Drugs that Suppress Hepatic Fat Synthesis in Starved-Refed BHE/cdb Rats also Have an Effect on Muscle Protein Synthesis (43648)

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Abstract. **Simultaneous lipogenesis and protein synthesis as influenced by LY79771, testosterone, or dehydroepiandrosterone in starved/refed rats were studied. Starved-refed BHE/cdb rats were injected with one of these compounds during the 2-day refeed period. Hepatic *de novo* fatty acid synthesis using tritium incorporation into fatty acids and protein synthesis using [^{14}C]phenylalanine incorporation into hepatic and muscle protein were determined. Hepatic lipogenesis was decreased by all three drugs and these drugs had a differential effect on protein synthesis. We did not observe a corresponding increase in protein synthesis in the liver when fat synthesis was decreased, but we did observe a corresponding increase in muscle protein synthesis. We concluded that in the acute hyperlipogenic state induced by starvation/refeeding, these drugs induced a reciprocal increase in muscle protein synthesis along with a suppression of fatty acid synthesis.** [P.S.E.B.M. 1993, Vol 204]

One of the current medical dictums today is that people who are overweight should attempt to lose their excess weight. Many do; however, almost as many regain their lost weight. The composition of the weight regained may not be identical to that which was lost. Formerly overweight people may regain the lost body fat before they regain the lost body lean. The regain process has not been well studied. There is a need to understand the process so that appropriate therapies can be developed that will be useful in helping the formerly obese human retain their lean body mass while resisting the regain of body fat.

One of the animal paradigms that is useful in studying the weight loss/regain process is the starvation/refeeding paradigm first described by Tepperman and Tepperman (1). Rats that are starved and refed a 65% glucose diet are characterized by a significant increase in lipogenic enzyme activity, *de novo* fatty acid synthesis, and liver lipid (1–9). Although rats seldom become obese after this treatment, the temporary change in lipogenesis after starvation makes it possible to study the weight recovery process. In addition, strains of rats compared at equivalent ages differ in the magnitude of their lipogenic response to starvation/refeeding (10, 11). Rats of the BHE/cdb strain have a greater response to this treatment than rats of the Wistar (10) or Sprague-Dawley (11) strain. BHE/cdb rats carry a genetic trait for non-insulin-dependent diabetes mellitus and are characterized by the development of a fatty liver well before their non-insulin-dependent diabetes mellitus develops (12). Although not obese, the BHE/cdb rat tends to have more body fat and less body

Table I. Composition of Diet

Ingredient	g/100 g total wt
Corn oil	5
Lactalbumin	10
Casein	10
Glucose	65
Cellufil[a]	4
Vitamin mix[b]	1.1
Mineral mix[c]	4.9

[a] Cellufil, hydrolyzed, U.S. Biochemical Corp., Cleveland, OH.
[b] AIN vitamin mix, U.S. Biochemical Corp.
[c] AIN mineral mix 76, U.S. Biochemical Corp.

[1] Data are from a thesis submitted by J. A. P. in partial fulfillment of the requirements for the M.S. degree.

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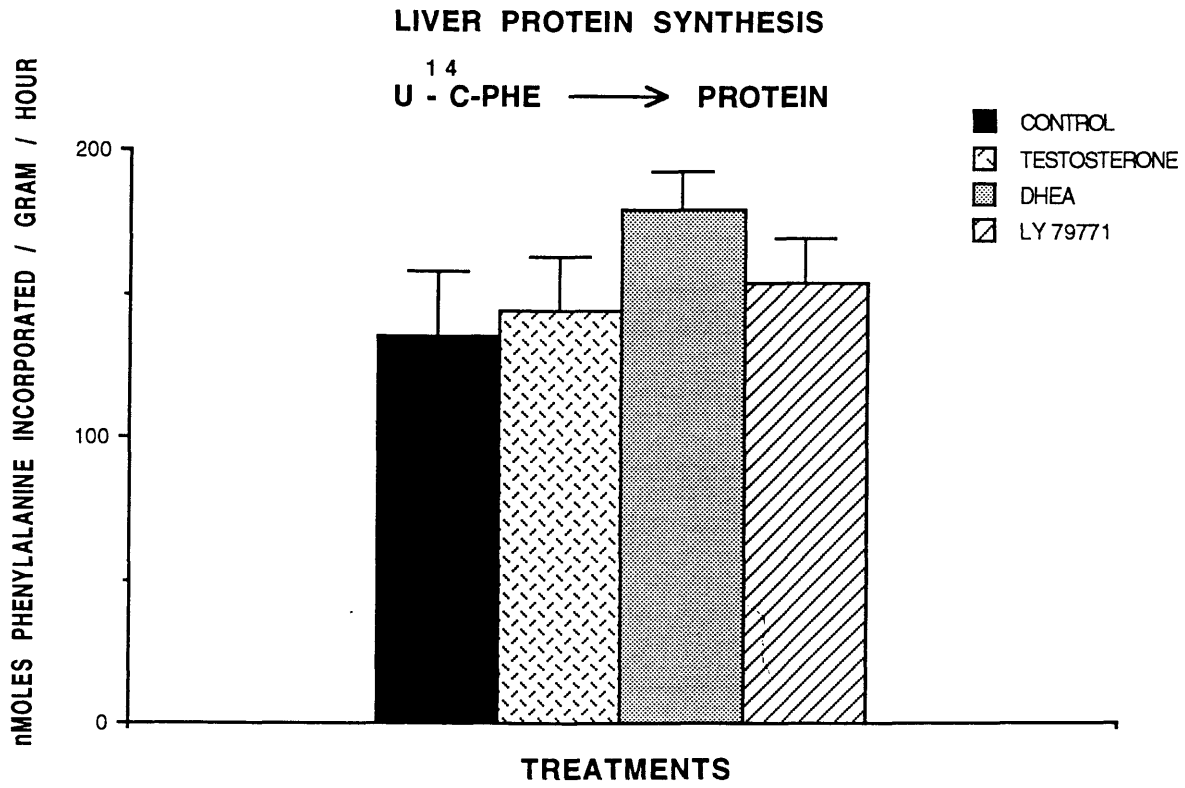

