Changes in hepatic lipids of mice infected with cysticerci of *Taenia crassiceps*

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Abstract

Nuclear magnetic resonance (NMR) spectroscopy was employed to investigate the effect of infection with *Taenia crassiceps* cysticerci on the lipid profile of mouse liver. Chloroform/methanol extracts of livers from infected mice showed lower concentrations of phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, total glycerophospholipid, triacylglycerol, total fatty acid (FA) and all measured FA components than those from controls. Furthermore, the ratios obtained on dividing concentrations of the FA components by that of total FA demonstrate that the concentration decreases caused by infection are less for polyunsaturated fatty acids (FAs) than for other FAs. Extracts of *T. crassiceps* displayed a similar lipid profile to that of host liver but contained a lower lipid content and a shorter average FA chain length.

Introduction

The cysticerci of *Taenia crassiceps* are an excellent experimental organism for studying cysticercosis, because of the immunological, molecular and biochemical similarities these cysticerci share with those of *T. solium* (Williams *et al.*, 1982; Larralde *et al.*, 1990; Sciutto *et al.*, 1990). Although *T. crassiceps* is primarily a parasite of wild rodents and canines, there have been a few reported cases of human cysticercosis caused by this species (Shea *et al.*, 1972; Gemmell *et al.*, 1983). During their existence in the mammalian host, cysticerci remove considerable amounts of nutrients and by doing so induce various metabolic disorders. In murine cysticercosis, carbohydrate metabolism is severly altered resulting in an increased rate of gluconeogenesis in the liver of infected animals (Corbin *et al.*, 1996, 1999). In addition, host body fat content was found to be significantly reduced (Crompton *et al.*, 1985). These observations indicate that the parasite exerts a systemic starvation effect on the host and suggest that in addition to changes in carbohydrate metabolism, lipid metabolism of the host must also be affected. The present study was designed to test this by analysing the hepatic lipid composition of mice with experimental cysticercosis. In recent years nuclear magnetic resonance (NMR) spectroscopy has emerged as an excellent alternative technique for analysing lipids as it is less tedious and time consuming than chromatographic methods (Christie, 1982). Major glycerophospholipids (GPLs), sterols and neutral lipids, as well as fatty acids (FAs) are represented simultaneously in the ^{1}H NMR spectrum and the average chain length and degree of unsaturation of FAs can also be obtained (Sze & Jardetzky, 1990; Pollesello *et al.*, 1993). Although ^{1}H NMR lipid profiles are complex, one and two dimensional NMR experiments have assigned nearly all of the lipid resonances visible in the one dimensional ^{1}H NMR spectrum (Sze & Jardetzky, 1990; Casu *et al.*, 1991; Adosraku *et al.*, 1994). In addition, quantitative NMR lipid data correlate well with traditional chromatographic results (Sparling *et al.*, 1989; Casu *et al.*, 1991). Based on the above information we decided to employ ^{1}H NMR spectroscopy for our analyses.

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Materials and methods

Infection and tissue collection

Swiss-Webster female mice, four months old, were randomly separated into two groups of 12 mice each. The mice in one group were infected via i.p. injection with 0.5 ml of *T. crassiceps* cysticerci (Manitoba strain) each. The remaining 12 mice served as controls. The mice were maintained in accordance with the principles of the Canadian Council on Animal Care as stated in *Guide to the Care and Use of Experimental Animals* and were allowed to feed on commercial pellets and water *ad libitum*. From 120

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to 122 days p.i., between 9:00 a.m. and 12:00 noon, several mice from each group were anesthetized intramuscularly with a mixture of ketamine and xylazine (87 mg kg^{-1} and 13 mg kg^{-1} respectively), their abdomens opened and the livers excised. The organs were rinsed with physiological saline, immersed in liquid N_2 and weighed. The animals were killed by cervical dislocation. Cysticerci, collected from the peritoneal cavity of infected mice, were rinsed several times in physiological saline, strained, weighed and frozen in liquid N_2. All samples were kept at -70°C until preparation of lipid extracts.

