# Dietary Supplementation with Ether-Linked Lipids and Tissue Lipid Composition

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**The goal of this investigation was to determine the effect of an alkylglycerol dietary supplement on the lipid composition of several major organs. Lipids from kidney, liver, and lung tissues of rats on a laboratory chow diet (controls) were compared to lipids from the same tissues of rats that had received oral supplements (300–600 mg/day) of 1-*O*-alkyl-2,3-diacetyl-*sn*-glycerol (alkyl groups were 65% 18:1 and 17% 16:1) for six days. Incorporation of the alkylglycerol into tissue lipids was indicated by both the presence of a neutral lipid in liver that had the same chromatographic migration as alkyldiacylglycerols and by a substantial increase (~150% of controls) in the octadecenyl group of the alk-1-enyl- and alkyl-glycerol side chains derived from total phospholipids of all three tissues. Compared to controls, there was a significant increase in the amount of alkylacylglycerophosphocholine in all three tissues of the alkylglycerol supplemented group. Total lipids, total phospholipid phosphorus, or the distribution of phospholipid classes (except for small differences in lung tissue) were not affected by the dietary supplement. The increase in ether lipids was offset by a corresponding decrease in the diacyl subclass in tissues from animals on the alkyldiacetylglycerol supplement. Our results indicate that the amount of ether-linked glycerolipids in rat tissues can be easily increased with dietary supplements of alkylglycerols.**
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Virtually all animal cells contain ether-linked aliphatic moieties as membrane lipid constituents. With few exceptions the *O*-alkyl moieties are most prominent in choline glycerolipids, whereas the *O*-alk-1-enyl chains are found primarily as ethanolamine plasmalogens. An exhaustive listing of available reports about the tissue distribution of ether-linked phospholipids in comparison to the diacyl types of lipids for tissues of various species has been compiled by Sugiura and Waku (1) and Horrocks (2). Although ether lipids were once thought to serve strictly as structural components of membranes, it is now apparent that some types of ether lipids possess biological activities. The most potent lipid mediator ever discovered is a novel ether-linked phospholipid subclass possessing the chemical structure 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (3–5). This phospholipid is commonly referred to as platelet-activating factor (PAF), although it elicits a wide variety of biological responses that involve many different types of cells (6–8).

Earlier studies have shown that orally administered fatty alcohols (9,10) or glycerol ethers (10–14) can be incorporated into the glycerol ether lipids of mammalian tissues. Incorporation into tissue glycerol ethers was based on either qualitative changes in composition of tissue alkyl/alk-1-enyl groups or the use of radioactive tracers. L-M fibroblasts grown in culture media supplemented with alkylglycerols were shown to contain increased amounts of glycerol ethers in their total phospholipids (15). However, except for the reported increase in the plasmalogen content of erythrocytes in one human subject (16) and no change in the plasmalogen content of rat tissues (12) after feeding a supplement of alkylglycerols, none of the earlier reports attempted to determine the quantitative levels of ether lipids found in tissues after feeding the glycerol ethers (or alcohols) to intact mammals. Because PAF can be biosynthesized from the precursor pool of 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (alkylacyl-GPC) *via* a remodeling pathway (8), we felt that it was important to see if this precursor pool size could be increased in mammalian tissues by the oral administration of an alkylglycerol diacetate. An increase in the PAF precursor pool size could have important biological ramifications because of the multiple cellular responses produced by this autacoid.

## MATERIALS AND METHODS

The dietary supplement of 1-*O*-alkyl-2,3-diacetyl-*sn*-glycerol (ADAG) used in these studies was a gift from Western Chemicals (Vancouver, Canada). Diacetyl derivatives of alkylglycerols were used as the dietary supplement in order to mimic the esterified forms of alkylglycerols that would occur in a normal diet. Analysis of the ADAG supplement by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) (17) revealed the supplement was >95% alkyldiacetylglycerols with the two major alkyl chains being 16:1 (17%) and 18:1 (67%); no other single species accounted for >5% of the total alkyl chains. Male CDF rats, seven weeks of age, were given 300-mg doses of carrier-free ADAG by stomach tube. The rats received one dose of ADAG on the first day of the experiment followed by two equivalent doses per day, 7 hr apart, on days two through five and a final single dose on day six. Rats were sacrificed six hours after the last dose of ADAG. Livers, lungs, and kidneys were removed and immediately frozen in liquid nitrogen until the time of lipid extraction. Both controls and the ADAG-fed rats (five animals per group) had access *(ad libitum)* to regular laboratory chow and water during the entire experimental period.