Figure 1. Rate of [U-^{14}C]phenylalanine incorporation (nmoles ^{14}C phe/g tissue/hr) in liver of starved/refed male BHE/cdb rats. Rats were injected intraperitoneally with 150 μM phenylalanine and 2 μCi [U-^{14}C]phenylalanine/100 g body wt. Each bar represents the mean ± SE, n = 8. No significant treatment effects were found with analysis of variance.

protein than rats of the Wistar or Sprague-Dawley strains (12, 13).

Bergen and Merkel (14) recently reviewed the literature on the use of pharmacologic partitioning agents to produce leaner meat animals. These drugs may be either growth hormone or β-adrenergic agonists, or anabolic steroids. When used by animal scientists studying growing animals, these agents have been shown to promote lean body growth while suppressing fat gain (14). These drugs are called partitioning agents because they partition nutrients and energy toward lean body mass accretion and away from fat accretion. Although these drugs are being developed for use in farm animals, they may also have a use in promoting the maintenance of lean body mass while suppressing the fat regain in formerly overly fat people. To test this possibility, we used three compounds: a β-agonist, LY79771 or [R-(R*,S*)]-α [[[3-(4-hydroxyphenyl)-1-methyl-propyl]amino]methyl]-benzenemethanol hydrochloride, an anabolic steroid, testosterone, and a steroid intermediate, dehydroepiandrosterone (DHEA), administered to starved/refed BHE/cdb rats. After 48 hr of refeeding, we measured the rate of *de novo* fatty acid synthesis using the incorporation of tritium into fatty acids and the rate of protein synthesis using the incorporation of radioactive phenylalanine into protein. We hoped that a comparison of these rates would allow us to determine whether these partitioning agents had simultaneous and opposite effects on these two anabolic processes. We found that these agents were effective in reducing hepatic *de novo* lipogenesis and enhancing muscle protein synthesis.

Methods and Materials

Male weanling BHE/cdb rats were obtained from the University of Georgia colony. Rats were housed individually in hanging wire mesh cages in a room controlled for temperature (22 ± 1°C), humidity (45–50%), and light (12:12-hr light:dark cycle). Unless otherwise indicated, food and water were available *ad libitum*. The animals were cared for according to the principles and policies for humane care as set forth by the American Association for Laboratory Animal Care. The work was conducted so as to conform to the regulations set forth in NIH Publication 88-23, 1985, NIH Guide for the Care and Use of Laboratory Animals. Four groups of five rats each were used. The rats were fed a stock diet (Purina laboratory animal chow; Ralston Purina, St. Louis, MO) until desired initial body weight was achieved (160–180 g). The rats were then starved for 48 hr and refed a 65% glucose diet (Table I) for 48 hr. Rats were weighed and food intakes

