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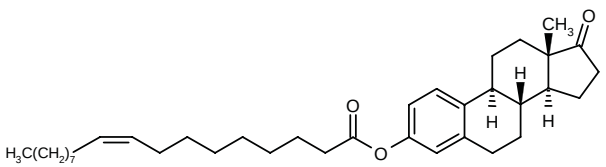
Oleoyl-Estrone

Antiobesity Drug

Estrone 3-Oleate

3-Oleoylestra-1,3,5(10)trien-17-one

3-[1-Oxo-9(*Z*)-octadecenyloxy]estra-1,3,5(10)-trien-17-one



$C_{36}H_{54}O_3$

Mol wt: 534.83

CAS: 180003-17-2

EN: 253430

Abstract

Oleoyl-estrone is a naturally occurring acyl-estrone that has slimming effects in lean and obese rats. When administered orally or intravenously, it decreases weight and fat content without causing any loss of body protein content. In obese rats, it also tends to normalize glucose and insulin levels. No significant side effects have been found with oleoyl-estrone, except for a mild estrogenic effect when administered intravenously. The oral formulation is more effective than the intravenous formulation and has no estrogenic effect. The compound has therapeutic potential as a new antiobesity and/or antidiabetic drug.

Synthesis

Oleoyl-estrone can be obtained by direct condensation of commercially available estrone (I) and oleoyl chloride (II) in anhydrous pyridine at 40 °C (1, 2). Scheme 1.

Introduction

Obesity has been defined as the pathological accumulation of fat reserves (3). It is a widespread disease with increasing severity that only recently has begun to be considered as such. The concurrent pathological traits of obesity (*i.e.*, hypertension, hypercholesterolemia, hypertriacylglycerolemia and type 2 diabetes mellitus) are responsible for the life-shortening nature of the disease. Food intake is controlled by the hypothalamus and modulated by signals from the intestine, by the levels of metabolites and insulin in blood, and also by ponderostat signals from the adipose tissue that inform the brain about the extent of fat reserves in the body. One of these ponderostat signals, leptin, is synthesized by adipocytes in proportion to fat mass and was considered to be a possible candidate for the treatment of obesity until it was found that leptin levels in humans are positively correlated with body mass index (BMI) and body fat percentage (4).

Steroid hormones are remarkably stable compounds that easily pass through the blood-brain barrier and fulfill different functions, such as being multifactorial messengers in the brain. Some steroids such as estrogens and androgens also have effects on body fat distribution. These findings prompted the study of steroid hormones as possible ponderostat signals. Oleoyl-estrone is an estrone fatty acid ester found naturally in all three lipoprotein fractions from rat plasma, although at different concentrations (Table I). It is the major estrone component in lipoproteins (5) and is synthesized in the white adipose tissue, where it is loaded into plasma lipoproteins and later is released to most tissues, where it is hydrolyzed to estrone (6). The fatty acid moiety of the molecule is a crucial factor in its effects (7). The comparison of lean and obese rats and humans revealed that estrone fatty acid levels in plasma are positively correlated with BMI, percentage of body fat, fasting insulin levels and serum leptin levels, but not with fasting glucose levels (Table II) (8). Moreover, plasma acyl-estrone levels (most of which consists of oleoyl-estrone) showed a linear correlation with BMI in nonobese women (BMI lower than 27), whereas obese women (BMI higher than 27) had plasma acyl-estrone levels higher than nonobese women but lower than expected had they retained a linear correlation with

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Scheme 1: Synthesis of Oleoyl-Estrone

