Altered renal diacylglycerol mass and fatty acid compositions in murine polycystic kidney disease: dietary effects

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The mass amounts and fatty acid compositions of the second messenger, diacylglycerol, have been shown to be altered in several disease states and in transformed cells. In the present study, the mass amounts and fatty acid compositions of renal diacylglycerol were determined in a mouse model of polycystic kidney disease. Kidney diacylglycerol (nmol/100 nmol phospholipid) was elevated by 21% in diseased mice compared to normal controls. Diseased kidneys were also characterized by higher levels of stearic (18:0) and arachidonic (20:4n-6) acids and lower levels of eicosenoic (20:1) and docosapentaenoic (22:5n-6) acids in the diacylglycerol fraction. Furthermore, the level and fatty acid compositions of kidney diacylglycerol in the mice with polycystic kidney disease were modified by altering dietary protein levels and lipid type. Compared with high protein diets, low protein diets were associated with both higher renal diacylglycerol levels (overall by 31%), and a retardation of renal cystic disease progression. Substituting dietary sunflowerseed oil with fish oil resulted in markedly lower levels of n-6 fatty acids and higher levels of n-3 fatty acids in kidney diacylglycerol. It remains to be determined what effect these changes in diacylglycerol level and type may have on protein kinase C-mediated events associated with biochemical defects found in polycystic kidney disease. These findings raise the possibility that diet-induced alterations of diacylglycerol mass and fatty acid compositions in vivo *could be utilized to modify intracellular signalling events that accompany certain pathophysiologic conditions.*

Keywords: diacylglycerol; kidney; low protein diet; dietary lipid; mouse; polycystic kidney disease

Introduction

Polycystic kidney disease (PKD) is characterized by several biochemical abnormalities, including altered epidermal growth factor activity, abnormal oncogene expression, vasoconstrictor-related abnormalities, and altered renal Na^+K^+ATPase activity.[1-7] Transmembrane signalling events related to these processes are mediated in part via the metabolism of membrane phospholipids, including their conversion to *sn*-1,2-diacylglycerol (1,2-DG) directly via phospholipase C action on phosphoinositides or phosphatidylcholine, or by the combined action of phospholipase D and phosphatidate phosphatase on phosphatidylcholine. The latter phospholipid is considered to yield a greater amount of DG for a more sustained period of time.[8] Other possible sources of DG may include triacylglycerol or de novo synthesis.[9-12] The 1,2 isomer of DG is required for protein kinase C (PKC) activation,[13-16] which results in protein phosphorylations associated with a plethora of biochemical actions. Activators of protein kinase C such as DG have also been demonstrated to influence the in vitro activity of proximal tubule cell Na^+K^+ATPase,[17] one of the enzymes that has altered activity in PKD.

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DG levels, fatty acid compositions, or both have been demonstrated to be altered in disease states such as myopathic hearts during the development of heart failure in hamsters,[18] in ischemic rat and dog hearts,[19,20] in T lymphocytes derived from mouse models of systemic lupus erythematosus,[21] and in *ras*-transformed kidney cells.[22] Using a lipogenic diet, it has been shown that DG levels and fatty acid compositions can be altered in normal rat liver by severely altering diet composition.[23] Fatty acid composition also influences the ability of DG to activate PKC.[24,25] The studies presented here demonstrate not only that renal DG levels and fatty acid compositions are altered in a mouse model of PKD, but also that moderate dietary changes (in protein level and lipid type) can modify both renal DG levels and their fatty acid compositions in these mice.

Materials and methods

The experimental animals used were the DBA/2FG-*pcy (pcy)* mouse, a model of autosomal dominant PKD,[26,27] and its normal counterpart, the DBA/2J mouse. Mice were housed individually in a temperature- (23° C), humidity- (60–65% relative humidity), and light- (12 hr light:dark) controlled environment.

Normal and diseased (*pcy*) male mice were weaned at 30 days and maintained on lab chow (Ralston Purina, St. Louis, MO, USA). At 120 days of age, mice were sacrificed after CO_2 anesthesia. The kidneys were immediately removed, frozen in liquid nitrogen, and stored at −60° C until further analysis as described below.