Preparation of lipid extracts and NMR samples

Each frozen sample of the liver or the larvae was transferred to a precooled mortar where it was pulverized in liquid N_2 with a pestle. The pulverized sample was then placed in a 50 ml beaker (precooled in an ice bath) with 20 ml of a mixture of chloroform (HPLC grade, hydrocarbon stabilized, from EM Inc.) and methanol (HPLC grade from BDH Inc.) (2:1, v/v) per gram of tissue as described by Folch *et al.* (1957) and homogenized with a Brinkman Polytron homogenizer while surrounded by an ice bath. The homogenized suspension was then filtered and the filtrate retained. The residue was re-extracted with the same volume of fresh chloroform-methanol (C/M) mixture used initially and the filtrates were pooled and washed twice with equal volumes of 0.5 M KCl in 50% methanol to remove non-lipid substances. After washing, the lipid rich chloroform layer was rotary evaporated to dryness under N_2. The residue was then suspended in 5 ml of benzene and again rotary evaporated to dryness under N_2 to azeotropically remove water. Resuspension of the residue in benzene and rotary evaporation was repeated a second time. The dried residue was then stored under N_2 at -20°C until preparation of NMR samples.

Each sample was prepared for NMR analysis by resuspending the dried residue in 2.5 ml of chloroform per gram of tissue with 2.5×10^{-3} g of tetrakis(trimethylsilyl)silane (TMSS) (Aldrich Chemical Co.) added as an intensity standard. The suspension was then centrifuged at 840 g for 10 min. For liver samples, 0.20 ml of supernatant was added to a 1.0 ml mixture of $CDCl_3$ (99.8 atom % D, CDN Isotopes) and CD_3OD (99.8 atom % D, CDN Isotopes) (2:3, v/v). For parasite tissue, to increase the concentrations of lipids in the final solution, 0.4 ml of the supernatant was added to 0.6 ml of CD_3OD. All samples were transferred to 5 mm NMR tubes for proton NMR analysis.

NMR spectroscopy and quantification

The ^{1}H NMR spectra of the lipid extracts were obtained at a temperature of 27°C using a Bruker AMX-500 spectrometer operating at 500.14 MHz. Spectra were accumulated locked on the methyl deuterons of CD_3OD, with a pulse interval of 10.2 sec at a flip angle of 78°. Off resonance presaturation, using a shaped gauss 1024 pulse at 59 dB, was applied to reduce the chloroform peak. The spectral width was 4504.50 Hz with 32 K data points used for acquisition. Chemical shifts are given relative to the C-18 methyl of cholesterol at 0.68 ppm. Peak assignments were based on published data (Sparling *et al.*, 1989; Sze & Jardetzky, 1990; Casu *et al.*, 1991; Adosraku *et al.*, 1993, 1994; Choi *et al.*, 1993). The peaks used in our analyses can be easily identified from the spectra presented in these publications as their spectra are very similar to those obtained in the current study.

Quantitative measurements were made by integration of lipid peaks relative to TMSS as an intensity standard. Integration was performed with a sub-routine from the software on either a Bruker AMX-500 spectrometer or a Bruker X-32 data station. Peaks areas were corrected for the number of protons contributing to each signal. Concentrations of individual lipid classes, total GPL, total FA and various FA components, in μmol g^{-1} wet weight, were calculated using these integration values and the recorded weight of the tissue. The sum of the methyl proton resonances at 0.86 ppm and 0.95 ppm was used as a measure of total FA content. The average FA chain (FAC) composition was also determined by calculating ratios in which the component concentration is relative to total FA concentration. These ratios, in effect, are the average number of times a given component is present in a FAC. In addition, the average FAC length was calculated using the following equation: Average FAC length = 2 + (CH_2 in -$(CH_2)_n$-: total FA) + (-CH_2CH_2COO : total FA) + (-CH_2COO : total FA) + 2(-CH=CH- : total FA) + (-CH_2CH=CH- : total FA) + (CH_2 in -CH=CH(CH_2CH=$CH)_n$: total FA) + 2(-CH=$CHCH_2CH_2COO$ of docosahexaenoic acid: total FA) + (-CH=$CHCH_2CH$=CH- of linoleic acid : total FA) + 2(-CH=$CHCH_2CH_2CH_2COO$ of arachidonic acid : total FA) where the 2 at the beginning of the formula is for the one methyl group and the one carbonyl group (C=O) per FA chain. Statistical analysis was performed using the SAS statistical computer program. Data were analysed using ANOVA where a value of $P \leqslant 0.05$ was deemed significant.