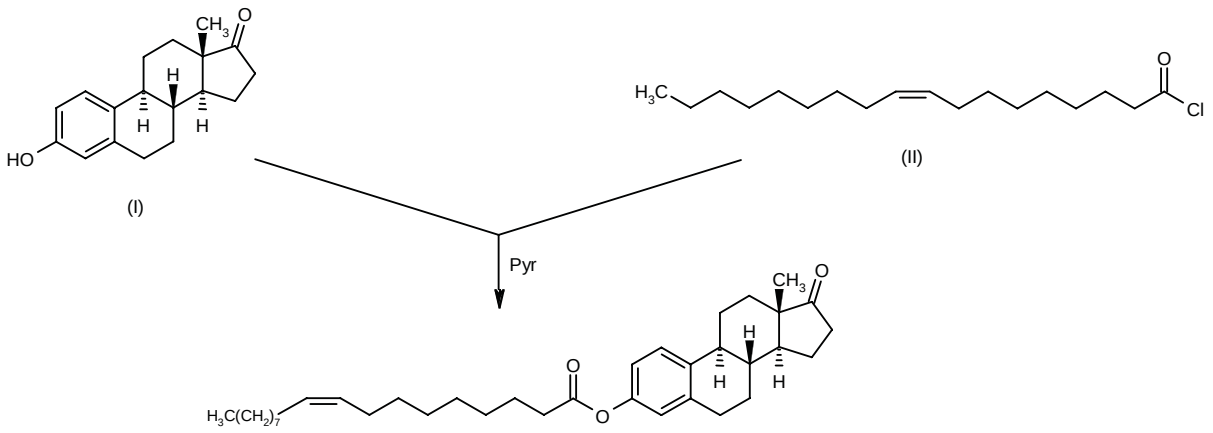

Table I: Rat lipoprotein composition (modified from ref. 5).

Lipoprotein fraction	TAG nmol/l	Free cholesterol nmol/l	Cholesterol esters nmol/l	Phospholipid nmol/l	Acyl-estrone pmol/l
Total plasma	56.9 ± 2.3	105.2 ± 2.3	399	346 ± 10	135 ± 22
LDPS	0.14 ± 0.01	5.1 ± 0.6	8.5	70.5 ± 2.0	7.3 ± 3.8
VLDL	29.5 ± 1.1	5.0 ± 0.6	2.0	16.3 ± 1.3	9.9 ± 2.0
LDL	19.3 ± 0.5	30.8 ± 4.9	50.4	38.2 ± 1.5	28.7 ± 12.7
HDL	3.0 ± 0.5	51.5 ± 3.9	374	250 ± 6	45.3 ± 25.3

LDL: low density lipoproteins; LDPS: lipoprotein-depleted plasma; HDL: high density lipoproteins; TAG: triacylglycerols; VLDL: very low density lipoproteins.

Table II: Plasma estrone-fatty acid ester levels in humans (modified from ref. 8).

	Lean men	Obese men	Lean women	Obese women
Mean BMI	24.4 ± 0.6	32.8 ± 0.7	20.3 ± 0.7	32.3 ± 0.7
% fat mass	17.51 ± 3.1	29.98 ± 2	20.43 ± 1.8	39.16 ± 1.5
Glucose (mmol/l)	5.31 ± 0.16	105.2 ± 2.3	346 ± 10	346 ± 10
Insulin (mU/l)	8.2 ± 0.8	5.1 ± 0.6	70.5 ± 2.0	70.5 ± 2.0
SI (min^{-1}/mU/l)	3.0 ± 0.3	5.0 ± 0.6	16.3 ± 1.3	16.3 ± 1.3
Leptin (μg/l)	4.3 ± 0.4	30.8 ± 4.9	38.2 ± 1.5	38.2 ± 1.5
Estrone-fatty acid ester	192 ± 13.9	51.5 ± 3.9	250 ± 6	250 ± 6

BMI: body mass index; SI: sensitivity to insulin.

BMI, thus suggesting that acyl-estrone function is altered in obesity (4).

Pharmacological Actions

Intravenous administration

The first evidence of the role played by oleoyl-estrone in body weight control came from the observation that i.v. injection of small amounts of liposomes containing oleoyl-estrone at doses of 0.1-2.5 μmol/day induced a steady weight loss in rats, especially at the beginning of treatment (9). On the other hand, weight increased in rats treated with estrone-containing liposomes. Weight loss with oleoyl-estrone was dose-dependent at doses ranging from 0.2-2 μmol/day. The only side effect found with oleoyl-estrone administration was a transient loss of appetite, which increased after a few days of treatment without increasing body weight (9). This loss of appetite was also dose-dependent; rats treated with a dose of 0.1 μmol/day·kg quickly recovered baseline feeding

Table III: Plasma parameters of lean Zucker rats fed a hyperlipidic diet supplemented with oleoyl-estrone (modified from ref. 14).

