Prevention of Obesity in A^{vy}/a Mice by Dehydroepiandrosterone

TERENCE T. YEN, JEAN A. ALLAN, DONOVAN V. PEARSON, JUNE M. ACTON, and **MARK M. GREENBERG,** The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46206

ABSTRACT

Dehydroepiandrosterone, a mammalian glucose-6-phosphate dehydrogenase inhibitor, prevented A^{vy}/a mice from becoming obese. Decreased accumulation of triacylglycerol accounted for a large portion of the weight difference between treated and control A^{vy}/a mice. Hepatic lipogenesis as measured by ^3H_2O incorporation into total lipid was less in the dehydroepiandrosterone-treated mice. Dehydroepiandrosterone did not suppress appetite and had no apparent toxic effects at the doses used, and its weight controlling effects were reversible upon withdrawal of treatment.

INTRODUCTION

Dehydroepiandrosterone (DHA), a steroid synthesized and secreted by the adrenal (1,2), is an inhibitor of mammalian glucose-6-phosphate dehydrogenase (G6PDH) (3-12), one of the enzymes supplying the NADPH required for fatty acid synthesis (13). Obese individuals excrete less DHA than the nonobese (14,15), and the urinary output of DHA increases during weight loss (15). These observations have led to the hypothesis that DHA may play a regulatory role in obesity (16). One possibility is that a higher concentration of DHA would decrease lipogenesis by lowering the availability of NADPH through the inhibition of G6PDH. However, there is no direct evidence to support this hypothesis.

Inhibition of erythrocyte and liver G6PDH by DHA has been demonstrated in vivo with humans and rats (17-20). In adrenal homogenates, apparent inhibition of G6PDH by DHA lowered the concentration of NADPH and thereby decreased glucose metabolism (21), an effect that could be reversed by addition of yeast G6PDH which is not inhibited by DHA (21). Furthermore, Ziboh et al. (22) reported that DHA inhibited synthesis of lipids from acetate by rat skin in vitro.

In spite of the work cited above, no direct evidence is available to suggest that DHA may be involved in the regulation of weight and the prevention of obesity. The data presented in this report show that DHA, possibly through an inhibition of hepatic lipogenesis, prevents viable yellow obese mice from becoming obese. A preliminary report of this investigation was published elsewhere (23).

MATERIALS AND METHODS

Two genotypes of mice of the *agouti locus* from our inbreeding colony were used in this study. They were the viable yellow obese mice (A^{vy}/a), genetically destined to become obese, and the black, recessive normal homozygotes

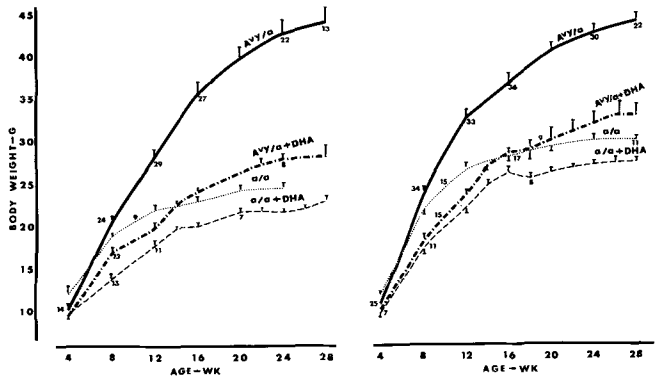

FIG. 1. Body weight of female (left panel) and male (right panel) A^{vy}/a and a/a mice given DHA, 500 mg/kg, p.o., in sesame oil three times weekly. The A^{vy}/a mice serving as controls were contemporary untreated mice combined with mice treated with sesame oil. The a/a mice serving as controls consisted of contemporary untreated mice only. All treatments started at the time of weaning and lasted the entire period shown in the graph. The curves illustrate mean body weight ± S.E. The numbers on the curves indicate tne number of mice for the point. If no number is shown, the number of mice is the same as that for the previous point.

