Increased Partial Pressures of Oxygen at Normal Barometric Pressure on Lipogenesis in Rat Tissues (37371)

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That enriched oxygen environments cause an increased fat synthesis provided the experimental animals have free access to food was shown by exposure of rats to 100% oxygen at 258 mm Hg, the Apollo cabin atmosphere (1). These results led to the conjecture that greater oxygen enrichment might further increase tissue biosynthetic reactions with still further energy conservation benefits. Thus, subsequent experiments were performed with 100% oxygen environments at barometric pressures of 350 mm Hg (1), 450 mm Hg (2) and 600 mm Hg (2). Further oxygen enrichment did result in greater enhancement of lipogenesis at 350 mm Hg, but, at 450 mm Hg, lipogenesis reached a plateau with no further increases. When the pressure of the 100% oxygen atmosphere was raised to 600 mm Hg, physiological impairment or tissue toxic changes occurred (3, 4), and lipogenetic values fell back to, or below normal values (2).

While the above-cited studies (1–3) implicate oxygen pressure as the causative agent, further experiments needed to be performed to eliminate the reduced barometric pressure as a factor in this response and to demonstrate that the observed changes of lipid metabolism resulted solely from an increase in oxygen tension.

This report presents the effects on the lipid metabolism of liver and adipose tissue slices obtained from rats previously exposed to increased partial pressures of oxygen at normal barometric pressure.

Materials and Methods. Aviator's oxygen and nitrogen were passed through a mixing vessel and into the environmental animal chamber at 10 liter/min at sea level barometric pressure. The percentages of dry gases at 1 atm were calculated (5) to correspond to the alveolar partial pressure of 100% oxygen at 258 mm Hg (pA_{O_2} = 171 mm Hg) and 350 mm Hg (pA_{O_2} = 273 mm Hg). These ratios were 30.5% O_2, 69.5% N_2 and 43.0% O_2, 57.0% N_2, respectively, when the alveolar partial pressure of CO_2 and water is assumed to remain constant at 40 and 47 mm Hg, respectively.

Gas samples of the in-going and the outgoing animal chamber gas mixtures were taken every 2 hr during the day. The samples were measured with a pH/Gas Analyzer, Model 113 (Instrumentation Laboratory Inc., Boston) to determine O_2 and CO_2 levels.

Male Sprague–Dawley rats (248–295 g) were divided into 3 groups: (a) a control group (stock) allowed free access to food and water and kept in an air environment at sea level barometric pressure; (b) 2 experimental groups exposed to gaseous environment containing mixtures of O_2 and N_2 for periods of time varying from 1 to 4 days at sea level barometric pressure and allowed free access to food and water; and (c) a control group (isolated) allowed free access to food and water and kept in air at sea level barometric pressure.

The stock control group rats were kept in the animal colony in groups of 4 with no special treatment prior to the experiment. The experimental and isolated control rats were placed in identical individual metal cages designed for daily food and water measurements and were placed in these cages 1 to 2 days prior to the experiment for training purposes. The exposure to altered gaseous environments was carried out in a plexiglass chamber described earlier (1). The chamber was opened daily for approximately 1 hr to clean cages and add fresh food and

TABLE I. Average Daily Food Consumption and Total Body Weight Change of Rats Exposed to Hyperoxic Environments for 1–4 Days and Their Respective Isolated Controls (Air at 760 mm Hg).

Experimental conditions	Exposure time (days)	Av daily food consumption (g)		Initial body wt (g)		Total wt change (g)	
		Isolated control	Oxygen treated	Isolated control	Oxygen treated	Isolated control	Oxygen treated
	1	24 ± 2[a]	22 ± 2	292 ± 4	293 ± 5	1 ± 2	2 ± 1
30% Oxygen	2	22 ± 1	22 ± 2	292 ± 4	295 ± 3	10 ± 2	8 ± 2
70% Nitrogen	3	22 ± 2	23 ± 2	274 ± 3	274 ± 2	11 ± 2	14 ± 2
760 mm Hg	4	27 ± 2	23 ± 2	274 ± 3	273 ± 6	12 ± 3	26 ± 5[b]
	1	21 ± 1	22 ± 2	244 ± 3	251 ± 4	1 ± 2	−5 ± 1
47% Oxygen	2	20 ± 2	22 ± 2	248 ± 4	254 ± 3	9 ± 6	5 ± 6
53% Nitrogen	3	26 ± 2	22 ± 2	253 ± 5	256 ± 4	11 ± 3	16 ± 2
760 mm Hg	4	22 ± 2	22 ± 2	248 ± 4	253 ± 3	13 ± 5	14 ± 2

