Zeitschrift für
Ernährungswissenschaft Z. Ernährungswiss. 22, 27–33 (1983)

Effects of feeding lysine-deficient diet on the metabolism of lipids in various tissues of rats*)

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Summary

32 weanling male albino rats were divided into 4 groups to study the effects of lysine-deficient wheat diet (AW) and AW supplemented with either 0.4 % lysine (LW) or 0.2 % carnitine (CW) as compared to casein diet on metabolism of lipids in various tissues. LW, CW and casein diet groups were pair-fed with AW group. Changes in total lipids, lipid components, individual fatty acids, mitochondrial content in liver, heart, skeletal muscles, lungs and adipose tissue were determined after 8 weeks of feeding. AW diet resulted in accumulation of lipids (mainly acylglycerols) in heart, liver, skeletal muscles and depletion in adipose tissue. The LW and CW diets reversed the effects of AW diet, the CW being more effective than LW diet. The LW and CW diets increased the relative proportion of C 14:0, C 16:0, C 16:1 and decreased that of $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ fatty acids which were decreased and increased, respectively, on the AW and casein diets. The fatty acids composition of adipose tissue was the same in all the groups. The AW diet increased the relative proportions of C 14:0, C 20:4 and decreased that of C 16:0, C 16:1, C 18:3 fatty acids in the lungs. Supplemented AW diet decreased the relative proportions of the former group and increased that of the later group including C 18:1 fatty acid also. The mitochondrial content of liver, heart, skeletal muscles and lungs was decreased on AW and reversed on LW and CW diets.

Zusammenfassung

32 abgesetzte männliche Albinoratten wurden in 4 Gruppen eingeteilt, um die Wirkung einer lysinarmen Weizendiät (AW), einer Weizendiät mit 0,4 % Lysin (LW) oder 0,2 % Carnitin (CW) sowie einer Caseindiät auf den Stoffwechsel von Lipiden in verschiedenen Geweben zu untersuchen. Nach 8 Wochen Fütterung der verschiedenen Diäten unter Anwendung der „paired feeding technique" wurden Veränderungen in den Gesamtlipiden, den Lipidbestandteilen, den einzelnen Fettsäuren und dem Lipidgehalt der Mitochondrien des Herzens, der Skelettmuskeln, der Lungen und des Fettgewebes der Tiere bestimmt. Die lysinarme Weizendiät (AW) bewirkte eine Lipidanreicherung (vor allem an Acylglyzerinen) im Herzen, in der Leber, den Skelettmuskeln sowie eine Lipidabnahme im Fettgewebe. Die Diäten mit 0,4 % Lysin (LW) oder 0,2 % Carnitin (CW) zeitigten die entgegengesetzte Wirkung, wobei CW wirkungsvoller erschien als LW. LW und CW erhöhten die relativen Anteile von $C_{14:0}$-, $C_{16:0}$- und $C_{16:1}$-Fettsäuren und verkleinerten diejenigen von $C_{18:1}$-, $C_{18:2}$- und $C_{18:3}$-Fettsäuren, während die Anteile unter der AW- und Caseindiät ab- bzw. zunahmen. Die Fettsäurezusammensetzung des Fettgewebes war bei allen Gruppen gleich. Die AW-Diät vergrößerte die relativen Anteile von

*) This work forms a part of thesis submitted by Miss Manju Thapar in 1982 to Punjab Agricultural University, Ludhiana, for M.Sc. (Biochemistry).

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$C_{14:0}$- und $C_{20:4}$- und verringerte die von $C_{16:0}$-, $C_{16:1}$- und $C_{18:3}$-Fettsäuren in den Lungen. Die AW-Ergänzungsdiäten verringerten die relativen Anteile von $C_{16:0}$-, $C_{16:1}$-, $C_{18:3}$- sowie auch der $C_{18:1}$-Fettsäuren. Der Lipidgehalt der Mitochondrien von Leber, Herz, Skelettmuskeln und Lunge verringerte sich unter der AW-Diät und erhöhte sich unter den LW- und CW-Diäten.

Key words: wheat, lysine, carnitine, lipids, mitochondria

Introduction

The feeding of diets deficient in lysine, such as wheat or rice not supplemented by extra protein, results in impairment of growth and accumulation of lipids in liver and heart (15, 16, 21) of experimental animals. The fatty livers induced by lysine-deficient diets can be prevented by supplementing such diets with lysine or carnitine (15, 16, 22). The present work was carried out to study the quantitative changes in lipid components under these feeding regimens.

