Some Factors Affecting Lipid Peroxidation in Mammalian Adipose Tissue

RIAZ AHMAD RIAZ, MUHAMMAD NASIR AHMAD AND ALI AKBAR
Department of Food Technology, University of Agriculture, Faisalabad, Pakistan

(Received 18th February, 1996, revised 7th September, 1996)

Summary: The present investigation is conducted to elucidate the factors contributing to lipid peroxides formation in adipose tissue, which is not only one of the principal tissue of the body, but also the one in which lipids are most abundant. Certainly age of animals as well as dietary lipids do affect the accumulation of lipid peroxides in adipose tissue. Tissue preparation techniques did not appreciably influence the formation of lipid peroxides. Different sources of albumin showed a marked influence in changing the peroxides during *in vitro* incubation of adipose tissue depending upon the nature of contaminants present in them. Definitely exposure of *in vitro* incubating tissues to either ultraviolet light or t-butyl hydroperoxide caused a substantial increase in lipid peroxide contents. When instead of fluorimetric TBA-technique, an alternative method was employed to monitor lipid peroxidation, it was noted with interest that ethane consistently increased in normal adipose tissue during *in vitro* incubation. Corn oil supplemented diet caused a substantial increase in ethane production in excised adipose tissue of rats. Incorporation of inosine, *t*-butyl hydroperoxide and albumin into the incubating medium also enhanced production of ethane from adipose tissues. It can be concluded that lipid peroxidation in tissue fragments and homogenates is ubiquitous and that the rate of peroxidation is altered by circumstances as mentioned above.

Introduction

Considerable advances have been made in elucidating the mechanism involved in the formation and degradation of lipid peroxides in living tissues [1]. The accumulation of lipid peroxides is governed by the balance between the processes responsible for their synthesis and degradation [2]. Although both processes are subject to variation, lipid peroxide level in the tissues of intact animals has generally been found to remain within narrow limits. However, the situation that arises in tissues incubated *in vitro* is considerably more variable. Not only are there gross changes in the availability of the substrates, the polyunsaturated fatty acids and molecular oxygen, another key factor is the composition of the incubation medium [3]. The recognition that the lipid peroxide induced changes have physiological significance has occurred largely within the last two decades [4], whereas the data on lipid peroxide formation in adipose tissues was obtained considerably earlier. In this study, therefore, the occurrence of peroxidation phenomenon in adipose tissue is re-investigated. Lipid peroxide is determined both immediately after excision and after an *in vitro* incubation period. Some of the difficulties encountered in applying TBA method to adipose tissue are overcome. Advantage is taken of the fact that when confronted with a tissue with a lipid dominated composition, it helps to use as assay in which TBA pigment is determined fluorimetrically after its extraction from an aqueous medium into butanol [5]. Attention was also given

to the influence of preparation of tissue (slicing vs homogenization), age of animal, presence of various source of albumins, oxidizing agent etc. on formation of lipid peroxides in adipose tissue. The formation of ethane, a low molecular weight hydrocarbon, as a product of lipid peroxide degradation is reported [6] to occur in various tissues. In this study an effort was made to monitor lipid peroxidation during *in vitro* incubation of adipose tissues by determining ethane.

Results and Discussion

It has frequently been found that when freshly excised tissues are subjected to the thiobarbituric acid (TBA) analysis a positive reaction is obtained [7]. There has been considerable uncertainty, however, as to the extent to which the substances responsible were present in the intact animal rather than being produced during the preparation of the tissue for analysis. The fluorimetric TBA analysis was used to assess the lipid peroxide content of freshly excised adipose tissue. The tissue preparations used were obtained by either a slicing procedure or a conventional homogenization. The results in Table-1 indicate that lipid peroxide contents (nmol/g tissue) were 15.1 ± 0.8 for sliced tissue and 17.1 ± 0.9 for homogenate of tissue. A similar trend in results was also observed in another replicate experiment. The peroxide values were somewhat higher after homogenization of adipose tissue. However, the fact that the results were so similar suggested that the preparation procedure was not critical and that, therefore, the chromogen was not formed during the isolation procedure.