[a] N = Average of from 6 to 10 rats ± standard error of the mean.
[b] $p < 0.01$, significantly different from isolated controls.

water. After daily cleaning of the animal chamber the experiment was continued by introducing oxygen alone into the chamber for 3–5 min until the desired O_2 level was obtained. The gas mixture was then taken from the mixing vessel. The relative humidity was maintained at 60–80%, CO_2 at less than 1% and temperature at 22–23°. The lighting was automatically set to turn on at 6 AM and off at 6 PM. All rats were fed a balanced diet[1] containing 6% fat, 24% protein, 60% carbohydrates and mineral and vitamin supplements.

All rats were killed by decapitation immediately after removal from the chamber. Blood for plasma collection was drained from the body stump directly into heparinized tubes which were frozen for subsequent analysis. An identical portion of each liver was removed and cut into 0.5-mm slices with a mechanical tissue chopper and epididymal adipose tissue was cut with scissors. One gram of each tissue was incubated at pH = 7.40 ± 0.02 for 3 hr in 10 ml of Kreb's-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 eleswhere (6). 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 by 1.0 ml of 2.5 N NaOH added to a removable plastic CO_2 collection well which had been inserted into the incubation flask prior to sealing (7). After incubation, the tissues were saponified overnight in 15 ml of a solution of 30% alcoholic KOH. Nonsaponifiable lipids, fatty acids and CO_2 fractions were separated and counted for radioactivity as described elsewhere (6).

Plasma lipids were extracted with chloroform–methanol (2:1, v:v) as described by Jover (8). Aliquots of the chloroform phase were taken to determine plasma triglycerides (8), total plasma lipids (9), total cholesterol (10), and inorganic P as phospholipids after digestion and oxidation (11). Plasma free fatty acids were determined by the method of Trout *et al.* (12). Plasma glucose was determined by the glucose oxidase method (13), and plasma corticosterone by the method of Peterson (14).

Results. Average daily food consumptions and weight gains are shown in Table I. A general trend is seen in weight gains in rats exposed to high oxygen environments after the 3rd and 4th day of exposure. Thus, while the isolated control rats gained approximately 12 g during this period, the experimental groups gained from 14 to 26 g of weight. However, only in 1 instance was the weight gain significantly different ($p < 0.01$) as

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

TABLE II. Fatty Acid Content of Liver and Adipose Tissue of Rats Exposed to Hyperoxic Environments for 1–4 Days and Their Corresponding Stock and Isolated Controls (Air at 760 mm Hg).

Experimental conditions	Exposure time (days)	Tissue fatty acid (% wet wt)			
		Liver		Adipose tissue	
		Isolated control	Oxygen treated	Isolated control	Oxygen treated
	1	1.60 ± 0.19[b]	1.87 ± 0.20	73.7 ± 1.0	77.2 ± 1.0
30% Oxygen	2	1.60 ± 0.19	1.75 ± 0.14	73.7 ± 1.0	75.4 ± 0.5
70% Nitrogen	3	[a]	[a]	76.1 ± 0.5	76.7 ± 0.6
760 mm Hg	4	[a]	[a]	76.9 ± 0.8	74.2 ± 1.4
	1	1.08 ± 0.11	1.33 ± 0.26	73.8 ± 1.9	76.4 ± 0.7
47% Oxygen	2	1.00 ± 0.15	1.24 ± 0.16	74.8 ± 1.7	75.9 ± 1.2
53% Nitrogen	3	1.20 ± 0.14	2.02 ± 0.14[c]	74.9 ± 1.8	75.5 ± 1.8
760 mm Hg	4	1.40 ± 0.13	2.06 ± 0.20[d]	74.8 ± 1.7	78.2 ± 0.7[e]
Stock Controls[f] (air)		1.18 ± 0.15		76.9 ± 0.8	

[a] Samples lost.
[b] N = Average of from 6 to 10 rats ± standard error of the mean.
[c] $p < 0.001$.
[d] $p < 0.01$.
[e] $p < 0.05$, significantly different from isolated control.
[f] 278 ± 3 g average weight.

judged by the t test: rats exposed to 30% O_2–70% N_2 for 4 days gained 26 g of weight compared to a gain of 12 g for the isolated controls.

