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Research Communications

Oleoyl-estrone induces the massive loss of body weight in Zucker *fa/fa* rats fed a high-energy hyperlipidic diet

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To test whether oleoyl-estrone plus a hyperlipidic diet affects body weight in Zucker fa/fa *rats, 13-week-old male Zucker obese (fa/fa) rats initially weighing 440–470 g were used. They were fed for 15 days with a powdered hyperlipidic diet (16.97 MJ/kg metabolizable energy) in which 46.6% was lipid-derived and 16.1% was protein-derived energy and containing 1.23 ± 0.39 μmol/kg of fatty-acyl esters of estrone. This diet was supplemented with added oleoyl-estrone to produce a diet with 33.3 μmol/kg of fatty-acyl estrone. Oral administration of oleoyl-estrone in a hyperlipidic diet (at a mean dose of 0.5 μmol · kg^{-1} · d^{-1}) resulted in significant losses of fat, energy and, ultimately, weight. Treatment induced the maintenance of energy expenditure combined with lower food intake, creating an energy gap that was filled with internal fat stores while preserving body protein, in contrast with the marked growth of controls fed the hyperlipidic diet. Treatment of genetically obese rats with a hyperlipidic diet containing additional oleoyl-estrone resulted in the loss of fat reserves with scant modification of other metabolic parameters, except for lower plasma glucose and insulin levels. The results agree with the postulated role of oleoyl-estrone as a ponderostat signal.* (J. Nutr. Biochem. 11:530–535, 2000)

Keywords: obesity; body weight; Zucker *fa/fa* rat; oleoyl-estrone; hyperlipidic diet; energy balance

Introduction

Obesity constitutes one of the most widespread metabolic disorders in our contemporary society. It is closely related to maturity-onset diabetes and other hormone and energy regulation dysfunctions;[1] in all cases, the loss of excess fat is an essential step for overall improvement in and reduction of morbidity and mortality.[2,3]

Oleoyl-estrone induces the dose-dependent loss of body fat reserves when injected chronically[4,5] or given orally. This effect is closely related to its molecular structure because the modification of the fatty acid or changes in the steroid moiety acutely diminish these effects.[6] The slimming effects of oleoyl-estrone are more deep and prolonged than the transient estrogen-induced decrease in body weight (BW).[7] Oleoyl-estrone does not interact with the estrogen receptor (unpublished results) and induces the dose-dependent loss of fat (sparing protein[4]) by decreasing food intake and maintaining energy expenditure.[5]

We have observed that oleoyl-estrone affects the BW of lean,[4] obese,[8] and cafeteria diet-fed rats.[9] This is accomplished by markedly reducing body fat while sparing protein.[4,5] Oleoyl-estrone treatment, however, maintains the energy homeostasis of the rat within physiological limits, with limited changes in plasma metabolites.[10] Circulating oleoyl-estrone, present mainly in the lipoproteins,[11] is closely correlated with body mass in normal-weight or overweight humans,[12] but not in the morbidly obese.[13]

The marked effects of oleoyl-estrone on body fat reserves is achieved through a decrease in food intake and the

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maintenance of energy expenditure at higher basal levels.[5] Treatment with oleoyl-estrone decreases both circulating insulin and leptin levels, but induces a marked counter-regulatory glucocorticoid and corticosterone-binding globulin response.[10,14] Oleoyl-estrone decreases the expression of the *Ob* gene[6] in lean but not in *fa/fa* rats,[15] and lowers the weight reference setting in lean but not in *fa/fa* rats.[16] This implies that although the mechanism of action of oleoyl-estrone does not require leptin, there is a close relationship between both hormones: Despite its levels being lowered by oleoyl-estrone, leptin induces the synthesis of oleoyl-estrone in adipocytes.[17]

We previously observed that oleoyl-estrone given orally in a powdered hyperlipidic diet strongly potentiated the loss of fat in normal-weight rats, exhausting their fat reserve but sparing protein (unpublished results). The Zucker *fa/fa* rat shows obesity and insulin resistance,[18] which makes them a choice animal model for the study of obesity. Because the Zucker obese rat tendency to accumulate fat is enhanced by lipid-rich hypercaloric diets,[19] in this study, we tested whether this trend may be reversed by the presence of oleoyl-estrone in the diet.

