Effect of catecholamines on lipolysis and esterification in vitro in adipose tissue of sheep fed low and high energy diets

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The purpose of this study was to determine effects of the catecholamines clenbuterol, norepinephrine, and isoproterenol (10^{-5} M), and dibutyryl-cyclic AMP (20 mmol/L on lipolysis and palmitate esterification in subcutaneous adipose tissue from sheep when fed a low energy diet, and then again when fed a high energy diet ad libitum. On the low energy diet, all catecholamines and cyclic AMP were lipolytic, and all inhibited palmitate esterification by 48% or more. When fed the high energy diet for 6 wk, only isoproterenol and cyclic AMP were lipolytic and inhibitory toward palmitate esterification, but less so than when sheep were fed the low energy diet. Blood plasma concentrations were not different for glucose, but free fatty acid greater and insulin concentrations lower for low energy-fed than for high energy-fed sheep. The mechanism for the altered catecholamine potency in ovine adipose tissue when sheep were fed the high energy diet is not clear; however, regulation of adrenergic receptors by changes in hormonal and/or metabolite balance because of diet were likely involved.

Keywords: catecholamines; lipolysis; esterification; adipose tissue; ovine; diet

Introduction

Fat deposition in mammalian adipose tissue ultimately occurs by triacylglycerol biosynthesis.[1] Research on glycerolipid biosynthesis in ovine adipose tissue has been minimal, and its hormonal regulation is not well understood. Compared with adipose tissue of other species, incubation of porcine adipocytes in the presence of the synthetic beta-adrenergic agonist, clenbuterol, did not stimulate lipolysis.[2] Thornton et al.,[3] however, observed stimulation of lipolysis by clenbuterol in isolated ovine adipocytes. Another synthetic beta-adrenergic agonist, isoproterenol, inhibited glycerolipid biosynthesis in porcine adipose tissue,[4] and norepinephrine inhibited it in rat[5] and porcine[6] adipose tissue. Whereas Payne and Masters[7] did not observe an effect of epinephrine on fatty acid esterification in ovine adipose tissue in vitro, McNamara and Hillers[8] observed decreased rates of esterification in adipose tissue of cows in lactation, a condition characterized by increased sensitivity to catecholamines.[8] Moreover, lactation is energy depleting, and would mimic differences in metabolism between high- and low-energy fed animals. Effectiveness of beta-adrenergic agonists, therefore, may be influenced by lactation and diet because not only were rates of basal and catecholamine-stimulated lipolysis increased[8–11] and lipid synthesis rates decreased[9,11,12] during lactation in bovine, but rates of lipolysis stimulated by epinephrine in vitro in adipose cells from high concentrate-fed dairy cows was about one-half that of cows fed a less energy-dense diet.[13]

The purpose of the present research was to compare effects of isoproterenol (ISO), clenbuterol (CLE), norepinephrine (NOR), and dibutyryl cyclic-3′, 5′-adenosine monophosphate (cA) on lipolysis and palmitate esterification in adipose tissue in vitro of wethers when

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first fed a maintenance energy diet, and then fed a high energy diet ad libitum.

Materials and methods

Six adult Suffolk wethers, approximately 2 yr old, were fed a low energy (LE) diet that consisted of alfalfa hay and poor quality grass to maintain an average body weight of 64.8 ± 1.8 (SEM) kg; wethers were fed this diet for about 5 wk before obtaining the first adipose tissue biopsy. These same wethers were then fed a high energy diet (HE) consisting of two-thirds corn and one-third dehydrated alfalfa for 6 wk, after which the second adipose tissue biopsies were obtained; wethers then weighed 80.6 ± 1.5 kg. To obtain biopsies, wethers were anesthetized by intravenous administration of 10 mL of pentabarbitol and samples of subcutaneous adipose tissue were taken adjacent to the tuber ischiadicum (tail head region). Adipose tissue biopsies were placed in Krebs-Ringer bicarbonate buffer (KRB), pH 7.4 at 37° C and brought immediately to the laboratory. Wounds were closed with absorbable sutures, and animals received antibiotics for 5 days following surgery. There was no post-surgical infection. Wethers were housed indoors in individual 1.22 × 2.44 m pens. Fresh water was always available as was feed when wethers were fed ad libitum.