The experimental rats had a significantly higher liver fatty acid content after 3 and 4 days of exposure to 47% O_2–53% N_2 atmosphere than did their isolated controls (Table II). No trend was noted in fatty acid content of adipose tissue although a significant increase was found in tissue obtained from rats after exposure for 4 days to a 47% O_2–53% N_2 gaseous environment.

Plasma corticosterone, glucose, free fatty acids and other lipid components were measured to monitor the health and degree of stress the environment may impose on the rats. No abnormal values were observed for all groups studied and these values are therefore not presented.

The conversion of acetate-2-^{14}C to CO_2 and fatty acids by liver slices varied daily within a given exposure profile as well as between the various exposures (Table III). The reasons for these variations are unknown but have also been reported earlier (1, 2). However, some general trends are shown. In 5 of the 8 experiments cited, the rats exposed to high oxygen tension had higher numerical values for conversion of acetate to CO_2 than did the isolated controls. In 2 of these cases (30% O_2–70% N_2, at 1 and 3 days), statistically significant increases were demonstrated. The conversion of acetate to nonsaponifiable lipids (not shown in table) by the oxygen-exposed rats remained relatively constant for all exposures and was comparable to the isolated controls. Liver slices from rats that were exposed to 47% O_2–53% N_2 for 3 and 4 days converted significantly more acetate to fatty acids than did liver slices obtained from isolated controls. For these experimental groups, 11–12% of the ^{14}C-acetate was recovered as ^{14}C-fatty acids per gram of liver slices compared to a value of about 4.5–6.0% found for the isolated controls.

The conversions of acetate to CO_2 by adipose tissue were comparable for the two different hyperoxic environments described in

TABLE III. Conversion of Acetate-2-^{14}C to $^{14}CO_2$ and ^{14}C-Fatty Acids by Liver Slices from Rats Exposed to Hyperoxic Environments for 1–4 Days, and Their Respective Isolated Controls (Air, 760 mm Hg).

Experimental conditions	Exposure time (days)	^{14}C recovered as $^{14}CO_2$ (%/g)		^{14}C recovered as $^{14}CO_2$-fatty acids (%/g)	
		Isolated control	Oxygen treated	Isolated control	Oxygen treated
	1	8.16 ± 0.70[a]	11.20 ± 0.97[b]	6.08 ± 1.63	5.94 ± 0.90
30% Oxygen	2	7.96 ± 0.69	7.05 ± 0.69	6.18 ± 1.58	6.82 ± 0.86
70% Nitrogen	3	7.75 ± 0.50	9.28 ± 0.50[b]	7.87 ± 0.53	10.75 ± 1.65
760 mm Hg	4	7.65 ± 0.50	8.00 ± 1.83	7.07 ± 0.83	5.66 ± 1.31
	1	10.22 ± 1.48	9.88 ± 0.86	5.76 ± 1.26	5.75 ± 1.66
47% Oxygen	2	12.03 ± 2.01	11.47 ± 1.34	4.74 ± 1.46	4.76 ± 0.99
53% Nitrogen	3	9.59 ± 1.20	10.88 ± 0.54	5.76 ± 1.27	11.89 ± 2.35[c]
760 mm Hg	4	9.42 ± 1.30	11.05 ± 0.76	4.56 ± 1.49	10.82 ± 1.43[b]
Stock Controls (air)		7.80 ± 0.87		4.98 ± 0.83	

[a] N = Average of from 6 to 10 rats ± standard error of the mean.
[b] $p < 0.05$, significantly different from isolated controls.
[c] $p < 0.01$.

