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EFFECTS OF CIMATEROL, A BETA ADRENERGIC AGONIST, ON LIPID AND PROTEIN METABOLISM IN RATS

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ABSTRACT

EFFECTS OF CIMATEROL, A BETA ADRENERGIC AGONIST, ON LIPID AND PROTEIN METABOLISM IN RATS

By

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Female rats (133 g) fed ad libitum for 4 wks high-carbohydrate purified diets containing 10 or 100 ppm cimaterol gained 45-75% more skeletal muscle and 25-31% less abdominal white adipose tissue than controls, with a significant acceleration in skeletal muscle gain evident within the first wk. To elucidate mechanism(s) of action of cimaterol in these rats, effects of cimaterol on energy balance, lipid and protein metabolism were examined.

Energy balance was unaffected by cimaterol, indicating that cimaterol does not simply increase energy expenditure to reduce body fat. Cimaterol failed to influence rates of fatty acid synthesis in liver and white adipose tissue in vivo or in vitro. Activities of fatty acid synthetase and malic enzyme in these tissues were also unaffected by cimaterol. Cimaterol stimulated lipolysis in vivo and in vitro, but failed to influence lipoprotein lipase activity in white adipose tissue.

Lipoprotein lipase activity was elevated 65-75% in extensor digitorum longus muscle after cimaterol administration in vivo, and also after addition of cimaterol in vitro indicating its direct effects on muscle.

Total body 3-methylhistidine content and urinary excretion were

measured in these rats in order to calculate fractional rates of accretion, degradation and synthesis of total body 3-methylhistidine containing proteins (actin and myosin). Consumption of a diet containing 100 ppm cimaterol for 1 wk elevated fractional accretion rates of 3-methylhistidine containing proteins by 120%, this resulted from a 25% decrease in fractional degradation rates, and a 32% increase in fractional synthesis rates of these proteins. In agreement with the increased fractional synthesis rates, there was a marked increase in RNA gain (165%) and concentration (20%) in hindlimb muscles of these rats. Plasma insulin, corticosterone and triiodothyronine concentrations were unaffected by cimaterol.

In summary, cimaterol stimulated lipolysis in white adipose tissue to reduce the deposition of fat, without affecting either de novo rates of fatty acid synthesis or lipoprotein lipase activity. Cimaterol also stimulated lipoprotein lipase activity in muscle to direct energy away from adipose tissue deposition toward skeletal muscle accretion. The marked increase in rate of accretion of 3-methylhistidine containing proteins was caused by decreased degradation and increased synthesis.

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Introduction

Deposition of excess fat is a problem not only for humans, but also for meat producing animals. Many people are trying to avoid consumption of products with excess fat for reasons related to consumer health and fitness. Thus, there is increased emphasis on production of leaner meat. Considerable research progress has been made in this area in the past several decades. Recent research approaches include the use of growth hormone, beta-agonists, and immunological techniques to improve the lean to fat ratio in livestock. Administration of exogenous growth hormone to increase muscle mass, and decrease adipose tissue mass, of livestock is under active investigation (37). Several beta adrenergic agonists that alter body composition, either by reducing only body fat deposition with little apparent effect on lean body mass or by simultaneouly decreasing body fat and increasing lean body mass accumulation, are under extensive investigation for their possible use for humans as antiobesity drug (5,112) and in the meat industry as a means to produce leaner meat (30). Another recent approach to reduced body fat deposition has been use of passive immunization with antibodies to fat cells. This approach may also have potential benefit for increased protein deposition (40). My research has focused on the effects of a beta-agonist, cimaterol on body composition and on possible mechanism(s) of action of this compound. I will, therefore, focus the literature review on beta-agonists.

Beta adrenergic agonists are synthetic compounds which act specifically at beta receptors and exert their action via increases in intracellular levels of cyclic AMP (38,68). These compounds are

unlike the naturally occurring catecholamines, epinephrine and norepinephrine, which can act on both alpha and beta receptors. The presence of beta receptors is thus a prerequisite for beta-agonist action. Species and age of the animal may influence the number of beta-adrenergic receptors present in a particular organ. For example, the number of beta receptors in adult rat liver is approximately 10 fold lower than in the liver of 7 day old rats (77), whereas the number of beta receptors in rabbit liver does not change with age (62). Beta receptors are functionally subdivided into beta 1 or beta 2. The proportion of these two receptors found within an organ in the body is a characteristic of that organ. For example, lung and uterine tissues have primarily beta 2 receptors. Stimulation of these receptors leads to tissue relaxation (67). Thus, beta 2 agonists, of which clenbuterol is an example, are useful for treatment of bronchial asthma (32). In contrast, the heart has predominantly beta 1 receptors, and activation of these receptors leads to a rapid increase in heart rate (67).

Various types of beta-agonists, which are known to function specifically at beta 1 or 2 receptor type, are now available. Recently, several beta agonists have been identified that alter body composition in animals. Among these compounds, agonists with specificity for beta 2 receptors are known to reduce deposition of body fat; some also promote the deposition of body protein. Beta-agonists currently under investigation include clenbuterol (9,30,31,34,36,80,95,97,110), cimaterol (10,11,24,60,85), ractopamine (3,12,79), L-640,033 (34,87,98), LY 104119 (112), BRL 26830 (4,5,6), BRL 35135 (34). Effects of catecholamines, and of these compounds, on

body composition are reviewed below.

Body composition

Initial studies were done by administering epinephrine and norepinephrine, the naturally occurring catecholamines. In pigs, daily injections of epinephrine (0.15 mg/kg body weight) increased nitrogen retention (29) with no appreciable change in fat deposition. Whereas in rats, administration of epinephrine and norepinephrine reduced fat deposition; effects of the catecholamines on muscle accretion were not studied (97). Based on these limited data it appears that the effects of catecholamines are not uniform between species. One difficulty faced in these studies is that epinephrine and norepinephrine have very short half lifes in the body (25). Consequently, a single daily injection may not be effective. Another difficulty is that these catecholamines interact with both beta and alpha receptors. This interaction results in complex responses, rather than specific effects on a single system. To circumvent these problems, specific agonists have been synthesized that have longer half lifes and act rather specifically with one receptor type.

Among the various beta-agonists that have been synthesized to alter body composition, some of these compounds (BRL 26830A and LY 104119, for example) reduce body fat deposition with little apparent influence on lean body mass accumulation. When BRL 26830 was fed to genetically (ob/ob) mice for 28 days, it prevented weight gain due to reduced body lipid content, with minimal effects on lean body mass (4). Similar effects with BRL 26830 are also observed in genetically obese (db/db) mice and in obese (fa/fa) rats (5). Another beta-agonist, LY 104119, has been shown to decrease body fat in obese

(A^{vy}/a) mice (112). Although fat gain is also slightly reduced in normal rats and mice given BRL 26830 and LY 104119, there are no changes in weight gain or carcass protein gain in response to these compounds (5, 112). Thus, these compounds are useful in reducing body fat in obese animals, but they do not improve lean body mass gain.

Other beta-agonists, such as clenbuterol, cimaterol, ractopamine and L-640033, are under active investigation because they increase lean body mass in association with reduced adipose tissue mass. Clenbuterol is the most extensively investigated beta-agonist to date in terms of altering body composition in meat animals. Clenbuterol, when fed to finishing lambs at 2 ppm level for 8 wks, decreased fat depth at the 12th rib by 37% and increased longismus muscle area and semitendinosis muscle weights by 42% and 23%, respectively. Body weight gain and feed efficiency were enhanced with no changes in quality grades (9). Similar reductions in fat deposition and improved muscle accretion have also been achieved by feeding diets containing clenbuterol to finishing steers (97), swine (30), broilers (31) and rats (36,95). In agreement with the studies on clenbuterol, other beta-agonists like ractopamine, L 640033 and cimaterol have been shown to also decrease adipose tissue deposition and increase muscle accretion (3,10,11,24,60,85,87,97).

The reasons that one group of beta-agonists reduces only body fat, whereas another group of beta-agonists reduces not only fat, but also increases lean body mass are not clear. It may be that the agonists have more or less affinity for a specific receptor type, or that they interact with different receptor types.

The mechanism(s) of action by which beta-agonists alter body composition has not been extensively evaluated. In the following sections I will review several possible mechanisms of action.

Energy balance

Decreased fat deposition in animals fed diets containing beta-agonists could occur as a direct result of increased energy expenditure, for example by stimulating brown adipose tissue metabolism, without a concomitant increase in enregy intake. BRL 26830A is known to directly stimulate brown adipose tissue thermogensis (5). Brown adipose tissue, due to its high thermogenic capacity (99), plays an important role in regulation of energy balance. When BRL 26830A is given as a single dose (0.16mg/mouse) to an ob/ob mouse, the rate of energy expenditure remained elevated for 21 h. After feeding the drug daily (9.5 mg/kg p.o) for 28 days, it prevented fat gain by increased energy expenditure, with no effect on food intake (4). Similar effects have also been observed in genetically obese (fa/fa) Zucker rats and in cafeteria fed obese mice (6). BRL 26830A and clenbuterol also increases energy expenditure in lean animals, but this increase in energy expenditure is compensated by increases in energy intake, resulting in minimal changes in body lipid content or weight gain (6,36). This increase in energy expenditure observed in animals treated with BRL 26830A is in large part caused by stimulation of brown adipose tissue metabolism (5). Similar effects on energy balance are also observed in animals treated with the beta-agonist, LY 104119 (112). Effects of cimaterol on energy balance are unknown.

Reduced fat deposition associated with increased protein accretion in response to beta-agonists is a mechanism to indirectly influence energy balance. The energy cost of fat and protein deposition is the increment of food energy (usually expressed as metabolizable energy) required to promote a defined increment in body protein or fat. Based on a number of studies, it is estimated that an intake of 2.25 kcal metabolizable energy is required for formation of 1 kcal of protein, while only 1.36 kcal is needed for the formation of 1 kcal of fat. These values correspond to efficiencies for protein formation of 48%, and for fat formation of 77% (94). Thus, decreased body fat deposition, and increased protein accretion, in response to beta-agonists may lead to overall decreases in energy efficiency of the animal.

Although an increase in energy expenditure may explain the reduced deposition of adipose tissue in animals treated with BRL 26830A, it cannot explain the associated increase in skeletal muscle deposition observed after administration of some of the other beta-adrenergic agonists (3,9,10,11,24,30,31,36,60,85,87,95,97,98). It is also likely the beta-agonists have direct effects on white adipose tissue metabolism, for example on fatty acid synthesis, lipoprotein lipase activity or lipolysis. These possibilities will be reviewed in the following section.

Lipid metabolism

Fatty acid synthesis. Beta-agonists might decrease the conversion of dietary carbohydrates to fatty acids as one mechanism to decrease body fat. Fatty acid synthesis from acetyl CoA in mammalian tissues requires the sequential action of two enzyme systems: acetyl CoA

carboxylase and fatty acid synthetase (101,107). Liver and adipose tissue contribute significantly to whole body fatty acid synthesis in rats and mice (54). Available data on effects of catecholamines and beta-agonists on the net flux through this pathway, and on the activities of these enzymes, in liver and adipose tissue are discussed below.

The actions of catecholamines on liver fatty acid synthesis is not well defined. It has been reported that epinephrine markedly decreases fatty acid synthesis in rat liver slices, and it was suggested that this inhibition was dependent on cAMP (78). Later, it was shown that norepinephrine inhibited fatty acid synthesis in rat liver through an alpha-adrenergic mediated phosphorylation and inactivation of acetyl CoA carboxylase (72). The beta-agonist isoproternol had no effect on carboxylase activity in this study. The contrasting observations in the above two studies may be due to the differences in body weights, 160-180 g (78) compared to 300-350 g (72). Maturation of the rat liver appears to be accompanied by a loss of functional beta-receptors (77) leading to catecholamine action in adult rat liver primarily through the alpha-receptor system (16,72,88). These effects were also confirmed by the changes observed in the presence of the beta-agonist isoproternol and the beta-antagonist propronalol.

In contrast to liver, epinephrine action in white adipose tissue is mainly mediated through beta-adrenergic receptors; epinephrine stimulates the phosphorylation of acetyl CoA carboxylase; this effect can be blocked by propanolol, a beta-antagonist (65,66). In vivo treatment of rats with epinephrine caused phosphorylation of adipose

tissue acetyl CoA carboxylase leading to inhibition of fatty acid synthesis (66). Beta-agonists like clenbuterol, isoproterenol, L-640033 and BRL 35135 also have been shown to decrease the in vitro incorporation of C^{14}-acetate into total lipids in white adipose tissue (34); incorporation of C^{14}-acetate specifically into fatty acids was not studied. In sheep adipose tissue, clenbuterol did not influence the incorporation of C^{14}-acetate, C^{14}-lactate or C^{14}-glucose into fatty acids in vitro (27). Caution must be exercised in interpreting these results. Incorporation of the exogenous C^{14}-labeled substrates used in the above studies does not provide a quantitative measure of lipogensis or fatty acid synthesis because of the presence of endogenous precursors (26). Use of tritiated water to measure rates of fatty acid synthesis would provide a measure of fatty acid synthesis that is independent of precursor source. A preliminary report suggests that the beta-agonist ractopamine may inhibit fatty acid synthesis in white adipose tissue as measured by tritiated water method (79).

Interscapular brown adipose tissue is capable of higher rates of fatty acid synthesis (3-4 times higher than liver or white adipose tissue) (106). Rates of fatty acid synthesis and the activity of acetyl CoA carboxylase in interscapular brown adipose tissue of cold-adapted rats were decreased in vivo by about 70% after injection of norepinephrine (43). Fatty acid synthesis was also inhibited in vitro in the presence of cAMP, indicating beta adrenergic mediated effects (86). Data are unavailable on effects of beta-agonists on fatty acid synthesis in brown adipose tissue.

In most of the reported studies with beta-agonists that alter body composition, animals have been fed high-carbohydrate diets (3,6,9,10,11,24,30,31,36,60,85,95,97). Therefore, an important contributor to fattening in these animals would be conversion of dietary carbohydrate to fatty acids. Measurements of rates of fatty acid synthesis in liver and adipose tissue under in vitro and in vivo conditions, and measurement of activities of lipogenic enzymes should provide data on one mechanism whereby fat deposition may be decreased in response to cimaterol.

Lipoprotein lipase. Lipoprotein lipase (LPL) catalyses the hydrolysis of plasma triacylglycerol and helps to transfer plasma triacylglycerol fatty acids from the circulation into adipose tissue for storage, and into muscle for metabolic fuel (28,103). This enzyme is synthesised in the parenchymal cells of a number of tissues, and is then secreted and transported to its functional site on the luminal surface of the capillary endothelial cells (27). Adaptive changes in lipoprotein lipase activity occur in different tissues in response to variations in the physiological state. These changes correlate with the altered rates of triacylglycerol fatty acid uptake into the respective tissue (103). Thus, another mechanism whereby beta-agonist might affect body composition is by altering activity of lipoprotein lipase (LPL).

Insulin has long been recognized as a major regulator of white adipose tissue lipoprotein lipase both in vivo and in vitro. Enzyme activity is increased several-fold after injection of insulin into fasted rats (17) and is strongly correlated with plasma insulin concentrations in a variety of nutritional states (28). This insulin-induced increase in lipoprotein lipase activity is potentiated

by glucocorticoids in vitro (8). Actions of catecholamines on insulin-induced LPL activity in white adipose tissue are not, however, well established. Insulin-induced increases in adipose tissue LPL activity in vitro are abolished by addition of epinephrine. It was suggested that epinephrine inactivated LPL before it was released from the adipocytes (7,8). These experiments demonstrate that epinephrine inhibits insulin-induced LPL activity, but it is not clear if the basal LPL activity would also be decreased by epinephrine. Recently it has also been shown that the degradation of lipoprotein lipase is increased in vitro by epinephrine (93). If catecholamines play an important role in regulation of white adipose tissue LPL activity, denervation of the tissue should affect LPL activity. But after microsurgical denervation of rat adipose tissue, LPL activity is similar to activity in non-operated tissue, eventhough the concentration of norepinephrine was at least ten times lower after denervation (51). Although there is some disagreement among the studies about the role of catecholamines in regulation of white adipose tissue LPL activity, under some conditions enzyme activity was reduced in the presence of epinephrine. Effects of beta-agonists on LPL activity in white adipose tissue have not been reported. Thus, the measurement of LPL activity in white adipose tissue should provide data on one mechanism whereby fat deposition may be reduced in response to cimaterol.

Unlike the tendency of catecholamines to depress white adipose tissue LPL activity, a large elevation in LPL activity in rat brown adipose tissue is observed 3-4 hours after injection of norepinephrine, and also after cold exposure ($4^{0}C$) for 28 days (21),

which would cause release of norepinephrine from nerve ending in brown adipose tissue. Neither insulin nor glucose could mimic the cold-induced increase in LPL activity, suggesting that in contrast to white adipose tissue, brown adipose tissue LPL activity is not stimulated by insulin, but rather by norepinephrine (21). This stimulation of LPL activity by norepinephrine is mimicked by the beta-agonist isoprenaline, and abolished by the beta-antagonist propranolol, indicating beta-adrenergic mechanism (21). These data on effects of catecholamines on LPL activity in white and brown adipose tissue suggest tissue specific changes in the regulation of LPL activity.

Relatively little is known about control of skeletal muscle LPL activity. Skeletal muscle represent about 45% of body weight (22), indicating its quantitative significance in utilizing considerable amounts of lipids as fuel through LPL activity.
Epinephrine increased the activity of the enzyme only in vastus deepest, whereas norepinephrine increased the activity in the vastus deepest and soleus muscles after single injections. In vastus superficial muscle catecholamines did not influence the enzyme activity (49). Reasons for these differences among various muscles in response to catecholamines are not clear. Data are not available on the effect of beta-agonists, including cimaterol, on LPL activity in rat tissues. It is possible that cimaterol may have differential effects on LPL by decreasing activity in white adipose and increasing activity in brown adipose tissue and muscle. Reduced enzyme activity in white adipose tissue would help to reduce the storage of lipids and stimulation of LPL activity in skeletal muscle would facilitate

availability of energy for increased muscle metabolism.