Materials and methods

Three groups of 13-week-old male Zucker obese (*fa/fa*) rats initially weighing 440–470 g were used. The rats were kept under standard conditions and were fed with a powdered high-energy hyperlipidic diet—control diet (HL-control)—(B&K, Sant Vicent dels Horts, Spain) that contained 22.18% fat, 17.23% protein, 4.93% fiber, 1.86% minerals, 36.31% starch, 3.43% sugars, and 6.60% water, with a gross energy content (measured using a bomb calorimeter; C-7000, IKA, Heitersheim, Germany) of 19.10 MJ · kg^{-1} and metabolizable energy content of 16.97 MJ · kg^{-1} (calculated from the standard values of its main components); 46.6% of the energy was derived from lipid, 37.2% from carbohydrate, and 16.1% from protein. Samples of this diet were used for the evaluation of naturally occurring acyl-estrone esters by means of extraction with anhydrous methanol (Panreac, Montcada, Spain) in a Soxhlet followed by saponification and radioimmunoassay of the estrone released.[20] The diet contained a mean of 1.23 ± 0.39 ($n = 5$) $\mu mol \cdot kg^{-1}$ fatty-acyl esters of estrone. A part of this diet was supplemented at origin with oleoyl-estrone (Salvat, Esplugues de Llobregat, Spain). The analysis of the supplemented diet (HL-OE) showed an acyl-estrone content of 33.3 ± 3.0 $\mu mol \cdot kg^{-1}$ ($n = 5$). Control and oleoyl-estrone-laced diets were given for 15 days to groups of 5 rats; the diet was given in the form of balls formed by kneading the powdered diet with a small amount of water. The weight of the rats was periodically measured, and their food consumption was determined from the differences in weight of the food balls, after correcting for humidity (comparing with similar balls left in cages with no rats). The dose of oleoyl-estrone ingested by the rats was computed from the food intake and acyl-estrone content of the diet.

At the end of the experiment, the rats were anesthetized with ethyl ether, and blood from heart puncture was used to obtain plasma. The rats were then killed by cervical dislocation and dissected, cleaned of intestinal contents, weighed again, and sealed in polyethylene bags that were subsequently autoclaved at 120°C for 2 hr; the whole rat was then minced to a smooth paste with a blender.

Plasma was used for the estimation of glucose, total cholesterol, triacylglycerols, and urea using a dry-chemistry strip auto analyzer (Spotchem, Menarini, Firenze, Italy) as well as for the estimation of 3-hydroxybutyrate (kit 907979, Boehringer Mannheim, Mannheim, Germany) and non-esterified fatty acids (kit 1383175, Boehringer Mannheim, Mannheim, Germany). Plasma samples were also used for the determination of total (i.e., mainly esterified) estrone[20] and insulin (rat insulin kit, Amersham, Amersham, UK). Plasma amino acids were determined with an ALPHA-PLUS (Pharmacia, Uppsala, Sweden) amino acid analyzer and a ninhydrin method.[21]

A group of 5 randomly selected rats (intact animals) were killed at the beginning of the experiment and used as Day 0 controls. They were neither exposed to the HL-control diet nor to oleoyl-estrone. These animals were processed as indicated for the experimental groups; their plasma was used for metabolite and hormone determinations and their bodies were also processed to a fine rat paste.

The rat carcass paste of all three groups was used for the estimation of the proportions of water (differential weighing after 24 hr at 100°C), lipid,[22] energy (bomb calorimeter), and nitrogen. The latter was measured as total N with a Carlo Erba NA-1500 elemental analyzer, and then converted into protein using a factor of 5.5.[23]

Energy intake was estimated from the mean amount of food consumed per day and per cage of 2 to 3 rats. Energy accrual was determined from the differences in energy content of intact, HL-control, and HL-OE rats when they were killed. Mean energy expenditure for the 15-day period was estimated as the difference between energy intake and energy accrual. For direct comparison between widely different BWs, the data for energy expenditure were corrected by an alometric index[24]; that is, $BW^{0.75}$.

Statistically significant differences ($P < 0.05$) between groups were determined using one- or two-way ANOVA programs followed by the Duncan test and Student's *t*-test.