Tissue slices were 0.5 mm thick and preincubated in KRB that contained 4.5% bovine serum albumin (BSA) (Armour Pharmaceutical Co., No 2295-00, Kankakee, IL) and 10 mmol/L glucose for 90 min at 37° C. The ratio of adipose tissue to preincubation medium was kept 1:10 (wt/vol) in all experiments by weighing tissue and adding a 10-fold volume of buffer. Preincubations were in Erlenmeyer flasks (250 mL) placed in a gyro-rotary water bath (2.5 RPS).

For adrenergic hormone comparisons, 90–110 mg of preincubated tissue slices were incubated in screw cap siliconized tubes (25 × 150 mm) that contained 3 mL of medium: KRB (pH 7.4, 37° C) with 4.5% BSA, 10 mmol/L glucose, 0.03 M theophylline, 1.0 μCi ^{14}C-palmitate (carboxylate-^{14}C-palmitic acid, NEC-075H, New England Nuclear, Boston, MA USA), 0.25 mmol/L palmitate, 0.56 mmol/L ascorbate, and hormone or cA. Concentrations of CLE (Boehringer Ingelheim, St. Joseph, MO), ISO, and NOR were 10^{-5} M, and 1000 μU/mL and 20 mmol/L were used for bovine insulin and cA, respectively. Maximal lipolytic responses occurred with 10^{-5} M ISO; therefore, 10^{-5} M was used for ISO, CLE, and NOR. For cA, 20 mmol/L resulted in maximal lipolysis. Insulin served as a positive control, and no-hormone incubations served as standard controls. All reagents, hormones, and agonists were purchased from Sigma Chemical Co. (St. Louis, MO USA) unless specified otherwise.

Tubes were sealed under an atmosphere of 5% CO_2 in oxygen and incubated in a gyro-rotary water bath (2.5 RPS) at 37° C for 3 hr (linear with time). After incubation, 1 mL of medium was frozen and stored at −20° C. Subsequent titrimetric analysis of free fatty acids[14] was done on all tubes to evaluate lipolysis as well as to correct for fatty acid pool dilution of substrate palmitate. With 0.25 mmol/L palmitate in the medium, fatty acid release was linear over the 3-hr incubation. The fatty acid concentration at 1.5 hr was calculated for each tube and used as the fatty acid pool to calculate specific activity.

Incubations were stopped by quickly rinsing tissue slices first with a mixture of KRB:BSA:water (66:15:18, vol:vol:vol) and second with a solution of KRB:water (66:33, vol:vol) (37° C) to remove excess ^{14}C-fatty acid and BSA, respectively. Reactions were stopped by placing slices in tubes (125 × 25 mm) that contained 10 mL of chloroform:methanol: water (1:2:.8, vol:vol:vol) as extraction solvent.[15] Details of lipid extraction and separation of residual fatty acid from glycerolipids by thin-layer chromatography have been described.[16]

Blood samples were obtained by jugular vein puncture from each wether when adipose tissue biopsies were obtained. Plasmas were harvested by using heparin as anticoagulent, and then stored at −70° C. Plasma samples were analyzed for free fatty acids titrametrically,[14] for glucose by using a diagnostic kit (No. 115, Sigma Chemical Co.), and for insulin by using an assay kit (Coat-A-Count No. TKIN1, Diagnostic Products Co., Los Angeles).

Statistical analysis employing analysis of variance procedures for one-way, balanced designs,[17] and computations were made by using the Statistical Analysis System[18] software package (SAS, Inc., Cary, NC USA). Lipolysis and palmitate esterification data were analyzed separately for hormone effects within each dietary energy regimen. Because the experimental design did not allow for measurement of interassay variation between experiments conducted on tissue from the two dietary energy regimens, no direct effects of dietary energy were considered. However, for each hormone studied, the lipolytic and esterification activities as percentages of the no-hormone control were calculated and used to compare effects of dietary energy by using the paired t test. Dietary energy was the independent variable for paired t test analyses of plasma concentrations of glucose, insulin, and free fatty acids.

Results

Effects of catecholamines on lipolysis in adipose tissue of wethers fed LE are illustrated in *Figure 1*. No-hormone control (C) and INS-treated tissue resulted in similar rates of fatty acid release (lipolysis). Incubation of adipose tissue with CLE only slightly stimulated lipolysis, but the effect was not significant when compared to C. Incubation with NOR and ISO re-