Lipolysis. The lipolytic system involves triglyceride lipase which catalyses breakdown of triglycerides to free fatty acids and glycerol (58). Lipolytic activity is commonly measured by release of glycerol or free fatty acids into the medium or plasma under in vitro and in vivo conditions, respectively. Beta-agonists might stimulate lipolysis and thereby increase the mobilization of stored lipids as one mechanism to reduce body fat.

Effects of catecholamines on lipolysis are well documented. When fat pads from young male rats were incubated in vitro in the presence of epinephrine (1.0 uM), lipolytic activity increased 3-4 fold, as measured by release of free fatty acids into the medium. This increase in release of free fatty acids is correlated with stimulation of intracellular cAMP concentration. Furthermore, beta-adrenergic blocking agents antagonise the effects of epinephrine on both cAMP levels and lipolysis (19). A number of other studies confirmed these effects using isolated fat cells or fat pads (15,63,74,102). Increases in cAMP dependent protein kinase is also observed in the presence of epinephrine in these studies. Infusion of catecholamines has also been shown to increase plasma levels of free fatty acids and of glycerol (41).

Consistent with the data on the effects of catecholamines on lipolysis, isoproternol at 1 uM concentration also increased the release of glycerol, cAMP and protein kinase suggesting beta-adrenergic stimulation of lipolysis (2). Similar increases in release of glycerol, in response to clenbuterol, LY 79771, L-640,033 and BRL 35135 have been reported (34,113). Available data on the

effects of catecholamines and beta-adrenergic agonists on lipolysis, strongly suggest that the beta-agonist cimaterol should also stimulate lipolysis in white adipose tissue. But, such studies have not been conducted. Results from the experiments I propose will provide evidence for increased mobilization of lipids as one of the mechanism to reduce deposition of fat in response to cimaterol.

Protein Metabolism

Overall rates of protein synthesis and degradation in muscle determine the state of protein balance in the tissue. Generally, during periods of rapid muscle growth rates of protein synthesis and degradation are both accelerated, with the increase in protein synthesis being greater than the increase in protein degradation (83). Under some conditions, however, such as during recovery phase from atrophy of immobilized muscle (47) and during work induced-hypertrophy (46), gain in muscle protein is caused by simultaneous inhibition of protein degradation and stimulation of protein synthesis. These changes represent the most efficient way for muscle accretion to be accelerated. The review of literature on the effects of catecholamines and beta-agonists on protein degradation and synthesis will provide insight into possible mechanisms of action of cimaterol to increase skeletal muscle protein deposition.

Protein Degradation. There is some evidence to suggest that catecholamines slow turnover of skeletal muscle proteins. Epinephrine at physiological concentrations (1 nM), lowered the release of alanine and glutamine from isolated rat epitrochlaris muscle (42). Dibutyryl cyclic AMP reproduced the effect of epinephrine and proponolol (a beta-antagonist), but not phentolamine (an alpha-antagonist), blocked

the effect of catecholamines, suggesting a beta-adrenergic process. It was concluded that epinephrine inhibited alanine and glutamine release from skeletal muscle by decreasing degradation of muscle proteins (42). But no direct evidence for a role for epinephrine in inhibiting degradation of muscle protein was provided in this study. Based on results from infusion of U-^{14}C leucine in pigs (50), and [^{15}N]leucine and [$^{2}H_3$]leucine in humans (81) it was also concluded that epinephrine or norephinephrine decreases protein degradation.

Consistent with the effects of catecholamines, isoporternol also lowered the release of amino acids such as alanine, threonine, phenylalanine, tyrosine, lysine, arginine, leucine and valine from perfused rat hemicorpus muscle preparations (71). Protein degradation, as measured by the release of phenylalanine in these preparations, showed a 20% reduction with addition of 1 uM isoproternol (71). It was also shown in mice, that daily injections of isoproternol increased the half life of parotid gland proteins (55). Clenbuterol administration to calves also decreased protein degradation, as measured by urinary excretion of 3-methylhistidine 110). It has been also suggested that clenbuterol may have rapid effects on reducing the protein turnover, based on the results calculated from measured rates of protein accretion and synthesis (95). All the data suggest that cimaterol may decrease protein degradation, but direct experimental evidence is lacking.

Protein Synthesis Data on effects of catecholamines and beta-agonists on protein synthesis are even less available than are data on protein degradation. Epinephrine has been reported to increase protein

synthesis in rat diaphragm muscle by increasing incorporation of ^{3}H-leucine and ^{3}H-tyrosine into protein (91). But stimulation of protein synthesis by epinephrine was only observed in diaphragms from hypophysectimized rats; protein synthesis in diaphragms from intact rats was not affected by epinephrine (91). Incorporation of C^{14}-labeled amino acids into total rat tibialis muscle protein is stimulated in vivo by chronic administration of isoproternol (0.3 mg/kg body weight) (33). Maximum stimulation occurs 2-3 hours after the fifth daily injection of isoproternol. This stimulation is greater during the first few days of treatment, and decreases gradually thereafter (33). Clenbuterol also increased fractional synthesis rates by 34% 1 h after the 7th daily injection, as measured by phenylalanine incorporation into mixed muscle proteins (36). Similarly, ractopamine, another beta-agonist, has been shown to increase fractional rates of protein synthesis in pigs (12). But, Reeds et al (95) reported that clenbuterol failed to stimulate the protein synthesis in rats. Others have reported contrasting results; both epinephrine and norepinephrine at a concentration of 0.1 ug/ml decreased incorporation of ^{14}C-amino acids into muscle protein (111). The inhibition occurred whether epinephrine was added in vitro or administered in vivo (111).

Reasons for the discrepancies among these studies are still unclear. These studies indicate that catecholamines or beta-agonists may stimulate, inhibit, or result in no change in protein synthesis. The type of compound studied, duration of administration, method of study (in vitro vs in vivo), the type of measurement (single injection vs constant infusion), time of the measurement after the dose, and

type of mesurement (whole body vs specific muscle) probably all contribute to the variable results.

From the above discussion it is possible that catecholamines and beta-agonists may exert significant regulatory actions on protein balance in skeletal muscle. Although there are discrepancies among the studies for the effects of catecholamines or beta-agonists on protein synthesis, all the studies indicate that these compounds decrease protein degradation. Therefore, an examination of the effects of cimaterol on protein degradation and synthesis associated with protein accretion will provide evidence for the mechanism of action to increase protein deposition, and also provide data about the extent of contribution of each to the increased accretion.

Dissertation Objective

The objective of my dissertation research was to examine the mechanisms of action of cimaterol in reducing adipose tissue deposition and increasing skeletal muscle accretion in rats. Therefore, the following studies on effects of cimaterol on lipid metabolism, and on protein metabolism, were conducted.

EFFECTS OF CIMATEROL, A BETA ADRENERGIC AGONIST, ON LIPID METABOLISM IN RATS

Introduction

Several beta adrenergic agonists have recently been identified that alter body composition in animals. Some of these compounds (BRL 26830A and LY 104119, for example) reduce body fat deposition with little apparent influence on lean body mass accumulation (5,112), whereas others such as clenbuterol and cimaterol not only decrease fat deposition but also increase lean body mass accretion. Clenbuterol, when fed to lambs, reduced fat thickness over the 12th rib by 37% and increased longissimus muscle cross sectional surface area by 41% (9). Similar shifts in body composition in response to clenbuterol or cimaterol have been reported in other species including cattle (97), chickens (31), pigs (60) and rats (36).

The mechanism of action of the beta-agonist BRL 26830A has been extensively studied. BRL 26830A increases energy expenditure by stimulating brown adipose tissue metabolism without a concomitant increase in energy intake; thus, animals treated with BRL 26830A retain less energy than control animals (5). Although an increase in energy expenditure explains the reduced deposition of adipose tissue in animals treated with BRL 26830A, it cannot explain the associated increase in skeletal muscle deposition observed after administration of some of the other beta adrenergic agonists (9,10,31,36,60,95,97). One hypothesis for the increased skeletal muscle deposition is that beta adrenergic agonists such as clenbuterol and cimaterol shunt dietary energy away from adipose tissue to skeletal muscle (97). Catecholamines and beta agonists influence a number of metabolic pathways in lipid metabolism, however, the specific effects of

clenbuterol and cimaterol on lipid metabolism have not been widely explored.

In most of the reported studies with beta-agonists animals have been fed high-carbohydrate diets (5,9,10,31,36,60,95,97,112). Therefore, an important contributor to fattening in these animals would be conversion of dietary carbohydrate to fatty acids. Under some conditions catecholamines inhibit acetyl coenzyme A carboxylase activity (66), a key regulatory enzyme in fatty acid synthesis. Thus, the reduced deposition of adipose tissue in animals given beta-agonists might be explained by inhibition of dietary carbohydrate conversion to fatty acids. Beta-agonists might also influence lipoprotein lipase activity (7,8). This enzyme facilitates transfer of fatty acids from circulating triglycerides into adipose tissue for storage and into muscle for metabolic fuel. Under certain physiological conditions, lipoprotein lipase activity in adipose tissue is reciprocally related to lipoprotein lipase activity in skeletal muscle (28). Thus, beta agonists may inhibit lipoprotein lipase activity in white adipose tissue to reduce storage of lipids and stimulate lipoprotein lipase activity in muscle to facilitate availability of energy for increased muscle metabolism. The stimulatory effect of catecholamines on white adipose tissue lipolysis is well established (38) and provides another potential mechanism whereby beta agonists may mobilize body lipids and thereby reduce adipose tissue deposition. I examined the effects of cimaterol, a beta-agonist that decreases fat deposition and increases skeletal muscle accumulation (10,11,60,85) on the above mentioned measures of lipid metabolism in rats. Body composition, energy balance, rates of

fatty acid synthesis, activities of fatty acid synthetase, malic enzyme and lipoprotein lipase, and rates of lipolysis were measured in rats administered cimaterol acutely or chronically.

Materials and methods

Animals and diets Female Sprague-Dawley rats (130-180 g) obtained from Harlan Industries, Indianapolis, IN, were housed individually at 23^{0}C in metal cages with wire-mesh floors. Room lights were on from 0700 to 1900 h. All animals were provided a nonpurified diet (Wayne Rodent Blox, Continental Grain Company, Chicago, IL) and water ad libitum for the first 2 days after arrival in the laboratory. Rats were then fed either a high-carbohydrate or a high-fat purified diet. The high-carbohydrate diet contained (in g/100 g): 66.0 g glucose,5.0 g corn oil, 20.0 g casein, 0.3 g methionine, 1.0 g vitamin mix (14), 0.2 g choline chloride, 3.5 g mineral mix (14) and 4.0 g cellulose. This diet provided 3.57 kcal metabolizable energy/g with 67% of metabolizable energy as carbohyrate, 13% as fat and 20% as protein. The high-fat diet contained (in g/100g): 18.2 g glucose, 19.6 g corn oil, 19.6 g tallow, 29.4 g casein, 0.44 g methionine, 1.47 g vitamin mix, 0.29 g choline chloride, 5.15 g mineral mix and 5.88 g cellulose. This diet provided 5.25 kcal/g with 13% of metabolizable energy as carbohydrate, 67% as fat and 20% as protein. The amounts of cimaterol (CL 263,780; anthranilonitrile, 5-[1-hydroxy-2-(isopropylamino)ethyl]-) added to these diets is indicated in the experimental design.

Experimental design. Experiment 1. This experiment was designed to examine effects of cimaterol on body composition and energy balance of rats fed the high-carbohydrate diet ad libitum. Rats were divided

into 4 groups. Group 1 was killed at the beginning of the experiment to obtain initial body composition values, groups 2, 3, and 4 were fed the high-carbohydrate diet containing 0, 10 or 100 ppm cimaterol, respectively. Food intake and weight gain were recorded twice weekly. Half the rats from each group were killed after 1 wk, and the remaining rats were killed after 4 wks. Carcasses were frozen for subsequent analysis.

Experiment 2. Effects of cimaterol on in vivo and in vitro rates of fatty acid synthesis and on activities of fatty acid synthetase and malic enzyme were examined in liver, parametrial white adipose tissue and interscapular brown adipose tissue.

Chronic effects of cimaterol on fatty acid synthesis were examined in rats fed the high-carbohydrate diet containing 0, 10 or 100 ppm cimaterol ad libitum for 1 or 4 wks. Animals from experiment 1 were used for these measurements. At the end of the 1 or 4 wk feeding period, rats were injected with 1 mCi of $^{3}H_{2}O$ (61) at approximately 0900 h and killed 15 minutes later. Blood, liver, total dissectable abdominal white adipose tissue and interscapular brown adipose tissue were rapidly collected. Tissues were frozen at -70^{o}C for subsequent determination of ^{3}H incorporation into fatty acids. An aliquot of plasma separated from blood was used to determine the specific radioactivity of water. Results were expressed as umoles ^{3}H incorporated into fatty acids per minute per total tissue. Tissues remaining after the measurement of rates of fatty acid synthesis were returned to the carcass for measurement of body composition and energy balance.

To verify the effects of cimaterol on fatty acid synthesis, activities of the lipogenic enzymes fatty acid synthetase and malic enzyme were measured in liver, white adipose tissue and brown adipose tissue of additional rats fed 0, 10 or 100 ppm cimaterol for 1 or 4 wks. Rats were killed between 0900 and 1100 h. Tissues were rapidly removed, weighed and aliquots were homogenized in 8 volumes of cold phosphate-bicarbonate buffer (70 mM $KHCO_3$, 85 mM K_2HPO_4, 9 mM KH_2PO_4, and 1 mM dithiothreitol), pH 8.0 (90) and centrifuged at 100,000g at 4^0 for 45 minutes. The resulting supernatent solution was stored at -70^0C for no longer than two days for subsequent measurement of the enzyme activites.

Acute effects of cimaterol on in vivo rates of fatty acid synthesis were examined after administration of cimaterol to rats that had been fed the high-carbohydrate diet ad libitum for 1 wk. Rats were injected intraperitoneally with 0.0 mg, 0.15 mg or 1.5 mg cimaterol in 0.2 ml saline at approximately 0900 h (time 0). These doses correspond to the daily amount of cimaterol consumed by rats fed the high-carbohydrate diet containing 0, 10, or 100 ppm cimaterol. After 15 minutes (time 15), each rat received 1 mCi ^3H_2O intraperitoneally, after another 15 minutes (time 30) all animals were killed. Blood, liver, white adipose tissue and brown adipose tissue were processed as described for the chronic in vivo trial. Meal fed rats show an increased lipogenic capacity (69). To increase the possibility of detecting an effect of cimaterol on rates of fatty acid synthesis, rats were trained to eat meals of the high-carbohydrate diet containing 0, 10 or 100 ppm cimaterol twice daily (a 1 h meal at

0900 h and a second at 1600 h) for 1 wk. Rats were injected with ^3H_2O (1 mCi/rat) 15 minutes after completion of the 0900 h meal, 15 minutes later rats were killed, blood and tissues were collected and processed for the determination of ^3H incorporation into fatty acids.

To determine effects of cimaterol on in vitro rates of fatty acid synthesis, rats were killed (0900 h) after being fed the high-carbohydrate diet ad libitum for 1 wk. Liver, parametrial white and interscapular brown adipose tissues were rapidly removed and placed in normal saline solution (0.9% NaCl). Liver slices (150-200 mg) were prepared using a Stadie-Riggs hand microtome. Thin distal portions of parametrial white adipose tissue (100-150 mg) and pieces of interscapular brown adipose tissue (60-100 mg) were cut, rinsed in saline, gently blotted and weighed. Liver slices and adipose tissue were immediately transferred to a flask containing 3 ml of Kreb's Ringer bicarbonate buffer, 40 uCi/ml, ^3H_2O, 10 mM glucose. Tissues were incubated for 2 h at 37^0C in the presence or absence of cimaterol (1 nM, 1 uM or 1 mM) in a shaking water bath with 95% O_2: 5% CO_2 gas mixture. Rates of fatty acid synthesis were also measured with the addition of 100 uU/ml and 100 mU/mlinsulin in the presence or absence of 1mM cimaterol. An aliquot of the final incubation media was used to determine specific radioactivity of media water. At the end of incubations, tissues were removed, rinsed with saline saponified and fatty acids were extracted. ^3H incorporation into fatty acids was determined. Data were expressed as umoles of ^3H incorporated into fatty acids per 2 h per 100 mg tissue.

Experiment 3. If cimaterol exerts its primary effect on body composition by inhibiting de novo fatty acid synthesis, then consumption of a high-fat diet which has been shown to inhibit de novo fatty acid synthesis (69) should circumvent effects of cimaterol on body composition. This hypothesis was tested by adding 0 or 147 ppm cimaterol to the high-fat diet. This dose of cimaterol is equal to the amount of cimaterol (per kcal energy) in the high-carbohydrate diet that contained 100 ppm cimaterol. Food intake and body composition measurements were performed as in experiment 1.

Experiment 4. Since uptake of fatty acids by adipose tissue and muscle from circulating triglycerides is facilitated by the enzyme lipoprotein lipase (28), the possible effects of cimaterol on modulation of lipoprotein lipase activity were examined. Chronic effects of cimaterol on lipoprotein lipase activity were determined in rats fed the high carbohydrate diet containing 0, 10 or 100 ppm cimaterol ad libitum for 4 wks. White and brown adipose tissue were removed and frozen immediately on dry ice. Both hindlimbs were also removed and frozen quickly in acetone-dry ice. All tissues were stored at -70^{0}C for subsequent measurement of lipoprotein lipase activity. Upon thawing, extensor digitorum longus (EDL), soleus muscles and remaining hindlimb muscles (removed from the leg bones and separated from the adhering adipose tissue) were selected as representative of white, red and mixed fiber types, respectively.