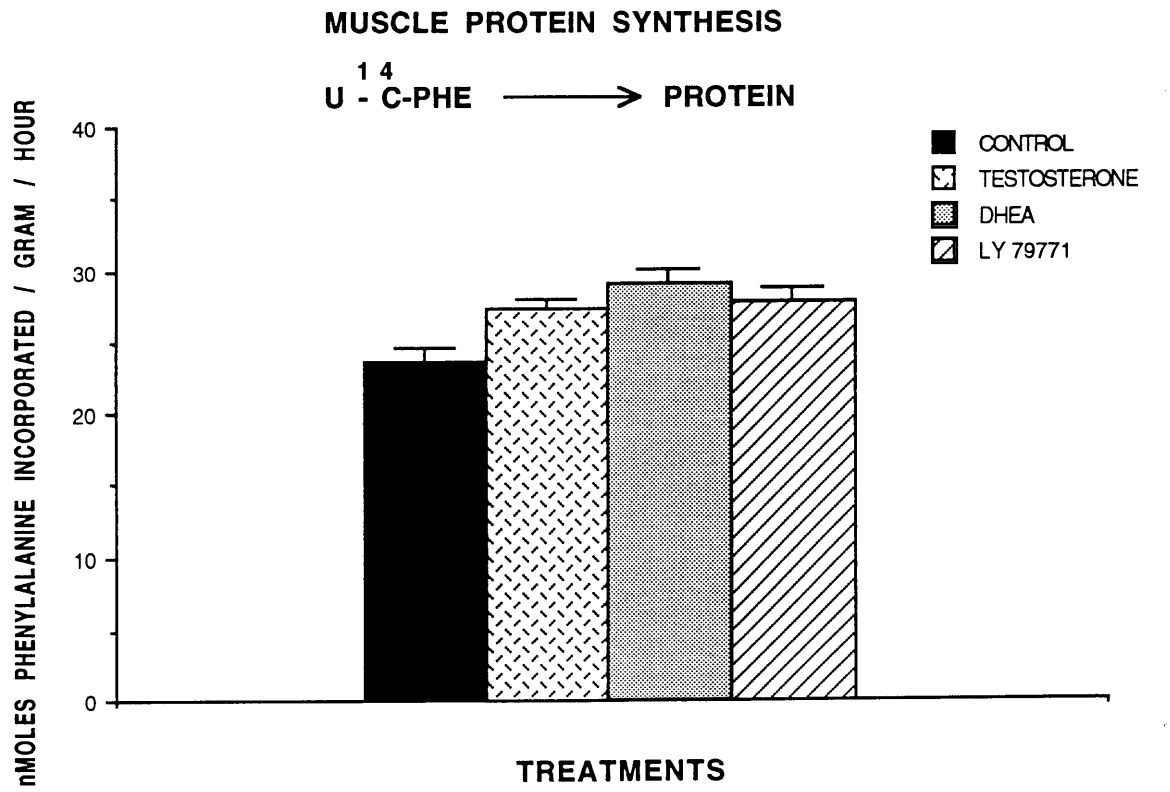

Figure 2. Rate of [U-^{14}C]phenylalanine incorporation (nmoles ^{14}C phe/g tissue/hr) in muscle of starved/refed male BHE/cdb rats. Rats were injected peritoneally with 150 μM phenylalanine and 2 μCi [U-^{14}C]phenylalanine/100 g body wt. Each bar represents the mean ± SE, $n = 8$. All treatments were significantly ($P < 0.01$) different from control by analysis of variance and Duncan's multiple range test ($\alpha = 0.05$).

Table II. Statistical Table of *In Vivo* Studies

	Muscle	Liver			Fat pad	
	^{14}C-PHE → Protein	^{14}C-PHE → Protein	^{3}H → Lipid	^{3}H → FFA	^{3}H → Lipid	^{3}H → FFA
Analysis of variance[a]						
Control	—	—	—	—	—	—
DHEA	$P < 0.01$	NS	$P < 0.01$	$P < 0.01$	NS	NS
LY79771	$P < 0.01$	NS	$P < 0.01$	$P < 0.01$	NS	NS
Testosterone	$P < 0.01$	NS	$P < 0.01$	$P < 0.01$	NS	NS
Duncan's multiple range test[b]						
Control	—	—	—	—	—	—
DHEA	$\alpha = 0.05$	NS	$\alpha = 0.05$	$\alpha = 0.05$	NS	NS
LY79771	$\alpha = 0.05$	NS	$\alpha = 0.05$	$\alpha = 0.05$	NS	NS
Testosterone	$\alpha = 0.05$	NS	$\alpha = 0.05$	$\alpha = 0.05$	NS	NS

[a] Probability of a significant treatment effect is indicated. NS indicates that the effect is not significant.
[b] Duncan's multiple range test with probability of difference from control at $\alpha = 0.05$.

were measured daily. During the 48-hr refeed period, the control group was injected with diluent (dimethylsulfoxide, 0.1 ml/100 g body wt) or the drugs were dissolved in diluent and injected at 0800 hr and at 1600 hr each day. The experimental groups were injected with either testosterone (0.027 mmol testosterone/100 g body wt), DHEA (0.30 mmol DHEA/100 g body wt), or LY79771 (0.96 mmol LY79771/100 g body wt). LY79771, a β-agonist, was a gift from Eli Lilly & Co., Indianapolis, IN. Dose-response curves (15–17) have been developed for this drug by scientists at Eli Lilly and we used the dose suggested by them. The other drugs were purchased from Sigma Chemical Co., St. Louis, MO. *De novo* lipid synthesis and protein synthesis were measured at the end of the refeed period.