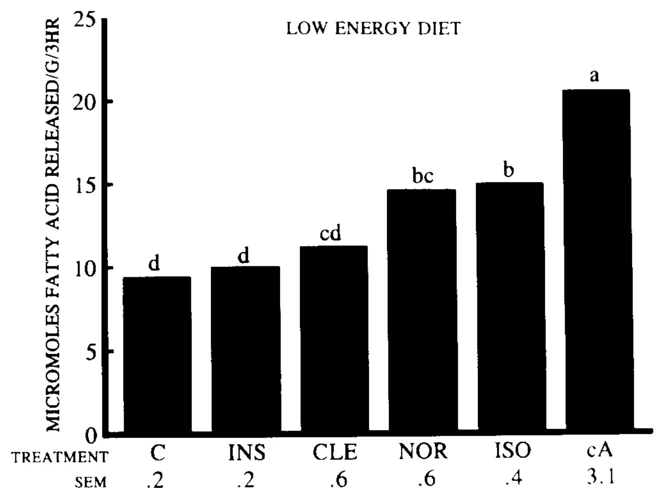

Figure 1 Lipolytic responses to no-hormone control (C), insulin (INS), clenbuterol (CLE), norepinephrine (NOR), isoproterenol (ISO), or dibutyryl-cAMP (cA) in adipose tissue from wethers fed a low energy diet. Standard error of the mean (SEM) is given for each hormone. Bars with uncommon superscripts are significantly different ($P < 0.05$).

sulted in marked stimulation of lipolysis ($P < 0.05$); however, the difference in lipolytic rates between NOR and CLE were less marked ($P < 0.1$). ISO-stimulated lipolysis was clearly greater than that of CLE ($P < 0.05$). The most active lipolytic agent was cA, which stimulated the release of 37% more fatty acid than did ISO ($P < 0.01$), and stimulated 2.2-fold greater fatty acid release than did C ($P < 0.001$). When fed HE *(Figure 2)*, cA remained the most potent lipolytic agent ($P < 0.01$). ISO also was lipolytic compared with C ($P < 0.05$); however, NOR was no longer lipolytic. Incubation of tissue with INS or CLE also did not affect lipolysis differently from C ($P > 0.05$). The major effect of changing from LE to HE, therefore, was the loss of NOR-stimulated lipolysis.

For both LE and HE wethers, rates of lipolysis in the C treatments appeared high, relative to values commonly reported for ovine as well as for other species. One possibility could be that 90-min preincubation caused an elevation in basal lipolysis. In previous studies, however, preincubation of porcine adipose tissue did not result in elevated basal lipolysis.[4]

Effects of catecholamines on palmitate esterification in adipose tissue of wethers fed LE are shown in *Figure 3*. No stimulation of esterification was observed with INS. However, incubation with CLE, NOR, ISO, or cA resulted in 51% ($P < 0.05$), 54% ($P < 0.05$), 59% ($P < 0.01$), and 48% ($P < 0.05$) inhibition of palmitate esterification compared with C, respectively. After wethers had consumed HE for 6 wk *(Figure 4)*, markedly different responses to these hormones and agents were observed. Esterification rates generally increased, but INS was still without stimulation. The inhibitory effects previously observed for CLE and NOR were completely lost by switching to HE. Only ISO and cA remained inhibitory.

To compare LE with HE for each hormone and

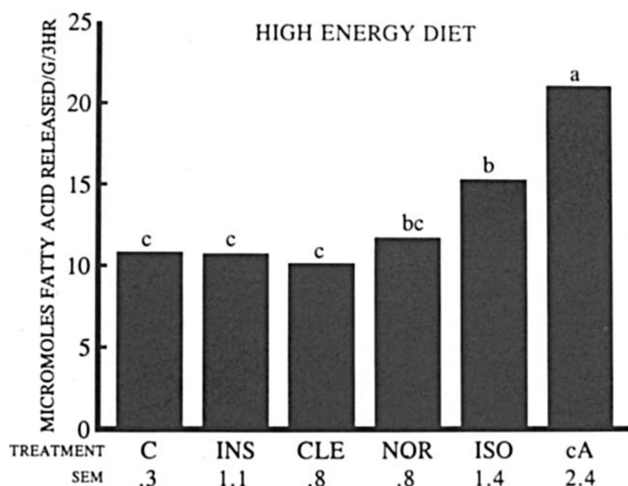

Figure 2 Lipolytic response to no-hormone control (C), insulin (INS), clenbuterol (CLE), norepinephrine (NOR), isoproterenol (ISO), or dibutyryl-cAMP (cA) in adipose tissue from wethers fed a high energy diet. Standard error of the mean (SEM) is given for each hormone. Bars with uncommon superscripts are significantly different ($P < 0.05$).