Results

Concentrations of metabolites

Mice infected with *T. crassiceps* contained an average of 14.46 ± 4.35 g of cysticerci at the time of dissection. The livers of these infected mice weighed less (1.14 ± 0.14 g) than those of controls (1.32 ± 0.13 g). The concentrations of various classes of lipids, total GPL, total FA and FA components in the liver and the parasite are presented in table 1. The livers from infected mice contained lower concentrations of phosphatidylethanolamine (PTE), phosphatidylcholine (PTC), phosphatidylinositol (PTI), total GPL, triacylglycerol (TAG), total FA and all of the FA components. Only the cholesterol (CTL) level was similar to that of uninfected controls. Cysticerci had lower concentrations of every lipid metabolite that was measured, with the exception of free FA which was not detectable in host liver.

Table 2 presents ratios (FA component : total FA) for individual FA components. Livers from infected animals possessed lower ratios of the methylene moieties of -$(CH_2)_n$- and -CH_2COO than those from the uninfected group. Infected mice, however, contained higher ratios of the following moieties which pertain to polyunsaturation: CH_2 in -CH=CH(CH_2CH=CH-)$_n$, -CH=$CHCH_2CH_2COO$

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Table 1. Concentration of lipid metabolites in liver of uninfected and infected mice and *Taenia crassiceps* cysticerci.

Lipid	μmol g^{-1} wet wt (mean ± S.D.)		
	uninfected	infected	cysticerci
Cholesterol	5.51 ± 0.31[a]	5.48 ± 0.30[a]	2.30 ± 0.04[*]
Phosphatidylethanolamine	8.17 ± 0.77[a]	7.04 ± 1.10[b]	0.37 ± 0.12[*]
Phosphatidylcholine	19.45 ± 1.06[a]	18.06 ± 0.98[b]	1.11 ± 0.20[*]
Phosphatidylinositol	2.08 ± 0.33[a]	1.73 ± 0.30[b]	NM
Total glycerophospholipids	42.52 ± 3.17[a]	38.21 ± 2.97[b]	3.37 ± 1.39[*]
Triacylglycerol	11.39 ± 2.94[a]	4.15 ± 1.09[b]	0.62 ± 0.31[*]
Total fatty acids	123.70 ± 11.97[a]	93.86 ± 6.00[b]	9.86 ± 1.79[*]
Fatty acid component			
$C\underline{H}_2$ in -$(CH_2)_n$-	1081.49 ± 119.42[a]	777.02 ± 45.71[b]	63.25 ± 10.83[*]
-$C\underline{H}_2CH_2COO$	111.42 ± 16.74[a]	80.17 ± 5.62[b]	NM
-$C\underline{H}_2COO$	106.99 ± 11.92[a]	77.80 ± 4.93[b]	4.88 ± 1.05[*]
-$C\underline{H}_2COOH$ (free)	NM	NM	2.30 ± 0.66
-$C\underline{H}$=CH-	172.72 ± 14.02[a]	136.25 ± 15.10[b]	10.52 ± 2.11[*]
-$C\underline{H}_2C\underline{H}$=CH-	132.59 ± 13.34[a]	95.11 ± 10.01[b]	8.34 ± 1.55[*]
$C\underline{H}_2$ in -CH=CH($C\underline{H}_2$CH=CH)$_n$	87.74 ± 7.88[a]	74.14 ± 11.70[b]	4.78 ± 0.88[*]
-$C\underline{H}$=CH$CH_2CH_2C\underline{O}O$ of DHA	8.26 ± 0.79[a]	7.39 ± 1.22[b]	0.49 ± 0.09[*]
-CH=CH$C\underline{H}_2C\underline{H}$=CH- of LA	18.15 ± 2.57[a]	14.61 ± 1.44[b]	1.11 ± 0.31[*]
-CH=CH$C\underline{H}_2CH_2CH_2COO$ of AA	11.86 ± 1.11[a]	10.35 ± 1.48[b]	NM

Different letters denote a significant difference between concentrations of lipids in uninfected and infected groups ($P \leqslant 0.05$). [*]Indicates a significant difference between concentrations of lipids in infected and cysticerci groups. In all groups n = 12; NM, not measurable. The underlined hydrogens (H) indicate those that were integrated. AA, arachidonic acid; DHA, docosahexaenoic acid; LA, linoleic acid.

of docosahexaenoic acid (DHA) and -CH=CHCH$_2$CH$_2$CH$_2$COO of arachidonic acid (AA). The ratios of FA components are also given for *T. crassiceps* and compared to those of the infected host (table 2). Cysticerci contained a shorter average FA chain length and lower ratios of all FA components for which measurement was possible in both liver and parasite.

