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Di(2-Ethylhexyl)Adipate (DEHA): Effect on Plasma Lipids and Hepatic Cholesterolgenesis in the Rat

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Di(2-ethylhexyl)adipate, DEHA (sometimes referred to as dioctyl adipate, DOA), is a widely used plasticizer in vinyl plastics that require low temperature flexibility (HUFF 1982). Examples of the types of products containing DEHA include vinyl packaging films for refrigerated and frozen foods, vinyl coated automotive fabrics, and garden hoses. DEHA is also used in polyvinyl hemodialysis tubings (HUFF 1982). In previous studies from this laboratory it was determined that another plasticizer, di(2-ethylhexyl)phthalate (DEHP), produces a number of changes in lipid metabolism in tissues from experimental animals (BELL 1982). In particular, DEHP is an inhibitor of sterologenesis in liver, brain, testis, and adrenal gland and induces a mild plasma cholesterol-lowering response (BELL 1982). Because of the structural similarity between DEHA and DEHP, the potential of DEHA to alter lipid metabolism was evaluated in rats fed 1% DEHA for up to 7 wk.

MATERIALS AND METHODS

Male rats (Upjohn:TUC (SD)spf, 200-225g) were individually caged and fed a stock rodent chow (Ralston Purina, #5001) or the chow supplemented with 1% di(2-ethylhexyl)adipate (DEHA), Eastman Chemical Products, Inc. (w/w). The DEHA was dissolved in diethyl ether for dispersal in the diet (BELL & NAZIR 1976); control diets received equivalent amounts of ether. The ether was permitted to evaporate under an exhaust hood before using the diets. Blood was drawn via cardiac puncture into heparinized syringes. Plasma total cholesterol was measured enzymatically with a commercially available kit (Calbiochem) whereas plasma unesterified (free) cholesterol was measured as the digitonide (SPERRY & WEBB 1950). Plasma triglycerides were measured using an automated technique (ROYER & KO 1972). Lecithin:cholesterol actyltransferase (LCAT, EC 2.3.1.43) was assayed in fresh plasma that was permitted to equilibrate with [4-^{14}C]cholesterol (60 Ci/Mol, New England Nuclear Corp.) as detailed previously (BELL 1983); the method provides a sensitive measurement of the absolute rate of plasma cholesterol esterification (LACKO et al. 1973). Lipid synthesis was measured at 37° in rat liver minces (500 mg) (BELL et al.1978) that were incubated for 90 minutes in 3.5 ml Krebs-Ringer-bicarbonate

buffer, pH 7.4, which contained 3.5 μCi [1-^{14}C]octanoic acid, sodium salt (sp. act 25.1 Ci/Mol, New England Nuclear Corp.). After incubation the tissues were extracted with $CHCl_3$ -MeOH (2:1, V/V) and the resulting extracts were washed as described by FOLCH et al. (1957). Portions of the extracts were fractionated by thin-layer chromatography on silica gel G-coated glass plates in a solvent system consisting of n-hexane:diethylether:acetic acid (146/50/4, V/V/V) (BELL & NAZIR 1976). Lipid bands corresponding to the phospholipids, triglycerides, and free fatty acids were scraped from the plates into vials and assayed for radioactivity by liquid scintillation counting (BELL & NAZIR 1976). Another portion of the lipid extracts was evaporated to dryness under N_2, dissolved in alcoholic-KOH, and heated to 60°C for 1 h to saponify the lipids (DALY 1971). The non-saponifiable lipid fraction was extracted with n-hexane (BELL 1976). The hexane extracts were chromatographed as above and the fraction cochromatographing with authentic cholesterol (BELL 1976) was scraped from the plates and assayed for radioactivity as above. Statistical analyses were performed using Student's *t*-test for independent samples.

RESULTS AND DISCUSSION

DEHA feeding resulted in alterations in plasma triglyceride and cholesterol levels in the rats (Figs. 1A and 1B). Cholesterol was lowered 15% at 2 and 5 wk but normalized by the seventh week. Plasma triglycerides appeared normal at 2 wk but fell 40% by 5 wks. By 7 wk, triglyceride levels were still somewhat below control values but the difference did not reach a level of statistical significance.