In the dietary study, male *pcy* mice were weaned and randomly divided into four groups. Water and diet were provided ad libitum. The groups of mice were fed semi-purified diets containing either a high (HP, 25% casein) or low level of protein (LP, 6% casein), and either sunflower-seed oil (SO) rich in the n-6 fatty acid, linoleic acid (18:2n-6), or a fish oil (FO) concentrate (MaxEPA, R.P. Scherer, Windsor, Ontario, Canada) rich in n-3 fatty acids (eicosapentaenoic acid, 20:5n-3, plus docosahexaenoic acid, 22:6n-3), in a 2 × 2 design. The FO-enriched diet contained 1% SO to supply adequate amounts of the essential fatty acid, 18:2n-6. Ethoxyquin was added as an anti-oxidant at the level of 0.05%.[28] The composition of the diets is detailed in *Tables 1 and 2*. Lipids were extracted[29] from the diets and the fatty acid compositions thereof were determined by gas-liquid chromatography (GLC) as described below for renal phospholipid and DG.

Table 1 Composition of experimental diets

Diet ingredient	% of diet by weight			
	HP-SO	HP-FO	LP-SO	LP-FO
Casein, vitamin-free	25	25	6	6
Cornstarch	25	25	44	44
Sucrose	30	30	30	30
Sunflowerseed oil	10	1	10	1
MaxEPA oil	—	9	—	9
Mineral mix	5.5	5.5	5.5	5.5
Vitamin mix	1	1	1	1
Inositol[a]	0.1	0.1	0.1	0.1
DL-methionine[a]	0.3	0.3	0.09	0.09
Choline chloride	0.2	0.2	0.2	0.2
Solka-floc(fiber)	3.3	3.3	3.3	3.3

[a]Included in vitamin mix.

Table 2 Fatty acid composition of SO- and FO-based diets

Fatty acid[a]	SO	FO
16:0	8.4	26.7
16:1	0.1	13.1
18:0	6.0	4.7
18:1	19.9	17.4
18:2n-6	53.7	9.6
18:3n-6	tr[b]	0.3
18:3n-3	0.2	1.1
18:4n-3	0.1	2.5
20:1	10.6	5.7
20:4n-6	tr	0.5
20:5n-3	tr	9.7
22:0	0.8	0.2
22:1	0.1	1.9
22:5n-3	tr	1.3
22:6n-3	tr	5.4

[a]Values expressed as mol percentage of total fatty acids present. Selected minor fatty acids have been omitted.
[b]tr, trace amounts, less than 0.1 mol percentage.

Kidney lipids were extracted by the standard or modified Bligh and Dyer extraction procedures,[30,31] using 0.05% butylated hydroxytoluene in the solvents as anti-oxidant. DG and total phospholipid were purified from the lipid extracts by thin-layer chromatography (TLC) on silica gel 60HR plates (Merck, British Drug House, Toronto, Ontario, Canada) using heptane/isopropyl ether/acetic acid (60/40/3, by volume) as the mobile phase.[32] The resolved bands were visualized under UV light after spraying the air-dried plates with 0.1% 8-anilino-1-naphthalene-sulfonic acid (Sigma Chemical Co., St. Louis, MO, USA) in H_2O.[33] These bands were scraped into test tubes containing appropriate amounts of monopentadecanoate (15:0) as internal standard, and transesterified at 80° C for 14–16 hr with 6% H_2SO_4 in methanol.[34] The derived fatty acid methyl esters (FAMEs) were extracted with petroleum ether, and purified by TLC using toluene as the mobile phase. The purified FAMEs were visualized, scraped from the plates and extracted in petroleum ether. FAMEs were analyzed by GLC as described,[34] using a DB-225 megabore column (Chromatographic Specialties Inc., Brockville, Ontario, Canada) at a temperature of 205° C. To account for background contamination, blank regions of the TLC plate corresponding to the lipid bands were treated exactly as the samples. The peak areas obtained by GLC for the blanks were then subtracted from the sample peak areas.