De novo lipogenesis was assessed using the methods of Lowenstein (18), Jungas (19), and Fain and colleagues (20, 21). Rats were injected intraperitoneally with 1 mCi $^{3}H_2O$ (sp act 100 μCi/mole; New England Nuclear, Boston, MA) per 100 g body wt. After 30 min,

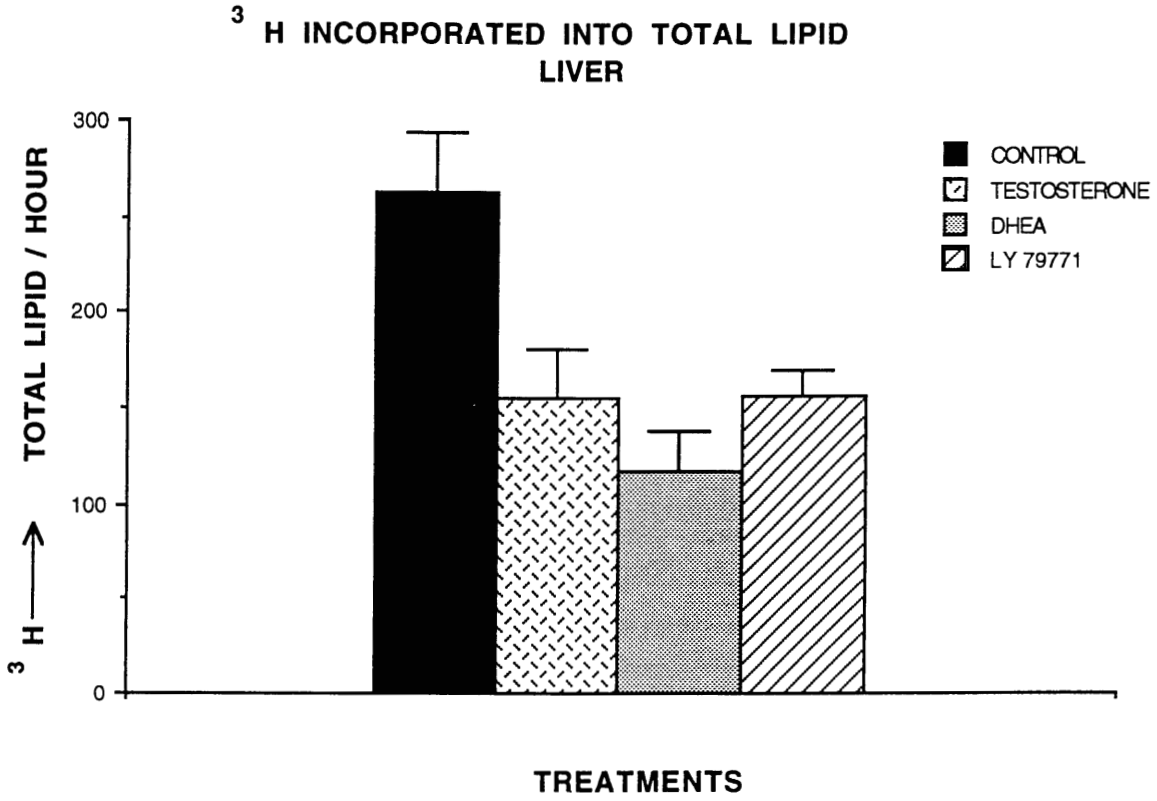

Figure 3. Rate of ^{3}H incorporation into lipid (nmol ^{3}H incorporated into lipid/g tissue/hr) in liver of starved/refed male BHE/cdb rats. Rats were injected intraperitoneally with 1 mCi ^3H_2O/100 g body wt. Each bar represents the mean ± SE, n = 10. All treatments were significantly ($P <$ 0.01) different from control by analysis of variance and Duncan's multiple range test (α = 0.05).

rats were anesthetized using an intraperitoneal injection of sodium pentobarbital (0.08 mg/100 g body wt). Fifteen minutes later, blood was drawn by heart puncture and the rats were sacrificed by pneumothorax. The liver, leg muscles, and epididymal fat pads were quickly excised, weighed, and frozen. Blood was centrifuged at 3500*g*, for 10 min at 4°C. Sera were used to determine the specific activity of the body water. Lipids from liver and fat pads were extracted and saponified by the procedures of Dole and Meinertz (22). The lipid extracts were counted in a scintillation solution containing 0.4% 2,5-diphenyloazole and 0.13% (2,5-phenyloxazolyl)-1-benzene in toluene. Counting efficiency for both fatty acids and total lipid samples was in the range of 55% to 58%. According to Jungas (19), the ^{3}HOH method yields an average of 0.87 atom of ^{3}H incorporated per carbon atom incorporated into long chain fatty acid. Thus, this factor was used to compute the results. Results were expressed as ηmol ^{3}H incorporated into total lipid or fatty acids per gram tissue per hour.

