.1 M perchloric acid. Dihydroxybenzylamine was used as internal standard to monitor recovery from the extraction step. Catecholamines were assayed by high performance liquid chromatography (h.p.l.c.) using electrochemical (amperometric) detection (Waters h.p.l.c. system) (Valet *et al.*, 1988).

Heat production

Heat production of dogs with or without yohimbine treatment was determined by indirect calorimetry as previously described by Vermorel *et al.* (1973). Several days before the measurements the dogs were trained to stay in a wired cage similar to the respiration chamber. On the measurement days fasting dogs (18 h) were housed in a 300 l air conditioned (20°C) open-circuit respiration chamber.

Gas exchanges were determined each minute with dual-channel paramagnetic O_2 (20–21%) and infrared CO_2 (0–1%) analysers. A computer programme was designed to account for the dead space of the chamber. Consequently, the variations in O_2 consumption and CO_2 production of dogs were recorded after a 4 and 3 min delay, respectively.

One hour after the dog had been introduced into the chamber, it received 0.5 mg yohimbine (or placebo) orally. Gas exchanges and heat production were monitored from 30 min before until 240 min after yohimbine (or placebo) administration.

Data analysis

Values presented are means ± s.e.mean. Student's paired *t* test was used for comparisons between matched pairs; differences were considered significant when *P* was less than 0.05. The Mann Whitney U test was used for analysis of unpaired data.

Results

Metabolic and endocrinological effects of yohimbine

Results are depicted in Figure 1 and Table 1. The placebo did not modify the plasma NEFA concentration. The higher dose of yohimbine induced an increase in plasma NEFA levels and a significant effect was already apparent 30 min after its

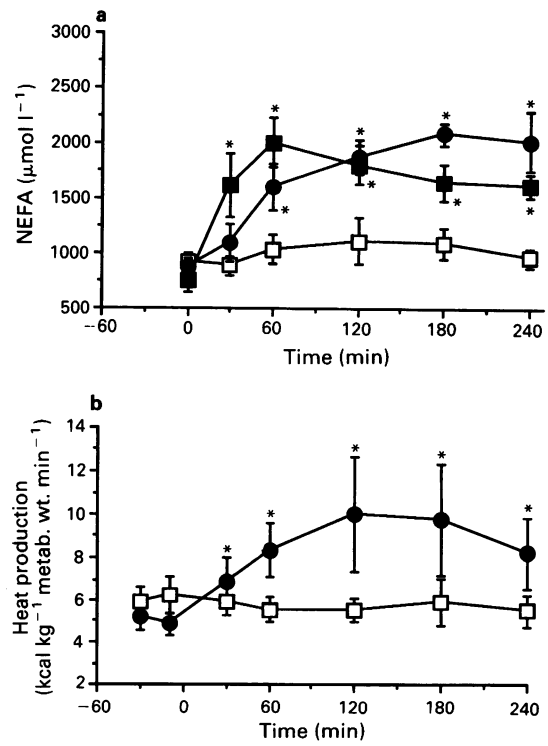

Figure 1 Effect of oral placebo or yohimbine administration (0.25 or 0.40 mg kg^{-1}) on non-esterified fatty acids plasma levels (NEFA) (a) and heat production (b) in 4 conscious dogs: yohimbine 0.25 mg kg^{-1} (●); 0.40 mg kg^{-1} (■); placebo (□). For heat production, each experimental point represents the average values over 10 min of heat production (derived from oxygen consumption measured every min). Values are mean with s.e.mean shown by vertical bars. *$P < 0.05$ when compared to corresponding values measured at time 0 min.

administration, the maximal increment being observed 30 min later. Plasma levels of NEFA remained significantly increased for at least 240 min. The lower dose of yohimbine (0.25 mg kg^{-1}) was only effective on the level of NEFA 60 min after its administration and the maximal effect was similar to that observed with 0.4 mg kg^{-1} (with regard to the highest levels reached and the duration of the action of the drug) (Figure 1).

Yohimbine also stimulated insulin secretion. The effect was transient since at the end of the experimental period, insulin values did not differ from those measured before yohimbine administration. The increase measured at time 60 min was greater with the higher dose (+183%) than with the lower dose (+89%). Yohimbine also promoted a small but significant (60 and 120 min after its administration) decrease in plasma glucose level. The effect, not observed with placebo, was similar whatever the yohimbine dose used.