Table IV. These conversions did not show any consistent difference from their respective isolated controls. Adipose tissue from rats exposed to 30% O_2–70% N_2 at 760 mm Hg for 3 and 4 days showed a significant increase in the conversion of acetate to fatty acids when compared to values found for isolated controls. Approximately 31% of the radioactivity was recovered in the fatty acids of adipose tissue obtained from isolated control rats compared to values of 45.4% and 38.2% found for rats exposed to 30% O_2–

TABLE IV. Conversion of Acetate-2-^{14}C to $^{14}CO_2$ and ^{14}C-Fatty Acids by Adipose Tissue from Rats Exposed to Hyperoxic Environments for 1–4 Days, and Their Respective Isolated Controls (Air, 760 mm Hg).

Experimental conditions	Exposure time (days)	^{14}C recovered as $^{14}CO_2$ (%/g)		^{14}C recovered as ^{14}C-fatty acids (%/g)	
		Isolated control	Oxygen treated	Isolated control	Oxygen treated
	1	2.87 ± 0.16[a]	4.02 ± 0.47[b]	29.5 ± 4.1	29.6 ± 4.7
30% Oxygen	2	2.75 ± 0.16	2.90 ± 0.25	31.5 ± 4.1	35.9 ± 3.4
70% Nitrogen	3	2.90 ± 0.14	2.30 ± 0.10[c]	30.9 ± 3.1	45.4 ± 3.9[d]
760 mm Hg	4	2.84 ± 0.15	2.81 ± 0.52	29.5 ± 3.1	38.2 ± 2.2[b]
	1	4.91 ± 0.33	4.33 ± 0.24	36.4 ± 5.2	40.7 ± 3.9
47% Oxygen	2	3.90 ± 0.18	3.65 ± 0.40	41.1 ± 3.1	55.4 ± 5.7[b]
53% Nitrogen	3	4.40 ± 0.20	3.84 ± 0.20	31.6 ± 7.8	53.8 ± 4.8[b]
760 mm Hg	4	3.90 ± 0.18	4.01 ± 0.32	44.3 ± 3.1	50.5 ± 5.7
Stock Controls (air)		3.47 ± 0.36		34.5 ± 2.6	

[a] N = Average of from 6 to 10 rats ± standard error of the mean.
[b] $p < 0.05$, significantly different from isolated controls.
[c] $p < 0.01$.
[d] $p < 0.001$.

70% N_2 for 3 and 4 days, respectively, representing an increase in lipogenesis of 30–47% above normal levels. An increase of 35–70% above control values was also found for lipogenesis in adipose tissue from rats exposed to 47% O_2–53% N_2 at sea level barometric pressures for 2 and 3 days.

Discussion. The process of lipogenesis is concerned with the conversion of glucose and intermediates such as pyruvate and acetyl-CoA to fatty acids. The nutritional state of the animal and its tissues is the main factor controlling the rate of lipogenesis. Thus, lipogenesis is high in the well-fed animal whose diet contains a high proportion of carbohydrate. It is depressed with a restricted caloric intake, on a high-fat diet, or when there is a deficiency of insulin. One might expect, then, if animals were exposed to unusual environments which might result in a decrease in food intake, that lipogenesis would be depressed. One such environment is the high O_2 composition of the atmosphere found during manned space flights.

Earlier reports showed that a 100% O_2 environment at a barometric pressure of 191 mm Hg caused rats to eat less food (1). Consequently a decrease in fatty acid synthesis was noted when compared with controls exposed to normal atmospheres and eating normal amounts of food. Step wise incremental increases in barometric pressure with concomitant increases in the partial pressure of alveolar oxygen brought fatty acid synthesis first to normal values and finally to values greater than normal. From these experiments, a conclusion is drawn that the change in fatty acid synthesis was the result of higher than normal O_2 alveolar pressures. However, due to the experimental design, the oxygen-enriched environments were always at subnormal barometric pressures.

The results presented here showed first that the enriched oxygen environment had no appreciable effect on food intake compared to the results obtained for rats kept under identical circumstances except for the oxygen composition of the animal chamber. With the same caloric intake, rats exposed for 4 days to the 30% O_2–70% N_2 environment gained more weight than controls. An earlier report (15) showed that rats exposed to 100% oxygen at 450 mm Hg continuously for 3 weeks also showed greater weight gains compared to control rats fed identical diets. Also confirmed by the present work was an increase in liver fatty acid content after increased oxygen exposure.