Analysis of diradylglycerol acetates[35] derived from diradylglycerol purified from *pcy* mouse kidneys indicated that approximately 90% of the fatty acids are derived from diacylglycerol moieties (at least 85% of which was the 1,2 isomer), with the remaining amount being derived mostly from alkylacylglycerol (unpublished observations). The diradylglycerol analyzed in this study is therefore referred to as DG; more than 75% of the total DG is 1,2-DG, the activator of PKC. The mass of total phospholipid and DG were calculated from the corresponding fatty acid amounts. Because these fractions do not contain exclusively diacyl

moieties, the conversion factors* used for total phospholipid and DG were 1.77 and 1.90 fatty acids per molecule, respectively.

All data are expressed as the mean ± standard error. The levels of renal DG are expressed in nmol/100 nmol total phospholipid, as disease affects the level of normal renal tissue, and consequently, the amount of total lipid. The data were analyzed by analysis of variance,[37] with differences being considered significant at $P < 0.05$.

Results

In the chow-fed animals, the overall level of renal DG (nmol/100 nmol phospholipid) was elevated by 21% in the *pcy* mice as compared to normals (*Figure 1*). Absolute levels (nmol/kidney) of renal DG were not different, while total renal phospholipid levels were lower (by 26%, $P < 0.01$) in *pcy* mice, resulting in a higher DG:phospholipid ratio. In addition to mass changes, the fatty acid compositions were also altered in kidneys derived from *pcy* mice compared with those derived from normals. Renal DG stearic (18:0) and arachidonic (20:4n-6) acids were higher, whereas eicosenoic (20:1) and docosapentaenoic (22:5n-6) acids were lower in *pcy* mice compared with controls (*Table 3*).

The levels of kidney DG (nmol/100 nmol phospholipid) were elevated by 31% in the *pcy* mice on the LP versus HP diets, but were not altered by lipid type (*Figure 2*). The absolute levels of renal DG and phospholipid were lower (by 26%, $P < 0.01$, and 38%, $P < 0.001$, respectively) in mice on LP diets; as the differences were greater for renal phospholipid, the DG:phospholipid ratio was higher in mice on LP compared with HP diets. In contrast, the fatty acid compositions of renal DG in *pcy* mice were markedly altered by substituting dietary FO for SO, while there was no effect of dietary protein restriction (*Table 4*). Replacement of dietary SO with FO resulted in alterations in the mol percentage of all major fatty acids except stearic acid (18:0). 18:2n-6 and arachidonic acid (20:4n-6) were markedly lower in renal DG derived from mice on FO diets, while 20:5n-3 and 22:6n-3 were considerably higher. Modestly higher levels of palmitic (16:0), palmitoleic (16:1), and oleic (18:1) acids in renal DG from FO-fed *pcy* mice were also noted.

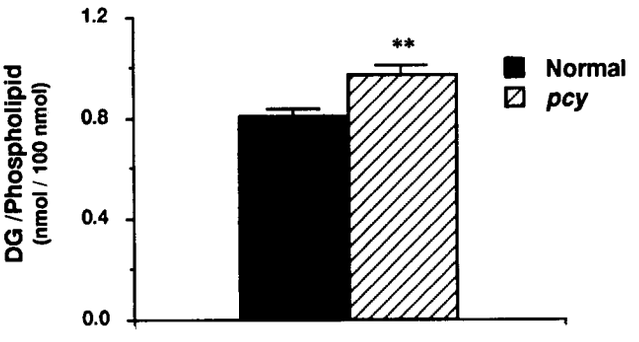

Figure 1 Renal DG levels in normal and *pcy* mice ($n = 6$). **Significantly different from normal, $P < 0.01$.