Oleoyl-estrone in diet	No oleoyl-estrone	Oleoyl-estrone 2.5 μmol/kg	Oleoyl-estrone 4.4 μmol/kg	Oleoyl-estrone 33.3 ± 3.0 μmol/kg
Glucose (mmol/l)	8.33 ± 0.23	8.51 ± 0.66	7.88 ± 0.18	6.95 ± 0.42
Urea (mmol/l)	7.35 ± 0.24	8.57 ± 0.62	6.94 ± 0.67	8.28 ± 0.19
Total proteins (g/l)	62.0 ± 1.6	65.1 ± 2.1	57.6 ± 1.6	58.6 ± 1.0
Triacylglycerols (mmol/l)	1.08 ± 0.29	0.56 ± 0.17	0.34 ± 0.03	2.39 ± 0.46
Total cholesterol (mmol/l)	1.58 ± 0.13	1.49 ± 0.09	1.51 ± 0.02	1.21 ± 0.23
Acyl-estrone (nmol/l)	98 ± 9	119 ± 29	195 ± 39	952 ± 44
Insulin (nmol/l)	0.42 ± 0.29	0.40 ± 0.11	0.17 ± 0.03	0.22 ± 0.04

habits, whereas rats receiving doses of 3.5 or 5.0 μmol/day·kg maintained lower food intakes for longer periods of time (1). Similar results were obtained when oleoyl-estrone was administered for 28 days to lean rats being fed a cafeteria diet (10) and obese Zucker fa/fa rats being fed a standard diet (11).

The decreases in body weight and food intake related to treatment with oleoyl-estrone were more pronounced in obese rats than in lean rats (43% and 22% of initial weights, respectively) (11). The study of samples of inguinal subcutaneous and periovaric white adipose tissue from lean Zucker rats treated with oleoyl-estrone revealed that the compound induced weight loss by decreasing the volume of adipocytes irrespective of tissue site (12). It was also found that lean rats (but not obese rats) maintained weight loss after the end of treatment with oleoyl-estrone, thus suggesting that the compound modified the animals' ponderostat setting (13). In all cases, weight loss was considered to be the result of a combination of lower food intake, maintenance of energy expenditure and mobilization of fat reserves, and although energy expenditure also decreased in obese rats treated with oleoyl-estrone, food intake decreased even more and created an energy gap fulfilled from body fat.

No significant changes in protein content were reported for lean and obese rats treated with chronic i.v. administration of oleoyl-estrone. Protein loss was estimated to be 8.9% of the initial protein content, which was in the range of body weight loss. This decrease was caused by the combination of unchanged urinary excretion, lower stool nitrogen levels and much lower protein intake (1, 11).

Oral administration

The finding that oleoyl-estrone is hydrolyzed to estrone, which could have opposite effects on body weight control, prompted the search for an alternative form of administration for oleoyl-estrone. Oral administration of a hyperlipidic diet supplemented or not with oleoyl-estrone to Zucker lean rats showed that oleoyl-estrone decreased food intake, fat content and body weight (up to 30% in 12 days) while increasing acyl-estrone levels in plasma. Only rats receiving the highest oral dose of oleoyl-estrone showed significant changes in plasma levels after 15 days of treatment, and no significant changes were found in plasma insulin levels (Table III). Most of the dietary estrone was excreted and no quick conversion of oleoyl-estrone into estrone was detected (14). Significant body weight loss was also found when oleoyl-estrone was orally administered to obese Zucker rats (Table IV) (15). Oral oleoyl-estrone was shown to be more effective than i.v. oleoyl-estrone, as the former induced higher weight losses at lower doses than the latter, possibly by a more natural incorporation of oleoyl-estrone through the intestine and by a limited counteracting effect of estrone. Further experiments with different oral doses of oleoyl-estrone administered to adult Wistar rats established that its slimming effects were dose-dependent (16). A more significant weight loss effect has been reported for oleoyl-estrone on male obese Zucker rats than on female rats; however, this difference did not affect other metabolic parameters and no estrogenization was observed (17).