A comparison was made of the lipid peroxide content of adipose tissue excised from rats of different ages. Since the epididymal adipose tissue differed in size, the units of measurement were crucially important to the comparisons made. Thus, although the total TBA chromogen in the tissues generally decreases with the age of the animal yet peroxide contents increased with age when expressed on the body weight of animal. The peroxide values (nmol/g tissue) as shown in Table-2 were 15.8 ± 1.2 before and 17.7 ± 0.5 after homogenization of adipose tissue of rat of six weeks old weighing 140 g whereas when adipose tissue obtained from a 7 months old rat (which

Table-1: The lipid peroxide content of freshly excised adipose tissue before and after homogenization.

	Lipid peroxide (nmol/g tissue)*	
	Tissue (sliced)	Homogenate**
Experiment 1	15.1 ± 0.80	17.1 ± 0.90
Experiment 2	14.7 ± 0.66	16.2 ± 1.38

* Mean (± SD) values of six separate determinations.
** Tissues were homogenized (1g/10 ml KRB) for 5 seconds at a moderate speed (setting No.3) using Polytron (PTA 10-35) homogenizer.

Table-2: The effect of age on the lipid peroxide content of freshly excised adipose tissue before and after homogenization

Animals		Lipid peroxide* nmol/g	
Age	Wt.	Tissue (sliced)	Homogenate
6 weeks	140 g	15.8 ± 1.2	17.7 ± 0.5
7 months	450 g	8.7 ± 0.7	9.8 ± 0.5

*Mean (± SD) values of 4 separate analyses.

weighed 450 g), the actual peroxide values were 8.7 ± 0.7 nmol/g of sliced tissue and 9.8 ± 0.5 nmol/g of homogenized fat tissue. In the present study, the amount of lipid peroxide detected in freshly excised adipose tissue was not appreciably influenced by the techniques used to prepare the tissue for the analysis. It is likely, therefore, that the level detected reflected the lipid peroxide present in the intact animals. The peroxide content of adipose tissue was equivalent to approximately 15 nmol/g tissue of malondialdehyde (See Table-1). Whether this should be considered to represent an equimolar amount of lipid peroxide remains uncertain: since the conversion of purified linolenic and arachidonic acid hydroperoxide to malondialdehyde under the conditions used in TBA method is approximately 10% efficient [8], a realistic estimate of lipid peroxide content of epididymal adipose tissue of the young rat may be as high as 150 nmol/g of tissue. The change in peroxide content of adipose tissue in relation to the age of animal is difficult to interpret because the dimensions of the tissue are susceptible to such gross changes. Whereas peroxide per gram decreased as the age of animal progressed from 6 weeks to 7 months, the total peroxide increased. Since it is now established that the fat cells of maturing rats are capable of differentiation, de-differentiation and proliferation [9], it is of some interest that a negative correlation between peroxidation and proliferation rate exists in many