Materials and methods

32 four-week-old male albino rats of Sprague-Dawley strain were divided into 4 groups of 8 animals each and maintained on experimental diets (table 1). 0.4 % lysine (LW) and 0.2 % carnitine (CW) supplemented wheat and casein diet groups were pair-fed with wheat diet alone (AW) group. The rats were caged separately. After 8 weeks, the rats of all the groups were killed and their liver, heart, skeletal muscles (thigh region), lungs and adipose tissue were removed and analysed for mitochondrial content (13), total lipids (8), total acylglycerols (23), phospholipids (1), cholesterol (25), total free fatty acids (19) and individual fatty acids on GLC. The protein content was determined (18) and the statistical analysis was also carried (20). Due to paucity of tissues, samples of heart, lungs and adipose tissue from two rats were pooled for analysis. Muscle and liver samples were analysed individually.

Results

The total lipids content of heart ($p < 0.01$), liver ($p < 0.01$) and skeletal muscles was elevated in rats fed wheat diet (AW) when compared to casein

Table 1. Components of wheat (AW), lysine (LW) and carnitine (CW) supplemented wheat and casein diets.

Ingredients	AW	LW	CW	Casein
Whole wheat flour	56.0	55.6	55.8	–
Casein	–	–	–	20.0
Corn starch	34.0	34.0	34.0	70.0
L-lysine	–	0.40	–	–
DL-Carnitine-Hydrochloride	–	–	0.20	–
*Vitamin B-Complex	1.0	1.0	1.0	1.0
Mineral mixture	4.0	4.0	4.0	4.0
Groundnut oil	5.0	5.0	5.0	5.0

* each 5 ml contained Vit. B_{12}, 6.25 µg; Vit. B_1, 3.75 mg; Riboflavine, 1.25 mg; Nicotinamide, 37.5 mg; Panthenol, 1.25 mg; Vit. B_6, 0.62 mg.
Vit. A, 3000 IU and Vit D_2, 300 IU were also added to each 100 g of diet.
The endogenous level of lysine in wheat and casein diets was 136 mg and 1.4 g and of carnitine 133.dt and 356.dt µg/100 g of diet, respectively.

Table 2. Effects of feeding AW, LW, CW and casein diets on the lipid composition (mean ± s.d. mg/g wet weight) of various tissues.

Tissue / *Diet*	*Total lipids*	*Acylglycerols*	*Cholesterol*	*Phospholipids*	*Free fatty acids*[1])
Liver					
AW	81.17 ± 8.29	21.90 ± 2.79	6.86 ± 0.11	11.85 ± 0.78	75.50 ± 1.42
LW	71.94 ± 8.00***	14.69 ± 1.64*	5.73 ± 0.54**	13.84 ± 1.15**	61.96 ± 6.35**
CW	60.84 ± 6.60*	18.12 ± 1.80**	6.09 ± 0.53	18.81 ± 0.65*	60.63 ± 3.88**
Casein	51.58 ± 2.94*	8.40 ± 0.17*	5.04 ± 0.26*	17.69 ± 0.81*	25.88 ± 2.00*
Heart					
AW	154.40 ± 5.05	19.68 ± 1.27	8.42 ± 0.36	13.18 ± 3.50	86.88 ± 1.74
LW	100.94 ± 9.90***	8.84 ± 0.46*	8.63 ± 0.37	10.93 ± 1.05	93.76 ± 8.79
CW	90.36 ± 8.79**	10.57 ± 0.82**	8.07 ± 0.36	13.86 ± 0.78	86.28 ± 1.26
Casein	70.28 ± 6.20*	4.73 ± 0.43*	6.49 ± 0.41**	9.48 ± 1.06	65.98 ± 5.17**
Skeletal muscles					
AW	29.86 ± 2.59	8.96 ± 0.78	3.14 ± 0.18	12.72 ± 0.83	12.05 ± 1.64
LW	27.24 ± 2.32	6.39 ± 0.53**	2.76 ± 0.22**	12.28 ± 1.40	11.19 ± 0.97
CW	25.93 ± 1.36	6.92 ± 0.29**	3.96 ± 0.13	14.17 ± 1.32	11.14 ± 0.71
Casein	21.32 ± 2.50	4.78 ± 0.73*	1.86 ± 0.17*	9.80 ± 0.29*	8.93 ± 0.36**
Lungs					
AW	74.73 ± 6.20	4.87 ± 0.32	9.18 ± 0.20	8.71 ± 0.77	45.22 ± 3.30
LW	107.63 ± 10.09**	8.97 ± 1.11*	10.55 ± 0.12	8.30 ± 0.95	46.37 ± 2.39
CW	92.77 ± 5.13***	3.55 ± 0.34**	9.82 ± 0.56	8.68 ± 0.36	48.11 ± 2.28
Casein	78.08 ± 5.66	3.19 ± 0.21**	7.59 ± 0.13	6.72 ± 0.58*	38.08 ± 2.78***
Adipose tissue					
AW	754.67 ± 12.26	403.49 ± 25.79	39.24 ± 1.22	–	47.73 ± 1.33
LW	767.14 ± 32.23	402.65 ± 27.34	38.02 ± 2.82	–	47.25 ± 1.41
CW	792.78 ± 44.19	438.42 ± 14.24	39.97 ± 1.87	–	48.62 ± 4.06
Casein	804.41 ± 37.36***	468.41 ± 16.37	21.75 ± 1.05*	–	62.59 ± 2.91**