Mechanism of Action

The slimming effects of oleoyl-estrone suggested the possibility of interaction with other compounds that also have effects on the body weight of rats, such as leptin, insulin and estrone. Leptin is a molecule with effects on body weight loss, appetite and thermogenesis similar to those reported for oleoyl-estrone. An association between oleoyl-estrone and leptin was first pointed out by the finding that i.v. administration of oleoyl-estrone inhibited leptin expression in lean rats (18). Obese Zucker rats (which express a mutated leptin receptor and show high levels of expression of leptin) lost weight after oleoyl-estrone administration without a concomitant decrease in leptin expression (19). Similar results were obtained in mice lacking a functional leptin pathway (20). *In vitro* experiments revealed that leptin promotes the uptake of estrone and its conversion to estrone esters by adipocytes (21). In obese Zucker rats, impairment of the leptin pathway resulted in a lower efficiency in the uptake of estrone and conversion to estrone esters and in the degradation of acyl-estrone from the bloodstream (6), together with the inability to maintain changes in the ponderostat setting induced by oleoyl-estrone administration

Table IV: Plasma parameters of obese Zucker rats fed a hyperlipidic diet supplemented with oleoyl-estrone for 15 days (modified from ref. 15).

Oleoyl-estrone in diet	Rats with no treatment	Rats with hyperlipidic diet	Rats with hyperlipidic diet supplemented with oleoyl-estrone
Glucose (mmol/l)	9.16 ± 1.19	15.40 ± 2.56	9.40 ± 1.20
Urea (mmol/l)	6.50 ± 0.66	7.93 ± 0.48	5.29 ± 0.61*
Triacylglycerols (mmol/l)	3.43 ± 0.15	3.86 ± 0.03	2.39 ± 0.46*
Nonesterified fatty acids (μmol/l)	251 ± 72	433 ± 41	440 ± 22
3-Hydroxybutyrate (μmol/l)	475 ± 159	347 ± 70	523 ± 104
Total cholesterol (mmol/l)	3.22 ± 0.32	4.02 ± 0.51	2.66 ± 0.24
Total amino acids (mmol/l)	4.74 ± 0.24	5.39 ± 0.20	5.93 ± 0.52
Insulin (nmol/l)	9.39 ± 2.69	12.70 ± 4.65	0.72 ± 0.26*
Total acyl-estrone (nmol/l)	179 ± 10	183 ± 11	1168 ± 129*

*$p < 0.05$ between rats with hyperlipidic diet and rats with supplemented hyperlipidic diet.

(13). Overall, these results suggested that the effect of oleoyl-estrone on body weight takes place downstream of the effect induced by leptin, and that there seems to be a negative feedback effect of oleoyl-estrone on leptin that requires a functional leptin receptor.

The effects of estrone on weight are the opposite of those induced by leptin. In rats, chronic i.v. administration of estrone increased body weight (9). Moreover, obese Zucker rats have higher plasma estrone levels than lean rats, and this is probably a major factor in the induction of obesity (6). Higher circulating estrone levels have been found in obese human subjects than in lean subjects (22). Lower plasma estrone levels have been reported in lean and obese Zucker rats actively losing weight after treatment with oleoyl-estrone (13, 19). Moreover, experiments conducted in rats showed that the ratio of esterified estrone versus free estrone in plasma slightly decreased after 6 h of starvation, partly due to an increase in free estrone levels (23). Apparently, contradictory results were reported after oral administration of a hyperlipidic diet enriched with oleoyl-estrone, which in rats induced more weight gain than in control rats being fed a nonsupplemented hyperlipidic diet. However, these effects were detected at oleoyl-estrone doses below those inducing slimming effects when administered i.v. to rats; therefore, the effects of oleoyl-estrone on body weight seem to vary depending on the method of administration (24).

The different effects of oleoyl-estrone and estrone on body weight in rats suggested that the mechanism of action of oleoyl-estrone is not directly related to the release of its estrogen moiety (9) and supported the hypothesis that the ponderostat setting depends on the balance between estrone and oleoyl-estrone levels in plasma (13). According to this model, a situation of low fat body content would promote the conversion from oleoyl-estrone to estrone that takes place in all rat tissues, thus increasing estrone levels in plasma and promoting fat deposition. In contrast, a high fat body content would increase estrone esterification in the white adipose tissue; this would increase plasma oleoyl-estrone levels and decrease plasma estrone levels, thereby promoting lipolysis (probably by adrenergic pathways) (25) and lipid oxidation in tissues such as skeletal muscle (26). Permanent exposure to high estrone levels could disrupt the estrone/oleoyl-estrone ponderostatic balance and induce obesity.