A large increase in the plasma noradrenaline level was observed 60 and 240 min after yohimbine administration. The two doses were effective but the increase was two fold higher with 0.40 mg kg^{-1} (at time 60 min) and in both cases, the effect persisted until the end of the experimental period. At 240 min, the plasma levels of noradrenaline were similar. The higher dose of yohimbine also increased plasma adrenaline only 60 min after its administration but the lower dose was without significant effect. Placebo administration did not modify the parameters studied (Table 2).

Effect of yohimbine on respiratory exchanges and heat production

Measurement of respiratory exchange and heat production was investigated only with 0.25 mg kg^{-1} yohimbine since this dose significantly increased plasma noradrenaline and NEFA levels with a minor effect on insulin release.

Placebo administration was without any effect on O_2 consumption, CO_2 and energy production. Yohimbine significantly increased the respiratory exchange and heat production as compared with the pre-administration values and with the placebo (Figure 1). Maximal responses were achieved between 120 and 240 min and reached 102 and 81% when compared to placebo values. Since the increase in O_2 consumption or CO_2 production was of the same order, there was no significant effect of yohimbine on the respiratory quotient which was approximately 0.75–0.77 before and after yohimbine or placebo administration.

Effect of yohimbine on blood pressure and heart rate

Yohimbine administration induced a progressive and significant rise in blood pressure. The maximal effect occurred 60 min after its administration and then progressively decreased (Table 1). The heart rate also increased; maximal values were measured 60 min after yohimbine administration and then, heart rate progressively decreased. The values measured 240 min after yohimbine did not differ significantly from pre-administration values. Placebo administration did not modify the parameters studied (Table 2).

Table 2 Effect of oral placebo administration on plasma glucose, insulin, noradrenaline and adrenaline levels and on cardiovascular parameters (heart rate, systolic and diastolic blood pressure) in 4 conscious dogs

Time (min)	0	60	240
Glucose (mmol l^{-1})	5.5 ± 0.3	5.7 ± 0.2	5.5 ± 0.1
IRI (μu ml^{-1})	15.5 ± 4.1	12.2 ± 3.2	16.3 ± 3.8
Noradrenaline (pg ml^{-1})	320 ± 80	370 ± 73	322 ± 72
Adrenaline (pg ml^{-1})	134 ± 37	133 ± 46	155 ± 16
Heart rate (beats min^{-1})	78 ± 9	71 ± 10	73 ± 10
SBP (mmHg)	165 ± 10	155 ± 9	157 ± 4
DBP (mmHg)	87 ± 8	78 ± 5	85 ± 3

IRI: immuno-reactive insulin; SBP: systolic blood pressure; DBP: diastolic blood pressure.
Values are mean ± s.e.mean.

Discussion

This study demonstrates that yohimbine, administered to the dog by an oral route at dosages commonly tested in man (Galitzky *et al.*, 1988; Berlan *et al.*, 1991), increases lipid mobilization, the activity of the adrenergic system and heat production over a period of at least 4 h. The lipolytic response promoted by yohimbine is linked to stimulation of the adipose tissue β-adrenoceptors since propranolol administration completely blocked the response (Taouis *et al.*, 1988). The present results confirm previous observations in the dog in which higher doses of yohimbine were administered by the intravenous route (Taouis *et al.*, 1988). Interestingly, the lower dose used in this study (0.25 mg kg^{-1}) promoted a lipomobilization of the same magnitude as that previously measured with a two fold higher dose given by the i.v. route, whereas plasma noradrenaline and insulin increments were about two fold less (Taouis *et al.*, 1988). Since, in the present study, the plasma NEFA level increase was of the same magnitude with the two different doses of oral yohimbine (Figure 1), while insulin secretion and noradrenaline (Table 1) release were dose-dependent, it is suggested that the β-lipolytic pathway is fully operative with 0.25 mg yohimbine.

Thermogenesis was also increased by yohimbine. The smaller dose used in the study promoted an increase (about 100%) of respiratory exchanges and heat production (Figure 1). This effect was observed over the experimental period and the basal level was not recovered within 4 h. This thermogenic action of yohimbine is attributable to the activation of the sympathetic nervous system (reflected by the increasing plasma noradrenaline levels). The thermogenic capacities of catecholamines and other sympathomimetic drugs have been demonstrated previously. Adrenaline and noradrenaline increase the metabolic rate in man (Jung *et al.*, 1979; Katzeff & Danforth, 1981). Ephedrine (a sympathomimetic drug) also stimulates thermogenesis at a dose of 0.25 mg kg^{-1} (Astrup *et al.*, 1985). A methylxanthine like caffeine is also effective in thermogenesis since this drug increases the resting metabolic rate in young men by 16% (Acheson *et al.*, 1980).