[1]) expressed as μ moles/g wet weight of tissue.

(–) could not be detected.

Comparisons were made between group AW and other groups using Student's "t" test and the level of significance designated, *$P < 0.01$, **$P < 0.05$; ***$P < 0.10$.

diet (table 2). In these tissues the AW-diet-induced lipidosis was mainly in acylglycerols ($p < 0.01$). The contents of cholesterol and free fatty acids (FFA) in liver and cholesterol, phospholipids and FFA in heart and skeletal muscles were also increased. The phospholipid content of liver was, however, decreased on AW diet. The total lipid content in the adipose tissue was less on AW than on casein diet and the decrease was due to lesser accumulation of acylglycerols and FFA. The cholesterol level was, however, significantly increased ($p < 0.01$) in this tissue on AW diet. The levels of acylglycerols, cholesterol, phospholipids and FFA were increased in the lungs on AW when compared on casein diet.

Supplementation of wheat diet with either 0.4 % lysine (LW) or 0.2 % carnitine (CW) lowered total lipids in liver (LW, $p < 0.10$; CW, $p < 0.01$), heart (LW, $p < 0.10$; CW, $p < 0.05$) and skeletal muscles, carnitine being more effective than lysine (table 2). Carnitine or lysine supplementation, amongst the total lipids, decreased the levels of acylglycerols, cholesterol, FFA in liver, acylglycerols in heart, acylglycerols and FFA in skeletal muscles. Phospholipids were increased in the liver of rats fed sup-

Table 3. Effects of feeding AW, LW, CW and casein diets on the fatty acids composition (% of total fatty acids) of various tissues.

Tissue Diet	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Liver									
AW	7.99	15.54	22.48	21.21	–	15.83	15.97	9.98	–
LW	9.80	20.34	34.31	19.61	–	9.80	6.13	t	–
CW	5.63	25.37	30.51	24.21	–	8.51	5.75	t	–
Casein	4.70	24.08	31.74	26.76	–	8.03	2.25	2.44	–
Heart									
AW	4.36	15.34	28.40	15.45	–	17.68	6.80	7.47	4.30
LW	7.98	18.47	32.91	20.49	–	12.51	4.64	3.00	–
CW	5.24	19.60	32.77	22.49	–	7.36	5.15	3.86	3.53
Casein	4.29	26.60	32.69	21.16	–	9.08	3.09	3.09	–
Skeletal muscles									
AW	2.46	1.42	20.66	7.90	6.73	35.38	19.57	5.88	–
LW	0.40	2.96	26.64	9.01	5.59	31.06	16.14	7.63	–
CW	1.49	2.81	27.57	11.70	4.06	30.59	15.81	5.97	–
Casein	2.66	1.87	26.67	9.00	5.56	30.24	16.80	7.19	–
Lungs									
AW	2.16	12.53	34.45	6.78	–	20.65	8.70	2.28	12.51
LW	t	3.38	45.29	9.93	–	26.30	9.90	2.76	2.44
CW	1.67	2.84	40.33	10.53	–	27.65	9.88	4.54	2.55
Casein	3.69	2.84	39.57	10.62	4.20	21.55	7.63	6.81	3.09
Adipose tissue									
AW	2.70	2.43	30.16	10.00	–	37.80	10.97	5.94	–
LW	2.22	2.47	32.98	6.62	–	38.68	12.74	4.29	–
CW	1.79	2.01	30.43	8.49	–	40.68	12.13	4.47	–
Casein	2.22	2.37	30.68	8.81	–	39.02	11.66	5.24	–

(t) traces, (–) absent

Table 4. Effects of feeding AW, LW, CW and casein diets on the mitochondrial content (mean ± S.D. μg mitochondrial protein/mg tissue homogenate protein) of various tissues.