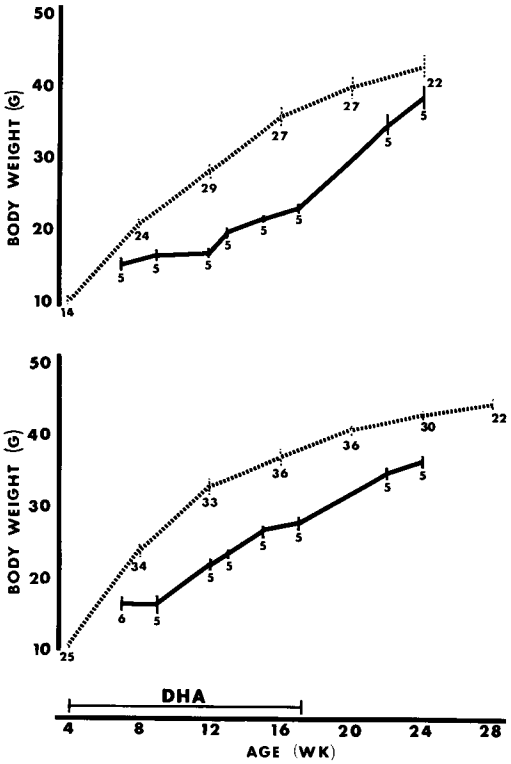

FIG. 2. Body weight of female A^{vy}/a mice (top panel) and male A^{vy}/a mice (bottom panel) given DHA, 500 mg/kg, p.o., in sesame oil three times weekly from 4 to 17 wk of age (solid line). At 17 wk of age, the treatment was discontinued. The A^{vy}/a mice serving as controls were contemporary untreated mice combined with mice treated with sesame oil (dotted line). The curves illustrate mean body weight ± S.E. The numbers on the curves indicate the number of mice for the point.

TABLE I

Food Consumption of DHA- and Vehicle-Treated A^{vy}/a Mice

Treatment	No. of mice	Food consumption g/mouse/day
Control	8♂ & 4♀	3.8 ± 0.1 (42)[a]
Control	5♂	3.5 ± 0.2 (25)
500 mg/kg, p.o.	7♂	4.7 ± 0.3 (12)
0.2% feed	5♂ & 5♀	3.6 ± 0.2 (57)

[a]Mean ± S.E. (no. of measurements).

(*a*/*a*) of the VY strain. Mice were fed Purina Laboratory Chow and had access to water ad libitum. Mice sacrificed for analysis had feed and water available until the time tritiated water was injected. The animal room was lighted from 6 am to 6 pm.

Procedures for analyzing triacylglycerol content and for measuring in vivo lipogenesis rates with tritiated water have been defined elsewhere (24). Chemicals and standards for all assays were from the same suppliers as described before (24). DHA was purchased from Searle Chemicals Inc., Chicago, IL. Emulphor EL-620 (General Aniline and Film, New York, NY), a polyoxyethylated vegetable oil, was used to facilitate the suspension of DHA in saline for parenteral injections.

Student's t-test was used in all statistical analyses of the data.

RESULTS

DHA given at 500 mg/kg, p.o., in sesame oil three times weekly controlled weight gain of both female and male A^{vy}/a mice (Fig. 1). The weights of the treated A^{vy}/a mice were only slightly greater than that of the control, lean *a*/*a* mice. Treatment of *a*/*a* mice with 500 mg/kg, p.o., DHA also reduced the weight gain of both female and male *a*/*a* mice (Figure 1), but the effect was not so pronounced as with A^{vy}/a mice.

The effect of DHA treatment was reversible. After the treatment was withdrawn, the weight of the mice increased and eventually approached that of mice not treated with DHA (Fig. 2). This was more apparent with the female mice than with the male.

DHA was also effective with both male and female A^{vy}/a mice when given in a diet containing 0.2% of the compound but was effective only with female A^{vy}/a mice when given at 150 mg/kg, p.o., thrice weekly or 10 mg/kg, i.p., thrice weekly. About 75 A^{vy}/a mice genetically destined to be obese were prevented from becoming obese by these treatments. The fact that parenteral injections of DHA are more effective than dietary DHA suggests that the compound is poorly absorbed.

The possibility of appetite suppression by DHA was investigated by monitoring the food consumption of the treated mice either periodically or throughout the entire treatment period (Table I). The food consumption of the DHA-treated A^{vy}/a mice was within or slightly above the normal range. Indeed, since the DHA-treated A^{vy}/a mice were lighter in weight, their food consumption on body weight basis was actually greater than that of vehicle-treated A^{vy}/a mice. A single injection of DHA at a dose as high as 32 mg/kg, i.p., also had no effect in an appetite-suppression test. The mice did not eat well when fed a diet containing 0.5% DHA. At that concentration in the diet, the dose would be equivalent to about 800 mg/kg of DHA per day. Kandutsch et al. (25) found androstanolone to suppress appetite at this dose.