In the present study, yohimbine, even at the lower dose, significantly increased both heart rate and blood pressure. This action could represent a major limitation for the potential use of yohimbine as an antiobesity drug. However, the stimulation of heat production largely exceeded the stimulant effect on heart rate and blood pressure. The thermogenic effect of yohimbine in dog (+100%) was also large when compared to the effect of other thermogenic drugs used in man (Jung *et al.*, 1979; Acheson *et al.*, 1980; Katzeff & Danforth, 1981). Indeed, the other parameters were also largely increased, particularly NEFA and noradrenaline plasma levels. The challenge of the utilization of this compound is now to determine whether the stimulation of thermogenesis and lipolysis could operate at lower doses of yohimbine which would be effective on thermogenesis without affecting cardiovascular parameters. It has been shown that in non-obese and obese human subjects, similar doses of oral yohimbine (0.20 mg kg^{-1}) induced lipid mobilization and sympathetic activation but were without effect on blood pressure, heart rate and plasma insulin or glucose levels (Galitzky *et al.*, 1988; Berlan *et al.*, 1991). It will be essential to check whether such a therapeutic dose is thermogenic in man.

The origin of the thermogenic action of yohimbine remains to be established. Since there is good evidence for an effect via

the activation of the sympathetic nervous system, it could be proposed that the β-adrenoceptor stimulation promotes a thermogenic effect by stimulation of the energetic metabolism in brown fat or in the muscles. In rodents, it has been demonstrated that ephedrine increases thermogenesis in brown adipocytes via β-adrenoceptor stimulation (Scheidegger *et al.*, 1984).

However, the presence of brown adipose tissue in adult dogs has never been established or investigated although this species has frequently been studied in the veterinary field (Joshua, 1970; De Bruijne, 1979) so a possible effect of noradrenaline on this tissue cannot be ruled out. In man, probably possessing lower amounts of brown adipose tissue, its involvement in thermogenesis has not been clearly demonstrated. The effect of ephedrine as a thermogenic agent arises from a major action on the skeletal muscle (Astrup *et al.*, 1985).

The effect of yohimbine on substrate utilization involved in thermogenesis activation could be deduced from the respiratory quotient. The respiratory quotient was not modified after yohimbine administration. Thus, the drug increased both lipid and carbohydrate oxidation. As proposed by Henny *et al.* (1987), lipolysis and triglyceride resynthesis processes in adipose tissue can also partly contribute to the thermogenesis through so-called futile cycles.

Drugs blocking α_2-adrenoceptors provoke insulin secretion through blockade of the α_2-adrenoceptors of the pancreatic β-cells. In the present study, yohimbine increased plasma insulin. Moreover, this effect on insulin secretion was less intense with the lower dose used while its effects on NEFA were not dose-related (Figure 1). This result confirms a previous investigation showing that the effect on insulin is transient while the effect on the plasma NEFA level is more sustained (Taouis *et al.*, 1988). It has been shown that in the dog, the insulin secretion induced by yohimbine is attributable to adrenergic activation since this effect is absent after β-blockade (Taouis *et al.*, 1988). It could be postulated that in fasting conditions (high plasma level of NEFA and low insulin and glucose levels), the increment in plasma NEFA levels predominates and that in these conditions, the insulinotropic action of noradrenaline will have a 'firing point' higher than that of its lipolytic action.

In man, it has been shown that yohimbine induces some similar pharmacodynamic effects to those observed in the dog. Moreover, in healthy male or female volunteers as well as in obese females a similar oral dosage of yohimbine did not induce either insulin or cardiovascular modifications (Galitzky *et al.*, 1988; Berlan *et al.*, 1991) whereas it did induce an increase in plasma NEFA and noradrenaline levels.