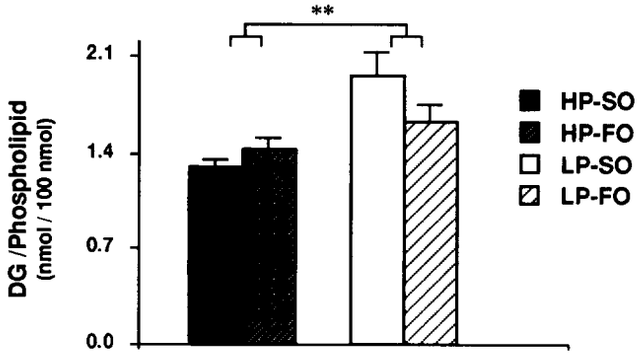

Figure 2 Effect of dietary protein level and lipid type on renal DG levels in *pcy* mice ($n = 5–7$). **$P < 0.01$, main effect of protein.

Table 3 Fatty acid composition of renal DG in normal and *pcy* mice

Fatty acid[a]	Normal	*pcy*
16:0	25.7 ± 1.3	24.8 ± 0.8
18:0	14.1 ± 0.8	15.9 ± 0.7*
18:1	13.1 ± 1.3	12.5 ± 1.3
18:2(n-6)	10.1 ± 0.5	9.7 ± 0.7
20:1	2.1 ± 0.1	1.4 ± 0.2*
20:2(n-6)	0.9 ± 0.1	1.0 ± 0.2
20:3(n-6)	0.9 ± 0.1	1.0 ± 0.1
20:4(n-6)	9.9 ± 0.6	12.8 ± 0.4**
22:4(n-6)	0.6 ± 0.0	0.5 ± 0.0
22:5(n-6)	1.8 ± 0.1	1.3 ± 0.1**
22:5(n-3)	1.0 ± 0.1	0.7 ± 0.1
22:6(n-3)	15.9 ± 0.9	13.8 ± 1.3

[a]Values represent means ± SE ($n = 6$). Results are expressed as mol percentage of total fatty acids present. Selected minor fatty acids have been omitted.
*$P < 0.05$, **$P < 0.01$, significantly different than DBA.

Discussion

This study represents the first report of altered renal DG (levels and fatty acid compositions) in PKD and its modification via dietary intervention. Changes in DG content in selected tissues affected by other disorders have previously been reported. For example, ischemic and reperfused dog and rat hearts were found to have higher levels of DG,[19,20] while myopathic hamster hearts had decreased DG levels during the development of heart failure.[18] T lymphocytes derived from a mouse model of systemic lupus erythematosus had lower levels of agonist-induced DG production,[21] while *ras*-transformed renal cells exhibited elevated levels of DG compared to normal kidney cells in culture.[22]

In the present study, the level of renal DG in *pcy* mouse kidneys was elevated compared with the level

*Conversion factor of 1.77 for phospholipid was derived from data from Aukema et al.;[36] calculations included consideration of the number of fatty acids in various phospholipids, including their subclasses. For DG, the conversion factor of 1.90 was derived from data (unpublished observations) that indicated that approximately 90% of the DG was diacyl (two fatty acids) and approximately 10% was alkylacyl DG (one fatty acid).

Table 4 Effect of dietary protein level and oil type on DG fatty acid composition in *pcy* mouse kidneys

Fatty acid[a]	HP-SO	HP-FO	LP-SO	LP-FO	Effect[b]
14:0	0.8 ± 0.1	2.0 ± 0.2	0.8 ± 0.1	2.4 ± 0.0	***
16:0	17.9 ± 1.0	21.6 ± 0.8	16.5 ± 1.0	21.0 ± 0.4	***
16:1	1.6 ± 0.4	4.2 ± 0.4	1.8 ± 0.2	5.0 ± 0.2	***
18:0	17.3 ± 1.0	15.3 ± 1.1	18.2 ± 1.4	17.5 ± 0.6	NS
18:1	11.3 ± 0.6	14.8 ± 0.6	12.8 ± 1.0	14.7 ± 0.5	**
18:2(n-6)	30.0 ± 1.1	13.8 ± 0.5	30.2 ± 0.5	12.6 ± 0.4	***
20:2(n-6)	2.3 ± 0.2	0.4 ± 0.0	2.3 ± 0.3	1.0 ± 0.4	**
20:3(n-6)	1.2 ± 0.2	1.2 ± 0.1	1.2 ± 0.2	1.0 ± 0.0	NS
20:4(n-6)	13.5 ± 0.8	8.8 ± 0.5	13.8 ± 0.6	8.7 ± 0.3	***
20:5(n-3)	0.2 ± 0.1	3.6 ± 0.2	0.3 ± 0.1	4.1 ± 0.2	***
22:4(n-6)	0.7 ± 0.1	0.1 ± 0.0	0.6 ± 0.1	tr	***
22:5(n-6)	2.0 ± 0.4	0.3 ± 0.1	1.8 ± 0.4	0.3 ± 0.0	***
22:5(n-3)	tr	1.2 ± 0.0	0.1 ± 0.0	1.3 ± 0.1	***
22:6(n-3)	0.7 ± 0.3	10.2 ± 1.0	1.3 ± 0.2	7.9 ± 0.8	***