TABLE II

Triacylglycerol (TG) Content and Incorporation of ^3H_2O into Total Lipid[a] in Liver and Carcass[b] of A^{vy}/a and a/a Mice

Treatment	N	Body weight (g)	μmoles TG / g body weight	Liver weight (g)	μmoles TG / g liver	μmoles ^3H_2O incorporated[a] into total lipid in carcass per g body weight	μmoles ^3H_2O incorporated[a] into total lipid in liver per g liver weight
			Male A^{vy}/a mice, 8 to 9 mo old				
Sesame oil, p.o.	7	49.6 ± 0.7[c]	358 ± 38	2.50 ± 0.06	75 ± 4	3.5 ± 0.1	57 ± 4
DHA, 500 mg/kg, p.o.	8	36.6 ± 1.5	179 ± 12	1.69 ± 0.07	20 ± 2	3.5 ± 0.2	33 ± 1
			Male a/a mice, 5 to 8 mo old				
None	8	29.9 ± 0.9	72 ± 8	1.23 ± 0.04	10 ± 1	2.6 ± 0.1	31 ± 4
			Female A^{vy}/a mice, 8½ to 9 mo old				
Sesame oil, p.o.	4	51.1 ± 3.3	442 ± 37	2.15 ± 0.21	61 ± 9	4.5 ± 0.6	61 ± 3
DHA, 500 mg/kg, p.o.	5	30.7 ± 1.2	202 ± 48	1.42 ± 0.06	19 ± 4	3.7 ± 0.4	42 ± 7
			Female a/a mice, 5 mo old				
None	4	23.1 ± 0.3	91 ± 6	1.03 ± 0.05	13 ± 3	2.7 ± 0.1	24 ± 1

[a]Lipogenesis rates in vivo were determined by measuring the incorporation of ^3H_2O into total lipid 1 hr after a trace amount of ^3H_2O was injected into the tail vein of the mouse. The rate of incorporation was calculated on the basis of dilution of ^3H_2O by total body water space which was estimated by the plasma radioactivity of each mouse, assuming water constitutes 90% of plasma (26).

[b]Carcass = entire body minus liver and most of the blood.

[c]All data are expressed as mean ± S.E.

The action of DHA on obesity would not appear to be due to a general toxic effect. The acute LD_{50} of DHA in mice is between 1 and 2 g/kg, s.c. Twenty A^{vy}/a and a/a mice on chronic DHA treatment were autopsied. No pathological changes were found that would contribute to the observed weight control.

For both male and female A^{vy}/a mice treated with DHA, carcass triacylglycerol contents were greatly reduced from that of the sesame oil-treated A^{vy}/a mice of the same sex (male: $p<0.0005$; female: $p<0.005$) (Table II).

DHA treatment reduced the liver weight (male: $p<0.0005$; female: $p<0.01$) but did not significantly change the liver weight/body weight ratio (Table II). DHA did, however, lower the hepatic triacylglycerol content of both male ($p<0.0005$) and female ($p<0.005$) A^{vy}/a mice (Table II).

From the data on the liver and carcass triacylglycerol content, it is possible to calculate the suppression of triacylglycerol deposition as a percentage of body weight change due to DHA. For male A^{vy}/a mice, 71% of the weight difference could be attributed to the difference in the amount of triacylglycerol deposited; for female A^{vy}/a mice, 65%. Since the female A^{vy}/a mice had higher triacylglycerol content than male A^{vy}/a mice of the same age (Table II), the inhibition of deposition due to DHA in terms of absolute amount of triacylglycerol was substantially greater in female A^{vy}/a mice than in male A^{vy}/a mice.

The lipogenesis rates of both the carcass and liver of A^{vy}/a mice were much higher than those of a/a mice (Table II). In both male and female A^{vy}/a mice, DHA significantly decreased the hepatic lipogenesis rates (male: $p<0.0005$; female: $p<0.05$) but did not affect the carcass lipogenesis rates (Table II).

DISCUSSION

A decrease of triacylglycerol content, both in the liver and in the carcass, accounts for most of the weight difference between treated and control mice. This decrease could have been caused by a lower rate of hepatic lipogenesis in the treated mice. Treatment with DHA was not found to be very effective in *ob/ob* mice, *db/db* mice and adult A^{vy}/a mice that were already obese (results not presented). We have demonstrated previously that *ob/ob* and *db/db* mice have higher lipogenesis rates even when they are young (24). In contrast, A^{vy}/a mice do not have higher hepatic lipogenesis rates until they mature. Perhaps treating A^{vy}/a mice from weaning allows sufficient time to prevent lipogenesis from becoming abnormally high with maturity.