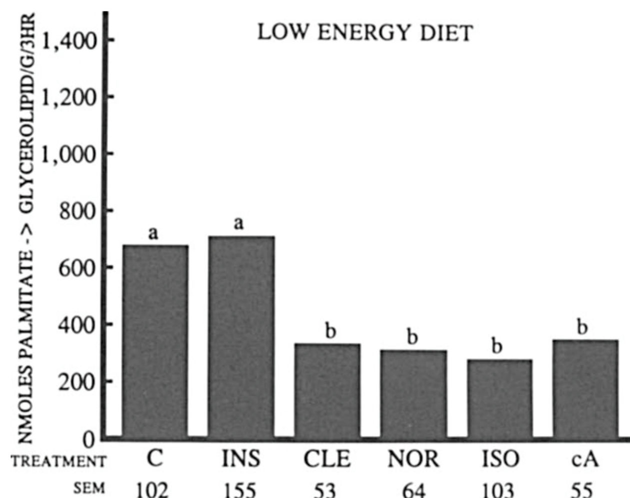

Figure 3 Palmitate esterification response to no-hormone control (C), insulin (INS), clenbuterol (CLE), norepinephrine (NOR), isoproterenol (ISO), or dibutyryl-cAMP (cA) in adipose tissue from wethers fed a low energy diet. Standard error of the mean (SEM) is given for each hormone. Bars with uncommon superscripts are significantly different ($P < 0.05$).

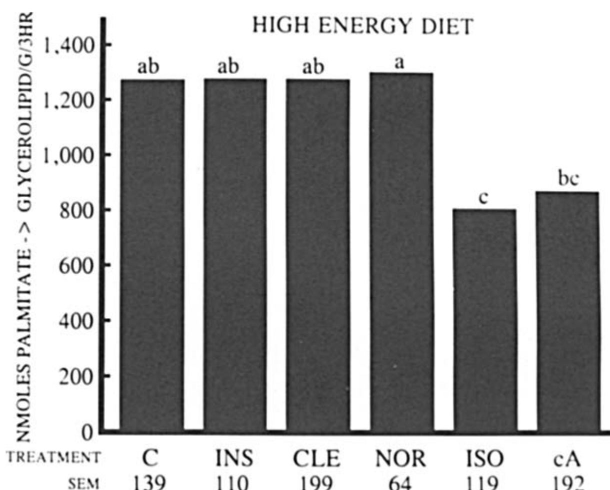

Figure 4 Palmitate esterification response to no-hormone control (C), insulin (INS), clenbuterol (CLE), norepinephrine (NOR), isoproterenol (ISO), or dibutyryl-cAMP (cA) in adipose tissue from wethers fed a low energy diet. Standard error of the mean (SEM) is given for each hormone. Bars with uncommon superscripts are significantly different ($P < 0.05$).

agent, lipolytic and esterification activities for each were expressed as percentages of their respective C values. For lipolysis, effects of dietary energy are shown in *Figure 5*. Only CLE and NOR were affected ($P < 0.01$) by dietary energy. There was, however, a tendency for lower lipolytic activities on HE. For esterification activity *(Figure 6)*, CLE ($P < 0.01$) and NOR ($P < 0.05$) effects were significantly altered by increasing the dietary energy. Inhibition effects of ISO and cA were blunted by HE, but more so for ISO ($P < 0.1$).

Concentrations of glucose, free fatty acids, and in-

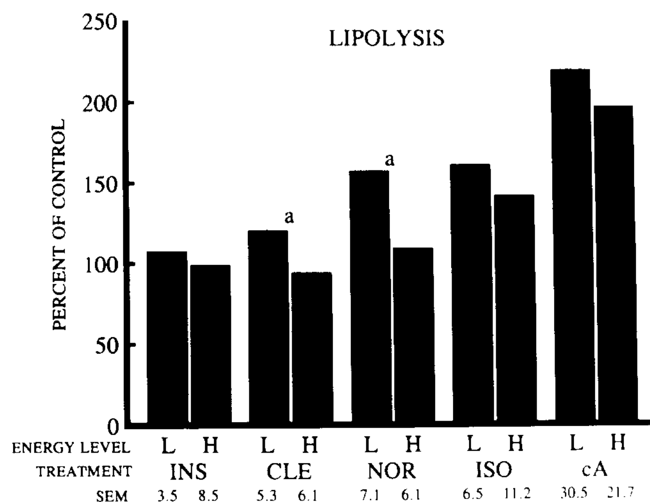

Figure 5 Effect of dietary low (L) or high (H) energy on lipolytic responses to hormones expressed as a percentage of no-hormone controls. Standard error of the mean (SEM) is given for each treatment. Superscripts indicate significant energy level effects for CLE ($P < 0.05$) and NOR ($P < 0.01$).

