Effects of Prolonged Continuous Exposure to 100% Oxygen at 450 mm Hg *in Vivo* on Lipid Synthesis in Rat Liver and Adipose Tissue Slices (35397)

D. D. FELLER, E. D. NEVILLE, AND K. S. TALARICO

Environmental Biology Division, Ames Research Center, NASA, Moffett Field, California 94035

In an earlier report from this laboratory, it was shown that short-term exposure (up to, but not exceeding 4 days) to hypobaric hyperoxic atmospheres had pronounced effects in rats on appetite, levels of certain blood constituents and, in particular, synthesis of fatty acids from acetate in slices of liver and adipose tissues obtained at sacrifice (1). Lipid synthesis was depressed as food intake was reduced at 191 mm Hg of 100% oxygen (normoxic). It was then demonstrated, interestingly enough, that despite a lower than normal food intake at 258 mm Hg of 100% oxygen (hyperoxic), fatty acid synthesis returned to normal values. Further increase in barometric pressure to 350 mm Hg (hyperoxic) resulted in conversion of acetate to fatty acids at values greater than controls. The conclusion drawn from these results is that 100% oxygen has a stimulatory effect upon fatty acid synthesis in liver and adipose tissue of rats exposed to hypobaric hyperoxic environments of 258 and 350 mm Hg.

Continuous exposure of rats to 100% oxygen at 450 mm Hg for 64 days as shown by Brooksby *et al.* (2) did not cause a significant difference in cumulative total food and water consumption or show a demonstrable histological and hematological difference in tissues when compared to control animals. However, Leon and co-workers (3) showed that, at higher partial pressures of 100% oxygen, food intake was reduced and toxic effects of oxygen were observed. Exposure of rats at 450 mm Hg, therefore, has two advantages: (i) a caloric intake of experimental animals equal to that of control animals; and (ii) a nontoxic hyperoxic environment. Exposure of rats to this environmental condition was the subject of the present study.

Methods. Male Sprague-Dawley rats (245–295 g) were divided into two groups: (i) an experimental group exposed to 450 mm Hg of 100% oxygen for 1, 2, or 3 weeks and allowed free access to food and water; and (ii) a control group pair-fed to the experimental group and maintained in standard laboratory metabolism cages in air at sea level barometric pressures. During the first day of pair-feeding, the controls were given an amount of food based on approximations from other exposure studies. Thereafter, the controls were fed the amount of food the experimental rats ate the previous day. Smaller rats were selected at the start of longer exposures in an attempt to have the rats from the various exposure times be of approximately the same weight at the termination of the experiments. An additional *ad libitum* control group (325 g) maintained at sea level conditions was also run. The rats were fed a balanced diet of 6% fat, 24% protein, and 60% carbohydrate, plus mineral and vitamin supplements. After exposure, all rats were decapitated at approximately 9:30 a.m. Plasma was collected for various assays and the *in vitro* incorporation of acetate-2-^{14}C into CO_2 and fatty acids by liver and adipose tissue was followed.

Each oxygen-exposed rat was housed in an individual plexiglass chamber of approximately 2800-cm^3 volume (4). By means of transfer tube and gate valve, the rats could be transferred into a clean chamber without exposing the rat to sea-level pressures. All chambers were cleaned and changed daily. A gas-flow rate of 800 ml/min was maintained as the optimum (2).

Analytical Procedures. After removal from

[1] Simonsen Maintenance Diet, Simonsen Laboratory, Gilroy, California.