The study reported in the present paper confirms earlier work (1), namely, that increased O_2 environment results in an increase in conversion of acetate to fatty acid in liver and adipose tissue. Table V shows a comparison of the results obtained from the 2 studies. The values in the table are expressed as a percent change in fatty acid synthesis of the oxygen-treated rats compared with 2 groups of controls: (a) the stock controls [referred to as *ad libitum* controls in the earlier study (1)] shown in columns A and C; and, (b) the isolated controls [referred to as pair-fed controls in the earlier study (1)] shown in columns B and D. All animals in each of the separate experimental groups regardless of the length of exposure time were pooled so that the comparisons consist of groups of rats totaling from 30 to 40 animals. In every case, the change in lipogenesis in liver and adipose tissue resulting from oxygen treatment was in the direction of an increase.

Several interesting observations are noted. When comparing the oxygen-treated experimental rats with the stock control rats (columns A and C), animals exposed to a partial pressure of oxygen of 258 mm Hg at a barometric pressure of 258 mm Hg (258/258) had a lesser per cent increase in lipogenesis than animals exposed to the same partial pressure of oxygen at a barometric pressure of 760 mm Hg (258/760). Animals exposed to a 350/350 regimen had a lesser per cent conversion in lipogenesis than animals exposed to a 350/760 regimen. A lesser per cent increase was found in liver and adipose tissue from rats exposed to the 258/258 regimen than from rats exposed to the 350/350 regimen, while in a 760 mm Hg barometric pressure atmosphere, rats exposed to an oxygen partial pressure of 258 mm Hg had a lesser per cent increase in

TABLE V. Summary of Effects of an Increased Oxygen Atmosphere on Lipogenesis.

Atmosphere oxygen partial pressure (mm Hg)	Total atmospheric barometric pressure (mm Hg)	Liver		Adipose tissue	
		Oxygen treated compared to:		Oxygen treated compared to:	
		Stock (A)	Isolated (B)	Stock (C)	Isolated (D)
		controls (%)	controls (%)	controls (%)	controls (%)
258	258[b]	8[a]	94	4	67
	760	46	7	8	23
350	350[b]	12	22	16	29
	760	67	60	45	30

[a] These values represent the per cent increase in conversion of ^{14}C-acetate to ^{14}C-fatty acids of oxygen-treated groups compared to corresponding control groups. Each value represents the mean of the entire group of 30 to 40 rats exposed 1, 2, 3, or 4 days to increased oxygen atmospheres.

[b] Calculated from earlier work (1) with permission of Aerospace Medicine. Comparisons made for animals listed in columns A and C had free access to food (*ad libitum* controls); animals listed in columns B and D were pair-fed controls.

lipogenesis than rats exposed to a partial pressure of 350 mm Hg.

When the comparisons were made between the oxygen-treated rats and the pair-fed rats shown in the first and third rows of columns B and D, the animals exposed to the 258/258 regimen had per cent increases in lipogenesis greater than other percentages found for all other groups. This group of control rats was a pair-fed group that received a restricted diet (1) and, therefore, the values for conversion of acetate to fatty acid were, as expected, markedly less than normal values obtained from animals that had free access to food at all times. This fact made the per cent increase value very high. With this exception, the patterns were identical to those described in the preceding paragraph.

The general conclusions drawn from this discussion are that the increase in lipogenesis resulting from a higher than normal atmospheric oxygen composition occurs both at reduced and at normal barometric pressures and that, to a point, the greater the oxygen enrichment, the greater the increase in lipogenesis. These facts have led to the unequivocal conclusion that the oxygen enrichment directly results in an increase in fatty acid synthesis.

These results encourage the study of the biochemical reactions at the molecular level that are controlled by oxygen tension and that are presumably responsible for the observations reported.

Summary. Male rats, fed *ad libitum,* were exposed to 30% and 47% oxygen environments (balance, N_2) at 1 atm for periods of time varying from 1 to 4 days. The rats exposed to increased oxygen tensions for 3 or 4 days gained more weight than controls. Fatty acid contents of liver and adipose tissue of the experimental group at 4 days of exposure were higher than the fatty acid contents found in these tissues for the control group. Conversion of acetate to fatty acids was found to be significantly higher in liver and adipose tissue of oxygen enriched animals. The conclusion is drawn that these findings are the result solely of an increase in alveolar oxygen tension.

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Received Jan. 12, 1973. P.S.E.B.M., 1973, Vol. 143.