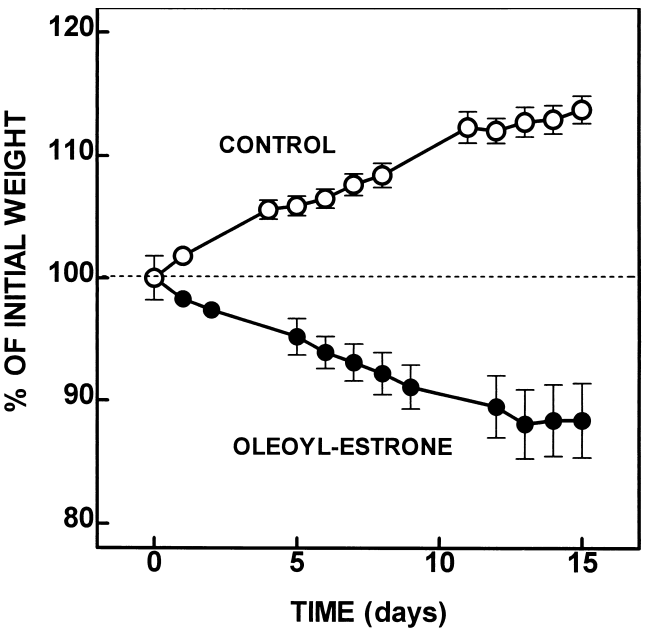

Figure 1 Changes in body weight in Zucker *fa/fa* rats induced by a hyperlipidic diet (CONTROL) containing oleoyl-estrone at a mean daily dose of 0.5 $\mu mol \cdot kg^{-1} \cdot d^{-1}$ (OLEOYL-ESTRONE). Values represent the mean ± SEM of 5 different animals per group and are expressed as a percentage of their initial weight. The differences between both groups were statistically significant ($P < 0.001$, ANOVA).

Table 1 Body composition changes of Zucker obese rats fed a hyprlipidic diet containing added oleoyl-estrone or not, compared with intact controls

Parameter	Initial (Day 0)			Day 15		Changes between Days 0–15	
	Intact	HL-control	HL-OE	HL-control	HL-OE	HL-control	HL-OE
Net body weight							
(g)	441.1 ± 11.2	463.5 ± 23.9	461.3 ± 17.8	526.4 ± 25.6	406.3 ± 11.1*	62.9 ± 5.0	−55.0 ± 15.0*
(%BW)						13.6	−11.9
Protein							
(%BW)	14.70 ± 0.33	*14.70*	*14.70*	16.09 ± 0.46	15.92 ± 0.80		
%[a]						*24.9*	*−3.9*
(g)	64.83 ± 1.64	*68.13 ± 3.51*	*67.81 ± 2.61*	84.03 ± 5.47	64.87 ± 6.32	*16.86 ± 2.78*	*−2.94 ± 4.60**
Lipid							
(%BW)	35.79 ± 1.06	*35.79*	*35.79*	38.15 ± 2.15	34.71 ± 1.60		
%[a]						*21.2*	*−14.2*
(g)	158.50 ± 4.00	*165.87 ± 8.55*	*165.09 ± 6.36*	201.11 ± 16.06	141.03 ± 7.33*	*35.23 ± 12.31*	*−24.06 ± 7.95**
Water							
(%BW)	42.75 ± 0.40	*42.75*	42.75	41.08 ± 0.9	42.5 ± 2.1		
%[a]						*6.8*	*−13.8*
(g)	192.9 ± 4.9	*202.8 ± 10.5*	*201.8 ± 7.8*	215.9 ± 9.9	172.5 ± 9.1*	*13.1 ± 6.3*	−29.3 ± 13.8*
Energy							
(kJ/g)	19.40 ± 0.32	*19.40*	*19.40*	20.87 ± 0.57	19.28 ± 0.82		
(MJ)	8.556 ± 0.217	*8.991 ± 0.464*	*8.949 ± 0.345*	11.208 ± 0.712	8.042 ± 0.299*		
%[a]						*22.7*	*−11.0*
(g)						*2031 ± 265*	*−993 ± 223**

Intact–rats receiving no treatment. HL-control–rats receiving the hyperlipidic diet. HL-OE–rats receiving the hyperlipidic diet with added oleoyl-estrone. BW–body weight.
Values are the mean ± SEM of 5 rats per group. Data in *italics* were estimated or calculated; all other data were experimental.
[a]Percentage of change in 15 days.
Significance of the differences between HL-control and HL-OE groups: $^*P < 0.05$.