Acute effects of cimaterol on lipoprotein lipase activity in white and brown adipose tissue and muscle were examined in rats that had recieved the high-carbohydrate control diet ad libitum for 1 week. Rats were injected intraperitoneally with cimaterol (0.0, 0.15 or 1.5

mg in 0.2 ml saline) at 0700 h and killed 4 h later. White and brown adipose tissue and both hindlimbs (EDL, soleus and total remaining hindlimb muscle) were removed for subsequent measurement of lipoprotein lipase activity.

To determine effects of cimaterol on skeletal muscle lipoprotein lipase activity in vitro, EDL and soleus muscles from rats fed the high-carbohydrate diet ad libitum for 1 wk were used. Rats were decapitated at approximately 0900 h. EDL (70-90 mg) and soleus (30-40 mg) muscle were clamped at both ends in situ to maintain their length and then removed carefully and placed in Kreb's Ringer bicarbonate buffer saturated with 95% O_2: 5% CO_2, pH 7.4. After gentle blotting, muscles were transferred to vials containing 2.0 ml of Kreb's Ringer bicarbonate buffer, 10 mM glucose, 0.1 U porcine insulin/ml, 3.0 % bovine serum albumin and amino acids at five times the concentrations of rat plasma (73). Muscles were incubated for 2 h at 37^{0}C in the presence or absence of 1mM cimaterol in a shaking water bath with 95% O_2: 5% CO_2 gas mixture. At the end of the incubations, muscles were removed, separated from the clamps, rinsed, blotted, weighed and prepared for lipoprotein lipase determination. An aliquot of the media was also obtained for measurement of lipoprotein lipase activity. To determine effects of the incubation per se on lipoprotein lipase activity, enzyme activity was measured in representative muscles at the start of the incubation.

Experiment 5. Effects of cimaterol on lipolysis in vivo and in vitro and on plasma concentrations of glucose and lactate were studied. Chronic effects of cimaterol were examined by measuring concentrations of plasma glycerol, free fatty acids, glucose and

lactate after feeding the high-carbohyderate diets containing 0, 10 or 100 ppm cimaterol for 4 wks. Animals from experiment 4 (i.e., those used to examine chronic effects of cimaterol on lipoprotein lipase activity) were used for these metabolite measurements. Rats were decapitated at 1100 h and blood was collected.

Acute effects of cimaterol on in vivo lipolysis were examined in rats that had been fed the high-carbohydrate diet ad libitum for 1 wk. Rats were injected intraperitoneally with 0, 0.15 or 1.5 mg cimaterol in 0.2 ml saline at approximately 0900 h. Half the rats receiving each dose of cimaterol were killed 15 minutes after injection and the remaining rats were killed 30 minutes after injection of cimaterol. Blood was collected for measurement of plasma glycerol, free fatty acids, glucose and lactate.

To determine effects of cimaterol on in vitro rates of lipolysis in white adipose tissue, rats were killed (0900h) after being fed the high-carbohydrate diet ad libitum for 1 wk. Thin distal portions of parametrial white adipose tissue (100-150 mg) were rapidly removed. Tissue was rinsed in saline (0.9% NaCl), gently blotted, weighed and transferred into a flask containing 3 ml of Kreb's Ringer bicarbonate buffer, pH 7.4, with 3% fatty acid free bovine serum albumin (fraction V) in the presence or absence of cimaterol (1 nM, 1 uM, 1 mM). Tissues were incubated at 37^0C for 1 h in a shaking water bath under an atmosphere of 95% O_2 : 5% CO_2. In a preliminary study, rates of lipolysis were shown to be linear for at least one h. At the end of incubation, glycerol and free fatty acid concentrations in the media were measured.

Analyses. Weights of selected tissues (abdominal white adipose tissue, right hindlimb muscle, EDL and soleus muscle) were determined after thawing the carcasses. Hindlimb muscle weights were determined after being stripped from the bones and separated from adipose tissue. Lengths of tibia and femur were measured using calipers. All tissues were returned to the carcasses except for the aliquots of homogenized tissue used for measuring rates of fatty acid synthesis. After removal of food residues from the stomach, carcasses were homogenized in water. Aliquots of the homogenate were used to determine body fat by chloroform:methanol extraction. Protein was determined by the method of Markwell et al (75) after the homogenate had been dissolved in 1 N sodium hydroxide. Another aliquot of the homogenate was dried at 50^{o}C and used to determine body energy by bomb calorimetery using a Parr Adiabatic Calorimeter (Parr Instrument Co., Moline, III).

Tissue weights, bone length and carcass energy of rats at the beginning of the experimental period were predicted from linear regression equations based on body weights, tissue weights, bone lengths and carcass energy of the initial group. Gains in tissue weight, bone length and carcass energy were calculated from observed values at the end of the experimental period minus predicted values at the beginning of the experiment. Carcass energy density, an indicator of relative proportions of fat and protein in the body, was calculated by dividing body energy content by body weight. Energy efficiency was calculated as body energy gain divided by metabolizable energy consumed during the 4 wk period. Energy expenditure was calculated as the difference between metabolizable energy intake and body energy gain.

To estimate rates of fatty acid synthesis from ^3H_2O, tissues (100-200mg) were saponified. Fatty acids were subsequently extracted and counted for radioactivity (70).

Fatty acid synthetase and malic enzyme activities were determined in liver, white adipose tissue and brown adipose tissue by established methods (90,92). Enzyme activities were expressed as rates of utilization of NADPH (fatty acid synthetase) or NADP (malic enzyme) per mg of cytosolic protein and per tissue per minute at 30^0C.

Lipoprotein lipase activity was measured by a modified method of Schotz (53). A weighed amount (100 mg) of minced white adipose tissue was homogenized for 1 minute at a setting of 5 (Potter-Elvehjem homogenizer) using glass and glass in 0.25 M sucrose-1 mM EDTA (pH 7.4) buffer with 20 U/ml heparin (1g tissue/8 ml buffer). After centrifuging the homogenate at 12000 g for 15 minutes at 4^0C, the fat-free, post-mitochondrial supernatent was recovered and stored at -70^0C for subsequent lipoprotein lipase assay. A second homogenization -centrifugation step increased recovery of lipoprotein lipase activity from white adipose tissue by only 8%. Therefore, I routinely employed only a single homogenization-centrifugation step for white adipose tissue. Muscle and brown adipose tissue were homogenized in detergent (59) to improve extraction of lipoprotein lipase. Tissues were weighed, minced and homogenized in 1:7 (w/v) 0.25 M sucrose-1 mM EDTA (pH 7.4) buffer containing 20 U heparin/ml, 1% bovine serum albumin and 0.2% deoxycholic acid. After centrifugation and aspiration of supernatent, the pellet was again homogenized and re-extracted. The two extracts were combined and stored at -70^0C for subsequent lipoprotein lipase assay.

Significant amounts of lipoprotein lipase activity (15-40%) were recovered during the second extraction of these tissues. Recoveries for muscle and brown adipose tissue lipoprotein lipase activities were 86% and 95%, respectively, for the combined two extractions compared to three extractions. Therefore, I routinely used two extractions for these tissues.

Lipoprotein lipase activity was determined using ^{14}C triolein prepared with human serum (53) as substrate. To control for nonspecific lipolysis the ^{14}C-triolein substrate was also prepared in the absence of serum. Only fatty acid release resulting from serum activation was specified as lipoprotein lipase activity. Using this assay system, at pH 8.0, enzyme activity was linear for 45 minutes of incubation and substrate (3.4 mM triolein) was not rate limiting. The amount of tissue used in the assay was in the linear range for lipoprotein lipase activity (amount varied with tissue depending on the amount of lipoprotein lipase activity). Since lipoprotein lipase activity was characterized by serum activation and NaCl inhibition, effects of 0.66 M NaCl on lipoprotein lipase activity was evaluated. Inhibition of lipoprotein lipase activity in the presence of 0.66 M NaCl was 92%, 90% and 82% in white adipose tissue, brown adipose tissue and muscle, respectively. Incubations were conducted for 30 minutes at 37^0C, and free fatty acids were extracted as described by Hietanen and Greenwood (53) and counted for radioactivity. Results were expressed as umoles of free fatty acids released per hour per tissue and per g tissue.

Glycerol was measured by enzymatic analysis using glycerokinase, pyruvate kinase and lactate dehydrogenase (Boehringer Manneheim,

Indianapolis, IN. cat.no. 148270). Free fatty acids were determined by the titrametric method according to Ko and Royer (64) with a standard curve developed using palmitic acid. Plasma glucose was measured, which involved glucose oxidase and peroxidase to produce a colored compound (Boehringer Manneheim, Indianapolis, IN. cat.no.189197). Lactate was determined enzymatically (56).

Data were analysed statistically using analysis of variance and the Bonferonni t test for post hoc treatment comparisons (44).

Results

Experiment 1. Effects of cimaterol on body composition and energy balance were examined in rats fed the high-carbohydrate diet for 4 wks. All rats remained healthy during the study with no visible adverse effects of cimaterol. An increase in rate of body weight gain was observed in rats fed cimaterol (Figure 1). The major weight gain response occurred during the first 2 wks when rats fed 10 or 100 ppm cimaterol gained 27 and 42 % more than control rats. By 4 wks rats fed cimaterol were no longer gaining body weight at an accelerated rate. Consumption of cimaterol stimulated weight gain of hindlimb muscle at an even faster rate than total body weight gain (Figure 1). In 4 wks rats fed 10 and 100 ppm cimaterol gained 56% and 75% more hindlimb muscle than control rats with a significant acceleration in gain evident within the first wk (80% and 124%). These increases in muscle gain represented increased protein deposition because protein concentration in hindlimb muscle was unchanged by cimaterol (Figure 1). Stimulation of muscle growth was generalized rather than selective for a specific fiber type because muscles containing predominantely red (soleus) or white (EDL) fibers responded similarly

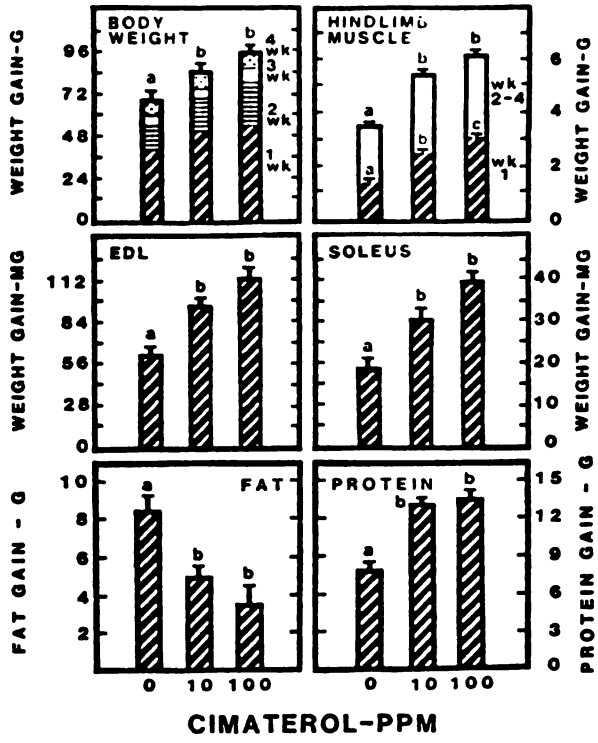

Figure 1. Effects of cimaterol on body composition. Gain in body weight, tissue weights, total body fat and protein in rats fed ad libitum for 4 wks a high-carbohydrate diet containing 0, 10 or 100 ppm cimaterol. The four segments of the bars for body weight gain represent gain per wk for each of the 4 wks. Gain in hindlimb muscle for the first wk is represented by the lower segment of the bar, the upper segment represents gain for the last 3 wks of the 4 wk study. Hindlimb muscle indicates total muscle stripped from one hindlimb; and EDL indicates extensor digitorum longus. The protein concentration in hindlimb muscle averaged 151 ± 6, 163 ± 3 and 168 ± 4 mg/g hindlimb muscle in rats fed for 1 wk 0, 10, or 100 ppm cimaterol, respectively, and 164 ± 6, 159 ± 7 and 170 ± 5 mg/g hindlimb muscle in rats for 1 wk 0, 10 or 100 ppm cimaterol respectively. Each point is the mean ± SEM of 10 rats. Different letters above the bars indicates significant differences among groups ($P<0.05$). Initial body weight, 131 ± 1.0 g; hindlimb muscle weight, 6.65 ± 0.13 ± g; EDL weight, 130 ± 1 mg; soleus weight, 55 ± 0.5 mg; total body fat, 9.3 ± 0.4 g: and total body protein 16.0 ± 0.7 g. Gains in body weight, tissue weight, total body fat and protein were calculated from the final values minus the predicted initial values based on initial body weight of each rat.

to cimaterol (Figure 1). Lengths of tibia and femur were unaffected by consumption of cimaterol (data not presented), indicating that effects of cimaterol on muscle accretion were not merely a consequence of increased bone length. A dose-dependent reduction (41-59%) in gain in total body fat content was observed in rats fed diets containing cimaterol (Figure 1). In agreement with the increased skeletal muscle gain, gain in total body protein was increased 70-76% in rats fed for 4 wks diets containing 10 and 100 ppm cimaterol (Figure 1). As a result of the shifts in fat and protein content and inturn tissue gain, cimaterol-fed rats were leaner than control rats even though they gained more body weight.

Energy intake was elevated in rats fed diets containing 10 ppm (5%) and 100 ppm (13%) cimaterol for 4 wks (Figure 2). This higher energy intake was evident within the first wk. There was a trend for a reduction in body energy gain and in the efficiency of energy retention in rats fed cimaterol, but the reductions were not statistically significant (Figure 2). A slight elevation in energy expenditure was observed in both treatment groups and closely paralleled the increase in energy intake (Figure 2).

Experiment 2. Effects of cimaterol on in vivo rates of fatty acid synthesis in liver and in white and brown adipose tissues were examined. Rates of fatty acid synthesis in the three tissues examined were not significantly affected by consumption of cimaterol for either 1 or 4 wks (Figure 3). Liver and interscapular brown adipose tissue weights were unaffected by treatment. Consequently, the same relationships are observed whether data are expressed per tissue or per g tissue. Consumption of cimaterol depressed weight of white

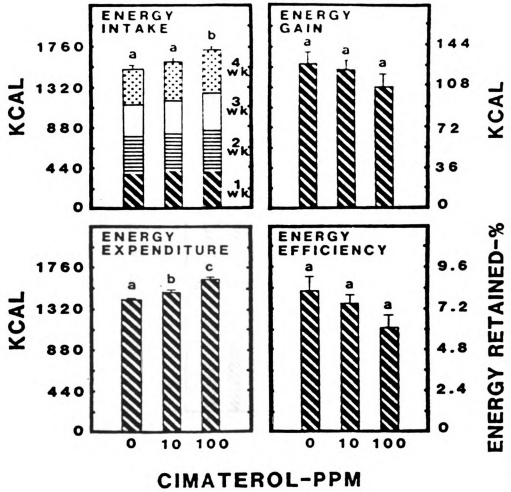

Figure 2. Energy balance in rats fed cimaterol. Rats were fed a high-carbohydrate diet containing 0, 10, or 100 ppm cimaterol ad libitum for 4 wks. The four segments of the bars for energy intake represent intake per wk for each of the 4 wks. Each bar is the mean $\pm$ SEM of 10 rats. Different letters above the bars indicates significant differences among the groups ($P<0.05$). Initial body energy was 238±7 kcal. Energy gain was calculated as final body energy minus the predicted initial body energy. Energy expenditure was calculated as the difference between metabolizable energy intake and energy gain. Energy efficiency equals energy gain divided by energy intake times 100.

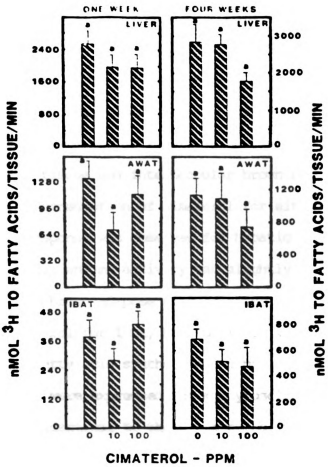

Figure 3. **Effects of cimaterol on rates of fatty acid synthesis. Rats were fed a high-carbohydrate diet containing 0, 10 or 100 ppm cimaterol ad libitum for 1 or 4 wks . AWAT indicates abdominal white adipose tissue and IBAT indicates interscapular brown adipose tissue. Values are means ± SEM for 10 animals. Different letters above the bars indicate significant differences between groups ($P<0.05$). Initial body weight of rats, 141±2 g and 131±1 g for 1 and 4 wk experiments, respectively. At the end of 1 or 4 wk feeding period, rats were injected with 1 mCi of ^3H_2O at 0900 h and killed 15 minutes later to measure the rates of fatty acid synthesis. Liver and IBAT weights were unaffected by treatment. Final liver weights averaged 6.63±0.13 g and 6.92 ±0.17 g in the 1 and 4 wk experiments, respectively. Final IBAT weights averaged 0.30 ±0.01 g and 0.42±0.05 g in the 1 and 4 wk experiments, respectively. Adipose tissue weights were affected by the treatment. Final weights of white adipose tissue averaged 2.14±0.11 g, 1.96±0.1 g, and 1.71±0.10 g in rats fed 0, 10 or 100 ppm cimaterol for 1 wk. Adipose tissue weights averaged 3.78±0.35 g, 3.05±0.16 g, and 2.89 ± 0.28 g in rats fed 0, 10 or 100 ppm cimaterol for 4 wks.**

adipose tissue. When rates of fatty acid synthesis were expressed per g white adipose tissue, rates were equal to or slightly higher than values for control animals (data not presented).