tissue [4]. The lipid peroxide assay used in the present study is indirect. Nevertheless if it is approximately correct, it may have considerable significance, since a modest degree of lipid peroxidation can affect adipose tissue functions in several ways. It may alter the fluidity and permeability of membranes, enhance prostaglandin synthesis, and decrease the thiol content of tissue [4]. It would appear from the above observations that freshly excised adipose tissue contains only modest amounts of lipid peroxide. Nor do the Kreb's Ringer based medium used to incubate adipose tissue show any strong oxidative activity, except when certain specific additions are made.In a number of experiments, no increase in the lipid peroxide content of adipose tissue pieces could be detected using the fluorimetric TBA method during its incubation in albumin-free medium. However, Table-3 shows that serum albumins of various origins differ in this respect. The increase in lipid peroxide contents of incubations containing adipose tissue slices can only be partly attributed to the peroxide associated with the albumin used [3]. Horse serum albumin and to lesser extent bovine serum albumin appear to stimulate the peroxidation of adipose tissue lipid, whereas human serum albumin appears to have the reverse effect. The oxidizing agent, *t*-butyl hydroperoxide considerably enhanced lipid peroxidation under these circumstances. It was further shown that the peroxidation reaction occurred in adipose tissue rather than in the medium: when fatty acids containing polyunsaturated acids were added to the medium in the absence of adipose tissue, lipid peroxidation did not occur [10]. In the earlier studies [11], evidence was obtained that the peroxidation reaction could be prevented if iron and citrate were eliminated from incubation medium. It is possible that the reduced peroxidation in the present study was due to the greater purity of the bovine serum albumin now available. In an experiment, adipose tissues were removed from 3 groups of rats raised from weaning stage on standard laboratory diet [41B], and standard diet when supplemented with 10% (w/w) either corn oil or soybean oil. After 6 weeks the rats in these 3 groups were only marginally different in weight but the lipid peroxide of freshly excised adipose tissue was lower in corn oil fed rats and higher for soybean oil fed rats, compared with that of control rats (Table-4). Subsequent

Table-3: The effect of various preparations of serum albumin on the lipid peroxide content of adipose tissue incubated in the presence and absence of t-butyl hydroperoxide.

Addition	Lipid peroxide (nmol/ml incubation)			
	Serum albumin			
	-	Bovine	Horse	Human
-	1.32	1.22	2.08	1.18
t-butyl hydroperoxide (100 μM)	1.80	1.70	4.00	2.10

*Average values of two separate analyses. When adiposa tissue (100 mg/ml KRB) was incubated for 60 min at 37° in the presence of bovine, horse and human serum albumins (2.5%; fraction v;Sigma Chemicals).

Table-4: The effect of dietary lipids on the lipid peroxide content of adipose tissues.

Supplemtnation of 41B diet	Weight of animals (g)	Lipid peroxide* (nmol/g tissue)	
		t_1	t_{60}
-	190-200	10.78 ± 1.25	13.23 ± 0.68
Corn oil (10%)	200-215	7.05 ± 0.92	8.60 ± 0.42
Soybean oil (10%)	200-225	18.35 ± 0.80	20.95 ± 2.00

*Mean values (± S.D.) of 4 separate determinations.
t, Time of incubation in min.

incubation of these excised tissues caused a slight increase in lipid peroxide contents in all 3 type of rats raised differently. The observed difference might be as consequence of the changes in the fatty acid composition of the stored lipids caused by supplementation of the diet with different oils as reported elsewhere [12]. Among the factors that were shown to promote lipid peroxidation *in vitro* was ultra violet light. Fig. 1 shows that TBA colour development progressed substantially and continuously over a period of several hours as compared to that of control counterpart. An alternative to the TBA method was also used to monitor lipid peroxide [13] during adipose tissue incubation in a limited number of experiments. Ethane production was determined periodically over a period of 11 hours. Fig. 2 shows that although the analysis was close to the limit of its sensitivity, lipid peroxidation due to the presence of adipose tissue could be consistently detected. In another such experiments, when adipose tissues (1g/10 ml) removed from rats fed for 4 weeks on standard diet or corn oil (10% w/w) supplemented diet were incubated in closed vials and then headspace gas phases were assayed for ethane over a period of seven hours, it was noticed that the corn oil supplement stimulated ethane production substantially whereas incorporation of albumin to