Further experiments should be undertaken to assess the possible usefulness of yohimbine (or, other new α_2-antagonists) in obesity therapy. The data from Peterson *et al.* (1988) showed that an increase in body fat stores is associated with depression of sympathetic nervous system activity in man. In addition, Klein *et al.* (1989) showed that long fasting periods even after 84 h, failed to increase or only moderately increased plasma noradrenaline levels. Taken together, these results indicate that drugs like yohimbine, able to increase sympathetic activity, lipid mobilization and thermogenesis during fasting or dietary therapy, should be of interest in the reduction of weight in obese subjects.

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References

ACHESON, K.J., ZAHORSKA-MARKIEWIZ, B., PITTET, P., ANANTHARAMAN, K. & JEQUIER, E. (1980). Caffeine and coffee: their influence on metabolic rate and substrate utilization in normal weight and obese individuals. *Am. J. Clin. Nutr.*, **33**, 989–997.

ASTRUP, A., BÜLOW, J., MADSEN, J. & CHRISTENSEN, N.J. (1985). Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. *Am. J. Physiol.*, **248**, E507–E515.

BERLAN, M., CARPENE, C., LAFONTAN, M. & DANG-LANG, L. (1982). Alpha2-adrenergic antilipolytic effect in dog fat cells: incidence of obesity and adipose tissue localization. *Horm. Metab. Res.*, **14**, 257–260.

BERLAN, M., GALITZKY, J., RIVIÈRE, D., FOUREAU, M., TRAN, M.A., FLORES, R., LOUVET, J.P., HOUIN, G. & LAFONTAN, M. (1991). Plasma catecholamine levels and lipid mobilization induced by yohimbine in obese and non-obese women. *Int. J. Obesity*, **15**, 305–315.

CAHILL, G.F., HERRERA, M.G., MORGAN, A.P., SOELDNER, J.A., STEINKE, J., LEVY, P.L., REICHARD, G.A. & KIPNIS, O.M. (1966). Hormone-fuel interrelationships during fasting. *J. Clin. Invest.*, **44**, 1751–1769.

CONNACHER, A.A., JUNG, R.T. & MITCHELL, P.E.G. (1988). Weight loss in obese subjects on a restricted diet given BRL 26830A, a new atypical beta-adrenoceptor agonist. *Br. Med. J.*, **296**, 1217–1220.

DE BRUIJNE, J.J. (1979). Biochemical observations during total starvation in dogs. *Int. J. Obesity*, **3**, 239–247.

DULLOO, A.G. (1988). Stimulation of thermogenesis in the treatment of obesity: a rational approach. *J. Obesity Weight Reg.*, **7**, 131–144.

DULLOO, A.G. & MILLER, D.S. (1987). Obesity – a disorder of the sympathetic nervous system. *World Rev. Nutr. Diet.*, **50**, 1–56.

FARREL, D.J. & WILLIAMS, V.J. (1989). The energy metabolism of rats during successive periods of weight gain and weight loss. In *Energy Metabolism of Farm Animals*. ed. Van der Honing, Y. & Close, W.H. pp. 41–44. The Netherlands: EAAP Publi. n°43 Produc. Wageningin.

GALITZKY, J., TAOUIS, M., BERLAN, M., RIVIÈRE, D., GARRIGUES, M. & LAFONTAN, M. (1988). Alpha2-antagonist compounds and lipid mobilization: evidence for a lipid mobilizing effect of oral yohimbine in healthy male volunteers. *Eur. J. Clin. Invest.*, **18**, 587–594.

HENNY, C., SCHUTZ, Y., BUCKER, A., MEYLAN, M., JEQUIER, E. & FELBER, J.P. (1987). Thermogenic effect of the new beta-adrenoceptor agonist RO 16-8714 in healthy male volunteers. *Int. J. Obesity*, **11**, 473–483.

JAMES, W.P.T., HARALDSOTTIR, J., LIDDEL, F., JUNG, R.T. & SMETTY, P.S. (1981). Autonomic responsiveness in obesity with and without hypertension. *Int. J. Obesity*, **5**, 73–78.

JENSEN, M.D., HAYMOND, M.W., GERICH, J.E., CRYER, P.E. & MILES, J.M. (1987). Lipolysis during fasting. *J. Clin. Invest.*, **79**, 207–213.

JOSHUA, J.O. (1970). The obese dog and some clinical repercussion. *J. Small Anim. Pract.*, **11**, 601–606.

JUNG, R.T., SMETTY, P.S., JAMES, W.P.T., BARRAND, M.A. & CALLINGHAM, B.A. (1979). Reduced thermogenesis in obesity. *Nature*, **279**, 322–323.