In agreement with the measurements of de novo fatty acid synthesis in vivo, activities of fatty acid synthetase in liver, abdominal white adipose tissue and interscapular brown adipose tissues were also unaffected by consumption of cimaterol for either 1 or 4 wks (Table 1). A similar response was observed for hepatic malic enzyme activity (Table 1). Malic enzyme activity was slightly depressed in white adipose tissue and brown adipose tissues after feeding the diet containing 100 ppm cimaterol for 1 wk, but not after 4 wks (Table 1). When the activities of fatty acid synthetase and malic enzyme in liver and brown adipose tissue were expressed per mg cytosolic protein, relative responses were identical to those expressed on a per tissue basis (data not presented). In white adipose tissue, the activities of fatty acid synthetase and malic enzyme expressed per mg cytosolic protein were actually elevated in cimaterol-treated rats (data not presented).

Effects of a single intraperitoneal injection of cimaterol on in vivo rates of fatty acid sythesis were examined (Figure 4). Rates of fatty acid synthesis in liver and white adipose tissue were unaffected by cimaterol, but rates of fatty acid synthesis were inhibited approximately 50% in brown adipose tissue of rats injected with cimaterol.

To increase the possibility of detecting an effect of cimaterol on rates of fatty acid synthesis, rats were trained to eat meals. As expected, rates of fatty acid synthesis were 2-3 fold higher in

Table 1. Effect of consuming cimaterol on lipoyenic enzymes.

Tissue	Enzyme	1 week Diet-ppm cimaterol 0	10	100	SE	4 week Diet-ppm cimaterol 0	10	100	SE
Liver	Fatty acid synthetase[1]	3.48[a]	2.94[a]	3.35[a]	0.14	6.29[a]	5.67[a]	6.21[a]	0.40
	Malic enzyme[1]	6.58[a]	6.93[a]	7.31[a]	0.38	12.30[a]	10.60[a]	10.45[a]	0.80
White adipose tissue	Fatty acid synthetase	0.25[a]	0.20[a]	0.19[a]	0.02	1.04[a]	1.13[a]	1.01[a]	0.09
	Malic enzyme	5.99[a]	5.21[ab]	4.61[b]	0.23	9.12[a]	9.62[a]	8.81[a]	0.09
Brown adipose tissue	Fatty acid synthetase	0.38[a]	0.32[a]	0.36[a]	0.02	1.53[a]	1.48[a]	1.40[a]	0.07
	Malic enzyme	2.54[a]	2.22[ab]	2.01[b]	0.10	4.65[a]	4.44[a]	4.05[a]	0.22

[1]One unit of enzyme activity is defined as utilization of one umole of NADPH or NADP per minute per tissue at 30°C. Values are means of 10 animals. Means for a given enzyme within wk 1 or within wk 4 with different superscript letters are different ($p<0.05$). Initial body weight 133 ± 1 g. Liver and interscapular brown adipose tissue weights were unaffected by treatment. Mean liver weights for the 1 and 4 wk experiments averaged 6.5 ± 0.2 g and 6.8 ± 0.2 g, respectively; interscapular brown adipose tissue weights for the 1 and 4 wk experiments averaged 0.31 ± 0.01 g and 0.39 ± 0.02 g, respectively. Mean weights for abdominal white adipose tissue from 1 wk experiment were: control, 1.8 ± 0.2 g; 10 ppm, 1.6 ± 0.1 g; and 100 ppm, 1.3 ± 0.1 g; and for the 4 wk experiment control, 3.85 ± 0.3 g; 10 ppm, 3.08 ± 0.2 g; and 100 ppm, 2.37 ± 0.2 g.

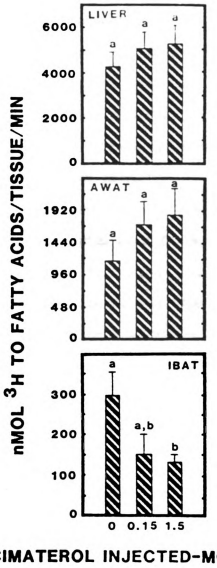

Figure 4. Acute effects of cimaterol on fatty acid synthesis. Effects of a single injection of cimaterol on in vivo rates of fatty acid synthesis in rats fed a high-carbohydrate diet. Rates of fatty acid synthesis were measured 15 to 30 minutes after intraperitoneal injection of cimaterol at 0900 h. AWAT indicates abdominal white adipose tissue and IBAT indicates interscapular brown adipose tissue. Values are means $\pm$ SEM of 10 animals. Different letters above the bars indicates significant differences among groups ($P<0.05$). Mean body weight, 153$\pm$1 g. Tissue weights; liver weight, 6.79$\pm$0.2 g; white adipose tissue weight, 3.18$\pm$0.24 g; and brown adipose tissue weight, 0.32$\pm$0.02g.

meal-fed rats (Table 2) than in ad libitum-fed rats (Figure 3). Even under these conditions of elevated rates of fatty acid synthesis, chronic administration of cimaterol failed to affect fatty acid synthesis in liver, white adipose tissue and brown adipose tissue (Table 2). Rates of fatty acid synthesis expressed per gram white adipose tissue were elevated in rats fed cimaterol because of the lower tissue weights (data not presented).

In agreement with the results of in vivo experiments, cimaterol failed to depress in vitro rates of fatty acid synthesis in liver or white adipose tissue (Figure 5). Rates of fatty acid synthesis were depressed by cimaterol in white adipose tissue incubated in media containing a high concentration (100 mU/ml) of insulin (Table 3). Cimaterol inhibited the in vitro rates of fatty acid synthesis in brown adipose tissue (Figure 5 and Table 3) as was also observed after acute injection of cimaterol (Figure 4).

Experiment 3. Consumption of high-fat diets depress de novo rates of fatty acid synthesis (69). To provide further evidence that effects of cimaterol on adipose tissue and muscle accretion do not require modulation of de novo fatty acid synthesis, rats were fed a high fat-diet containing cimaterol. Consumption of cimaterol increased body weight gain (20%) and hindlimb muscle gain (66%), and reduced gain in white adipose tissue (66%) (Figure 6). Energy density of the weight gain, an indicator of relative proportions of fat and protein gain, was approximately 32% lower in rats fed 100 ppm cimaterol. These results paralleled those obtained when the high-carbohydrate diet was fed (Figure 1) and support the suggestion

Table 2. Rates of fatty acid synthesis in meal-fed rats

	Diet-ppm cimaterol			
	0	10	100	SE
Liver	8387	7680	6745	406
Abdominal white adipose tissue	4825	4223	3374	357
Interscapular brown adipose tissue	636	522	397	53

Nanomoles tritium incorporated into fatty acids per tissue per minute. Values are means of 10 animals. None of the treatment effects were significant ($p<0.05$). Initial body weight averaged 141 ± 2g. Food was available only between 0900-1000 h and between 1600-1700 h daily. After 1 wk rats were injected intraperitoneally with 1 mCi of ^3H_2O at 1015 h. After another 15 minutes rats were killed to measure rates of fatty acid synthesis. Liver and brown adipose tissue weights were unaffected by treatment and averaged 5.39 ± 0.13 g and 0.30 ± 0.02 g respectively. Mean weights for white adipose tissue; control, 1.17 ± 0.11 g; 10 ppm, 0.94 ± 0.14 g; and 100 ppm, 0.58 ± 0.06 g.

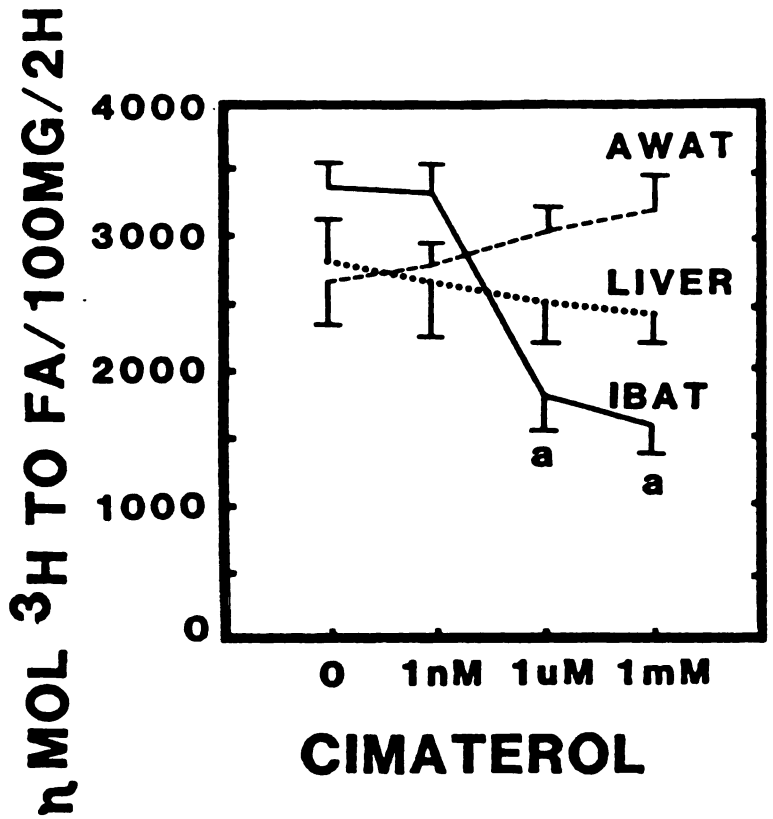

Figure 5. Rates of fatty acid synthesis in vitro. Each point is the mean ± SEM of 12 observations; tissues from each of 12 rats were divided into 4 pieces and incubated at each of the four concentrations of cimaterol. Mean body weight of rats was 211±2g. Letter a indicates that the value is significantly lower than the control value ($P<0.05$).

Table 3. In vitro rates of fatty acid synthesis in the presence of insulin.

	100 uU insulin/ml		100 mU insulin/ml		
	concentration of cimaterol				
	0	10^{-3}M	0	10^{-3}M	SE
Liver	2249	2319	2355	2611	0.10
Abdominal white adipose tissue	2698	2801	4796[b]	3397[a]	0.17
Interscapular brown adipose tissue	4465	1729[a]	8551[b]	3700[ab]	0.24

Nanomoles tritium incorporated into fatty acids per 100 mg per 2 h. Each value is the mean of 12 observations; tissues from each of 12 rats were incubated at each of the four concentrations of insulin and cimaterol. Mean body weights of rats was 211±2 g. Superscript a indicates significant effect of drug within one concentration of insulin ($P<0.05$). Superscript b indicates significant effect of concentration of insulin within control or cimaterol treated tissue ($P<0.05$).

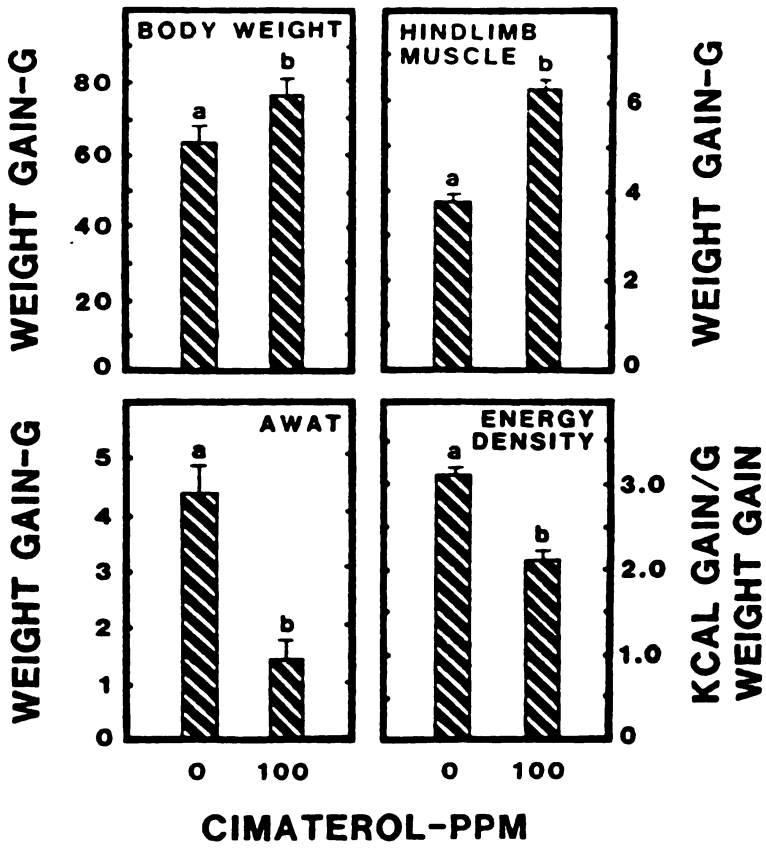

Figure 6. Body composition in rats fed a high-fat diet. Gain in body weight, tissue weight and energy density in rats fed a high-fat diet containing, 0 or 100 ppm cimaterol ad libitum for 4 wks. AWAT indicates abdominal white adipose tissue. Values are means $\pm$ SEM for 10 rats. Different letters above the bars indicate significant differences between the groups ($P<0.05$). Initial mean body weight averaged 169 ± 4 g; muscle weight, 7.98 ± 0.13 g; AWAT weight, 2.2 ± 0.17 g; and energy density, 1.78 ± 0.27 kcal/g body weight.

that cimaterol does not influence body composition via alterations in fatty acid synthesis.

Experiment 4. Since uptake of fatty acids by adipose tissue and skeletal muscle from circulating triglycerides is facilitated by lipoprotein lipase (28). Modulation of lipoprotein lipase activity by cimaterol as a potential mechanism to influence body composition was examined. Chronic consumption of cimaterol (4 wks) failed to influence lipoprotein lipase activity in white adipose tissue when the results were expressed per total tissue weight (Figure 7). Lipoprotein lipase activity per g white adipose tissue was elevated in rats fed cimaterol because tissue weights were depressed. Chronic consumption of cimaterol failed to influence lipoprotein lipase activities in brown adipose tissue regardless of method of expression. As expected, lipoprotein lipase activity per gram muscle was lower in EDL than in soleus with an intermediate activity in total hindlimb muscle. These activities are consistent with their white, red and mixed fiber types, respectively. Lipoprotein lipase activities, expressed per tissue, in both EDL and in total hindlimb muscles were elevated by 67% when the diet containing 100 ppm cimaterol was consumed. This elevation in activity resulted from increased weights of these muscles after consuming cimaterol for 4 wks coupled with a trend for stimulation of activity per gram muscle. Lipoprotein lipase activity in soleus muscle was not significantly affected by cimaterol (Figure 7).

Effects of a single intraperitoneal injection of cimaterol on activities of lipoprotein lipase are shown in Table 4. Lipoprotein lipase activity in white adipose tissue was unaffected by cimaterol

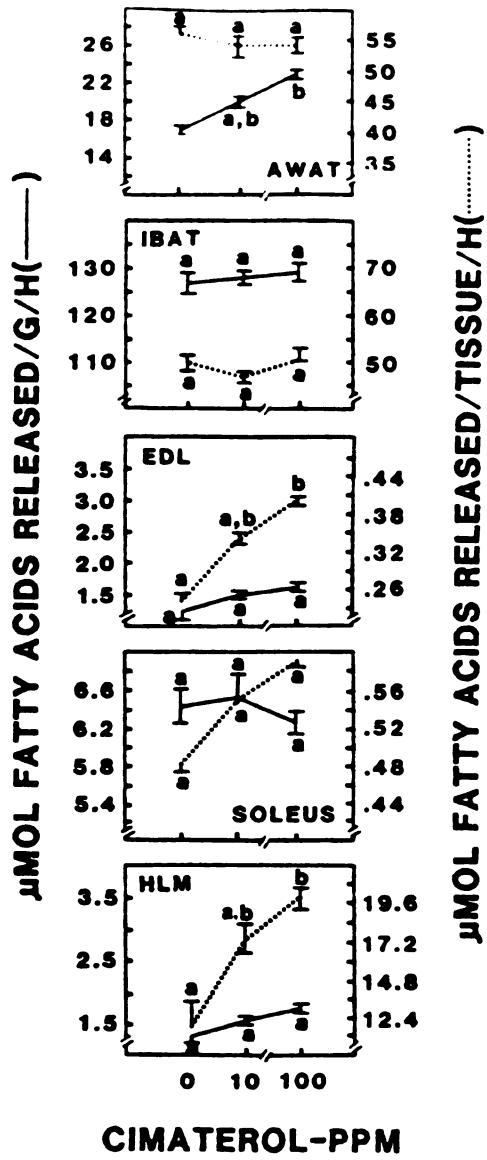

Figure 7. Effects of cimaterol on lipoprotein lipase activity. Rats were fed a high-carbohydrate diet containing 0, 10 or 100 ppm cimaterol ad libitum for 4 wks. Values are expressed per g tissue and per tissue. AWAT indicates abdominal white adipose tissue; IBAT, interscapular brown adipose tissue; EDL, extensor digitorum longus. Values are means of ± SEM of 10 rats. Different letters by points on the lines indicate significant differences among the groups ($P<0.05$). Initial mean body weight 131± g. Rats were killed at 1100 h to measure the lipoprotein lipase activity.

Table 4. Acute effects of cimaterol on lipoprotein lipase activity.

	Cimaterol injected-mg			
	0	0.15	1.5	SE
Abdominal white adipose tissue	31.2[a]	32.9[a]	33.9[a]	2.9
Interscapular brown adipose tissue	17.2[a]	25.2[b]	27.2[b]	0.7
Extensor Digitorum Longus muscle	0.09[a]	0.13[ab]	0.16[b]	0.01
Soleus muscle	0.36[a]	0.34[a]	0.33[a]	0.02
Total hindlimb muscle	14.57[a]	14.82[a]	17.78[a]	0.88

Micromoles of free fatty acids released per tissue per h. Means with different superscript letters are significantly different ($P<0.05$). Values are means of 8-10 rats per group. Rats, fed the high-carbohydrate control diet for 1 wk, were injected intraperitoneally with cimaterol at 0700 h and killed after 4 h. Mean body weight, 175 ± 1 g; white adipose tissue weight, 1.9 ± 0.1 g; brown adipose tissue weight, 265 ± 31 mg; extensor digitorum longus weight, 152 ± 4 mg; soleus weight, 69 ± 1 mg; and total hindlimb muscle weight, 7.8 ± 0.1 g.

whereas brown adipose tissue lipoprotein lipase activity was elevated by 60% in rats injected with 1.5 mg cimaterol. A marked increase (75%) in lipoprotein lipase activity was also observed in EDL muscle, but not in soleus, in response to injection of 1.5 mg of cimaterol. There was a trend for an increase (22%) in lipoprotein lipase activity in total hindlimb muscle of rats injected with cimaterol, although the increase was not statistically significant.