Protein synthesis was determined in the same rats used for assessing lipogenesis using the method of Garlick *et al.* (23) with modifications by Jepson *et al.* (24). There were also other modifications that are described below. Rats were injected intraperitoneally with 2 μCi [U-^{14}C]phenylalanine (sp act 450 μCi/mmol; New England Nuclear) and 150 μM cold phenylalanine/100 g body wt. The injection was made at the 30-min time point of the ^3H_2O incubation. The incorporation time of the [U-^{14}C]phenylalanine was, therefore, 15 min. Blood was drawn by heart puncture. Muscles (combined gastronemis, plantaris, and soleus) and liver were quickly removed. The muscle tissue was flash-frozen between copper plates precooled in liquid nitrogen. The muscle was prepared this way to facilitate subsequent homogenization and extraction of labeled protein.

Portions of frozen tissues (0.2–0.6 g) were homogenized and precipitated in 6 ml of cold 2% (w/v) $HClO_4$ and centrifuged at 2800*g* for 15 min. Supernatant and precipitate were separated. Saturated tripotassium citrate (3 ml) was added to the supernatant, resulting in the precipitation of $HClO_4$. The sample was centrifuged at 2800*g* for 15 min and supernatant used to determine the specific activity of free phenylalanine in the tissue. The precipitate was washed three times with 2% $HClO_4$. The precipitate was then incubated with 10 ml of 0.3 *M* NaOH for 1 hr at 37°C. The protein was reprecipitated with 2 ml of 20% $HClO_4$ and spun at 2800*g* for 15 min. The precipitate was washed three times with 2% $HClO_4$. Protein was then hydrolyzed in 5 ml of 6 *N* HCl for 24 h at 110°C. HCl was removed by evaporating to dryness. The amino acids were resuspended

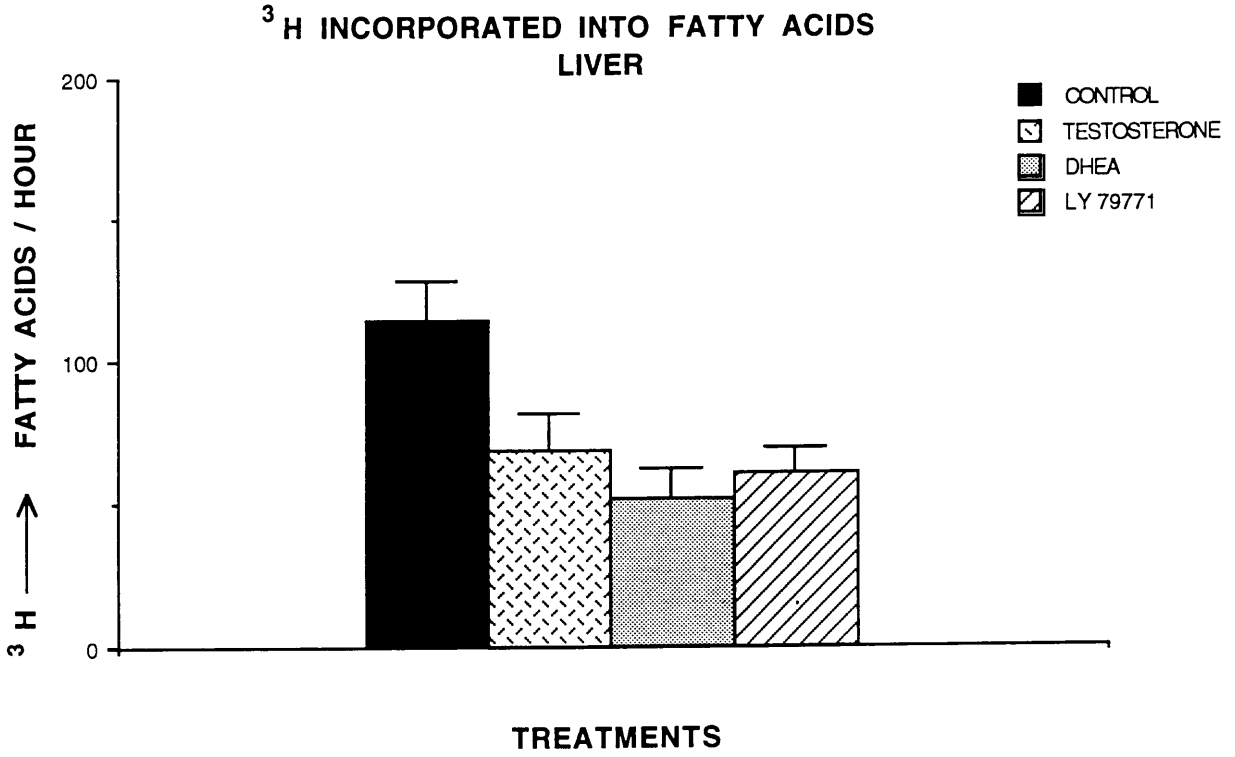

Figure 4. Rate of ^{3}H incorporation into fatty acids (nmol ^{3}H incorporated into fatty acids/g tissue/hr) in liver of starved/refed male BHE/cdb rats. Rats were injected intraperitoneally with 1 mCi ^3H_2O/100 g body wt. Each bar represents the mean ± SE, n = 10. All treatments were significantly ($P < 0.001$) different from control by analysis of variance and Duncan's multiple range test ($\alpha = 0.05$).