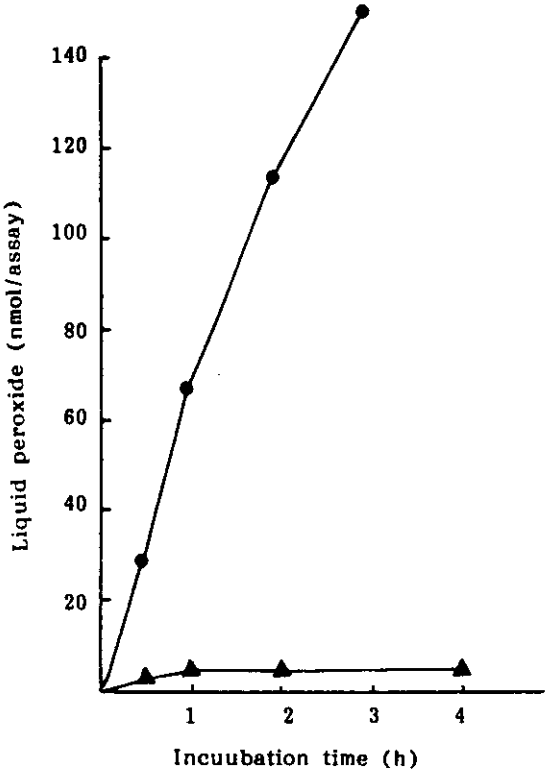

Fig. 1: The effect of ultraviolet irradation on lipid peroxide formation in adipose tissue *in vitro*.
Adipose tissue (100 mg/ml medium) in albumin free Krebs Ringer phosphate at 37° and exposed to ultraviolet light. Lipid peroxide was determined during different intervals by fluerimeric TBA method after butanol extraction from UV light-exposed ⊖ and unexposed ● adipose tissue.

the incubating medium further enhanced ethane production in both counterpart controls (see for details in Table-5). Since adipose tissue contains xanthine oxidase [14], the possibility was investigated to enhance this endogeneous enzyme activity by the incorporation of its substrate into the incubation medium. This strategy was explored in an experiment of long duration (upto 18 hours) in which ethane formation was used to provide an indirect assessment of lipid peroxidation.

Table-6 shows that the addition of inosine (1 mM) to the incubating medium with adipose tissue

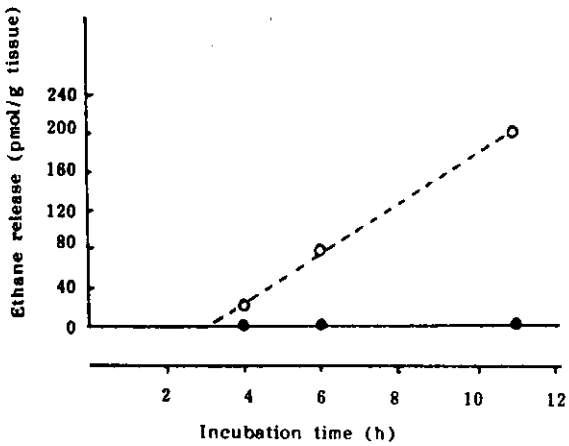

Fig. 2: Ethane release from the adipose tissue incubated *in vivtro*.
Adipose tissue (1 g/10 ml Krebs Ringer phosphate containing 2% albumin) was incubated at 37° in a closed vial (O------O----O). In control (●—●—●); the tissue was inactivated by adding sulphuric acid (0.5 ml, 1.5 M). Analyses were carried out on headspace gas samples obtained after various intervals.

Table-5: The effect of a corn oil-supplemented chow diet on ethane release from rat adipose tissue of homogenate.

Incubation medium	Ethane release* (pmol/g tissue)					
	41B diet			Corn oil (10%) -supplemented 41B diet		
	Sampling intervals (h)			Sampling intervals (h)		
	0	3.5	7	0	3.5	7
KRB	0	72	330	0	360	1030
KRB + 2% albumin	0	836	1540	0	697	1690

*Average values of two analyses.

Table-6: The effect of inosine incorporation into the incubation medium on ethane release from adipose tissue in the presence or absence of an organic hydroperoxide.

Addition	Ethane release (pmol/g tissue)*			
	KRB time (h)		KRB + Inosine (1mM) time (h)	
	8	18	8	18
-	72	156	240	408
t-Butyl hydroperoxide (100 μM)	102	186	336	552

*No ethane release was recorded when t-butyl hydroperioxide (100 μM) was added to tissue-free medium.