Discussion

This study demonstrates that cysticerci of *T. crassiceps* cause marked changes in the lipid profile of the liver of their mammalian host. Among the numerous changes detected, the most pronounced was the depletion of TAG, a class of neutral lipids whose primary function in the mammalian cell is to serve as one of the principal sources of energy (Marinetti, 1990; Mathews & van Holde, 1990). Given that TAGs are composed of three FAs esterified to a glycerol backbone, it is not surprising that the concentration of total FA and each FA moiety investigated also decreased in the liver of infected mice. Cysticerci, incapable of *de novo* FA and sterol synthesis, (Barrett, 1983; Smyth & McManus, 1989) acquire, from the host, water soluble components such as choline, glycerol, short

Table 2. Composition of fatty acids in liver from uninfected and infected mice and in *Taenia crassiceps* cysticerci.

FA component	Ratio (FA component : total FA) mean ± S.D.		
	uninfected	infected	cysticerci
$C\underline{H}_2$ in -$(CH_2)_n$-	8.66 ± 0.18[a]	8.28 ± 0.13[b]	6.46 ± 0.06[*]
-$C\underline{H}_2CH_2COO$	0.88 ± 0.04[a]	0.85 ± 0.02[a]	NM
-$C\underline{H}_2COO$	0.86 ± 0.02[a]	0.83 ± 0.01[b]	0.50 ± 0.04[*]
-$C\underline{H}_2COOH$ (free)	NM	NM	0.23 ± 0.42
-$C\underline{H}$=CH-	1.40 ± 0.06[a]	1.45 ± 0.12[a]	1.07 ± 0.08[*]
-$C\underline{H}_2C\underline{H}$=CH-	1.07 ± 0.04[a]	1.01 ± 0.07[a]	0.89 ± 0.09[*]
$C\underline{H}_2$ in -CH=CH($C\underline{H}_2$CH=CH)$_n$	0.71 ± 0.07[a]	0.79 ± 0.11[b]	0.49 ± 0.03[*]
-$C\underline{H}$=CH$CH_2CH_2C\underline{O}O$ of DHA	0.07 ± 0.01[a]	0.08 ± 0.01[b]	0.05 ± 0.01[*]
-CH=CH$C\underline{H}_2C\underline{H}$=CH- of LA	0.14 ± 0.02[a]	0.15 ± 0.01[a]	0.11 ± 0.02[*]
-CH=CH$C\underline{H}_2CH_2CH_2COO$ of AA	0.09 ± 0.01[a]	0.11 ± 0.02[b]	NM
		Average chain length	
	17.44 ± 0.16[a]	17.20 ± 0.39[a]	12.91 ± 0.58[*]

[a,b]Different letters denote a significant difference between ratios of fatty acid (FA) components in uninfected and infected groups ($P \leqslant 0.05$). [*]Indicates a significant difference between ratios of FA components in infected and cysticerci groups. In all groups n = 12; NM, not measurable. The underlined hydrogens (H) indicate those that were integrated. AA, arachidonic acid; DHA, docosahexaenoic acid; LA, linoleic acid.