Insulin enhances the synthesis and release of leptin in humans (27), suggesting that alteration in insulin function is a key factor defining severe obesity. Intravenous administration of oleoyl-estrone decreased plasma insulin levels and increased liver glycogen levels in both lean and obese Zucker rats (18, 19). Insulin resistance found in obese Zucker rats was overcome by oral administration of oleoyl-estrone, which decreased plasma glucose and insulin levels until reaching normal values (17). Furthermore, recovery of glucose levels after i.v. oleoyl-estrone treatment and oral glucose load was faster in obese rats than in lean rats, suggesting that oleoyl-estrone enhances the insulin response to glucose (28).

Glucocorticoids induce insulin resistance and decrease thermogenesis, thereby contributing to the maintenance of body fat reserves and obesity. Chronic i.v. administration of oleoyl-estrone increases plasma glucocorticoid levels in lean and obese rats, although the latter maintain high glucocorticoid levels after the end of treatment (13, 18, 19). Furthermore, oleoyl-estrone decreases the expression of corticosterone-binding globulin in the liver and induces a loss of corticosterone binding in plasma, liver and white adipose tissue from lean and obese rats (29). This, combined with the finding that the decreases in weight, food intake and levels of glucose, insulin and leptin in plasma induced by oleoyl-estrone were much higher in adrenalectomized lean rats, supported the hypothesis that glucocorticoids play a protective role against overexpression of oleoyl-estrone and other hormones (30). The anorexigenic effects induced by oleoyl-estrone appear to be mediated by pathways other than corticotropin-releasing hormone (CRH) and neuropeptide Y, and the increase in glucocorticoid levels could be caused by extrahypothalamic production of CRH or ACTH (31, 32).

Table V: Distribution of labeled estrone or oleoyl-estrone among organs and tissues of obese Zucker rats 10 min after injection (modified from ref. 34).

Tissue/organ	Estrone (%)*		Oleoyl-estrone (%)*	
	Lean	Obese	Lean	Obese
Blood	0.99 ± 0.6	2.36 ± 1.04^{+}	14.90 ± 4.09‡	41.91 ± 4.37$^{+\ddagger}$
Lung	0.94 ± 0.19	0.89 ± 0.39	2.02 ± 0.46	2.03 ± 0.27
Spleen	0.12 ± 0.02	0.05 ± 0.02	2.26 ± 0.81‡	4.59 ± 0.71‡
Liver	23.98 ± 3.92	16.19 ± 3.07	30.53 ± 3.95	15.72 ± 4.33
Kidney	0.44 ± 0.04	0.38 ± 0.15	0.43 ± 0.11	0.30 ± 0.05
Intestine	5.31 ± 0.72	3.03 ± 0.38	1.23 ± 0.28‡	1.73 ± 1.02
Heart	0.17 ± 0.02	0.23 ± 0.09	1.29 ± 0.36‡	0.27 ± 0.04^{+}
Muscle	16.43 ± 2.08	17.50 ± 4.09	8.59 ± 2.31‡	4.04 ± 0.73‡
Skin	9.29 ± 0.60	6.00 ± 0.53^{+}	4.11 ± 0.83‡	4.45 ± 1.11
White adipose tissue	36.26 ± 4.45	48.62 ± 3.42	27.63 ± 4.49	18.79 ± 3.41‡
Brown adipose tissue	1.40 ± 0.33	0.75 ± 0.17	3.19 ± 1.10	0.03 ± 0.02‡
Uterus	0.35 ± 0.14	0.05 ± 0.02	0.05 ± 0.01‡	0.04 ± 0.01
Ovaries	0.12 ± 0.01	0.08 ± 0.03	0.06 ± 0.00‡	0.05 ± 0.01
Adrenals	0.08 ± 0.01	0.08 ± 0.03	0.08 ± 0.02	0.04 ± 0.01
Hypophysis	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01‡	0.01 ± 0.00
Brain	0.51 ± 0.06	0.33 ± 0.08	0.12 ± 0.03‡	0.90 ± 0.40
Whole rat	96.4	96.52	94.9	97.05

*Percentage of injected radioactivity. $^{+}p < 0.05$ between lean and obese groups. $^{\ddagger}p < 0.05$ between estrone and oleoyl-estrone groups.