To determine if the elevation in lipoprotein lipase activity in skeletal muscle observed after administration of cimaterol to rats was a consequence of direct action of cimaterol on skeletal muscle, enzyme activity was measured in EDL (white) and soleus (red) muscles incubated in the absence or presence of cimaterol. Lipoprotein lipase activity in the control muscles was essentially unchanged during the 2 h incubation (Figure 8). Release of lipoprotein lipase activity into the incubation media was stimulated by cimaterol. In EDL, 1 mM cimaterol elevated enzyme activity by 66% (tissue and media combined) (Figure 8). Although there was a trend for stimulation of lipoprotein lipase activity in soleus muscle in response to cimaterol, activity was not significantly elevated.

Experiment 5. Lipolysis, estimated in vivo by the concentration of free fatty acids in plasma, was stimulated after consuming cimaterol for 4 wks indicating a persistent lipolytic activity of cimaterol (Figure 9). Both plasma free fatty acids and glycerol concentrations were elevated following a single intraperitoneal injection of cimaterol (Figure 10). Increases in plasma free fatty acid and glycerol concentrations were noted within 15 minutes of injection, and tended to be even higher after 30 minutes. Lipolysis

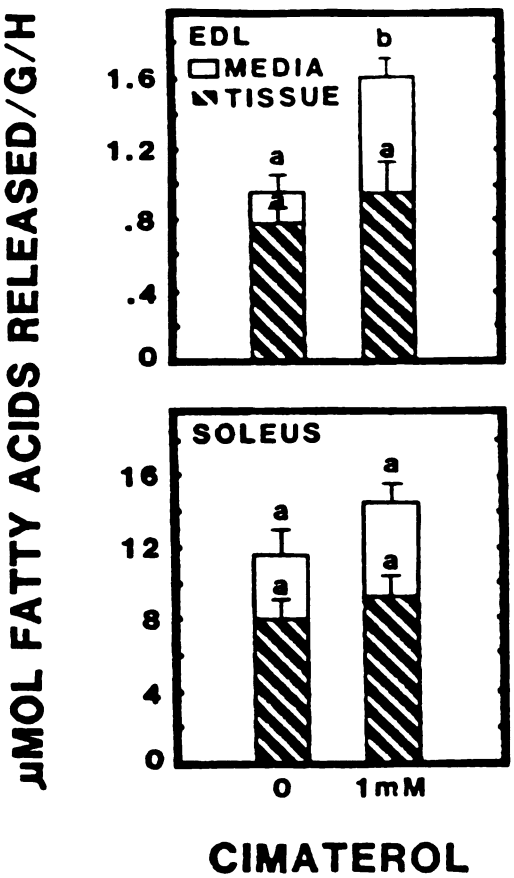

Figure 8. Lipoprotein lipase acivity in vitro in muscle. Each bar is the mean ± SEM of 12 rats. Muscles from one hindlimb were incubated for 2 hrs at 37^{0}C in absence of cimaterol and muscles from the other hindlimb incubated with 1 mM cimaterol. Body weight of the rats that had been fed a high-carbohydrate diet for 1 wk ranged from 80 to 100 g. EDL muscle weight ranged from 80 to 100 mg and soleus muscle weight ranged from 30 to 40 mg. Lipoprotein lipase activity of representative muscles prior to incubation averaged 0.79±0.09 for EDL muscle and 8.0 ±0.3 uM free fatty acids released per gram per hour for soleus muscle. Different letters above the bars indicates significant differences between groups ($P<0.05$).

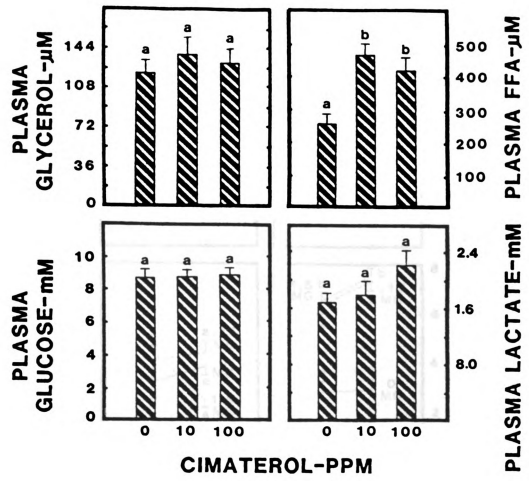

Figure 9. Effects of cimaterol on plasma metabolites. Plasma metabolites in rats fed ad libitum for 4 wks a high-carbohydrate diet containing 0, 10 or 100 ppm cimaterol. Values are means ± SEM of 10 rats. Different letters above the bars indicates significant differences among the groups. Initial body weight 131±1.0 g.

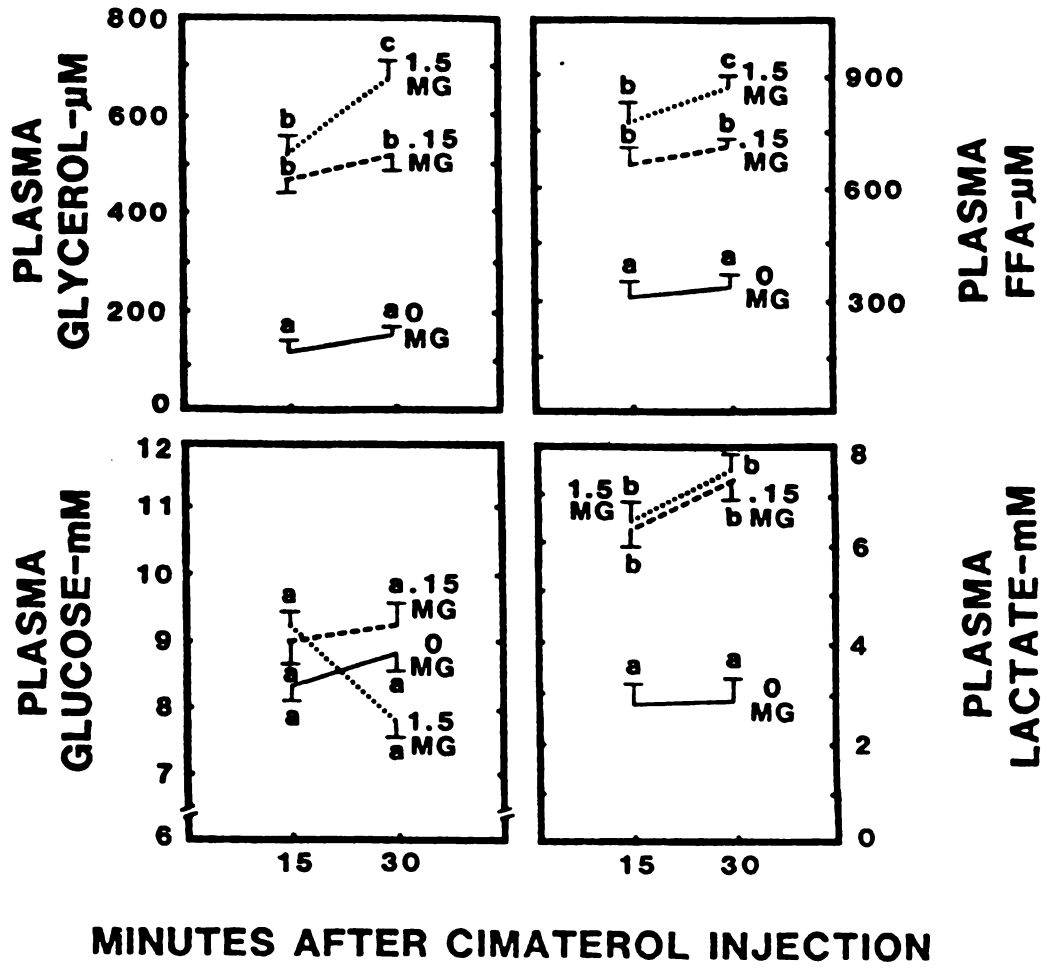

Figure 10. Acute effects of cimaterol on plasma metabolites. Effects of a single injection of cimaterol (0, 0.15 or 1.5 mg) on plasma metabolites in rats fed a high-carbohydrate diet. Values are means ± SEM of 10 animals. Different letters above the points indicates the significant diffrences among the groups either at 15 or 30 minutes after injections ($P<0.05$). Mean body weight 187±12 g.

in white adipose tissue, as measured in vitro by release of free fatty acid and glycerol, was stimulated by cimaterol in a dose dependent manner (Figure 11).

Plasma lactate was increased following the injection of cimaterol (Figure 10) but was unaffected after consuming cimaterol for 4 wks (Figure 9). Glucose concentrations in plasma were unaffected by cimaterol (Figure 9 and 10).

Discussion

Results of the present study demonstrate that the beta adrenergic agonist cimaterol increases skeletal muscle accretion and concomitantly depresses adipose tissue deposition in rats. These data parallel responses of several other species to selelected beta adrenergic agonists (9,10,31,36,60,95, 97). Effects of cimaterol on body composition were clearly evident within the first wk of administration (Figure 1, Table 1), as has also been observed in rats that recieved clenbuterol (96). Cimaterol exerted a less dramatic influence on body composition beyond the first wk of treatment.

Cimaterol depressed fat gain and accelerated protein gainin these rats with no appreciable change in overall energy balance (Figure 2). Although consumption of cimaterol increased energy intake of the rats (Figure 2), this increase in intake appeared to be an indirect response to the heavier body weights because energy intakes of the treatment groups were not increased when expressed per gram body weight (data not shown). Others have reported increases, no change or decreases in energy intake depending on the specific agonist used (5,9,10,31,36,60,95,97,112). Considering that beta-agonists activate brown adipose tissue thermogenesis in rats (5), and that the energetic

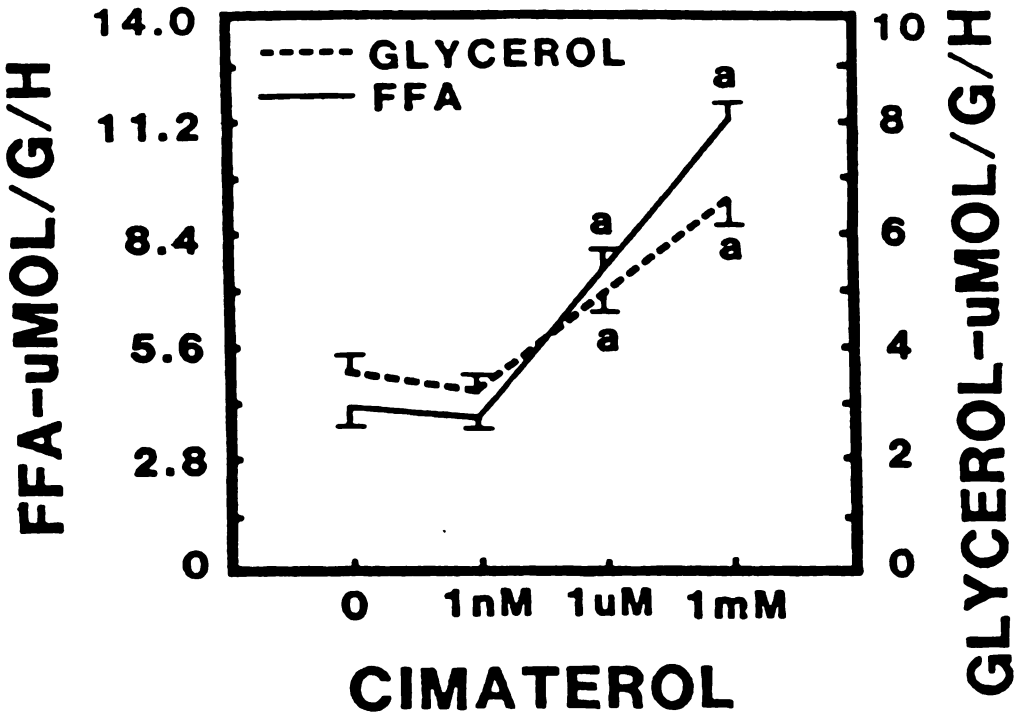

Figure 11. Rates of lipolysis in vitro in white adipose tissue. Each point is the mean ± SEM of 12 observations. Adipose tissue from each of 12 rats was devided into 4 pieces and incubated at one of four concentrations. The letter a by point indicates that the value is significantly higher than the respective control value ($P<0.05$). Mean body weight of rats 165±2.0 g.

efficiency for deposition of protein (48%) is normally considered to be much lower than for deposition of the same amount of energy as fat (77%) (94), I have predicted that the efficiency of energy retention would be lower in rats fed cimaterol than in control rats. Higher doses of cimaterol than used in the present study will activate brown adipose tissue thermogensis and lower the efficiency of energy retention in mice (unpublished). The pathways responsible for the observed changes in body composition appeared to be more sensitive to cimaterol than are the thermogenic pathways.

My working hypothesis was that cimaterol would inhibit conversion of dietary carbohydrates to fatty acids as one mechanism to direct energy away from adipose tissue toward skeletal muscle. Results of the present study do not support this hypothesis. In vivo rates of fatty acid synthesis in liver and white adipose tissue, assessed by using ^3H_2O, were unaffected by cimaterol, even in meal-fed rats where high rates of de novo fatty acid synthesis were observed (Table 2). Furthermore, activities of two adaptive lipogenic enzymes, fatty acid synthetase and malic enzyme (Table 1), and in vitro rates of fatty acid synthesis (Figure 5) were also unaffected by cimaterol, except for depressed fatty acid synthesis in white adipose tissue in the presence of high concentrations of insulin (Table 3). I conclude that the reductions in fat content and the increases in protein content in rats fed cimaterol are not caused by suppressed conversion of dietary carbohydrates to fatty acids. This conclusion is supported by the observation that cimaterol also decreased white adipose tissue gain and increased skeletal muscle gain in rats fed a high-fat diet where de novo fatty acid synthesis would be suppressed (69).

The failure of cimaterol to affect the rates of fatty acid synthesis in liver is probably related to the lack of functional beta-receptors in liver of adult rats (77). This is supported by the finding that cimaterol also failed to stimulate hepatic glycogenolysis, as indicated by the unchanged plasma glucose concentrations in rats injected with cimaterol (Figure 10). Although catecholamines have been shown under certain conditions to inhibit the actvitiy of acetyl coenzyme A carboxylase and glucose conversion to fatty acids in the presence of high concentrations of insulin in white adipose tissue (20,66), I found no evidence that cimaterol inhibited fatty acid synthesis in vivo in white adipose tissue under the conditions where fat deposition was depressed. Cimaterol increased concentrations of plasma lactate, a major lipogenic substrate (26); this substrate may have permitted rats fed diets containing cimaterol to maintain rates of fatty acid synthesis in white adipose tissue.

Rates of fatty acid synthesis and lipolysis in adipose tissue are inversely related in rats fed a high carbohydrate meal or fasted (100). There are several possibilites to explain why fatty acid synthesis was not depressed as lipolysis was stimulated in rats fed cimaterol. Beta-receptor activation has recently been shown to stimulate by 5-10 fold the transport of fatty acids from adipocytes (1). Stimulated efflux of fatty acids generated during lipolysis would limit accumulation and subsequent feedback inhibition of de novo fatty acid synthesis. Another possibility is that cimaterol selectively activated lipolysis without affecting enzymes involved in fatty acid synthesis. Data are available to show that cAMP-dependent

activation of glycogen phosphorylase and of hormone sensitive lipase can occur independently (57).

De novo fatty acid synthesis in brown adipose tissue is thought to contribute minimally to body fattening, but rather to supply a readily utilizable fuel for brown adipose tissue thermogensis (106). Rates of fatty acid synthesis in brown adipose tissue were inhibited by cimaterol in vitro and also after acute in vivo administration (Figure 4, 5). This contrasts with findings in white adipose tissue and may be related to high concentrations of beta-receptors in brown adipose tissue (104). Fatty acid synthesis in brown adipose tissue became resistant to action of cimaterol within 1 wk, perhaps due to desensitization of the beta-receptors after chronic exposure to the agonist (45).

Lipolysis was stimulated in vivo by cimaterol as evidenced by an increase in plasma free fatty acid concentration (Figure 9, 10). Plasma free fatty acid concentrations correlate well with their turnover rates and with body fat content (13). Therefore, the elevated concentrations of free fatty acids observed in rats fed cimaterol, even after 4 wks when body fat gain was significantly reduced, is indicative of persistent lipolytic activity of cimaterol. The in vitro results indicate that cimaterol has direct lipolytic effects on adipose tissue in rats (Figure 11). Stimulation of lipolysis in vitro by beta agonists such as cimaterol and clenbuterol may, however, be species-dependent because Mersmann (80) has shown that clenbuterol fails to stimulate lipolysis in vitro in pigs although it increases plasma glycerol and free fatty acid concentrations.

Lipoprotein lipase activity in white adipose tissue was unaffected by either acute or chronic exposure to cimaterol (Table 4, Figure 7). This observation agrees with the conclusions of Hansson et al (51) and Chernick et al (23) that lipoprotein lipase activity in rat adipose tissue is unlikely to be regulated by adrenergic mechanisms although Ashby et al (7,8) reported inhibition of insulin stimulated lipoprotein lipase activity in adipose tissue in response to epinephrine. We conclude that reduced adipose tissue gain observed in cimaterol fed rats did not result from inhibition of lipoprotein lipase activity.