in 3 ml of 0.5 mmol/liter sodium citrate, pH 6.3. One milliliter of supernatant was counted to determine the incorporation rate. Results are expressed as nmoles of [U-^{14}C]phenylalanine incorporated into protein per gram of tissue per hour (23).

Statistical Analysis

Statistical evaluation was done by using a one-way analysis of variance followed by Duncan's multiple range test using SAS techniques (25).

Results

When animals were starved for 48 hr, the mean weight of the rats was 169 ± 2 g. During the refeed period, the average amount of food consumed per day per rat (mean ± SE, n = 5) was 16.1 ± 0.5 g, 16.4 ± 1.2 g, 16.5 ± 0.9 g, and 16.6 ± 0.5 g for control, testosterone, DHEA, and LY79771 treatments, respectively. The different agents had no effect on food intake when compared with the control group. The average weight regain during the refeed period was 31 ± 1 g, 31 ± 2 g, 29 ± 2 g, and 28 ± 3 g for control, testosterone, DHEA, and LY79771 groups, respectively. There were no effects of the agents on the amount of weight regained. The mean liver weight was 10.28 ± 0.46 g, 10.91 ± 0.52 g, 10.55 ± 0.13 g, and 10.81 ± 0.26 g for control, testosterone, DHEA, and LY79771 treatment groups, respectively. Liver weight was not affected by the different treatments. The fat pad mean weight was 1.10 ± 0.03 g, 0.98 ± 0.04 g, 1.14 ± 0.6 g, 1.11 ± 0.05 g for control, testosterone, DHEA, and LY79771, respectively. Fat pad weights were not affected by the different treatment. Muscle weights were 2.24 ± 0.12 g, 2.23 ± 0.12 g, 2.19 ± 0.16 g, and 2.17 ± 0.20 g for control, testosterone, DHEA, and LY79771 treatment groups, respectively. The means were not different between control and treatment groups. The agents had no effect on any of the weights of the organs or tissues examined.

The rate of incorporation of phenylalanine into protein in the liver (Fig. 1) was increased slightly by the drug treatments, but none of the treatment effects was significant ($P > 0.05$). However, the rate of phenylalanine incorporation into muscle tissue (Fig. 2) was affected by the various treatments ($P > 0.05$). Table II shows statistical comparisons for these *in vivo* studies.

The rate of *de novo* lipid synthesis in the liver (Fig. 3) was significantly higher in the control group than in the treated groups. Lipid synthesis was 50% less in the experimental groups. *De novo* fatty acid synthesis in the liver (Fig. 4) was significantly higher in the control group than in the treatment groups. Fatty acid synthesis was 50% less in the treated groups. *De novo* lipid and