KATZEFF, H. & DANFORTH, E.J. (1981). The thermogenic response to norepinephrine, food and exercise in lean man during overfeeding. *Clin. Res.*, **29**, 663A.

KLEIN, S., PETERS, E.J., HOLLAND, O.B. & WOLFE, R.R. (1989). Effect of short and long-term beta-adrenergic blockade on lipolysis during fasting in humans. *Am. J. Physiol.*, **257**, E65–E73.

LANDSBERG, L. & YOUNG, J.B. (1978). Fasting, feeding and the regulation of the sympathetic nervous system. *N. Engl. J. Med.*, **298**, 1295–1301.

LIVAVIVATHANA, U., CAMPBEL, R.G. & BRODOWS, R.G. (1978). Control of insulin secretion during fasting in man. *Metabolism*, **27**, 815–821.

MA, S.W.Y. & FOSTER, D.O. (1986). Starvation induced changes in metabolic rate, blood flow and regional energy expenditure in rats. *Can. J. Physiol. Pharmacol.*, **64**, 1232–1258.

MILAVEC-KRISMAN, M. & WAGNER, H. (1978). Effect of the adrenergic agonists isoprenaline and noradrenaline and the alpha-blocking agents dihydroergotamine and phentolamine on the lipolysis in isolated fat cells of the rat, guinea pig, dog and man. *Biochem. Pharmacol.*, **27**, 2305–2312.

PETERSON, M.R., ROTHSCHILD, M., WEINBERG, C.R., FELL, R.D., MELEISH, K.R. & PFEIFER, M.A. (1988). Body fat and the activity of the autonomic nervous system. *N. Engl. J. Med.*, **318**, 1077–1083.

RUMPLER, W.V., SEARLE, J.L., BODWELL, C.E. & CONWAY, J.M. (1989). The long and short term effects of caloric restriction on

energy expenditure in human subjects. In *Energy Metabolism of Farm Animals*. ed. Van der Honing Y. & Close, W.H. pp. 275–278. The Netherlands: EAAP Publi. n°43 Produc. Wageningin.

SCHEEN, A., CESSION-FOSSION, A., SCHEEN-LAVIGNE, M. & LUYCKX, A. (1981). Diminution de la sécrétion d'adrénaline au cours de la diète protéique chez l'obèse. *Ann. Endocrinol.*, **42**, 531–536.

SCHEIDEGGER, K., O'CONNELL, M., ROBBINS, D.C. & DANFORTH, E.J. (1984). Effects of chronic beta-receptor stimulation on sympathetic nervous system activity, energy expenditure and thyroid hormones. *J. Clin. Endocrinol. Metab.*, **58**, 895–903.

TAOUIS, M., BERLAN, M., MONTASTRUC, P. & LAFONTAN, M. (1987). Characterization of dog fat cell adrenoceptors: variation in alpha-2 and beta-adrenergic receptors distribution according to the extent of the fat deposits and the anatomical location. *J. Pharmacol. Exp. Ther.*, **242**, 1041–1049.

TAOUIS, M., BERLAN, M., MONTASTRUC, P. & LAFONTAN, M. (1988). Mechanism of the lipid-mobilizing effect of alpha2-adrenergic antagonists in the dog. *J. Pharmacol. Exp. Ther.*, **247**, 1172–1180.

VALET, P., DAMASE-MICHEL, C., TAOUIS, M., MONTASTRUC, J.L., COTONAT, J. & MONTASTRUC, P. (1988). Acute release of catecholamines on circulating blood cell adrenoceptors and metabolic indices in the dog. *Fundam. Clin. Pharmacol.*, **2**, 267–276.

VERMOREL, M., BOUVIER, J.C., BONNET, Y. & FAUCONNEAU, F. (1973). Construction et fonctionnement de 2 chambres respiratoires du type "circuit ouvert" pour de jeunes bovins. *Ann. Biol. Anim. Biochem Biophys.*, **13**, 659–681.

YOUNG, J.B. & LANDSBERG, L. (1977). Suppression of the sympathetic nervous system during fasting. *Science*, **196**, 1473–1476.

ZAHORSKA-MARKIEWICZ, B., KUCIO, C. & PISKORSKA, D. (1986). Adrenergic control of lipolysis and metabolic responses in obesity. *Horm. Metab. Res.*, **18**, 693–697.

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