Diet	Liver	Heart	Skeletal muscles	Lung
AW	178.00 ± 11.25	394.29 ± 21.18	24.74 ± 2.22	53.20 ± 5.09
LW	223.43 ± 22.11*	401.67 ± 31.84	27.91 ± 2.75	62.22 ± 2.08**
CW	261.15 ± 21.66*	412.98 ± 16.88	27.73 ± 2.30	73.71 ± 9.09***
Casein	238.89 ± 16.34*	535.48 ± 28.73**	31.24 ± 1.42**	89.63 ± 5.89*

Comparisons were made between group AW and other groups using student's "t" test and the level significance designated * $P < 0.01$, ** $P < 0.05$, *** $P < 0.10$.

plemented diet. The LW or CW diet, however, increased total lipids in the adipose tissue and lungs. The LW diet increased the levels of acylglycerols and cholesterol while CW diet decreased acylglycerols content in the lungs.

The LW and CW diets increased the relative proportions of C 14:0, C 16:0, C 16:1 and decreased that of C 18:1, C 18:2, C 18:3 fatty acids in liver, heart and skeletal muscles, which were decreased and increased respectively on AW and casein diet. The CW was more effective than LW diet (table 3). The fatty acids composition of adipose tissue was almost the same in the 4 groups. The AW diet increased relative proportions of C 14:0, C 20:4 and decreased that of C 16:0, C 16:1, C 18:3 fatty acids in the lungs when compared with casein diet. The supplementation of wheat diet decreased the relative proportions of C 14:0, C 20:4 and increased that of C 16:0, C 16:1, C 18:1, C 18:3 fatty acids, the carnitine being more effective than lysine.

The AW diet decreased the mitochondrial content (MC) of liver, heart, skeletal muscles and lungs (table 4). Supplementation of wheat diet with lysine or carnitine increased MC in liver ($p < 0.01$), lungs (LW, $p < 0.05$; CW, $p < 0.10$), heart and skeletal muscles. Apparently, CW influenced MC more than LW diet.

Discussion

The wheat diet which is deficient in lysine provided approximately 5 % protein and the lysine and carnitine content were 10 and 37 %, respectively, of those in the casein diet. The feeding of wheat diet caused accumulation of lipids in heart, liver and skeletal muscles and depletion (acylglycerols and to some extent FFA) in the adipose tissue and this effect was reversed by supplementing this diet with either lysine or carnitine (table 2). It showed that the disturbed metabolism of lipids on wheat diet can be corrected by supplementing the diet with lysine or carnitine. Lysine is the precursor of carnitine (2, 3, 6, 7, 11, 12, 14, 21) and the carnitine, so biosynthesized in the liver, is transported mainly to the tissues which depend upon fatty acid oxidation as an energy source such as heart, skeletal muscles and liver (5). Carnitine enhances the oxidation of

long-chain fatty acids (9) by transporting them to the site of oxidative enzymes in the mitochondria (4, 10, 17). The carnitine deficiency due to dietary lysine deficiency, therefore, can impair fatty acid oxidation and cause lipid accumulation in these tissues. The accumulation of lipids and especially of acylglycerols in liver, heart and skeletal muscles on AW diet in the present study seems to be due to impaired fatty acids oxidation and mobilization of fat from the adipose tissue.

Acetyl CoA is generated within the mitochondria (24) and transported out of it with the help of carnitine (4). Since long-chain fatty acids are synthesized extramitochondrially from acetyl CoA and malonyl CoA (24), the carnitine may enhance the synthesis of long-chain fatty acids. The increased proportions of C 14:0, C 16:0, C 16:1 and decreased proportions of C 18:1, C 18:2, C 18:3 fatty acids in liver, heart and skeletal muscles of rats fed LW or CW diet (table 3) may be due to increased synthesis of C 14:0, C 16:0 and C 16:1 fatty acids in these tissues. The lipid composition changes in lungs in the 4 groups were peculiar and needs further investigation.

The data regarding changes in MC (table 4) showed either degeneration of mitochondria or less mitochondria biogenesis in liver, heart, skeletal muscles and lungs in rats fed AW diet. It seems that carnitine and its precursor lysine besides involved in the transport of fatty acids across the mitochondrial membrane also provide favourable conditions for mitochondria biogenesis.

Acknowledgement

Authors are thankful to Dr Rattan Singh, Prof-cum-Head, Department of Biochemistry, Punjab Agricultural University, Ludhiana, for providing research facilities.

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(Received September 13, 1982)

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