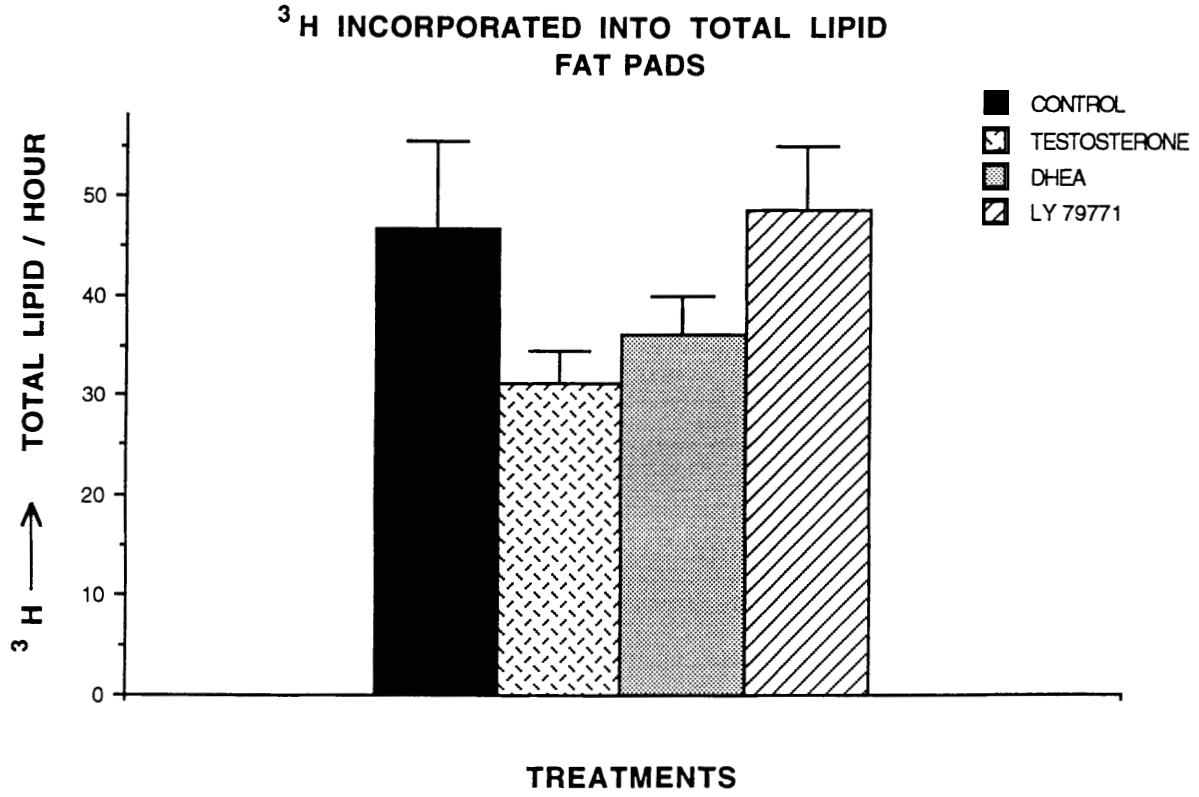

Figure 5. Rate of ^{3}H incorporation into fatty acids (nmol ^{3}H incorporated into fatty acid/g tissue/hr) in epididymal fat pads of starved/refed male BHE/cdb rats. Rats were injected intraperitoneally with 1 mCi ^3H_2O/100 g body wt. Each bar represents the mean ± SE, n = 10. No significant treatment effects were found with analysis of variance.

fatty acid synthesis (Figs. 5 and 6) in the fat pad was not affected by the various treatments. This effect was not surprising because the most responsive organ to starvation/refeeding is the liver (1–9). The lipogenic responses of LY79771-treated rats to starvation/refeeding were consistent with previous reports (26).

Discussion

The purpose of this work was to determine whether partitioning agents developed for potential use in the rearing of meat animals might have an additional use in suppressing the fat regain of animals subjected to starvation/refeeding. The starvation/refeeding paradigm was used to simulate the situation in humans who have lost weight through semistarvation only to regain this lost weight once they resume normal eating (27–31). This work was an extension of our earlier work (26) on LY79771 in which we showed that this drug could inhibit the fat regain of starved/refed BHE/cdb rats made overfat through the feeding of a high fat diet or made hyperlipogenic through the use of the starvation/refeeding paradigm. Others have also shown that LY79771 has an antiobesity effect through its action as a lipolytic agent (15–17, 32). The ultimate objective was to explore the possible usefulness of such drugs to help formerly obese people remain lean and to help them develop a "normal" partitioning of energy between fat and protein synthesis.

In the present study, we compared not only the effects of LY79771 on *de novo* fatty acid synthesis *in vivo*, but also its effect on the incorporation of labeled phenylalanine into protein. We wanted to determine whether a reciprocity existed between these two processes in the acute short-term experiment. In other words, during the acute phase of weight regain after weight loss, do these drugs truly partition (or repartition) the flow of energy and nutrients away from fat synthesis and toward protein synthesis? Will protein synthesis increase if fatty acid synthesis is decreased or fatty acid turnover increased? We then wanted to know whether a steroid such as testosterone or a steroid hormone precursor, DHEA, would have a similar effect. Earlier studies using DHEA had also shown a suppressant effect of this steroid on fatty acid synthesis (11, 33, 34), but *de novo* protein synthesis in a hyperlipogenic animal has not been reported. Thus, what is new in the present work is the simultaneous measurement of *de novo* fatty acid and protein synthesis in drug-treated or control hyperlipogenic animals. The mode of action of each drug was known to differ, yet it was hoped that any change in fat synthesis might be reciprocally linked to a change in protein synthesis.