Pharmacokinetics

Experiments conducted with tritium-labeled oleoyl-estrone showed that the compound disappears rapidly from the bloodstream. A half-life of 250 seconds was calculated for oleoyl-estrone in blood, and only 3.2% of all labeled compound was present in blood at 10 min postinjection. Oleoyl-estrone was quickly taken up by tissues, and 10 min after injection most label was detected in the liver (62.5%), spleen (7.04%) and lungs (3.53%). In most tissues, oleoyl-estrone is degraded by esterases within minutes, as demonstrated by the fact that most oleoyl-estrone is present in its intact form in blood and estrogen is present in tissues such as brown and white adipose tissue, lung, uterus and ovaries (33). *In vitro* experiments with rat adipocytes revealed that the percentage of intact oleoyl-estrone after 10 min of incubation is higher in the nuclei of cells than in the whole tissue (46 ± 7% *vs.* 18 ± 7%, respectively) (9).

The distribution of labeled estrone or oleoyl-estrone administered i.v. to lean and obese rats showed significant differences. Most free estrone was detected in white adipose tissue, liver and muscle of lean rats and in white adipose tissue, muscle and liver of obese rats. Furthermore, most free oleoyl-estrone was found in liver and white adipose tissue of lean rats and in blood and adipose tissue of obese rats. The percentage of labeled oleoyl-estrone found in blood was much higher in obese rats than in lean rats (Table V) (34).

Oral administration of labeled oleoyl-estrone resulted in a different tissue distribution. At 1 h after administration, most label was found in the blood, stomach, muscle and liver (Table VI), and the high concentration of labeled compound found in the hypothalamus suggested that this organ plays a key role in the function of oleoyl-estrone as a ponderostat signal (13). Most oleoyl-estrone (71%) was absorbed through the intestine in its intact form and was carried into passing plasma lipoproteins (mainly in the HDL fraction), although significant percentages were absorbed in the form of free estrone (17%) or hydrophilic esters of estrone (12%). The high percentage of absorbed unaltered oleoyl-estrone suggested that a larger proportion of this ester was transferred into the physiologically active compartment, which would be one of the reasons for the more potent slimming effect found with oral administration. In the liver, most oleoyl-estrone remained unaltered, whereas most free estrone was converted to hydrophilic esters and later partly eliminated through the feces. Thus, the liver seems to act as a

Table VI: Distribution of labeled estrone after a single oral dose of 250 mmol of labeled oleoyl-estrone (modified from ref. 35).

Organ/tissue	Labeled estrone (pmol/g tissue)
Stomach	1303 ± 219
Duodenum	311 ± 50
Jejunum	263 ± 59
Hypothalamus	211 ± 94
Blood	173 ± 34
Ileum	114 ± 51
Liver	93 ± 11
Brown adipose tissue	40 ± 10
Large intestine	24 ± 2
White adipose tissue	17 ± 6
Skeletal muscle	11 ± 2
Rest of the brain	3 ± 1

barrier against free estrone, and failure to perform this function has been suggested as one cause of dietary estrone-induced obesity (35).

Conclusions

Oleoyl-estrone is a ponderostatic compound that tends to have a dose-dependent normalizing effect on body weight and fat content through normal physiological mechanisms already present in the body. It decreases weight without inducing significant changes in the body protein content, thus leading to a situation very different from that found with starvation or hypocaloric dieting, where initial nitrogen wasting and difficulties in maintaining nitrogen balance and body protein are common (1).

Treatment with oleoyl-estrone is essentially safe. When administered to young rats, it delayed their growth rate but induced no changes in plasma glucose, urea and triacylglycerols or total cholesterol levels (36). No significant side effects have been reported for oleoyl-estrone when administered to adult rats, although it has been suggested that the compound could induce estrogenic side effects via its conversion to estrone and, later, to 17β-estradiol. However, a recent report has described that binding of oleoyl-estrone to the α estrogen receptor (usually associated with estrogenic effects) is negligible and only mild estrogenic effects are found with i.v. administration of the compound to female rats. Oral oleoyl-estrone induced no estrogenic effects even at doses higher than those administered intravenously (37).

In conclusion, oral oleoyl-estrone shows great potential as a possible antiobesity drug. Its counterbalancing effects on the weight increase induced by high estrone levels are especially relevant when bearing in mind that estrone is widely present in foodstuffs included in Western diets (24). The effects of oral oleoyl-estrone on glucose and insulin levels also suggest a potential use of the agent in the treatment of type 2 diabetes. Future clinical trials to be conducted in humans will provide new information on this promising new drug.

Source

Oleoyl-Estrone Developments, SL (ES) codeveloped with Manhattan Pharmaceuticals (US).

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