In contrast to white adipose tissue where lipoprotein lipase activity was unaffected, enzyme activity in skeletal muscle was stimulated after administration of cimaterol to rats (Table 4, Figure 7). This increase in enzyme activity was also demonstrable in vitro (Figure 8), indicating that cimaterol has direct actions on skeletal muscle. The most pronounced effects of cimaterol were observed in EDL where white fibers predominate. Stimulation of lipoprotein lipase activity in EDL by cimaterol may be an adaptive response to a shift from carbohydrate utilization to fat as a metabolic fuel for the tissue. Soleus muscle, which contains a high proportion of red fibers, and utilizes mainly fat as an energy source (105), has higher lipoprotein lipase activity per gram tissue than EDL. This may have contributed to the lesser percentage increase in soleus muscle lipoprotein lipase activity than in EDL. Since EDL muscle has a lower concentration of beta adrenergic receptors, than does soleus (109), it would appear that postreceptor events mediated the selective

stimulation of lipoprotein lipase in EDL and the equal percentage increases in weight of the two muscles.

A large elevation in activity of lipoprotein lipase was observed in brown adipose tissue within 4 h after in vivo injections of cimaterol (Table 4). Similar responses have been shown in brown adipose tissue after injection of norepinephrine (21). But after chronic exposure to cimaterol, lipoprotein lipase activity in brown adipose tissue was no longer elevated (Figure 7). This is similar to the response of fatty acid synthesis in brown adipose tissue where it was inhibited only after acute but not after chronic administration of cimaterol, again suggesting desensitization of the beta receptors after chronic exposure to the agonist (45).

In summary, cimaterol altered body composition by reducing fat gain and increasing protein gain with minimal changes in overall energy balance. Reduced adipose tissue gain in rats fed cimaterol resulted from increased mobilization of stored triglycerides by stimulation of lipolysis with no major influence on either de novo fatty acid synthesis or the activity of lipoprotein lipase in white adipose tissue. On the other hand, increased skeletal muscle gain was associated with elevated lipoprotein lipase activity, indicating a preferential utilization of fatty acids as fuel in muscle of rats treated with cimaterol.

EFFECTS OF CIMATEROL, A BETA ADRENERGIC AGONIST, ON PROTEIN METABOLISM IN RATS.

Introduction

Clenbuterol and cimaterol, beta adrenergic agonists, increase skeletal muscle accretion in rats (36,76,95) and other species (9,10,11,31,60,97). The mechanism(s) responsible for these beta agonist-induced increases in skeletal muscle mass have not yet been clarified. Cimaterol stimulates lipoprotein lipase activity in rat skeletal muscle (Figures 7,8 and Table 4). This would facilitate availability of energy for increased muscle metabolism, but would not directly stimulate muscle accretion. There is some evidence that catecholamines slow skeletal muscle protein turnover. Epinephrine or the beta-adrenergic agonist isoproterenol decreases release of alanine and glutamine from isolated rat epitrochlaris muscle preparations during a 3 h incubation period, and release of phenylalanine from rat hemicorpus preparations during a 3 h perfusion (42,71). These effects in the epitrochlaris preparation were blocked by the beta-antagonist propranolol (42). Reeds et al (95) concluded that the beta-agonist clenbuterol probably also decreases skeletal muscle protein turnover in rats; a conclusion based on the observation that addition of clenbuterol to the diet for 4 days stimulated fractional accretion rates of skeletal muscle without detectable increases in fractional synthesis rates of muscle proteins. But others have reported that clenbuterol increases by 34% fractional rates of phenylalanine incorporation into mixed skeletal muscle proteins 1 h after the the seventh daily injection (36), and that isoproterenol stimulates incorporation of C^{14}-labeled amino acids into tibialis muscle protein 2-3 h after the fifth daily injection (33). Thus, effects of beta-agonists on protein metabolism remain unclear. The timing of the

measurement relative to administration of the beta adrenergic agonist appears important.

Stimulation of skeletal muscle accretion by beta-agonists in rats is most pronounced during the first wk of administration (Figure 1 and ref 95). Therefore, protein metabolism likely changes with time after administration of beta-agonists. The present study was, therefore, designed to evaluate the temporal relationships between administration of a beta-agonist, cimaterol, protein accretion, and protein turnover in rats. Urinary excretion of 3-methylhistidine was selected as the indicator of protein turnover, or more specifically as an index of turnover of 3-methylhistidine containing proteins (114). 3-Methylhistidine, found exclusively in actin and myosin, is quantitatively excreted in urine of rats, and as such has been used as an indicator of skeletal muscle protein turnover (because more than 90% of total body 3-methylhistidine is in skeletal muscle) (114). Although the non-skeletal muscle pool of 3-methylhistidine containing proteins is small, this pool turns over faster than the skeletal muscle pool, and thus provide a disproportionately large fraction of urinary excretion of 3-methylhistidine (82). Consequently, urinary excretion of 3-methylhistidine best provides an index of turnover of total body 3-methylhistidine containing proteins, not exclusively those in skeletal muscle. I, therefore, measured the total body pool size of 3-methylhistidine containing proteins and urinary excretion of 3-methylhistidine at selected times to calculate effects of cimaterol on turnover of total body 3-methylhistidine containing proteins. Total body protein, hindlimb muscle protein, RNA and DNA content, and

concentrations of plasma amino acids, insulin, triiodothyronine and corticosterone were also measured.

Materials and methods

Animals and diets. Female Sprague-Dawley rats (100-110 g), obtained from Harlan Industries, Indianapolis, IN, were housed individually at 23^{0}C in metal cages with wire-mesh floors. Room lights were on from 0700 to 1900 h. All animals were provided a nonpurified diet (Wayne Rodent Blox, Continental Grain Company, Chicago, IL) and water ad libitum for the first 2 days after arrival in the laboratory. Rats were then fed a purified high-carbohydrate diet for 6 days and then divided into experimental groups. The high-carbohydrate diet contained (in g/100 g): 66.0 g glucose, 5.0 g corn oil, 20.0 g casein, 0.3 g methionine, 1.0 g vitamin mix (14), 0.2 g choline chloride, 3.5 g mineral mix (14) and 4.0 g cellulose. This diet provided 3.57 kcal metabolizable energy/g with 67% of metabolizable energy as carbohyrate, 13% as fat and 20% as protein. The amount of cimaterol (CL 263,780; anthranilonitrile, 5-[1-hydroxy-2-(isopropylamino)ethyl]-) added to these diets is indicated in the experimental design.

Experimental design. Rats were divided into 4 groups. Group 1 (10 rats) was killed at the beginning of the experiment to obtain initial body composition values; groups 2, 3, and 4 (30 rats per group) were fed for 7, 14, or 29 days the high-carbohydrate diet containing 0, 10 or 100 ppm cimaterol, respectively, and then killed. Blood was collected and plasma was stored at -70^{0}C for subsequent measurement of amino acids and hormones. Hindlimbs were separated and quickly frozen in dry ice and acetone. Carcasses were frozen for subsequent

analysis. Food intake and weight gains were recorded daily during the first wk, and twice weekly during the following 3 wks. Urine was collected daily with a funnel below each cage into the bottles containing 6 N HCL as preservative during the first wk, and for two days each wk during the following 3 wks. At the end of each 24 h collection, urine was filtered and stored at -20^{0}C for subsequent 3-methylhistidine analyses.

To determine acute effects of cimaterol on plasma amino acids and hormones, rats fed the high-carbohydrate control diet for 1 wk were injected intraperitoneally with saline, 0.15 or 1.5 mg cimaterol in 0.2 ml saline at 0700 h and killed at 1100 h. These doses correspond to the daily amount of cimaterol consumed by rats fed diets containing 0, 10, or 100 ppm cimaterol. Plasma, separated from the blood, was stored at -70^{0}C for subsequent amino acid and hormone analyses.

Analyses. Weights of the carcass (skeletal muscle and associated bone after removing the dissectable adipose tissue) and of the rest of the body (head, skin, tail, removable adipose tissue and viscera) were recorded. Abdominal white adipose tissue (total dissectable adipose tissue from the abdomen) and right hindlimb muscle weights were also recorded separately. Hindlimb muscle weights were determined after being stripped from the bones and separated from adipose tissue. These tissues were returned to the carcasses or the rest of the body, except for aliquots of homogenized hindlimb muscle used for measuring protein, RNA and DNA.

Carcasses and the rest of the body (after removal of residues from intestines) were homogenized in eight volumes of cold distilled

water. Aliquots of these homogenates were used to determine protein and 3-methylhistidine content.

Protein content was determined by the procedure of Markwell (75), after the homogenates had been dissolved in 1 N NaoH at 100^{o}C. RNA was determined by a modified Schmidt-Thannhauser method as described by Munro & Fleck (1969), and DNA by the method of Burton (18) as modified by Richards (96).

To analyse urinary 3-methylhistidine, urine was hydrolyzed in 6 N HCL for 2 h at 110^{0}C. HCL was removed by rotary evaporation, and the hydrolysate was dissolved in 0.2 M pyridine. Initial separation of 3-methylhistidine was by the method of Haverberg et al (52) using pyridine elution chromatography with Dowex 50-X8 mesh columns. The fraction containing 3-methylhistidine was collected in 1 M pyridine, dried by rotary evaporation, redissolved in distilled water and anlyzed by the method of Ward (108) using HPLC and UV detection. The eluent was water-methanol (60:40) at a flow rate 1.5 ml.min^{-1}. Chromatographic separations were performed using a HPLC column (100 x 4.6 mm I.D) packed with uBondapack C_{18}, particle size 5 um. Retention time for the 3-methylhistidine peak averaged 7 min. Peak heights of the samples were recorded, and the amount of 3-methylhistidine in samples was calculated based on the peak height of standard 3-methylhistidine. Triplicate standard 3-methylhistidine solutions were also subjected to all the steps (hydrolysis, ion-exchange columns and HPLC) and used to calculate recovery of 3-methylhistidine, which under these conditions averaged 75%.

For the measurement of body pools of 3-methylhistidine, aliquots of homogenates from carcass and the rest of the body were treated with

trichloroacetic acid to achieve a final concentration of 10%. The mixture was held at 4^0C for 10 minutes to allow precipitation of proteins. This preparation was then centrifuged (1100 g), and the precipitated proteins were washed twice with ice-cold 10% trichloroaceticacid. To extract lipids, the protein precipitate was washed twice with ethanol:ether (1:1) followed by ether (84). The residue was then dried to constant weight. Hydrolysis of the residue was carried out in 6 N HCL (10 mg/ml) for 20 h at 110^0C in sealed ampules. Samples were then processed as described for urine for measurement of 3-methylhistidine.

Plasma amino acid concentrations were determined after derivatization with phenylisothiocyanate to produce phenylthiocarbamyl amino acids. These amino acid derivatives were then analyzed by HPLC with a C_{18} reverse phase column (Waters PICO.TAG system, Waters division of Millipore, Milford, MA 01757). Plasma insulin and corticosterone were determined by standard radioimmunoassay procedures (Novo Laboratory, DENMARK and Endocrine Sciences, Tarzana, CA, respectively). Plasma triiodothyronine was measured by the method of Nejad et al (89).

Calculations. Cumulative gains in tissue weights, protein, RNA, DNA and 3-methylhistidine content were calculated from observed values at the end of the experimental periods minus predicted values at the beginning of the experimental periods. Values at the beginning of an experimental period were predicted from ratio of tissue weights, protein, RNA, DNA or 3-methylhistidine content to body weights, or hindlimb weights, in rats killed at the start of each treatment period. Gains during wk 2, and wks 3 and 4 were calculated from

observed values at the end of experimental period minus the predicted initial values from the corresponding treatment groups at the beginning of wk 2 and wk 3. That is, values obtained from rats fed diets containing 0, 10 or 100 ppm cimaterol and killed at the end of wk 1 and wk 2 were utilized to predict the initital values for each respective group.

Fractional accretion rates of 3-methylhistidine containing proteins were calculated from the total body gain in 3-methylhistidine per day divided by the average 3-methylhistidine pool size during that period, and expressed as a percentage. Fractional accretion rates of 3-methylhistidine were assumed to equal fractional accretion rates of 3-methylhistidine containing proteins because the concentration of 3-methylhistidine in protein was unaffected by the treatment. Fractional degradation rates of total body 3-methylhistidine containing proteins were calculated from the urinary excretion of 3-methylhistidine per day divided by the average 3-methylhistidine pool size during that period, and expressed as a percentage. Fractional synthesis rates were obtained by the addition of fractional accretion and degradation rates (FAR= FSR-FBR).

Data were analysed statistically using analysis of variance, and the Bonferonni t test for post hoc treatment comparisons (44).

Results

Rats fed diets containing 100 ppm cimaterol for 1 day consumed less energy than control rats, but then increased their energy intake to levels above those of control rats by day 3 (Figure 12). This elevated energy intake was closely associated with increased body weight, which was also detectable by day 3. The major weight gain

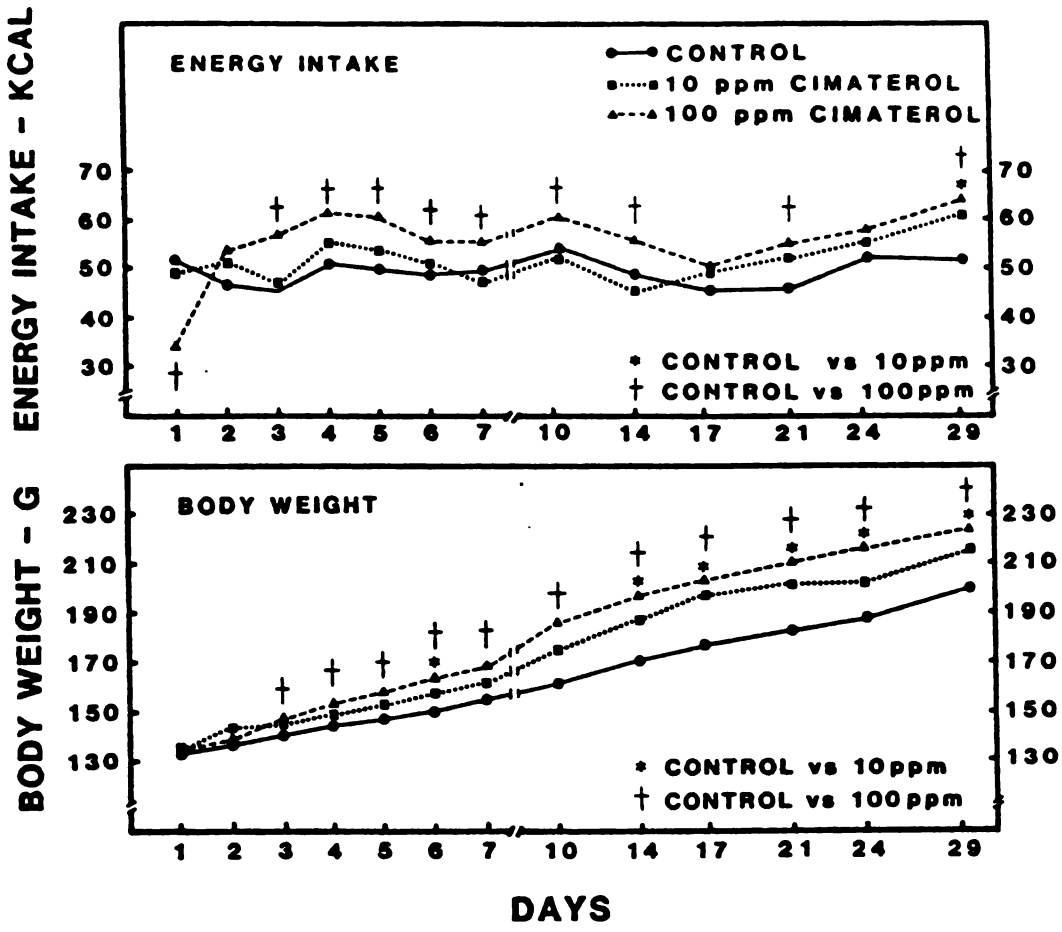

Figure 12. Energy intake and body weights in rats fed cimaterol. Rats were fed ad libitum for 4 wks a high-carbohydrate diet containing 0, 10, or 100 ppm cimaterol. Each point is the mean ± SEM of 10 rats. Asterisks and + above a point indicates that values for rats fed diets containing 10 and 100 ppm cimaterol, respectively, are significantly different from the control value ($P<0.05$).

response occurred during the first 2 wks when rats fed diets containing 10 or 100 ppm cimaterol gained 45 and 80% more than control rats (Figure 13). After being fed diets containing cimaterol for 2 wks, rats no longer gained body weight at an accelerated rate.

Consumption of cimaterol stimulated weight gain of carcass, which was primarily skeletal muscle and associated bone, at an even faster rate than total body weight gain (Figure 13). Rats fed 10 and 100 ppm cimaterol gained 45% and 65% more carcass weight than control rats during the 4 wk experiment with the greatest acceleration in gain evident within the first wk (80% and 131%). Gain in hindlimb muscle followed a similar pattern (Figure 13). Gain in weight of the rest of the body (all components of the body excluding carcass) was unaffected by cimaterol, confirming that most of the body weight gain was associated with increased skeletal muscle accretion. A dose-dependent reduction in gain in abdominal white adipose tissue weight was observed in rats fed diets containing cimaterol (Figure 13). Unlike the increased gain in carcass where major changes were observed only within the first wk, gain in abdominal white adipose tissue was significantly reduced in response to cimaterol beyond the first wk.

In agreement with the observed changes in tissue gains, gain in protein in rats fed 10 and 100 ppm cimaterol was also markedly elevated in total body (169-212%), carcass (191-236%) and hindlimb muscles (61-130%) within the first wk (Figure 14). Effects of cimaterol on protein gain in the carcass were minimal beyond the first wk. Protein gain in the rest of the body (ROB) was not significantly increased during the 4 wk experimental period (Figure 14).