Results

The mean oleoyl-estrone intake by HL-controls was 0.06 $\mu mol \cdot kg^{-1} \cdot d^{-1}$, and that of HL-OE rats only 10-fold higher: 0.5 $\mu mol \cdot kg^{-1} \cdot d^{-1}$.

Figure 1 shows the changes in BW experienced by control and oleoyl-estrone-treated rats. In 15 days, controls fed the hyperlipidic diet increased about 0.91% per day, whereas in oleoyl-estrone treated rats, BW decreased at the rate of 0.79% per day; at the end of the experiment, treated rats weighed about 77% of that of the controls. This loss of weight was mainly due to the loss of fat, as presented in *Table 1*, where the composition of HL-controls and HL-OE rats is compared with that of intact rats. In 15 days, HL-control rats increased their initial protein content by one fourth, but the losses observed in HL-OE were not different from zero. In contrast, HL-controls increased their lipid reserves by 21% but HL-OE rats lost 14% of their calculated initial fat. The changes in total carcass energy content followed the lipid pattern: HL-controls increased 22% and HL-OE rats lost 11% of the calculated initial energy.

The estimated energy balances are presented in *Table 2*. Oleoyl-estrone induced a marked decrease (about 30%) in food intake in HL-controls. This energy imbalance was only partially covered with internal reserves, which resulted in lower energy expenditure in HL-OE rats than in HL-control rats. In HL-controls, 32% of the energy ingested was stored as body fat or protein (i.e., 48% of the estimated energy expenditure), In contrast, HL-OE rats supplied 34% of the current energy budget (energy expenditure) from internal stores, meaning that food intake provided only two thirds of the energy needed for sustenance, with the rest being drawn, essentially, from the fat stores. In any case, the energy expenditure of HL-OE rats was 32% lower than that of HL-controls; this was maintained even after correcting for size: 24% lower in treated rats.

Table 3 shows metabolite and hormone levels in the plasma of rats on Day 15. There were statistically significantly lower values for urea, triacylglycerols, and insulin, and higher values for total estrone in HL-OE rats compared with HL-controls. HL-controls showed higher triacylglycerol levels than intact rats. Oleoyl-estrone treatment tended to regularize glucose, urea, triacylglycerols, and cholesterol levels, which were more similar to those in the intact rat than the HL-control rat.

Table 2 Energy balance of Zucker obese rats fed a hyperlipidic diet with or without added oleoyl-estrone

Parameter	HL-control	HL-OE
Energy intake		
(g/day)[a]	21.21 ± 0.31	6.28 ± 0.84*
(W)[b]	5.21 ± 0.08	1.59 ± 0.21*
Energy accrual		
(W)	1.68 ± 0.22	−0.82 ± 0.18*
Energy expenditure		
(W)	3.53	2.41
$(mW/g^{0.75})$[c]	33.6	25.4

[a]Expressed as dry weight; mean ± SEM of two cages; energy content of 21.22 kJ/g.
[b]Energy data have been presented in watts ($J \cdot s^{-1}$) for easier comparison within a comparable time frame.
[c]For this calculation, the body weight used was the mean of initial plus final body weights.
Significance of the differences between groups: $^*P < 0.05$.

Table 3 Plasma metabolites in Zucker obese rats fed a hyperlipidic diet containing added oleoyl-estrone or not

Parameter	Intact	HL-control	HL-OE
Glucose (mM)	9.16 ± 1.19	15.40 ± 2.56	9.40 ± 1.20
Urea (mM)	6.50 ± 0.66	7.93 ± 0.48	5.29 ± 0.61*
Triacylglycerols (mM)	3.43 ± 0.15	3.86 ± 0.03†	2.39 ± 0.46*
NEFA (μM)	251 ± 72	433 ± 41	440 ± 22
3-Hydroxybutyrate (μM)	475 ± 159	347 ± 70	523 ± 104
Total cholesterol (mM)	3.22 ± 0.32	4.02 ± 0.51	2.66 ± 0.24
Total amino acids (mM)	4.74 ± 0.24	5.39 ± 0.20	5.93 ± 0.52
Insulin (nM)	9.39 ± 2.69	12.70 ± 4.65	0.72 ± 0.26†,*
Total (acyl) estrone (nM)	179 ± 10	183 ± 11	1168 ± 129†,*

Values are the mean ± SEM of 5 rats per group.
Intact–rats receiving no treatment. HL-control–rats receiving the hyperlipidic diet. HL-OE–rats receiving the hyperlipidic diet with added oleoyl-estrone. NEFA–non-esterified fatty acids.
Statistical significance of the differences, versus intact rats: †$P < 0.05$; between HI-control and HL-OE: *$P < 0.05$.