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chain FAs, micelles of long chain FAs and monoacylglycerols (MAG) released from hydrolysed chylomicrons. Evidence for the uptake of free FAs by cysticerci is demonstrated in this study by the presence of a high concentration of unesterified FA, as indicated by $-CH_2COOH$ (free), in these metacestodes. Although intracellular hydrolysis of endogenous TAG and GPL from the cysticerci tissues could contribute to the levels of free FA detected, the inability of cestodes to catabolize FAs seems to suggest that the hydrolysis of these lipids would be a minor process. Indeed this appears to be the case, as Mills *et al.* (1981) report very low levels of MAG, diacylglycerols (DAG) and lysophosphatidyl-choline/-ethanolamine (deacylated phosphatidyl-choline/-ethanolamine) in the tissues of *T. crassiceps*. Albumin bound FAs may also become accessible to the parasite. Hustead & Williams (1977) demonstrated that *T. crassiceps* is capable of internalizing albumin and therefore it is likely that albumin bound FAs, derived from adipose tissue, are also captured by the cysticerci. The parasite probably does not acquire very long chain FAs in this manner as this kind of FA is not prominent in mammalian plasma. Instead, these FAs must be synthesized by cestodes through the elongation of acquired shorter chain FAs (Barrett, 1983; Frayha & Smyth, 1983; Smyth & McManus, 1989). The inability of cestodes to perform FA desaturation offers a possible explanation for our observation that *T. crassiceps* cysticerci possess fewer double bonds per FAC than host liver. Short to medium chain FAs ranging from 5 to 12 carbons are readily soluble in blood without the aid of solubilizing plasma proteins (Shiau, 1987). Preferential absorption of these FAs could readily account for the shorter average FAC length of 12.91 carbons for the cysticerci, compared to 17.20 carbons per FAC for host liver.

Fatty acids from the TAG core of chylomicrons in infected hosts are much more likely to be utilized than those from controls because of uptake by the parasite and also because of depleted peripheral tissues arising from the starvation effect induced by the parasite. Thus the resulting chylomicron material would arrive at the liver more depleted in TAG which must, in part, account for the lowered hepatic concentrations of TAG and FA observed in this study. In addition to the removal of FAs, the cysticerci also acquire substantial amounts of glucose from the host. In response, the liver attempts to correct this metabolic disturbance by mobilizing glycogen stores and accelerating the gluconeogenic process (Corbin *et al.*, 1996, 1999). As a consequence, the utilization of glucose in secondary pathways, such as FA synthesis, is inhibited (Goodridge *et al.*, 1986; Katz, 1986; Sugden *et al.*, 1993). Thus with decreased hepatic FA synthesis, FA esterification and TAG synthesis are also expected to be markedly diminished. With the reduction in the amount of FA found in the liver of infected animals, it can be assumed that FAs are no longer available in excess to be channelled into ketogenic processes. The lower concentration of hepatic β-hydroxybutyrate in mice infected with *T. crassiceps* (Corbin *et al.*, 1996) supports this hypothesis. It seems then that the FAs available in the liver were used for energy generation; continued complete oxidation of FA is essential for this organ as it derives most of its energy from this process (Seifter & Englard, 1994).

Although the concentration of each FA component in the liver of infected mice was significantly lower than that of controls, this was not the case for the ratios obtained on dividing these concentrations by that of total FA. The ratios for CH_2 in $-(CH_2)_n$ and $-CH_2COO$ groups were reduced, indicating that there were on average fewer of these components per FA chain than in uninfected mice. On the other hand, the higher ratios for CH_2 in $-CH=CH(CH_2CH=CH)_n$ and those components normally ascribed to DHA and AA (see tables 1 and 2) denote that there were more of these moieties per FAC than in uninfected animals. In general, this means that the concentration decreases caused by infection are less for polyunsaturated FAs than for other FAs. This is consistent with the knowledge that although oxidation of polyunsaturated FAs occurs, they are a less important source of energy (Smith *et al.*, 1983). Further, these observed alterations in FA composition with infection are similar to descriptions of changes in hepatic FA composition of fasting animals, with the exception that the increased amount of polyunsaturated FA per FAC was not as pronounced as in fasted animals (Cunnane, 1988; Chen & Cunnane, 1992). A possible mechanism for preferential retention/utilization of specific FAs in the liver has been discussed (Gavino & Gavino, 1991). Enhanced biosynthesis of polyunsaturated FAs does not seem to be a likely explanation for our results; in particular this can be seen in the case of AA considering that linoleic acid (LA), the essential FA required for its production, was depleted to the same degree as total FA. Further, with the reduced quantity of hepatic FA in infected mice it seems highly probable that in these animals, similar to in fasting animals, there will be an inhibition of desaturation pathways (Smith *et al.*, 1983) which also infers that increased biosynthesis of polyunsaturated FAs is unlikely in these animals.