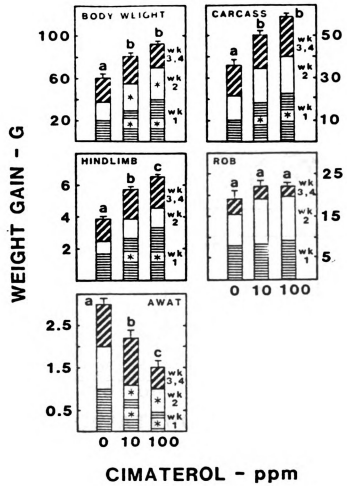

Figure 13. Effects of cimaterol on body weights and tissue weights. Rats were fed ad libitum for 1, 2 or 4 wks a high-carbohydrate diet containing 0, 10 or 100 ppm cimaterol. The lower two segments of the bars represent gain per wk for each of the first two wks. The upper segment represents gain for the last 2 wks of the 4 wk study. Carcass indicates total skeletal muscle and the associated bone; hindlimb, muscle stripped from one hindlimb; ROB, rest of the body excluding carcass; and AWAT, abdominal white adipose tissue. Each point is the mean ± SEM of 10 rats. Bars within a panel with different letters are significantly different ($P<0.05$). Asterisks within bars indicate that the value is significantly different from the corresponding control value within that wk ($P<0.05$). Initial body weight, 133±2 g; carcass weight, 61±1 g; hindlimb muscle, 5.1±0.1 g; rest of the body, 61±1 g and abdominal white adipose tissue, 0.87±0.08 g. Gains in tissue weights were calculated from the final values minus the predicted initial values based on initial body weight of each rat.

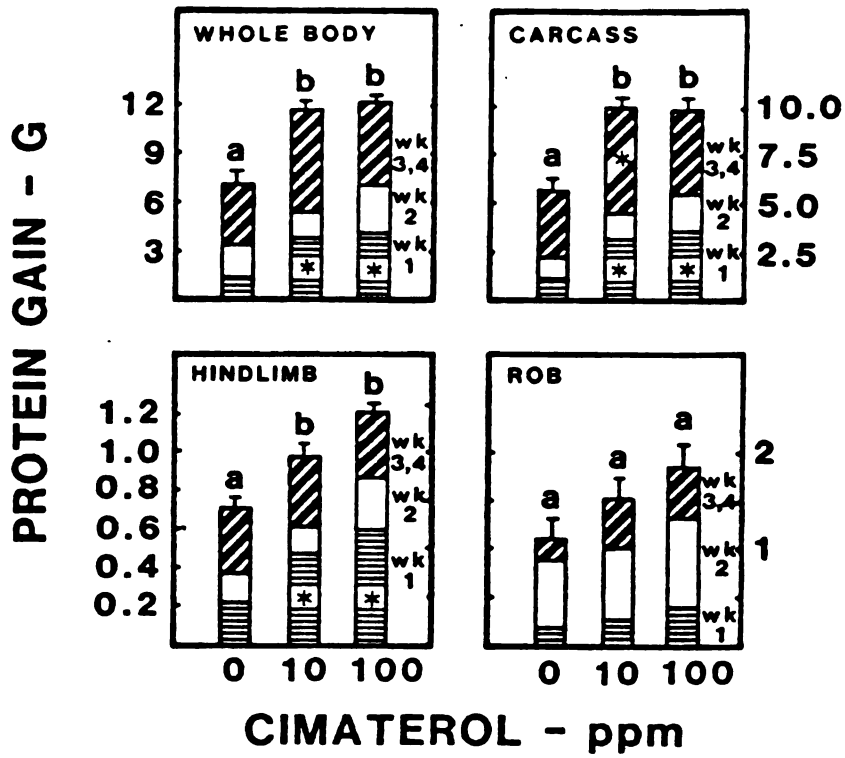

Figure 14. Gain in protein in rats fed cimaterol. Gain in protein content of total body, carcass, hindlimb muscle and rest of the body (ROB) of rats fed ad libitum for 1, 2 or 4 wks a high-carbohydrate diet containing 0, 10, or 100 ppm cimaterol. The lower two segments of each bar represents gain per wk, and the upper segment represents gain for the last 2 wks of the 4 wk study. Each bar is the mean ± SEM of 10 rats. Bars within a panel with different letters are significantly different ($P<0.05$). Asterisks within a bar indicate that the value is significantly different from the corresponding control value within that period ($P<0.05$). Initial total body protein, 15.96±0.7 g; carcass protein, 8.02±0.5 g; hindlimb muscle protein, 0.79±0.09 g; and rest of the body protein, 7.9±0.0.4 g. Gain in protein was calculated as final body or tissue protein minus the predicted initial body or tissue protein.

There was a dramatic increase in RNA gain (84-200%) in hindlimb muscle evident within first wk in response to 10 and 100 ppm cimaterol (Figure 15). Effects of cimaterol on RNA gain were less pronounced after 2 and 4 wks. DNA accumulation during the first wk did not respond to cimaterol, but did increase after 2 to 4 wks. As a result of the changes in RNA, DNA and muscle weight during the first wk, the concentration of RNA per g hindlimb muscle was 20% higher, and that of DNA 11% lower, in rats fed the diet containing 100 ppm cimaterol than in control rats. Effects were minimized after 2 or 4 wks (Figure 15).

The amount of 3-methylhistidine in the whole body of rats fed cimaterol increased in proportion to the increase in protein accumulation (Figure 16). More than 90% of the 3-methylhistidine present in the body was in the carcass, with no significant change in proportion with consumption of cimaterol (Figure 16). Concentrations of 3-methylhistidine in carcass and rest of the body averaged, 3.25 $\pm$ 0.11 and 0.540 $\pm$ 0.03 umoles per g protein, respectively, and were unaffected by cimaterol.

Fractional accretion rates of 3-methylhistidine containing proteins increased 40-120 % in response to cimaterol during wk 1 (Figure 17), in agreement with the increase in protein gain during this period (Figure 14). No major treatment-induced changes in fractional accretion rates were evident beyond wk 1.

Excretion of 3-methylhistidine in urine was selected as an indicator of the rates of degradation of 3-methylhistidine containing proteins (82,114). Urinary excretion of 3-methylhistidine, per 100 g body weight, was lowered 33% the first day rats consumed diets containing cimaterol (Figure 18). This effect of cimaterol persisted

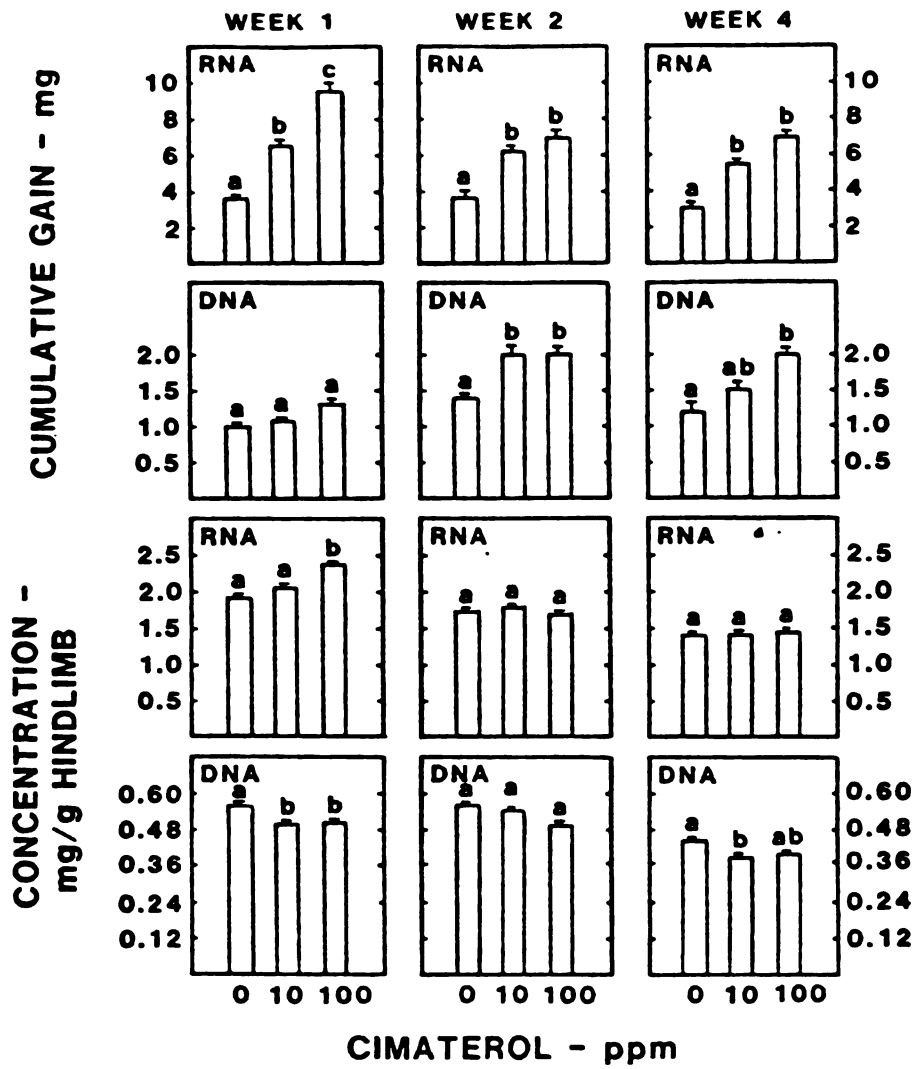

Figure 15. Effects of cimaterol on RNA and DNA. Cumulative gain in RNA and DNA content, and RNA and DNA concentrations, in hindlimb muscle of rats fed ad libitum for 1, 2 or 4 wks a high-carbohydrate diet containing 0, 10 or 100 ppm cimaterol. Each bar is the mean ± SEM of 10 rats. Bars within a panel with differrent letters are significantly different ($P<0.05$). Initial hindlimb RNA content, 10.2±0.5 mg; DNA content, 2.8±0.1 mg; RNA concentration, 2.0±0.1 mg/g; and DNA concentration, 0.56 ±0.02 mg/g hindlimb muscle. Gain in RNA and DNA contents were calculated from the final values minus the predicted initial values based on initial body weight and hindlimb muscle weight of each rat.

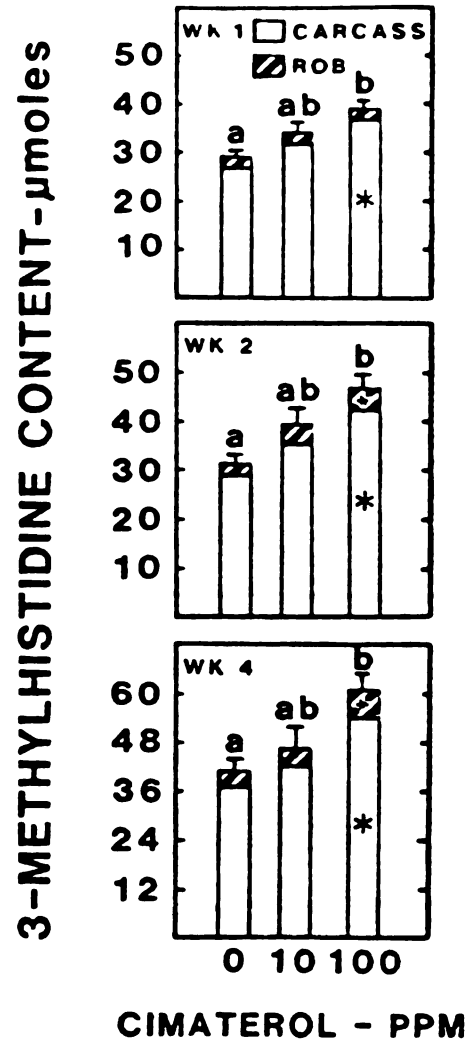

Figure 16. 3-Methylhistidine content in the body of rats fed cimaterol. 3-Methylhistidine content of total body, carcass and rest of the body of rats fed ad libitum for 1, 2 or 4 wks a high-carbohydrate diet containing 0, 10, or 100 ppm cimaterol. Total height of each bar indicates the amount of 3-methylhistidine present in the whole body. Two segments of each bar represent 3-methylhistidine content of carcass and rest of the body (ROB). Each value is the mean ± SEM of 10 rats. Bars within a panel with different letters are significantly different ($P<0.05$). Asterisks within bars indicate that the value is significantly different from the corresponding control value ($P<0.05$). Initial 3-methylhistidine content for total body, 21.0±2.0 umoles; carcass, 19.7±1.9 umoles; and rest of the body, 1.3±0.1 umoles.

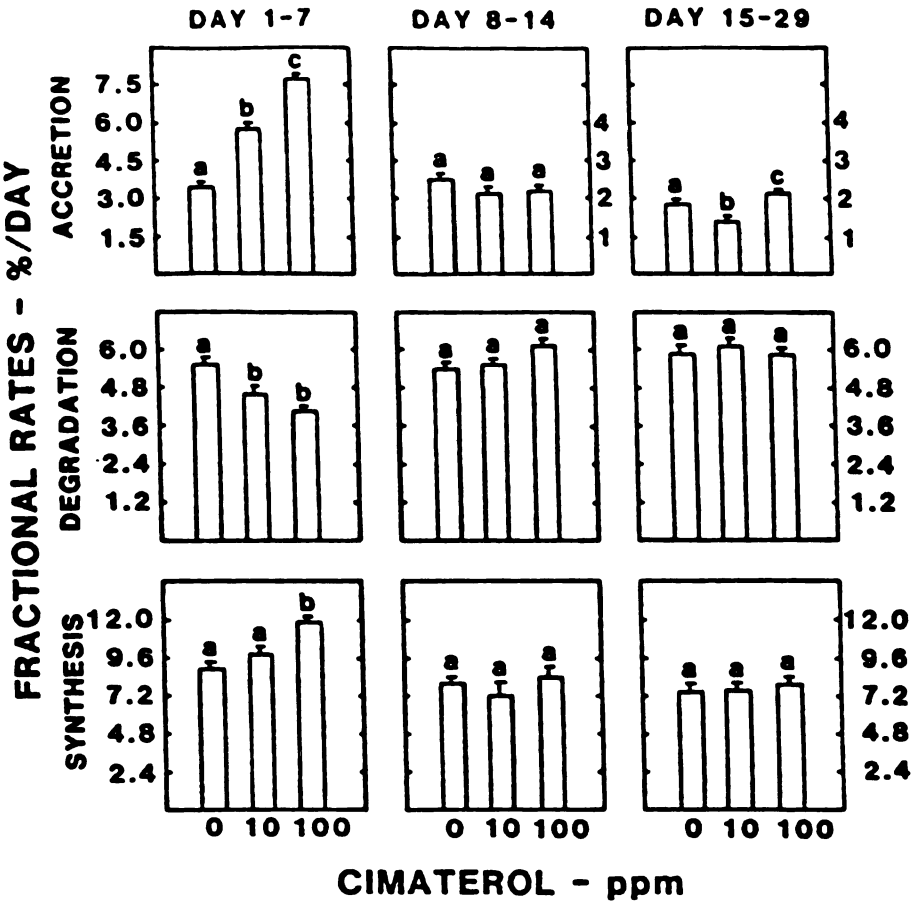

Figure 17. Fractional accretion, degradation and synthesis rates of total body 3-methylhistidine containing proteins. Rats were fed ad libitum for 1, 2 or 4 wks a high-carbohydrate diet containing 0, 10, or 100 ppm cimaterol. Each bar is the mean ± SEM of 10 rats. Bars within a panel with different letters are significantly different ($P<0.05$). Gain in 3-methylhistidine content was calculated as final body 3-methylhistidine minus the predicted initial body 3-methylhistidine. Fractional accretion rates were calculated from the average body gain in 3-methylhistidine per day divided by the average 3-methylhistidine pool size during that period, and expressed as a percentage. Fractional accretion rates of 3-methylhistidine were assumed to equal fractional accretion rates of 3-methylhistidine containing proteins, because the concentration of 3-methylhistidine present in protein was unchanged with treatment. Fractional degradation rates were calculated from the average urinary excretion of 3-methylhistidine per day divided by the average 3-methylhistidine pool size during that period, and expressed as a percentage. Fractional synthesis rates were obtained by the addition of fractional accretion and degradation rates (FAR = FSR -FBR).

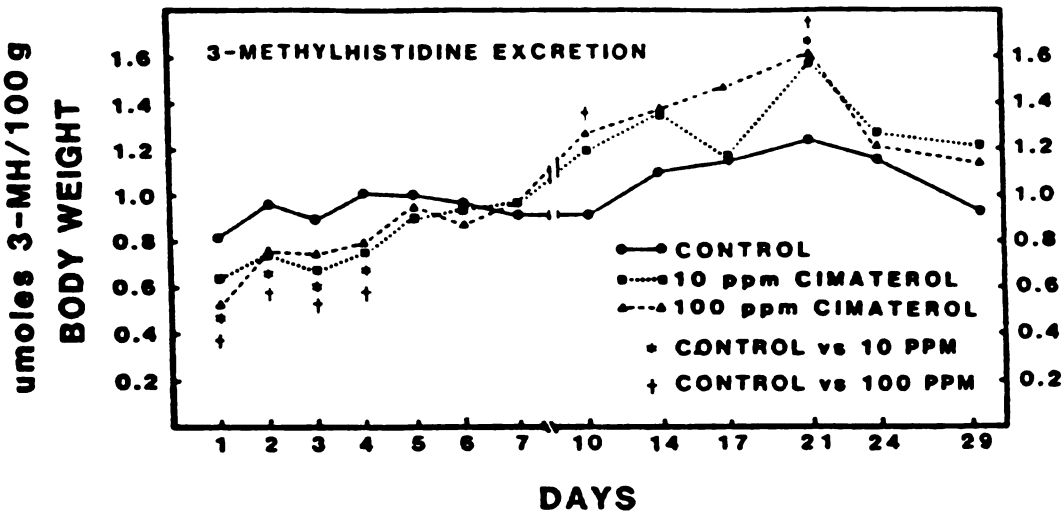

Figure 18. Effects of cimaterol on 3-methylhistidine excretion. Urinary 3-methylhistidine excretion of rats fed ad libitum for 4 wks a high-carbohydrate diet containing 0, 10, or 100 ppm cimaterol. Each point is the mean ± SEM of 10 rats. Asterisk and + above a point indicates the values for rats fed diets containing 10 and 100 ppm respectively, respectively, are significantly different from the control value ($P<0.05$).

for the first 4 days; thereafter, 3-methylhistidine excretion in cimaterol-treated rats was equal to or higher than values in control rats.