the chamber, the rats were decapitated, and plasma was collected and frozen for subsequent analysis. A portion of the liver was removed and cut into 0.5-mm slices with a mechanical tissue chopper; and epididymal adipose tissue was cut with scissors. One g of each tissue was incubated at pH 7.40 ± 0.02 for 3 hr in 10 ml of Krebs–bicarbonate buffer containing 0.01 *M* succinate, 0.011 *M* glucose, and 10 μCi of 0.001 *M* sodium acetate-2-^{14}C as described elsewhere (5). The gas phase of the incubation-flask was 95% O_2, 5% CO_2. The reactions were terminated by adding 0.2 ml of 10 *N* H_2SO_4 to the incubation media. The CO_2 liberated was absorbed with 1 ml of 2.5 *N* NaOH added to a removable plastic CO_2 collection well which was inserted prior to sealing the incubation flask (6). After incubation, the tissues were washed and lipids were saponified by heating the tissues overnight in a solution of alcoholic-KOH over a steam bath. The nonsaponifiable tissue lipids were removed with hexane extractions. Tissue fatty acids were extracted with aliquots of hexane after acidification with 6 *N* HCl. The ^{14}C content of fatty acids, nonsaponifiable lipids, and CO_2 was determined (5).

Plasma lipids were extracted with chloroform–methanol (2:1, v:v) as described by Jover (7). Aliquots of the chloroform phase were taken to determine plasma triglycerides (7), total plasma lipids (8), total cholesterol (9), and inorganic P as phospholipids after digestion and oxidation (10). Plasma free fatty acids were determined by the method of Trout *et al.* (11). Plasma glucose was determined by the glucose oxidase method (12), and plasma corticosterone by the method of Peterson (13).

Results. The average daily food consumption (Table I) for the 1-, 2-, and 3-week exposures was 30.1, 28.6, and 29.3 g/day, respectively. The oxygen-exposed rats gained more weight than did their respective pair-fed controls in all exposures. The oxygen-exposed rats gained 33, 50, and 59 g during the 1-, 2-, and 3-week exposures, respectively, while the pair-fed rats gained 23, 19, and 27 g for these respective time periods.

Figure 1 shows that the oxygen-exposed rats had a substantial decrease in food intake during the first week of exposure. The food intake approached preexposure levels during the second week and returned to preexposure levels during the third week. However, during

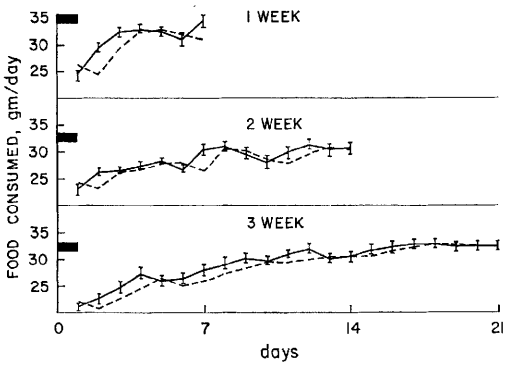

FIG. 1. The daily food consumption of rats exposed to 450 mm Hg of 100% oxygen or 760 mm Hg of air for 1, 2, and 3 weeks. The inset bar along the ordinate is the normal daily food intake *ad libitum* at sea-level conditions. Initial and final mean weights of the rats are recorded in Table I. (—) oxygen-exposed; (- -) pair-fed controls in air; $N = 6 \pm$ SEM.

TABLE I. Average Daily Food Consumption and Total Body Weight Change of Rats Exposed to 100% Oxygen at 450 mm Hg for 1, 2, and 3 Weeks and Their Respective Pair-Fed Controls (Air at 760 mm Hg).

Exposure time (weeks)	Av daily food consumption (g)		Rat wt (g)			
			Initial		Final	
	Pair-fed	Experimental	Pair-fed	Experimental	Pair-fed	Experimental
1	29.0 ± 0.5[a]	30.1 ± 1.1	295 ± 5	295 ± 5	318 ± 6	328 ± 11
2	27.9 ± 0.5	28.6 ± 1.2	270 ± 3	268 ± 3	289 ± 5	318 ± 10[b]
3	28.3 ± 0.6	29.3 ± 1.1	247 ± 4	245 ± 4	274 ± 6	304 ± 7[b]

[a] $N = 6 \pm$ SEM.

[b] $p < 0.05$, significantly different from final weight of pair-fed controls.

TABLE II. Changes in Plasma Glucose and Plasma Lipids of Rats Exposed to 450 mm Hg of 100% Oxygen for 1, 2, or 3 Weeks and Their Respective *ad Libitum* and Pair-Fed Controls (air at 760 mm Hg).