Plasma amino acids in intact rats and the changes induced by hyperlipidic diet and oleoyl-estrone ingestion are shown in *Figure 2*. Hyperlipidic diet induced increases in branched-chain amino acids (significant for valine), and a decrease in glycine when compared with intact rats. The citrulline + ornithine versus arginine ratio in HL-control rats (1.3 ± 0.1) was not different from that in intact rats (1.7 ± 0.4). The oleoyl-estrone-laced hyperlipidic diet, however, induced more changes in the amino acid pattern: There were significant differences between HL-OE and intact rats for glutamate + glutamine, serine, and glycine (higher in HL-OE) and there were significant differences between HL-OE and HL-controls for valine, phenylalanine, and arginine (lower in HL-OE) and glutamate + glutamine, serine, and glycine (higher). The citrulline + ornithine versus arginine ratio in HL-OE rats (2.2 ± 0.6) was higher (n.s.) than that in intact and HL-control rats.

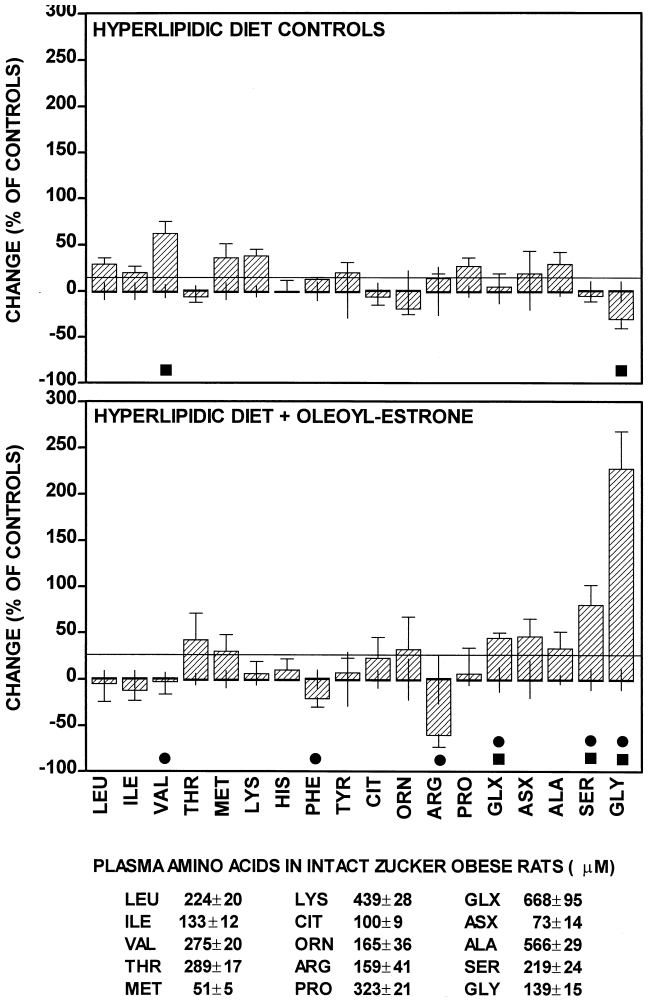

Figure 2 Plasma aminograms of Zucker *fa/fa* rats fed a hyperlipidic diet with added oleoyl-estrone at a mean daily dose of 0.5 $\mu mol \cdot kg^{-1} \cdot d^{-1}$ or without. The columns show the differences in percentage of the mean data for the corresponding amino acid of intact rats; the SEM values are indicated by a T-type line. The SEM values of intact rats are also shown as straight uncrossed lines. The mean value of change for the sum of all amino acids is represented by a thin line parallel to the origin. As a reference, the absolute concentrations of plasma amino acids in intact rats (in μM) are given at the bottom of the Figure. Statistical significance of the differences between means (one-way ANOVA plus Duncan test) for control and oleoyl-estrone groups: The effect of oleoyl-estrone on amino acids was significant ($P < 0.05$) for Val, Phe, Arg, Ser, and Gly. The specific differences versus intact rats ($P < 0.05$) have been indicated by a black square, and those between hyperlipidic diet-controls and those receiving oleoyl-estrone by a black circle.