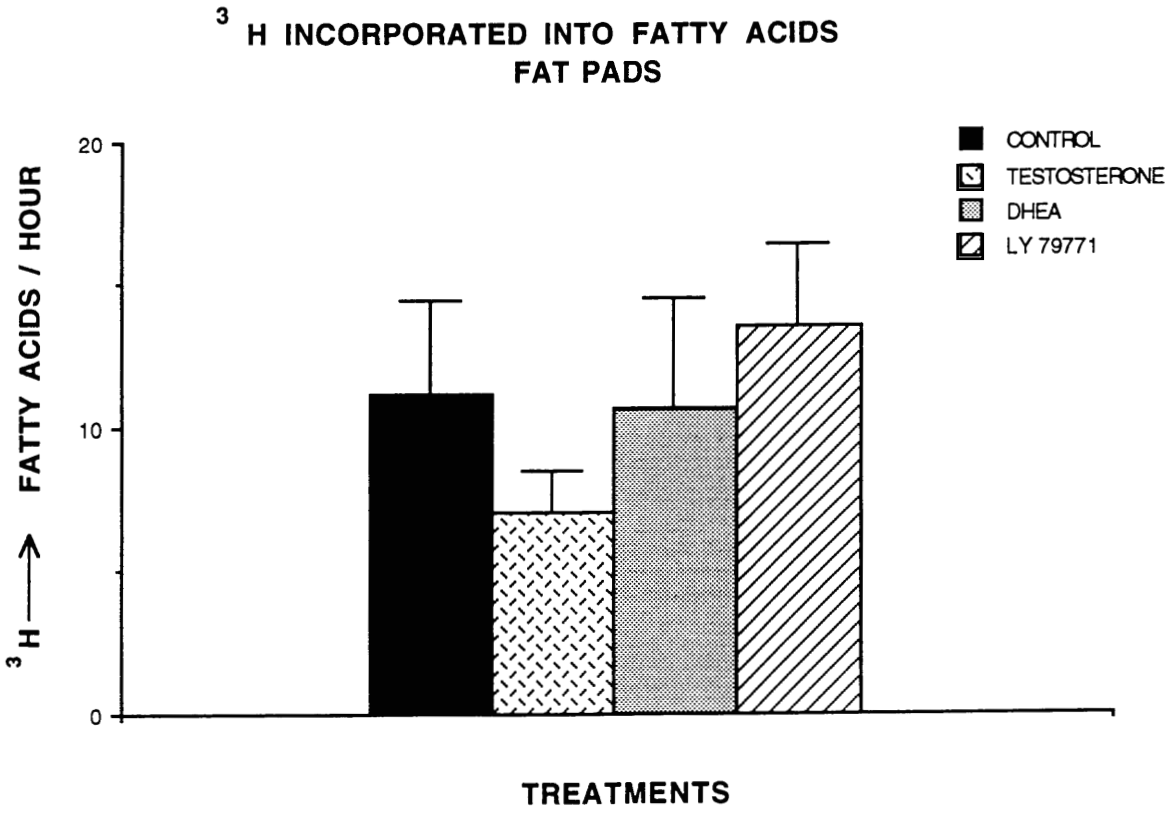

Figure 6. Rate of ^{3}H incorporation into lipid (nmol ^{3}H incorporated into lipid/g tissue/hr) in epididymal fat pads of starved/refed male BHE/cdb rats. Rats were injected intraperitoneally with 1 mCi ^3H_2O/100 g body wt. Each bar represents the mean ± SE, $n = 10$. No significant treatment effects were found with analysis of variance.

All three of the drugs affected *de novo* hepatic fatty acid synthesis while having little effect on peripheral fatty acid synthesis. More than likely, these effects were due to the fact that the major tissue involved in the hyperlipogenic response to starvation/refeeding is the liver. It is this tissue that receives the nutrients from the gut via the portal blood and that must actively metabolize these substrates. Although the fat pads do synthesize some of their stored lipid *de novo* in the starved/refed rat, the majority of their lipid is received from the liver. Hence, by comparison to the liver, the rate of incorporation of tritiated water into lipid or fatty acids was approximately 20% of that found in the liver. On the other hand, amino acids flowing to the liver from the gut are usually passed on through this organ to all the cells and tissues of the body. Thus, the rate of incorporation of phenylalanine into hepatic protein would be expected to be low while peripheral, i.e., muscle, incorporation into protein would be higher. Although we observed a "slight" increase in hepatic protein synthesis in the testosterone-treated rats compared with the control rats, the difference was not statistically significant. In the muscle tissue, there was an increase in the rate of protein synthesis showing a reciprocal effect. Since muscle mass comprises 40% of the total body weight, the effect by the agents used could be of great benefit by partitioning nutrient energy to lean body mass and away from fat synthesis. In these young animals (approximately 70 days of age), the fat mass would have been less than 10% of the total, while the liver was 5% of the total body mass. Given these figures (15% of the body mass to synthesize lipid versus 40% to synthesize protein), it appears that these drugs have exerted a reciprocal effect on these two processes. Long-term studies have shown that body composition (fat and protein) is affected in a reciprocal manner when partitioning agents are used (14, 35–37). These partitioning agents now have been shown to affect the synthesis rates of protein and lipid in a short-term study. This may help explain how partitioning agents exert their effect on changing body composition. Future work will have to incorporate longer term paradigms to determine not only synthesis, but all aspects of lipid and protein metabolism with respect to body composition change in the postobese state and the effects of drugs thereon.

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