Plasma constituents	*Ad libitum* controls (sea level)	(week): 1		2		3	
		Pair-fed	Experimental	Pair-fed	Experimental	Pair-fed	Experimental
Glucose (mg/100 ml)	142 ± 4[a]	134 ± 3	131 ± 2	151 ± 4	143 ± 7	140 ± 4	150 ± 3
FFA (μEq/liter)	357 ± 33	416 ± 28	338 ± 27	396 ± 23	347 ± 26	385 ± 34	330 ± 27
Total lipid (mg/100 ml)	331 ± 31	239 ± 18	334 ± 60	188 ± 19	345 ± 36[b]	225 ± 7	250 ± 12
Cholesterol (mg/100 ml)	50 ± 3	56 ± 4	58 ± 4	44 ± 3	55 ± 6	51 ± 3	54 ± 4
Triglycerides (mg/100 ml)	123 ± 11	88 ± 14	102 ± 7	40 ± 5	106 ± 7[c]	74 ± 8	81 ± 9
Phospholipids (mg/100 ml)	97 ± 7	84 ± 14	92 ± 6	56 ± 5	100 ± 8[c]	71 ± 4	79 ± 5
Corticosterone (μg/100 ml)	37 ± 6	58 ± 5	59 ± 6	47 ± 8	37 ± 3	46 ± 6	54 ± 3

[a] $N = 6 \pm$ SEM.
[b] $p < 0.01$, significantly different from pair-fed controls; [c] $p < 0.001$.

the third week, the food intake for the oxygen-exposed rats was less than that for rats of comparable weight maintained at sea-level conditions. This is shown by the initial weight and food intake of the 1-week group which had an initial weight of 295 g and a food intake of 35 g/day at sea-level conditions compared to a weight of 295-304 g and a food intake of 32–33 g/day for the oxygen-exposed rats during the third week. The pair-fed controls were essentially *ad libitum* controls by the end of 3 weeks of exposure. The 3-week pair-fed controls weighed 274 g at the end of pair-feeding and consumed 32–33 g/day of food during the third week. This is comparable to an initial weight of 270 g for the 2-week group with a food intake of 32–33 g/day at sea-level conditions.

The principal changes in plasma constituents (Table II) were observed after 2 weeks of exposure and pair-feeding when the pair-fed controls had a significantly lower plasma total lipid, triglyceride, and phospholipid level than that of the oxygen-exposed rats. Generally, the plasma glucose and plasma lipids of the oxygen-exposed rats were comparable to those rats fed *ad libitum* at sea-level conditions.

The pair-fed controls had a significantly lower liver fatty acid content during the 1- and 2-week exposures than did the oxygen-exposed rats (Table III). There was a trend in the pair-fed controls to increase their fatty acid content as their food ration approached *ad libitum* conditions. In contrast, no difference was noted in the fatty acid content of adipose tissue between the oxygen-exposed rats and the pair-fed controls.

The conversion of acetate-2-^{14}C to $^{14}CO_2$ and ^{14}C-fatty acids by liver and adipose tissue is shown in Table IV. No noticeable change in $^{14}CO_2$ production was observed between the pair-fed controls and the oxygen-exposed rats except in the liver at the end of 3 weeks. The recovery in the oxygen-exposed rats was 16.4% compared to 11.5% in the pair-fed controls for this time period.

The conversion of acetate to fatty acids by liver from the oxygen-exposed rats was comparable to sea level *ad libitum* controls, but generally higher than in the pair-fed controls (Table IV). Conversion for pair-fed controls was 1.80% at 1 week, 2.10% at 2 weeks, and 4.05% at 3 weeks, respectively, compared to

TABLE III. Fatty Acid Content of Liver and Adipose Tissue of Rats Exposed to 100% Oxygen at 450 mm Hg for 1, 2, and 3 Weeks and Their Corresponding *ad Libitum* and Pair-Fed Controls (air at 760 mm Hg).