LCAT activity was measured in plasma from rats fed DEHA for 5 wk. Although plasma free (unesterified) cholesterol was significantly reduced by about 18% (Table 1), the absolute rate of cholesterol esterification was not affected by DEHA feeding. Esterification rates averaged 28.2 and 26.4 μmoles cholesterol esterified/l plasma/h in the control and DEHA-treated animals, respectively, (Table 1).

The effect of DEHA feeding on the incorporation of [^{14}C]octanoate into various classes of lipid was studied in liver minces from rats fed 1% DEHA for 7 wk (Table 2). Incorporation into cholesterol was reduced about 66% ($p < 0.05$) with DEHA feeding whereas incorporation into phospholipids, triglycerides, and free fatty acids was not significantly affected.

Diesters of phthalic acid and of adipic acid are commonly used as plasticizers in the production of flexible plastic products. Experimental evidence obtained from studies in rodents and other animals indicates that di(2-ethylhexyl)phthalate (DEHP), the most widely used phthalate plasticizer, exerts a variety of toxic effects in biological systems (BELL 1982; GANGOLLI 1982; SETH 1982; WARREN et al. 1982). As a result, interest in assessing the biological activity of other plasticizers has developed. In the present

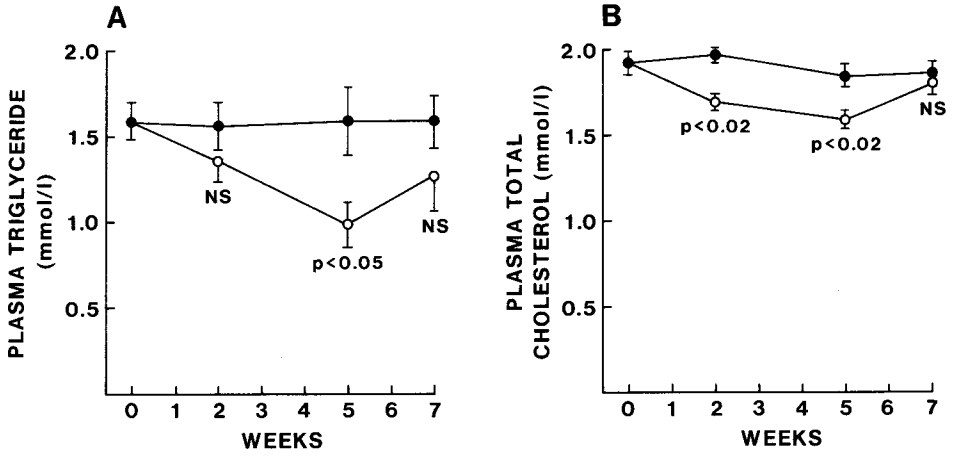

Figure 1. The effect of 1% DEHA feeding on plasma lipid levels in the rat. Rats were fed the stock chow diet alone (Control) or supplemented with 1% DEHA for up to 7 wk. All data points are means ± SEM of 8 animals except for the 7 wk DEHA points which are the mean of 5 animals. P values denote treatment means differing significantly from the corresponding control means by Student's independent t-test; NS, not significantly different.

A. Plasma triglyceride (Control -●-●-; DEHA, -O-O-)

B. Plasma total cholesterol (Control -●-●-; DEHA, -O-O-)

Table 1. Effect of 1% DEHA Feeding on Plasma Unesterified Cholesterol and the Activity of Lecithin:Cholesterol Acyltransferase (LCAT)[a]

	Plasma Unesterified Cholesterol (mmol/l)	Esterification of Plasma Cholesterol by LCAT (μmol/l/hr
Control	0.40 ± 0.02[b]	28.2 ± 1.8
DEHA	0.33 ± 0.01	26.4 ± 1.2
	$p < 0.01$[c]	NS[d]

[a]Unesterified cholesterol and LCAT activity were measured in plasma from rats fed the stock chow diet alone (Control) or supplemented with 1% DEHA for 5 wk.