Lipids were extracted from the tissues by the method of Bligh and Dyer (18). TLC of neutral lipids was carried out on plates coated with 250 μm layers of Silica Gel G developed in a solvent system of hexane/diethyl ether/acetic acid (85:15:1, v/v/v). Alkyldiacylglycerols are easily separated from cholesterol esters, triacylglycerols, and fatty acids in this neutral lipid TLC system. Phospholipids were separated by TLC on 250 μm layers of silica gel H using a solvent system of chloroform/methanol/acetic acid/water (50:25:8:2, by vol). For preparative

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Abbreviations: ADAG, 1-*O*-alkyl-2,3-diacetyl-*sn*-glycerol; GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine; HPLC, high-performance liquid chromatography; PAF, platelet-activating factor; TLC, thin-layer chromatography.

TABLE 1

**Distribution of Phospholipid Classes in Selected Tissues of Controls and Rats Fed Alkyldiacetylglycerol (ADAG)[a]**

| Tissue | Diet | Sphingomyelin + LysoGPC | Diradyl-GPC | Phosphatidylinositol/serine | Diradyl-GPE |
|---|---|---|---|---|---|
| Kidney | control | 14.5 ± 0.4 | 44.5 ± 0.8 | 9.1 ± 0.7 | 32.1 ± 0.6 |
| | ADAG-fed | 15.5 ± 0.4 | 43.0 ± 0.8 | 10.7 ± 0.6 | 30.8 ± 0.4 |
| Liver | control | 6.2 ± 0.7 | 61.5 ± 2.3 | 4.2 ± 0.8 | 25.7 ± 0.9 |
| | ADAG-fed | 4.9 ± 0.7 | 62.8 ± 0.5 | 4.3 ± 0.5 | 28.0 ± 0.8 |
| Lung | control | 11.9 ± 0.2 | 52.9 ± 0.6 | 11.6 ± 0.2 | 23.5 ± 0.4 |
| | ADAG-fed | 13.2 ± 0.1[b] | 49.5 ± 0.4[b] | 13.4 ± 0.3[b] | 23.9 ± 0.6 |

[a] All values represent mean mole percent ± S.E. from tissues of five rats in each group, except for one lung extract sample that was lost in the control group.

[b] Values are significantly different from corresponding controls ($P < 0.01$).

purposes, the developed TLC plates were exposed to $NH_3$ fumes (3–4 min), then sprayed with a solution of 0.1% 2, 7-dichlorofluorescein in ethanol and viewed under ultraviolet (UV) light to locate the separated phospholipid bands. The phospholipids were extracted from the silica gel (18) for subsequent analysis. For quantitative analysis of phospholipid classes the developed TLC plates were charred with $H_2SO_4$ and the amount of phosphorus in the separated phospholipids was determined (19). The percentage of octadecenyl (18:1) groups in the alkyl- and alk-1-enyl-glycerol ethers derived by Vitride reduction, benzoylation, and HPLC of the total phospholipid fractions was determined by earlier methodology (17). Quantitation of the subclasses in diradyl-*sn*-glycero-3-phosphoethanolamine (GPE) and diradyl-GPC was performed *via* phospholipase C hydrolysis, benzoylation of the diradylglycerols, and HPLC of the benzoate derivatives, as previously described (20).

All data are expressed as means ± S.E. and statistical significance between groups was based on Student's t-test (only P values of <0.05 were considered significant).