Exposure time (weeks)	Tissue fatty acid (% wet wt)			
	Liver		Adipose tissue	
	Pair-fed	Experimental	Pair-fed	Experimental
1	1.50 ± 0.40[b]	3.22 ± 0.44[c]	77.8 ± 1.7	78.7 ± 1.2
2	1.80 ± 0.14	2.39 ± 0.12[d]	75.1 ± 1.2	78.3 ± 0.9
3	1.92 ± 0.28	2.50 ± 0.33	74.8 ± 1.4	77.0 ± 0.8
Ad libitum controls[a] (air)	2.96 ± 0.65		76.0 ± 0.8	

[a] 325 g average weight.
[b] $N = 6 \pm$ SEM.
[c] $p < 0.05$, significantly different from pair-fed controls; [d] $p < 0.01$.

values of 3.05, 5.76, and 5.37%, respectively, for the oxygen-exposed rats.

The conversion of acetate to fatty acids by adipose tissue from the oxygen-exposed rats was significantly greater for all exposures than that of the *ad libitum* rats at sea-level conditions (Table IV). The oxygen-exposed rats converted approximately 10% of the acetate to fatty acids while the sea-level controls converted approximately 7%. The oxygen-exposed rats also converted more acetate to fatty acids than did the pair-fed controls for all exposures. The differences were significantly greater at the 1 and 2 week periods. However, during the third week when the pair-fed controls ate amounts of food comparable to *ad libitum* controls, the pair-fed group converted more acetate to fatty acids than did sea-level controls. There was no significant difference between the oxygen-exposed rats and pair-fed controls at 3 weeks.

Discussion. Earlier reports (1) have shown that rats exposed to a hypobaric–hyperoxic environment convert more acetate to fatty acids than do pair-fed controls. In those experiments, the exposure times were limited to 4-days duration. The hyperoxic environment of much longer duration (1, 2, and 3 weeks) permits the daily measurement of food intake for times that extend beyond the adjustment period, as well as allow observations of growth (wt gain) and changes in tissue composition. The fatty acid content of a sample of epididymal adipose tissue from rats exposed to the oxygen-rich environment showed slightly higher, but not significantly different, values than those from rats eating the same amounts of daily food rations (*ad libitum* and pair-fed controls) but exposed to a normal gaseous and barometric environment. No measurement of the change of total amounts of adipose tissue, *per se*, or total body fat was made, but the animals exposed to 100% oxygen at 450 mm Hg did show a significant weight gain of 12% more than control rats. The livers of the oxygen-exposed rats also had fatty acid contents significantly higher than those of the pair-fed group. These results strongly suggest that the oxygen-enriched environment alters the utilization of foodstuffs in a manner which more efficiently conserves energy. The greater-than-normal amounts of conversion of acetate to fatty acids in tissues obtained from rats exposed to 350 mm Hg of 100% oxygen for up to 4 days lends credence to this hypothesis (1).

The conversion of acetate to fatty acids by liver from the pair-fed controls is probably not maximal and reflects periods of fasting. The less-than-normal daily food requirement was rationed on a lumped daily basis. It is possible the rats ate their ration in less than 24 hr and then went through a period of fasting. This would depress liver lipogenesis, particularly in the early exposures where food intake was much less than normal. Contrary to liver, adipose tissue from the pair-fed

TABLE IV. Conversion of Acetate-2-^{14}C to $^{14}CO_2$ and ^{14}C-Fatty Acids by Liver and Adipose Tissue from Rats Exposed to 100% Oxygen at 450 mm Hg for 1, 2, and 3 Weeks and Their Corresponding *ad Libitum* and Pair-Fed Controls (air at 760 mm Hg).