At lower doses, DHA was more effective on female A^{vy}/a mice than on male A^{vy}/a mice. Since female A^{vy}/a mice are more obese than male A^{vy}/a mice (as shown by the triacylglycerol content), a reduction of the same percentage of triacylglycerol content would affect the weight of a female A^{vy}/a mouse more than that of a male A^{vy}/a mouse.

The control of obesity of A^{vy}/a mice is another piece of evidence suggesting that DHA may play a physiological role in regulating weight. Pharmacologically, we believe that this is the first demonstration of control of obesity with a chemical agent that probably acts on some step of metabolism (G6PDH?) instead of on appetite. Further studies are necessary to determine the mechanism responsible for these effects of DHA which may point the way to the metabolic control of human obesity.

ACKNOWLEDGMENTS

The authors wish to thank Mr. W.R. Gibson and Drs. C.G. Culbertson and P.N. Harris for undertaking the toxicology and pathology studies. The authors are also indebted to Drs. E. Farkas and R.J. Kraay for their advice.

REFERENCES

1. Nieschlag, E., D.L. Loriaux, H.J. Ruder, I.R. Zucker, M.A. Kirschner, and M.B. Lipsett, J. Endocrinol. 57:123 (1973).
2. Doouss, T.W., S.J.M. Skinner, and R.A.F. Couch, Ibid. 66:1 (1975).
3. Marks, P.A., and J. Banks, Proc. Nat. Acad. Sci. 46:447 (1960).
4. McKerns, K.W., and E. Kaleita, Biochem. Biophys. Res. Comm. 2:344 (1960).
5. Criss, W.E., and K.W. McKerns, Biochim. Biophys. Acta 184:486 (1969).
6. Raineri, R., and H.R. Levy, Biochemistry 9:2233 (1970).
7. Oertel, G.W., and I. Rebelein, J. Steroid Biochem. 1:93 (1970).
8. Oertel, G.W., P. Menzel, and D. Bauke, Clin. Chim. Acta 27:197 (1970).
9. Oertel, G.W., and P. Benes, J. Steroid Biochem. 3:493 (1972).
10. Beitner, R., and Z. Naor. Biochim. Biophys. Acta 286:437 (1972).
11. Lopez-S, A., and A. Rene., Proc. Soc. Exp. Biol. Med. 142:258 (1973).
12. Setchenska, M.S., E.M. Russanov, and J.G. Vassileva-Popova, FEBS Lett. 49:297 (1975).
13. Lane, M.D., and J. Moss, in "Metabolic Pathways," Vol. 5, Edited by H.J. Vogel, Academic Press, New York, NY, 1971, p. 23.
14. Šonka, J., and J. Gregorova, Acta Univ. Carolinae 1:89 (1963).
15. Lopez-S., A., and W.A. Krehl, Lancet 2:485 (1967).
16. Šonka, J., and J. Gregorova, J. Physiol., Paris, 56:650 (1965).
17. Willmer, J.S., and T.S. Foster, Can. J. Biochem. 43:1375 (1965).

18. Lopez-S., A., and W.A. Krehl, Proc. Soc. Exp. Biol. Med. 126:776 (1967).
19. Tepperman, H.M., S.A. De la Garza, and J. Tepperman, Am. J. Physiol. 214:1126 (1968).
20. Hilgertová, J., and J. Šonka, Horm. Metab. Res. 5:286 (1973).
21. Tsutsui, E.A., P.A. Marks, and P. Reich, J. Biol. Chem. 237:3009 (1962).
22. Ziboh, V.A., M.A. Dreize, and S.L. Hsia, J. Lipid Res. 11:346 (1970).
23. Yen, T.T., J.A. Allan, D.V. Pearson, J.M. Acton, and M.M. Greenberg, Fed. Proc. 33:1156 (1977).
24. Yen, T.T., J. Allan, P.L. Yu, M.A. Acton, and D.V. Pearson, Biochim. Biophys. Acta 441:213 (1976).
25. Kandutsch, A.A., D.L. Coleman, and S.E. Alpert, Experientia 28:473 (1972).

[Revision received January 5, 1977]