Discussion

Treatment with oleoyl-estrone induces the dose-dependent loss of BW, essentially fat,[4,5] of both normal-weight and obese rats.[8] The oral administration of oleoyl-estrone induces a more marked response than the intravenous injection of the hormone in liposomes because the fat-shedding effects of oleoyl-estrone are partially counteracted by the estrone freed by hydrolysis.[25] The administration of moderate doses of oleoyl-estrone in the food, however, induces the rapid loss of body fat without apparent ill-effects, but maintaining plasma glucose and other energy parameters within the physiological range.[10,16] This includes the decrease of both leptin and insulin levels,[10] and the maintenance of energy expenditure well above the values of energy intake.[5]

Oleoyl-estrone directly affects insulin[26] and adrenergic pathways;[27] its synthesis is modulated by leptin[28] and reflects the body fat mass, at least in humans.[12] Its effects on food intake are dose-dependent[29] and are not mediated by neuropeptide Y.[30] Probably, the mechanism of action of oleoyl-estrone implies direct effects on lipolysis,[27] leptin function, and nuclear receptor-mediated actions.[4]

The Zucker *fa/fa* rat tends to accrue inordinate amounts of fat (but also of protein) when exposed to hypercaloric diets,[19] which results in a massive increase in body mass. The results presented here agree with this trend because the rate of growth neared 1% per day in controls. The presence of oleoyl-estrone in the diet resulted in a marked decrease in food consumption, in agreement with what happened in animals in which the hormone was injected;[4] this limited the dose of oleoyl-estrone ingested to only 0.5 μmols per kg of rat per day, a dose much lower than the standard for intravenous infusion (3.5 $\mu mol \cdot kg^{-1} \cdot d^{-1}$). In spite of this

difference and the presence of a high-energy diet, treated rats lost weight and energy, essentially fat. This was accomplished with minimal protein losses despite a drop of 12% in BW in 15 days. These results agree with those observed in lean rats treated with oral oleoyl-estrone (unpublished results), and support the hypothesis that the mechanism of action of oleoyl-estrone does not involve leptin (the Zucker *fa/fa* rat has a defective leptin function because the leptin receptor is not operative).[31]

The loss of fat is accomplished by draining about one third of the energy needed from internal stores, but maintaining two thirds sustained by food intake. This allows a fine regulation of glucose levels, with a marked lowering of insulin levels, which contrasts with the well-known insulin resistance of Zucker obese rats;[32,33] in oleoyl-estrone-treated *fa/fa* rats, the parallel decrease of insulin and glucose compared with HL-controls indicates that oleoyl-estrone counteracts the marked diabetogenic effect of the hyperlipidic diet, increasing insulin efficiency. Plasma lipids decreased under oleoyl-estrone treatment. The net loss of fat and maintenance of homeostatic parameters, especially the lowering of insulin resistance maintaining glucose levels, hint at the feasibility of the eventual use of oleoyl-estrone for the treatment of obesity and type II diabetes in humans.[13] Oleoyl-estrone treatment enhances the insulin response to a glucose challenge, improving the glucose response in both lean and obese rats.[34]

The lower urea and sustained availability of amino acids in oleoyl-estrone-treated rats despite an active mobilization of internal reserves and a significant loss of BW and energy agrees with the limited loss of protein. This protein-sparing effect is a feature of oleoyl-estrone effects on BW and energy.[4,5] The amino acid data suggest an imbalance in the operation of the urea cycle because despite accrual of other intermediates, HL-OE rat arginine levels dropped more than 60% compared with intact rats and 65% compared with HL-controls. The massive accumulation of glycine in HL-OE rats suggests that the hepatic glycine cleavage system[35] is affected, or else that glycine synthesis by the kidney is enhanced. The increased levels of glutamate + glutamine, serine, and other nonessential amino acids support the second hypothesis. In contrast, branched-chain amino acid levels tended to be lower than in HL-controls, which agrees with a more active lipid (β-oxidation) metabolic utilization.

The results presented here show a powerful slimming effect of oleoyl-estrone in genetically obese Zucker *fa/fa* rats. This occurs in spite of consumption of a hyperlipidic diet, with scant modification of energy homeostasis parameters, lowering of insulin resistance, and sustained drainage of fat reserves sparing protein.

Acknowledgments

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