Exposure time (weeks)	^{14}C recovered as $^{14}CO_2$ (%)				^{14}C recovered as ^{14}C-fatty acids (%)			
	Liver		Adipose		Liver		Adipose	
	Pair-fed	Experimental	Pair-fed	Experimental	Pair-fed	Experimental	Pair-fed	Experimental
1	12.2 ± 0.6[b]	14.9 ± 1.4	6.36 ± 0.48	6.39 ± 0.31	1.80 ± 0.56	3.05 ± 0.74	7.57 ± 0.95	11.21 ± 0.87
2	18.5 ± 1.2	13.7 ± 1.9	6.91 ± 0.30	6.79 ± 0.25	2.10 ± 0.20	5.76 ± 1.00[c]	5.60 ± 0.72	9.50 ± 0.80[d,e]
3	11.5 ± 1.3	16.4 ± 1.2[d]	7.84 ± 0.36	8.07 ± 0.18	4.05 ± 0.62	5.37 ± 0.54	9.52 ± 1.26	10.49 ± 1.20[e]
Ad libitum[a] controls (air)	15.4 ± 0.8		6.71 ± 0.64		4.74 ± 0.83		7.26 ± 0.79	

[a] 325 g average weight.
[b] $N = 6 \pm$ SEM.
[c] $p < 0.05$, significantly different from pair-fed controls; [d] $p < 0.001$.
[e] $p < 0.05$, significantly different from *ad libitum* controls.

controls did not show a depressed lipogenic activity when compared to *ad libitum* controls. The magnitude of the lipogenic response to feeding frequency (meal feeding versus free access) has been shown to be different for these two tissues (14).

Exposure of rats to 100% oxygen at 450 mm Hg for extended periods of time (1–3 weeks) increases above normal the conversion of acetate to fatty acids in adipose tissue. These findings complement the results reported earlier of shorter duration exposure, where the high oxygen environments reestablished base line conversion values, and in fact, exceed normal values.

Summary. Male rats, fed *ad libitum*, were exposed to 100% oxygen at 450 mm Hg for periods of time varying from 1 to 3 weeks. After exposure, blood lipid assays were performed and *in vitro* incorporation of acetate-2-^{14}C into CO_2 and fatty acids by slices of liver and epididymal adipose tissue was measured. The oxygen-exposed rats gained a greater amount of weight over the exposure period than did their isocalorically, pair-fed controls. Plasma lipid values obtained from oxygen-exposed rats were higher than those obtained from the pair-fed group. Fatty acid contents in liver and adipose tissue of the oxygen-exposed rats were higher than those obtained from the pair-fed control rats. Conversion of acetate to fatty acids was found to be significantly higher in adipose tissue of O_2-exposed rats than that of nonexposed controls. A similar difference was not found in the case of slices of liver obtained from the same two groups of rats. The possibility that the oxygen-enriched environment alters the utilization of foodstuffs in a manner which more efficiently conserves energy by the storage of greater amounts of fat and/or greater synthesis of lipids is discussed.

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1. Feller, D. D., Neville, E. D., and Talarico, K. S., Proc. Pac. Slope Biochem. Conf. Seattle, Wash., 1969 50.

2. Brooksby, G. A., Dennis, R. L., and Staley, R. W., Acrosp. Med. 37, 243 (1966).

3. Leon, H. A., Brooksby, G. A., Chackerian, M.

J., and Staley, R. W., Aerosp. Med. Prepr. (1967).

4. Quattrone, O. D., and Staley, R. W., J. Appl. Physiol. **21**, 741 (1966).

5. Feller, D. D., and Neville, E. D., Amer. J. Physiol. **208**, 892 (1965).

6. Neville, E. D., and Feller, D. D., Anal. Biochem. **11**, 144 (1965).

7. Jover, A., J. Lipid Res. **4**, 228 (1963).

8. Bragdon, J. H., J. Biol. Chem. **190**, 513 (1951).

9. Abell, L. L., Levey, B. B., Brodie, B. B., and Fendall, F. E., J. Biol. Chem. **195**, 357 (1952).

10. Fiske, C. H., and Subbarow, Y. J., J. Biol. Chem. **66**, 375 (1925).

11. Trout, D. L., Estes, E. H., Jr., and Friedberg, S. J., J. Lipid Res. **1**, 199 (1960).

12. Fales, F. W., Russell, J. A., and Fain, J. N., Clin. Chem. **7**, 289 (1961).

13. Peterson, R. E., J. Biol. Chem. **225**, 25 (1957).

14. Leveille, G. A., Proc. Soc. Exp. Biol. Med. **125**, 85 (1967).

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