[b]Values are means ± SEM of 8 rats/group.

[c]Differs significantly from the control mean using Student's independent *t*-test; NS, not significantly different from the control mean ($p > 0.05$).

Table 2. Effect of 1% DEHA Feeding on the Incorporation of [^{14}C]Octanoate into Cholesterol, Free Fatty Acids, and Glycerolipids in Rat Liver Minces (dpm/g wet wt)[a]

	Cholesterol	Phospholipid	Triglyceride	Free Fatty Acid
Control	5915	48310	183990	16215
	±1330[b]	±5960	±17175	±3630
DEHA	2010	34480	165095	21065
	±540	±3050	±17590	±4430
	$p < 0.05$[c]	NS[d]	NS	NS

[a]Livers from rats fed the stock chow diet alone (Control) or supplemented with DEHA for 7 wk were used to prepare minces for incubation with [1-^{14}C]octanoate.

[b]Values are means ± SEM of 8 control rats and 5 DEHA-fed rats.

[c,d]Differs significantly from the control mean using Student's independent *t*-test; NS, not significantly different from control mean ($p > 0.05$).

studies, di(2-ethylhexyl)adipate (DEHA), the most commonly used adipate plasticizer, was evaluated for its effects on certain aspects of lipid metabolism in rats over a 7 wk period. In rats fed 1% DEHA, there was a transient hypocholesterolemic and hypotriglyceridemic response which was pronounced at 5 wk but which essentially disappeared by 7 wk (Figs. 1A and 1B). Plasma cholesterol and triglyceride lowering effects have been observed in rats fed the phthalate ester DEHP for up to 4 wk. Whether or not the lipid-lowering responses seen with DEHP are also reversed at later time periods is unknown since studies reported to date have not exceeded 4 wk (REDDY et al. 1976; BELL et al. 1978; YANAGITA et al. 1978; BELL 1982).

The hypocholesterolemic effect of DEHA was not associated with changes in the activity of LCAT, the enzyme responsible for the esterification of cholesterol in plasma (BELL 1983a) and one considered to be involved in the removal of cholesterol from tissues (GLOMSET 1979). Although plasma free (unesterified) cholesterol was significantly lowered by DEHA-feeding (0.40 vs 0.33 mmol/l, $p < 0.01$), the rate of cholesterol esterification by LCAT, as determined by a sensitive isotopic method, was similar in control and DEHA-fed rats (Table 1). These results are similar to our results in DEHP-fed rats in which plasma cholesterol lowering also occurred without an effect of LCAT activity (BELL et al. 1978). Other species however may not necessarily respond similarly since phthalate plasticizers have been reported to be inhibitors of LCAT in human plasma (LAGENTE et al. 1978; 1979).

Previous studies which showed that hepatic sterologenesis from [^{14}C]acetate was inhibited by 2 wk of DEHA feeding in the rat (BELL 1983b) have been confirmed in the present study and extended to demonstrate that inhibition of sterologenesis persists throughout periods of DEHA exposure for up to 7 wk (Table 2). Additionally, the inhibition of hepatic sterologenesis observed in the present study using [^{14}C]octanoate circumvents the possibility of an apparent inhibition which could arise if [^{14}C] acetate were to be diluted by changes in endogenous acetate pools (DIETSCHY & BROWN 1974) as a result of DEHA metabolism in vivo. This point is especially pertinent since adipate, a metabolite of DEHA (TAKAHASHI et al. 1981) appears to undergo β-oxidation to some extent (RUSOFF et al. 1960; TAKAHASHI et al. 1981) and is therefore a potential source of acetyl units for sterologenesis.

Data from the present study suggests that the plasma cholesterol-lowering effect of DEHA seen at 2 and 5 wk is not a simple reflection of an inhibition of hepatic cholesterolgenesis because plasma cholesterol levels normalized by 7 wk despite the fact that hepatic sterologenesis was reduced 50%; other factors which can affect plasma cholesterol levels such as cholesterol excretion and plasma lipoprotein turnover may be involved as well.

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