## RESULTS AND DISCUSSION

Except for an unexplained lower percent weight gain by rats fed the ADAG supplement (5.3 ± 1.7% *vs* 18.5 ± 0.8% for the controls), the glycerol ether supplement was well tolerated by the animals with no other visible indications of problems or morbidity. There were no significant differences in the amounts (as percent of tissue wet weight) of total lipids extracted from the tissues of the control *vs* the ADAG-fed rats (liver, 4.61 ± 0.08 *vs* 4.85 ± 0.17; kidney, 4.41 ± 0.13 *vs* 4.56 ± 0.23; and lung, 3.43 ± 0.17 *vs* 3.66 ± 0.15; for control *vs* ADAG-fed, respectively). Total tissue phospholipids (as mg P per g wet weight) were also not statistically different between the control and ADAG-supplemented animals (liver, 1.08 ± 0.06 *vs* 1.21 ± 0.04; kidney, 0.95 ± 0.05 *vs* 0.99 ± 0.01; and lung, 0.74 ± 0.03 *vs* 0.80 ± 0.03; for control *vs* ADAG-fed, respectively).

TLC analysis of the neutral lipids from the three tissues revealed livers from the ADAG-fed group contained significant amounts (estimated at 2–3% of the total lipid mass) of a lipid component having the same chromatographic mobility as alkyldiacylglycerols. This lipid component was also detected (in lesser amounts) in the lung lipids of the ADAG-fed rats but was present in no more than trace amounts in the lipids of these three tissues from control animals. These results indicate the alkylglycerol portion of the ADAG supplement was incorporated into the liver and lung lipids. Only trace amounts of this neutral lipid could be detected in the kidney lipids from both groups of rats. There was no evidence for the presence of alkyldiacetylglycerols in the TLC analysis of tissue neutral lipids from ADAG-fed rats.

The dietary supplement of ADAG had little effect on the distribution of phospholipid classes in the three rat tissues (Table 1). There were small, but statistically significant, changes noted in three of the phospholipid classes from lungs of the ADAG-fed group; sphingomyelin/lysoGPC and phosphatidylinositol/serine were increased and diradyl-GPC was decreased when compared to controls. The biological significance, if any, of these small changes is unknown. HPLC analysis of alkyl- and alk-1-enyl-glycerol dibenzoates, derived from total

TABLE 2

**Effect of Dietary Supplements of Alkyldiacetylglycerol (ADAG) on the 18:1 Content of Ether Groups in the Total Phospholipid Fraction of Selected Rat Tissues[a]**

| Tissues and glycerol ether subclasses | % of glycerol ether chains as 18:1 | |
|---|---|---|
| | Control | ADAG-fed |
| Kidney | | |
| alk-1-enylacyl | 19.2 ± 0.4 | 32.6 ± 0.4 |
| alkylacyl | 44.2 ± 1.2 | 61.1 ± 1.0 |
| Liver | | |
| alk-1-enylacyl | 18.8 ± 2.0 | 38.9 ± 1.0 |
| alkylacyl | 45.2 ± 1.9 | 78.7 ± 3.4 |
| Lung | | |
| alk-1-enylacyl | 14.1 ± 0.6 | 26.0 ± 0.1 |
| alkylacyl | 42.2 ± 1.9 | 64.5 ± 0.8 |

[a] The total phospholipid fractions from the same tissues as in Table 1 were analyzed for the composition of the aliphatic ether chains as described in Materials and Methods. All values represent the mean percent ± S.E. (n=3) and were significantly higher ($P < 0.001$) in 18:1 for all tissues from rats that were fed alkyldiacetylglycerol.

TABLE 3

**Effect of Dietary Alkyldiacetylglycerol (ADAG) on the Distribution of Ether Subclasses in the Diradyl-GPE and Diradyl-GPC Phospholipids of Selected Rat Tissues[a]**

| Tissues and glycerol ether subclasses | % of phospholipid class | | | |
|---|---|---|---|---|
| | Control | | ADAG-fed | |
| | GPE | GPC | GPE | GPC |
| Kidney | | | | |
| alk-1-enylacyl | 20.0 ± 1.0 | 2.3 ± 0.1 | 21.3 ± 0.9 | 3.9 ± 0.2[b] |
| alkylacyl | 0.9 ± 0.1 | 1.7 ± 0.1 | 2.2 ± 0.2[b] | 6.3 ± 0.2[b] |
| Liver | | | | |
| alk-1-enylacyl | 3.3 ± 0.2 | 0.4 ± 0.0 | 4.9 ± 0.2[b] | 0.6 ± 0.1 |
| alkylacyl | 0.1 ± 0.0 | 0.3 ± 0.0 | 3.4 ± 0.6[b] | 3.5 ± 0.5[b] |
| Lung | | | | |
| alk-1-enylacyl | 41.3 ± 1.0 | 1.6 ± 0.1 | 47.1 ± 1.1[c] | 2.6 ± 0.2[c] |
| alkylacyl | 1.1 ± 0.1 | 2.6 ± 0.1 | 2.9 ± 0.2[b] | 10.1 ± 0.4[b] |