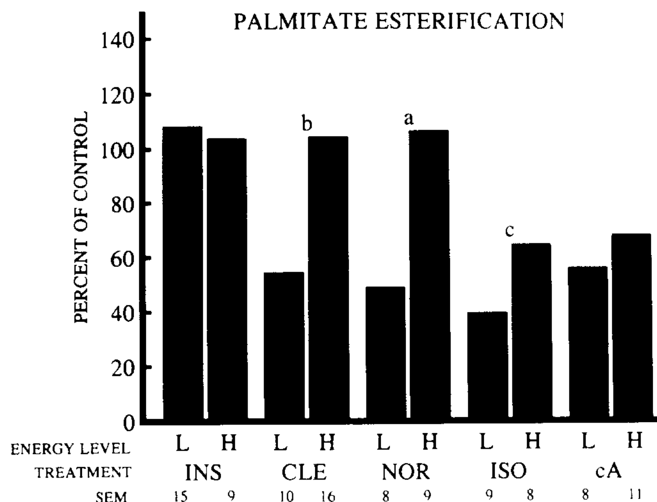

Figure 6 Effect of dietary low (L) or high (H) energy on palmitate esterification responses to hormones expressed as a percent of no-hormone controls. Standard error of the mean (SEM) is given for each treatment. Superscripts indicate significant energy level effects for NOR ($P < 0.01$), CLE ($P < 0.05$), and ISO ($P < 0.1$).

Table 1 Effects of low and high energy diets on plasma concentrations of glucose, free fatty acids, and insulin

Item	Dietary energy regimen: Low	Dietary energy regimen: High	Probability value
Glucose (mg/dL)	62.8 (1.7)[a]	64.9 (1.7)	0.42
Free fatty acid (μEq/L)	352.8 (35.7)	267.8 (19.5)	0.06
Insulin (μIU/mL)	14.6 (2.2)	35.7 (10.8)	0.08

[a]Values in parentheses are standard errors of the mean.

sulin in blood plasma of wethers are given in *Table 1.* Plasma glucose was not different for wethers fed LE or HE. Plasma insulin concentrations were greater ($P < 0.1$) for wethers fed HE, indicating that the animals responded to the greater influx of nutrients by maintaining constant plasma glucose. Plasma free fatty acid concentrations were greater ($P < 0.1$) for wethers fed LE, indicating that greater mobilization of stored fatty acid occurred on this dietary regimen than on HE.

Discussion

Results of the present study indicate that several catecholamines stimulate lipolysis in ovine adipose tissue in vitro. The mechanism of catecholamine stimulation of lipolysis involves beta-adrenergic receptor binding that elicits an increase in cAMP production,[19,20] which in turn results in the long-accepted phosphorylation cascade, activating hormone-sensitive lipase.[21] A comparison of different catecholamines and analogs has not been reported for ovine adipose tissue. However, in isolated swine adipocytes, CLE did not stimulate lipolysis as much as epinephrine did,[22] whereas others have demonstrated a lack of lipolytic stimulation by CLE.[4,23] Smith and McNamara[24] observed greater lipolytic responses to ISO than epinephrine in adipose tissue of dairy cows 30 days prepartum but not at 120 days postpartum when 10^{-5} M agonist was used. In the present study, CLE was marginally lipolytic. In contrast, Thornton et al.[3] observed marked stimulation of glycerol release from isolated adipocytes by CLE in vitro. The reason for this discrepancy is not clear; however, we used tissue slices and Thornton et al.[3] used isolated adipocytes. The change in lipolytic response to NOR that we observed by feeding wethers HE appeared similar to the decrease in epinephrine stimulation observed in bovine adipocytes from cows when diets were changed to a higher energy regimen.[13] Smith and McNamara[24] observed less lipolytic sensitivity to catecholamines in bovine adipose tissue of nonlactating than early lactation cows; because lactation imposes an energy drain, lactation could be comparable to animals consuming a low energy diet.

Early studies by Payne and Masters[7] did not show an effect of epinephrine on fatty acid esterification in ovine adipose tissue in vitro. In the present study, catecholamines inhibited this process by one-half or more, but only when wethers were fed LE. When wethers were fed HE, NOR and CLE lost their inhibitory abilities. The present study could not determine

the amount of energy consumption needed to influence sensitivity to catecholamines; therefore, the lack of affect of epinephrine on esterification observed by Payne and Masters[7] may have been the result of their sheep having consumed enough energy daily to preclude an effect on esterification in vitro.