Fractional degradation rates of 3-methylhistidine containing proteins, expressed per total body pool of 3-methylhistidine containing proteins, were 25% lower in rats fed cimaterol during first wk than in control rats (Figure 17), with no effects of cimaterol beyond 1 wk. Fractional synthesis rates of 3-methylhistidine containing proteins, calculated from fractional accretion and degradation rates, were elevated by 32 % in rats fed cimaterol for 1 wk, with no effects of cimaterol thereafter (Figure 17).

Plasma amino acid concentrations 4 h after an injection of cimaterol were markedly reduced (Figure 19). Similar reductions in total amino acid levels in plasma were reported 2 h after injection of isoproterenol (33) and also during a 3 h perfusion of the isolated rat hemicorpus with isoproterenol (71). This reduction in plasma amino acids was most pronounced for the branched chain amino acids valine, isoleucine and leucine in agreement with results obtained with isoproternol (71). But, by the end of wk 1 (Figure 19), 2 and 4 (data not presented) plasma amino acid concentrations were unaffected by cimaterol.

Consumption of diets containing cimaterol did not affect plasma insulin or triiodothyronine concentrations (Table 5).

Discussion

It is now well documented that several beta-agonists, including cimaterol, can increase skeletal muscle mass in a number of species (9,10,31,36,60,76,97). The present findings show that these effects

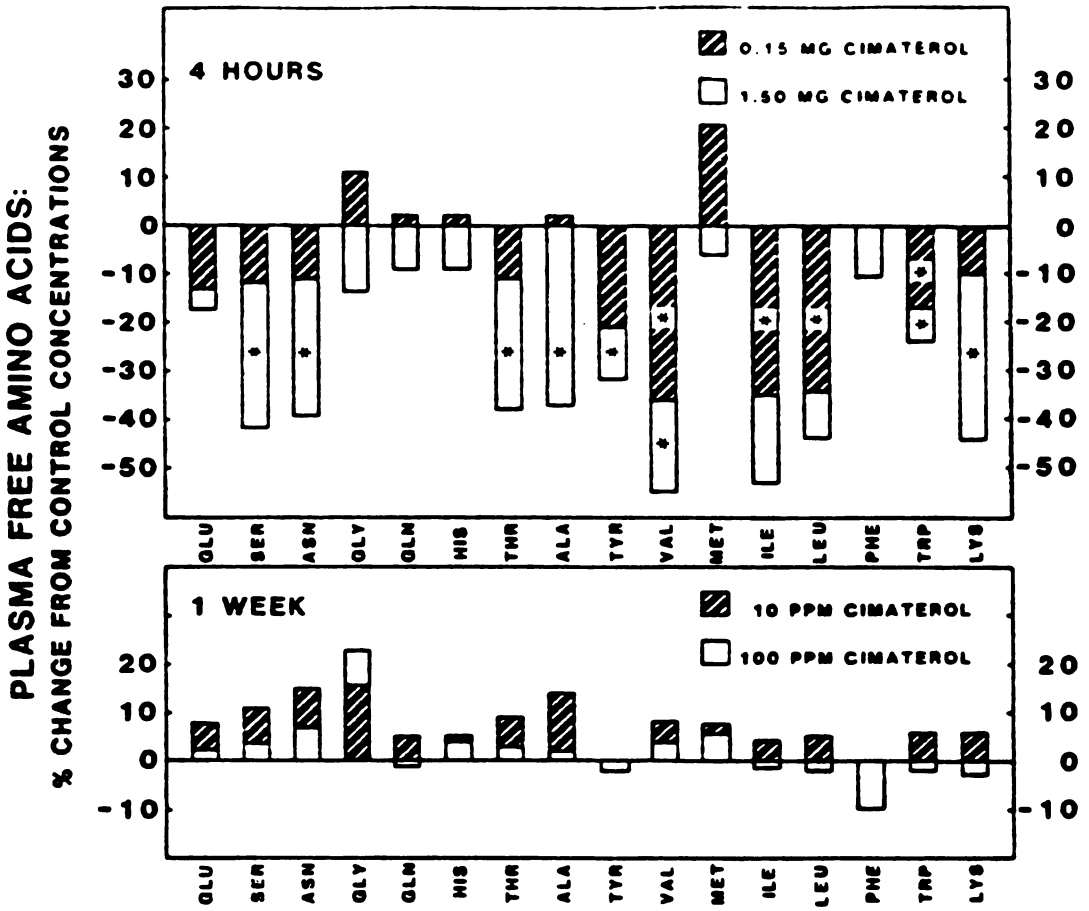

Figure 19. Effects of cimaterol on plasma amino acids. Plasma amino acid concentrations in rats 4 h after a single injection of cimaterol, and also after feeding for 1 wk a high-carbohydrate diet containing 0, 10, or 100 ppm. Body weights of rats injected with cimaterol averaged 175± 1 g. Values are means of ± SEM of 10 animals, and are expressed as percentage changes from the control value for each amino acid. Asterisks within a bar indicate that the value is significantly different from the control value ($P<0.05$). Control values (umoles/dl) 4 h after injection of saline and 1 wk after feeding the control diet were for GLU, 7.3±0.6, 7.4±0.6; SER, 16.3±1.1, 19.3±1.4; ASN, 5.5±0.5, 6.2±0.4; GLY, 10.6±0.8, 10.6±0.6; GLN, 41±3, 41±3; HIS, 4.4±0.2, 4.6±0.4; THR, 18.2±1, 37±5; ALA, 26.5±1.2, 33±1.7; TYR, 4.2±0.3, 5.2±0.6; VAL, 14.4±0.9, 14.9±1.2; MET, 4.3±0.4, 7.4-0.7; IL, 6.7±0.5, 6.7±0.6; LEU, 11.3±0.8, 11.0±0.7; PHE, 3.6±0.2, 3.3±0.2; TRP, 6.6±0.2, 7.7±0.5 and LYS, 35±4, 41±3, respectively.

Table 5. Effects of cimaterol on plasma hormones.

	Diet-ppm cimaterol			
	0	10	100	SE
		Insulin - uU/ml		
Acute-4 h	54	63	56	4
Day 7	81	72	64	6
Day 14	74	70	62	6
Day 29	75	54	54	7
		Triiodothyronine - ng/ml		
Acute- 4 h	1.5	1.5	1.3[a]	0.04
Day 7	2.7	2.7	2.6	0.08
Day 14	2.0	1.9	1.8	0.10
Day 29	1.7	1.7	1.7	0.08
		Corticosterone - ug/100ml		
Day 25	62	40	40	7.1

Rats fed a high-carbohydrate control diet for 1 wk, were injected intraperitoneally with saline, 0.15 or 1.5 mg cimaterol in 0.2 ml saline at 0700 h and killed after 4 h. Blood was collected from rats killed after 4 h of injections and at the end of 1, 2 or 4 wk of feeding cimaterol in the diet. Plasma, separated from blood stored at -70^{0}C until analyses. Values are means of 10 rats per group. Letter a indicates that the value is significantly different from control value ($P<0.05$).

are very pronounced upon initial exposure to the beta-agonist, but wane with time likely in part at least due to desensitization of beta-recptors (45). Total body protein gain was stimulated by 169-212% the first wk rats were fed diets containing cimaterol, with virtually all the increase associated with skeletal muscle. Beyond 1 wk, cimaterol failed to have major stimulatory effects on skeletal muscle gain. These results agree with a recent report demonstrating that clenbuterol fed to rats for 4 days stimulated fractional rates of skeletal muscle deposition by 40%, with no further significant stimulation evident after 11-25 day exposure to the agonist (95). Effects of cimaterol on rat skeletal muscle lipoprotein lipase activity, and on brown adipose tissue lipoprotein lipase activity and de novo rates of fatty acid synthesis, are also most pronounced immediately after initial exposure to cimaterol (Figure 4 and Table 4). However, lipolysis in white adipose tissue of rats appears to remain elevated even after 4 wks of exposure to cimaterol (Figure 9). The mechanism(s) reponsible for these apparent time dependent differences in sensitivity of metabolic pathways to cimaterol remain to be elucidated.

During the first wk of exposure to cimaterol, fractional accretion rates of 3-methylhistidine containing proteins (actin and myosin) increased up to 120% (Figure 17), with virtually all the increase confined to the carcass. Based on daily measurement of 3-methylhistidine excretion in urine and on total body pool size of 3-methylhistidine containing proteins, fractional degradation rates of 3-methylhistidine containing proteins averaged 25% lower in rats fed diets containing 100 ppm cimaterol for 1 wk than in control rats.

During the first day of exposure to cimaterol fractional degradation rates of 3-methylhistidine containing proteins were depressed by 33%, indicating a rapid onset of action. By day 7 fractional degradation rates were no longer depressed in rates fed cimaterol (data not presented). These data are consistent with the finding that cimaterol depressed plasma amino acid concentrations 4 h after a single administration, but not after feeding for 1 wk, and illustrate the importance of considering the timing of measurement relative to cimaterol administration. The average depression in turnover of 3-methylhistidine containing proteins of 25% in rats fed cimaterol for 1 wk is in reasonable agreement with the calculated value of a 55% reduction in fractional degradation rates of gastrocnemius and soleus muscle proteins in rats fed clenbuterol for 4 days reported by Reeds et al (95). These data, together with results of earlier in vivo and in vitro studies (33,42,71), indicate that one mechanism of action of beta adrenergic agonists to rapidly stimulate skeletal muscle protein accretion is by slowing protein turnover.

From the measurements of fractional accretion and degradation rates of 3-methylhistidine containing proteins it was possible to calculate fractional synthesis rates of these proteins. This approach, although indirect, has the advantage of providing an estimate of fractional synthesis rates integrated over time, unlike the use of radiolabeled amino acids which measures rates of protein synthesis over a period of only several hours. Fractional synthesis rates of 3-methylhistidine containing proteins calculated by this approach were elevated by 32% in rats fed diets containing cimaterol for 1 wk, with no effect evident beyond 1 wk. In agreement with the

increase in fractional synthesis rates, there was a marked increase in RNA gain and concentration in hindlimb muscles of rats that consumed cimaterol for 1 wk (Figure 15). Others have also noted increases in skeletal muscle RNA in response to clenbuterol and cimaterol (11,95). As noted in the introduction, beta agonist-induced increases in protein synthesis have been observed when measurements were made within several hours after injection of the agonist (33,36). Reeds et al (95) failed to observe an increase in fractional synthesis rates of muscle proteins in rats that consumed cimaterol for 4 days, but they measured protein synthesis during the light period when food intake, and consequently clenbuterol intake, would be expected to be low. A stimulation in fractional synthesis rates of muscle protein may have been observed if they had measured protein synthesis after feeding diets containing clenbuterol for only 1 or 2 days and during the dark period when rats normally consume most of their food.

Accelerated skeletal muscle growth in young animals is generally caused by increases in rates of fractional synthesis rates with lesser increases in rates of fractional degradation rates (83). Cimaterol, however, appears to function by simultaneously stimulating fractional synthesis rates and depressing fractional degradation rates of 3-methylhistidine containing proteins. Such action maximizes accretion of protein with minimal changes in rates of synthesis and degradation; accretion increased by 120% in rats fed cimaterol for 1 wk with only a 32% increase in fractional synthesis rates coupled with a 25% decrease in fractional degradation rates. Similar reciprocal changes in protein synthesis and degradation have been observed during

work-induced hypertrophy (46) and during the recovery phase from atrophy of immobilized muscle (47). Under these conditions, increased net gain in protein is also caused by stimulation of fractional rates of protein synthesis and inhibition of protein breakdown.

It is unknown whether beta-agonists influence protein accretion directly, or indireclty via release of hormones (39). Several lines of evidence point to direct effects of beta-agonists on skeletal muscle. Isoproterenol inhibits protein degradation as measured by release of phenylalanine from rat hemicorpus preparations (71), and release of alanine and glutamine from isolated rat epitrochloris muscle preparations (42). Cimaterol stimulates lipoprotein lipase activity in rat EDL muscle in vitro (Figure 8). The beta agonist-induced increase in protein accretion can occur the absence of changes in plasma insulin, triiodothyronine or corticosterone (Table 5 and ref 36,76). Cimaterol will supress hyperinsulinemia in lambs (11) and mice (24), but there is no evidence that this is essential prerequesite for accelerated muscle accretion. The specific mechanisms whereby cimaterol depresses protein turnover and concomitantly accelerates protein synthesis remain to be elucidated.

Cimaterol, when fed to rats, improved skeletal muscle accretion and decreased adipose tissue deposition. The present findings show that these effects of cimaterol were rapid, and wane with time due to either desensitization of beta receptors (47) or to some post receptor effects. A gradual increase over time in dose of cimaterol to these rats might overcome at least part of the resistance that develops. Alternatively, intermittant removal of cimaterol from the diet might be effective. It would be interesting to see if these manipulations would have larger effects on body composition than the observed changes in the present study. Cimaterol altered the body composition without influencing energy balance indicating that it is not merely redirecting energy from fat to protein deposition.

In an effort to elucidate the mechanism of action of cimaterol to reduce deposition of adipose tissue, effects of cimaterol on fatty acid synthesis, lipoprotein lipase activity and lipolysis were examined in these rats. Cimaterol failed to influence in vivo or in vitro rates of fatty acid synthesis in either white adipose tissue or liver. Consequently, decreased body fat content in response to cimaterol did not result from decreased rates of de novo rates of fatty acid synthesis. This conclusion is supported by the observation that cimaterol also decreased white adipose tissue gain and increased skeletal muscle gain in rats fed a high-fat diet where de novo fatty acid synthesis would be suppressed (69). Reduced fat deposition observed in rats consumed cimaterol was also not caused by reduced transfer of fatty acidsf rom circulating triglycerides, because in white adipose tissue lipoprotein lipase activity was unaffected by cimaterol.

Cimaterol stimulated lipolysis in vitro in white adipose tissue and in vivo. Consequently, cimaterol decreased fat gain by increasing mobilization of stored lipids. The specific mechanism involved in stimulating lipolytic activity by cimaterol remains to be studied. Although it is known that beta adrenergic stimulation of lipolysis is mediated by increases in cAMP and protein kinase activity which activate hormone sensitive lipase, it has not been demonstrated whether cimaterol also mediates its effects in a similar manner.

Fatty acid synthesis in brown adipose tissue was inhibited by cimaterol, suggesting different control mechanisms for fatty acid synthesis in brown adipose tissue and in white adipose tissue. This may be related to the presence of more beta-receptors in brown adipose tissue than white adipose tissue, but there were sufficient receptors in white adipose tissue to activate lipolysis. Other possibilities such as different types of receptors in brown adipose tissue and white adipose tissue, differences in intracellular pools of cAMP among tissues, or enzymes under different control mechanisms may be involved in these tissue-specific selective responses.

Effects of cimaterol on fatty acid oxidation and transport of circulating free fatty acids into muscle would provide more complete evidence in terms of how cimaterol can redirect energy from fat deposition to protein accretion. But such experiments have yet to be conducted.

In skeletal muscle, lipoprotein lipase activity was elevated in response to cimaterol, demonstrating a capacity for the increased transfer of fatty acids from circulating triglycerides probably for increased utilization of energy from fat. Cimaterol also stimulated

lipoprotein lipase activitiy in brown adipose tissue. These responses in skeletal muscle and brown adipose tissue are in contrast to the lack of effect on white adipose lipoprotein lipase activity and indicate a selective regulation of this enzyme in different tissues. The specific mechanisms whereby cimaterol stimulates muscle lipoprotein lipase activity remain to be elucidated; change in enzyme activation, turnover and synthesis, specific types of receptor (beta 1, beta 2 or mixed) that mediate these effects, and measurement of cAMP and protein kinase activity are among the factors that need to be explored. Very little is currently known about the beta adrenergic regulation of this enzyme activity in muscle.

The mechanism of action of cimaterol in increasing protein accretion was examined using 3-methylhistidine as an indicator of protein turnover. Data from the present findings indicate that cimaterol induced increases in skeletal muscle protein accretion by decreasing fractional protein degradation rates, and increasing fractional synthesis rates. This effect of cimaterol is rapid, as evidenced by reduced fractional degradation of 3-methylhistidine containing proteins even on the first day that rats consumed cimaterol. Consistant with decreased degradation and increased synthesis rates, plasma amino acid concentrations were also depressed 4 h after the administration of cimaterol. Supporting the observed stimulation in fractional synthesis rates, there was a marked increase in RNA gain and concentration in hindlimb muscles of rats that consumed cimaterol. Additional studies are needed to examine the possibility that cimaterol has direct effects on protein synthesis. Results from my experiments provide a clear indication that

examination of protein synthesis should be done soon after the administration of cimaterol. Measurement of protein degradation under in vitro conditions by the release of 3-methylhistidine from muscle protein in the presence of cimaterol would show whether cimaterol can directly affect turnover of myofibrillar proteins. One could also measure tyrosine release under similar conditions to see if other skeletal muscle proteins were affected by cimaterol (50). Such studies coupled with others on the effects of cimaterol on protein synthesis in vitro would provide valuable insights into the mechanism of action of cimaterol on protein metabolism in muscle.

Plasma insulin, corticosterone and triiodothyronine concentrations were unaffected by cimaterol suggesting that cimaterol influences the pathways of lipid and protein metabolism directly rather than by indirect action. This conclusion is supported by findings of the present study that cimaterol stimulated lipolysis in white adipose tissue and lipoprotein lipase actvity in skeletal muscle in vitro.

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