[a]Ether-containing subclasses of diradyl-GPE and diradyl-GPC were determined as described in Materials and Methods from the same tissues as in Table 1. All values are means ± S.E. (n=5, except as noted in Table 1). Values for the diacyl subclasses (not shown) are the difference between 100 percent and the sum of the two ether subclasses. Values that are significantly higher than corresponding data for controls are indicated by (b) = $P < 0.001$ and (c) = $P < 0.01$. Diacyl subclasses, except for diacyl-GPE of the kidneys, were significantly lower ($P < 0.01$) in tissues of the animals fed ADAG.

phospholipids of the tissues, showed that the ether chains were highly enriched in the 18:1 moiety in rats that were fed ADAG as compared to controls (Table 2). The increased content of 18:1 in the ether-linked moieties was so dramatic that we felt it was necessary to analyze the total phospholipid fractions of tissues from only three rats of each group. Also, others have fed unlabeled alkylglycerols to rats and reported a shift in the qualitative makeup of the tissue glycerol ether groups toward the composition of the dietary alkylglycerol (10,12). In one study, only the level and composition of the alk-1-enyl group of ethanolamine plasmalogens from several different rat tissues was analyzed (12) and in the other instance the chain length composition of both *O*-alkyl and *O*-alk-1-enyl groups were analyzed but only from liver (10). Our results (Table 2) support the findings of both of these papers.

We next examined the amounts of ether-containing subclasses present in the diradyl-GPE and diradyl-GPC phospholipid classes. Except for the diradyl-GPC of lung, the amounts of diradyl-GPC and diradyl-GPE were not significantly different between the tissues of controls and those of the rats on the ADAG-supplemented diet (mg P/g wet weight times the percent of the phospholipid class, Table 1); therefore, the percent distribution of subclasses shown in Table 3 is representative of actual changes in their relative mass (Table 3). In agreement with earlier results (1,2), these three tissues from both groups of rats showed a general pattern of higher concentrations of plasmenyl (alk-1-enylacyl-) ether lipid in the GPE class, whereas the GPC class was enriched in the plasmanyl (alkylacyl-) type. Except for the alk-1-enylacyl-GPE of kidney and the alk-1-enylacyl-GPC of liver, the plasmenyl subclasses were slightly increased in the tissues as a result of dietary supplementation with ADAG. In every instance, the concentration of the alkylacyl- subclasses was significantly increased, compared to controls, in both GPC and GPE phospholipid fractions from tissues of rats that received the ADAG supplement (Table 3). The precursor of PAF in the remodeling pathway, alkylacyl-GPC, was increased by a factor of almost four-fold (kidney and lung) to ten-fold (liver) in the three tissues of the ADAG-fed rats. The increase in alkylacyl and alk-1-enylacyl subclasses of the diradyl-GPE and diradyl-GPC fractions was compensated for by decreases in the corresponding diacyl subclasses (see legend of Table 3). We are unaware of any other experiments in which attempts were made to quantitate the levels of alkylacyl glycerolipids in tissues after feeding alkylglycerols to intact mammals. The exact mechanism for incorporation of the alkylglycerol portion of the dietary ADAG into rat tissues is presently unknown. However, one possible explanation is *via* hydrolysis of the acetate groups from ADAG by intestinal and/or other tissue lipases followed by phosphorylation of the resulting alkylglycerol (21,22) to produce alkylglycerophosphate. This compound serves an intermediate in the *de novo* biosynthesis of alkylacyl-GPE, and alkylacyl-GPC (23). The increased levels of alkylacyl-GPC (the precursor of PAF) found as a result of oral administration of alkyldiacetylglycerols in the current study could have a very pronounced influence on biological responses elicited by PAF and eicosanoid mediators. Future experimentation in this project is designed to explore this possibility.

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