Although studies on catecholamine effects on esterification in ovine adipose tissue have been lacking, effects on lipogenesis have been reported. Acetate conversion to fatty acids was significantly decreased in ovine adipocytes incubated with CLE[3] as well as with cimaterol.[25] Catecholamine effects on adipose tissue lipogenesis have been studied in other species as well. For example, Miller et al.[26] observed decreased fatty acid biosynthesis and decreased activities of lipid biosynthetic enzymes after exposure of bovine adipose tissue to CLE in vitro. In swine adipose tissue, CLE, ISO, cimaterol, and ractopamine inhibited lipogenesis,[23] as did cA.[2] Mersmann[27] reported epinephrine-induced inhibition of lipogenesis in swine adipose tissue, but only when theophylline was present.

Insulin did not stimulate esterification in adipose tissue of wethers fed LE or HE. Others have also observed a lack of lipogenic stimulation in ovine adipose tissue in vitro.[25]

Catecholamines were shown to inhibit glycerolipid biosynthesis in swine[4] and rat adipose tissue.[28] Determining mechanisms of this inhibition, for example by reversible phosphorylation,[1] was not possible because the inhibition was dependent on assay conditions. In the present study, assay conditions were based on the ability of ISO to stimulate lipolysis.

The loss of lipolytic stimulation and esterification inhibition by CLE and NOR when wethers were changed to HE suggests that gastrointestinal factors and/or hormone/metabolite balance in vivo had altered adipose cell responsiveness to these catecholamines in vitro. When we changed from LE to HE, plasma concentrations of insulin increased and concentrations of fatty acids decreased, which demonstrated a change in hormone/metabolite balance. In dairy cows, total beta-adrenergic receptor number increased when non-lactating animals proceeded into lactation,[29] which later was shown to cause greater sensitivity of adipose tissue to catecholamine-stimulated lipolysis,[8–11] and inhibited lipid synthesis.[9,11,12] In the present study, total beta-adrenergic receptor number may have decreased when animals were fed HE because sensitivity to catecholamines was lost or blunted. Also, because ISO was affected to a much lesser degree than were CLE and NOR, changes in adrenergic receptor numbers and/or binding affinities may have occurred with receptor types or subtypes with more or less affinity for CLE, NOR, or ISO. Both alpha- and beta-adrenergic receptors were found in ovine adipocytes,[30] and $alpha_2$ receptors were shown to be modulated by insulin[30]; $alpha_2$-receptor interactions produce less cAMP and lower rates of lipolysis.[20] This may partially explain effects observed for NOR because it has some alpha-agonist activity. For CLE, which is thought to only bind to beta-adrenergic receptors, an alternative mechanism was likely responsible for the loss of CLE effects when wethers were fed HE. The greater insulin concentrations in plasma of wethers fed HE, therefore, may have caused an up-regulation of $alpha_2$ adrenergic receptors that could not have been overcome by CLE or NOR, but could have been by ISO. Also, because beta-adrenergic receptors are classified into subtypes, for example $beta_1$ and $beta_2$[31] and more recently $beta_3$,[32] the possibility exists that different potencies for different subtypes by the agonists used in the present study were confounded by fewer or greater numbers of subtypes when dietary energy was altered. Moreover, differences in hormone/metabolite balance in vivo may have affected the ability of adipose cells to respond to each catecholamine's stimulation of cA production. For example, Mills et al.[33] observed marked differences in cA accumulation and lipolysis in adipocytes by different lipolytic agents in hypothyroid compared with euthyroid rats. Insulin in vivo, on the other hand, likely caused a greater deficit in cA by mechanisms carried over in vitro,[34] so as to result in continued loss of cA that was only overcome by addition of cA in vitro, or by its production in response to the very potent beta-agonist ISO.

In conclusion, the beta-adrenergic agonists, CLE, NOR, and ISO, were all lipolytic and inhibitory towards esterification in ovine adipose tissue in vitro when the animals were maintained on a low energy diet. When dietary energy was increased and food consumption was ad libitum, lipolysis was neither stimulated nor was palmitate esterification inhibited by CLE or NOR. Effects of ISO and cA were essentially maintained with HE, but blunted compared with LE. The mechanism for these observations was not obvious, but likely involved changes in adrenergic receptor number and/or binding.

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