[a]Values represent means ± SE (n = 5–7). Results are expressed as mol percentage of total fatty acids present. Selected minor fatty acids have been omitted.
tr, trace amounts, less than 0.1 mol percentage.
[b]***$P < 0.001$, **$P < 0.01$, main effects of oil type; NS, not significant.

found in normal mouse kidneys. DG mass was also modified by protein restriction, but not by lipid type. It remains to be determined if the higher DG levels are due to increased DG production (via phospholipase C, phospholipase D and phosphatidate phosphatase, triacylglycerol lipase, or de novo) or reduced DG metabolism (via DG kinase or DG lipase). In PKD, alteration of these metabolic pathways may reflect variant transmembrane signalling associated with PKC-mediated phosphorylations, including altered growth factor activity, oncogene expression, Na^+K^+ATPase activity, or vasoconstrictor-related events.[1–7] These pathways could be influenced by, or result in, the alterations in renal DG levels in *pcy* mice compared with normals, and in *pcy* mice on LP diets compared with HP diets, as found here. In this regard, it is noteworthy that dietary protein restriction retards cyst development in *pcy* mouse kidneys.†

Dietary lipid type (n-3 versus n-6), but not protein level, modified fatty acid composition of renal DG in *pcy* mice. These results are of interest because DG fatty acid compositions can influence the activation of PKC. The in vitro addition of DG species enriched in n-3 fatty acids were found to be less potent than those containing 18:0 and 20:4n-6 in activating PKC derived from the rat spleen.[25] It is these latter two fatty acids that are enriched in renal DG derived from *pcy* mice compared with normals. Future studies will need to assess the relation, if any, of the altered fatty acid compositions of renal DG as observed here, to altered PKC-mediated protein phosphorylations and/or associated biochemical events in PKD. Previous studies suggest that replacement of n-6 with n-3 fatty acids has little or no effect†[38] on PKD progression in these mice.

With respect to the metabolic origin of the DG found in normal or diseased kidneys, an enrichment of stearoyl-arachidonoyl DG suggests that the phosphoinositides could be a relatively greater source of the renal DG in *pcy* mice compared with normal mice; the phosphoinositides are the most enriched in these two fatty acids in these mouse kidneys.‡ The fatty acid profiles, however, suggest that they could not be solely derived from either individual phospholipids or from triacylglycerol. The fatty compositions of renal DG resemble a mixture of phospholipid§ and triacylglycerol.[36] This is consistent with reports that suggest that DG may be derived from phosphatidylcholine, phosphoinositides, triacylglycerol, or from de novo synthesis.[9–12,17]

In conclusion, we have demonstrated increased levels and altered fatty acid compositions of renal DG in mice with PKD as compared with normal mice. In addition, modest dietary manipulations effected changes in both the DG levels and the fatty acid compositions of this putative second messenger in kidneys of mice with PKD. The effects of these changes in DG level and/or type on PKC activity, and the possible effects on the retardation of disease progression in *pcy* mice remains to be elucidated. These findings raise the possibility, however, of modifying intracellular signalling events in various disease states by altering DG mass and fatty acid compositions in vivo via dietary manipulations.

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