(1g/10 ml) appeared to almost double the rate of ethane production which further increased by incorporation of *t*-butyl hydroperoxide under these circumstances.

Although the formation of ethane is indirectly related to lipid peroxidation [13], it has certain advantages. Because this gas is not only stable and chemically inert but also conveniently separated from *in vitro* incubation system and can be measured by a sensitive method of gas chromatography. This method which was first time applied in this preliminary study, was found capable of detecting very minute quantity of ethane present in headspace gas produced during adipose tissue (pmol/g) incubation.

Experimental

The epididymal fat pads were excised from male albino rats killed by decapitation. These rats were raised on either standard diet or when it was supplemented 10% oil (soybean or corn). The adipose tissues were collected in warm isotonic saline (0.9%) and finally either sliced thinly with help of a pair of scissors or homogenised (1 g/10 ml Kreb's Ringer bicarbonate buffer for 5 seconds at a moderate speed setting No.3 employing Polytron PTA-10-35 homogenizer. The incubation medium consisted of 2 ml of Kreb's Ringer buffer into which the saline washed sliced tissues (100 mg/ml) were introduced. The *in vitro* incubations (the duration of which are quoted in Figures and Tables) were carried out at 37°C in a CO_2:O_2 (5:95) atmosphere in a reciprocating water bath. Lipid peroxide in sliced (or homogenized) preparation of adipose tissue was determined by a modification of fluorimetric TBA-method [5] which increased the sensitivity more than 10-fold. In this fluorimetric method, fluorescence units were recorded using an excitation wavelength of 515 nm and emission wavelength of 553 nm in Perkin-Elmer LS-5 spectrofluorimeter when sample for analysis was in butanol phase obtained by centrifuging (at 3000 rpm for 20 minutes) a thoroughly mixed *n*-butanol (4 ml) into the product of normal TBA reaction (3 ml). An other alternative method for monitoring lipid peroxidation also employed in which headspace gas (0.5 ml each), obtained from closed vial at different suitable intervals during *in vitro* incubating adipose tissues under various conditions, was immediately analysed at ambient temperature when carrier gas (N_2) flow was 20 ml/minute in GC model Pye 104 equipped with a glass column (1.5 m x 0.5 mm) containing Durapak OPN Porasil C packing (Phase Separations Ltd. UK).

References

1. R.A. Riaz, Ph.D. Thesis University of Wales, UK (1984).
2. R.A. Riaz and J.I. Davies, *Pak. J. Chem. Soc.*, **15**, 132 (1993).
3. R.A. Riaz, *Pakistan J. Agr. Sci.*, **31**, 98 (1994).
4. Y.A. Vladimirov, V.I. Olenev, T.B. Suslova and Z.P. Cheremisine, *Adv. Lipid Res.*, **17**, 173 (1980).
5. K. Yagi, *Biochem. Med.*, **15**, 212 (1976).
6. C.A. Riely, G. Cohen and M. Lieberman, *Science*, **183**, 208 (1974).
7. K.M. Wilbur, F. Bernheim and O.W. Shapiro, *Arch. Biochem. Biophys.*, **24**, 305 (1949).
8. H. Ohkawa, N. Ohishi and K. Yagi, *J. Lipid Res.*, **19**, 1053 (1978).
9. A. Cryer, *Trends in Biochem. Sci.*, **5**, 196 (1980).
10. W.G. Blackard, M.F. Ball and F.L. Engel, *J. Clin. Invest.*, **51**, 549 (1962).
11. A.E. Reynold and G.F. Cahill Jr., Handbook of Physiology p. 349, American Physiological Society, Washington (1965).
12. P.W. Larking and E.R. Nye, *J. Nutr.*, **22**, 291 (1975).
13. H. Sies and A. Wendel, Functions of Glutathione in Liver and Kidney, p. 176, Springer-Verlag, Germany (1978).
14. M.F. Grahn, J.E. Souness and J.I. Davies, *Mol. Physiol.*, **1**, 119 (1981).