A particularly intriguing aspect of our results is the observed increase in the contribution of the component normally attributed to AA to the composition of total FA. Another possible contributor to this increment would be a boost in the production of a number of eicosanoids which also contain this structural unit, namely prostaglandins E_2 and I_2 and thromboxane A_2. Eicosanoids synthesis in mammalian cells is most commonly via phospholipase A_2 which is activated by hormonal or inflammatory stimuli. The AA hydrolysed from membrane phospholipids by this enzyme enters a cascade of microsomal oxygenation reactions catalysed by prostaglandin G/H synthase to produce the prostaglandins and thromboxanes or enters the lipoxygenase pathway to generate leukotrienes or related hydroxy acids. Once synthesized, eicosanoids are released from the cell and act locally by binding to nearby target cells where they modulate numerous physiological and immunological processes (Mathews & van Holde, 1990; Smith *et al.*, 1991; Keppler, 1994). The inflammation of the peritoneum and marked leucocytosis which accompanies *T. crassiceps* infection (Freeman, 1962, 1964) also extends to the host liver (Chernin & McLaren, 1983). Therefore it would not be surprising if the host produced increased amounts of these compounds.

Many parasitic helminths, including cestodes, are also capable of converting AA into various eicosanoids (Belley & Chadee, 1995). However, the inability of cestodes to

desaturate FAs renders them incapable of synthesizing AA from LA. Therefore any AA in the parasite must be acquired from the host as a solubilized free FA. Although in this experiment levels of AA in *T. crassiceps* cysticerci were not measurable, chromatographic experiments performed by Mills *et al.* (1981) did detect AA in this parasite, albeit at low concentrations. The low levels of AA could be due to high rates of eicosanoid synthesis and immediate secretion by the parasite which would account for the marked immunomodulation of the host (Sciutto *et al.*, 1995; Villa & Kuhn, 1996).

The concentrations of PTC, PTE, PTI and total GPL were all lower in liver of infected mice than in controls. This cannot be attributed to direct absorption of these lipids by the parasite as there is no evidence that cestodes are able to perform this function. However, cestodes are known to absorb GPL precursors as mentioned above and this must be considered for possible influence on hepatic GPL concentrations. As in mammals, cestode GPLs function primarily as structural membrane constituents (Mills *et al.*, 1981; Barrett, 1983; Frayha & Smyth, 1983; Smyth & McManus, 1989). Precursors such as FAs, choline, phosphocholine (PC), ethanolamine, phosphoethanolamine (PE), inositol and glycerol are, within the parasite, eventually constructed into membrane GPL by the cytidine 5′-diphosphate (CDP) linked cytidyltransferase catalysed condensation of DAG with choline, ethanolamine or inositol. Phosphatidylserine (PTS) and cardiolipin are also formed by CDP pathways in cestodes. Given the high rates of membrane turnover and asexual reproduction, cysticerci of *T. crassiceps* must acquire considerable amounts of GPL precursors to maintain continued membrane biosynthesis. Thus, parasite active uptake of membrane precursors must be contributing to hepatic GPL reduction. Further, of these hepatic membrane precursor concentrations, those of FAs are probably the most influential in determining hepatic GPL levels. With fewer FAs in the liver of the infected host, the availability of DAG, a key participant in GPL synthesis (Ansell & Spanner, 1982; Vance, 1991), becomes limited. When DAG is not available to participate in the CDP-choline 1,2-DAG PC transferase reaction, PTC synthesis is impeded and choline and PC start to accumulate in the cell; this was actually observed in an earlier study on this host–parasite system (Corbin *et al.*, 1996). PTE and PTI may also be synthesized by the condensation of CDP-ethanolamine and myo-inositol with DAG and CDP-DAG, respectively. Therefore the limited supply of DAG in the liver would also contribute to the reduced concentration of PTE and PTI found in infected mice. Since individual GPL are no longer in excess, the rates of head group exchange and interconversion may be reduced which would result in lower concentrations of other GPLs, including PTS. This phospholipid is not present at high enough levels in the liver or the cysticerci to be quantified by ^{1}H NMR. It has, however, been detected in cestode tissues by other analytical means (Mills *et al.*, 1981; Frayha & Smyth, 1983) indicating that these parasites do remove PTS precursors from the host. The diminished decarboxylation of PTS would lead to further reduction of PTE.

It can be concluded that *T. crassiceps* cysticerci do indeed drastically alter the lipid profile of host liver and that NMR spectroscopy is an appropriate technique for analysing such complex systems.

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