THE EFFECT OF HIGH AND LOW ERUCIC ACID RAPESEED OIL ON ENERGY METABOLISM IN CHICKS

By Thomas Clandinin[1], Sheila M. Innis[2] and Ruth Renner[2]

[1]University of Toronto, Toronto and [2]University of Alberta, Edmonton, Canada

INTRODUCTION

Previous studies (1) have shown that isocaloric substitution of 20 parts HER for SFO in the chicks' diet under conditions of equalized nutrient intake results in decreased fat deposition and efficiency of energy utilization when energy gained per unit of energy consumed was used as the criterion of efficiency. When HER containing diets were fed ad libitum to the chick at lower dietary oil levels analogous changes in energetic efficiency were indicated (2).

In the diet of the rat substitution of HER for SFO has been associated with lower energetic efficiency and increased rate of oxygen consumption both attributed to uncoupling of oxidative phosphorylation (3). Impaired energy utilization has been observed for the rat in some (4, 5) but not all (6) short-term studies of oxidative phosphorylation by cardiac mitochondria isolated from rats fed diets containing HER. Prolonged dietary feeding of HER and LER alters the efficiency of oxidative metabolism of several mitochondrial substrates in vitro (7, 8). Other evidence suggesting that dietary rapeseed oils induce transitions in mitochondrial function arises from studies to determine the mechanism of triglyceride accumulation in cardiac tissue of rats fed diets containing HER, (reviewed by Rocquelin, 9).

Some minor degree of cardiac fat accumulation may occur in the growing chick fed HER containing diets (10), however, only factors that directly affect the metabolic mechanisms of energy conservation could conceivably be responsible for the reduced energetic efficiences observed for longer term feeding of HER containing diets (1). The following studies were therefore conducted to determine whether energy utilization in chicks fed diets containing rapeseed oil is associated with dietary fat induced transitions in mitochondrial metabolism and concomitant alterations in metabolic conservation of energy. In addition the effect of feeding rapeseed oils on the fatty acid composition of mitochondrial lipids was examined.

MATERIALS AND METHODS

Chicks were fed diets containing 20 parts of HER (36.5% $C_{22:1}$), LER (2.5% $C_{22:1}$) or SFO. The composition of the diets fed together with the method used in their formulation has been reported previously (1). The chicks were housed in electrically heated, thermostatically controlled battery brooders in a temperature-controlled laboratory. Two experiments in which each diet was fed ad libitum to quadruplicate groups of ten male crossbred (Dominant White x White Plymouth Rock) chicks from 4 to 28 days of age were conducted. In addition, in each experiment quadruplicate groups of ten chicks were pair-fed diets containing 20 parts of HER, LER or SFO from 4 to 27 days, their feed intakes being restricted to that of chicks fed diets containing 20 parts HER. The methods of processing excreta, conducting chemical analyses for moisture, nitrogen, combustible energy, fat and chromium oxide and for computing metabolizable energy, from the data, have been described previously (1).

Ad libitum fed chicks were killed by cervical dislocation at 28 days of age. As each group of ten chicks was sacrificed, the hearts were immediately excised, surplus fat and atrial tissue removed and mitochondria isolated from the ventricular muscle by the established procedures described by Dow (11, 12). Oxygen uptake studies were completed within 1 hr. of having sacrificed the first chick of the group concerned. The rate of oxygen utilization and ADP/O ratio were measured with pyruvate (10mM), malate (2 mM) plus malonate (10 mM) as substrates for oxidative phosphorylation in a reaction mixture previously described (8, 13). Three repeated analyses from each mitochondrial isolate were made, however, only determinations of separate mitochondrial isolates contributed to the degree of freedom for statistical analysis.

The chicks pair-fed were killed by cervical dislocation, the hearts immediately excised, cardiac mitochondria isolated and immediately fractionated with digitonin in order to prepare the inner mitochondrial membrane matrix compartment isolated as described by Schnaitman and Greenawalt (15). The inner membrane matrix compartment was used as the source of membrane lipids for further study. For fatty acid analysis of membrane lipids aliquots of the inner mitochondrial membrane matrix fraction (100 mg protein/ml) were extracted in 20 ml of chloroform-methanol (2/1) with ethoxyquin (50 ug) added, followed by chloroform-methanol-aqueous ammonia (35/5/2) (16). Extracts were used for purification of membrane phospholipids by thin layer chromatography. Individual phospholipids were separated from total phospholipids on silica gel H plates using the following solvent systems; chloroform:methanol: 25% aqueous ammonia (14/6/1) and chloroform:methanol:glacial acetic acid:water (80/13/0.3), and identified by co-chromatography with authentic compounds. Fatty acid methyl esters of membrane lipid extracts were prepared with boron trifluoride-methanol reagent (17). Methyl-esters were analyzed by gas-liquid chromatography using three meter glass columns (3 mm I.D.) packed with silar-5 CP (10% w/w) coated on acid-washed chromosorb W (80-100 mesh). The results were analyzed by analyses of variance and treatment differences were evaluated by Duncan's multiple range rest (14).

RESULTS AND DISCUSSION

Analyses of variance showed that when fed ad libitum, chicks fed diets containing HER consumed fewer calories, grew at a slower rate and had larger hearts than chicks fed comparable diets containing either LER or SFO (Table 1). These results also illustrate that chicks fed diets containing LER ate less and grew at a slower rate than SFO chicks, but, heart size was not significantly different.

TABLE 1

WEIGHT GAIN, ENERGY CONSUMPTION, ENERGETIC EFFICIENCY AND HEART SIZE OF CHICKS FED THE EXPERIMENTAL DIETS AD LIBITUM FOR 24 DAYS

Diet	Weight gain (g)	Energy consumption[1] (kcal)	Energetic efficiency (g gain/kcal consumed)	Heart size[2] (mg/g)
HER	740^{a}[3]	3670^{a}	$.202^{a}$	6.96^{a}
LER	842^{b}	4075^{b}	$.206^{b}$	6.26^{b}
SFO	895^{c}	4280^{c}	$.210^{b}$	5.86^{b}

1 Calculated using determined metabolizable energy values for the diets
2 mg heart/g body weight
3 Values are averages of duplicate experiment. Values without a common superscript are significantly different ($P<0.05$).

Data on body fat deposition and energetic efficiency (Table 2) clearly show that the reduction in energetic efficiency, when either HER or LER is substituted isocalorically for SFO in the diet of the chick, is not limited to myocardial tissue and may be a more widespread phenomena affecting whole body metabolism. Specifically, the results of the pair-feeding studies show that chicks fed diets containing HER deposited less fat and utilized energy less efficiently (kcal gained/kcal consumed) than chicks fed diets containing either LER or SFO. Energetic efficiency and fat deposition of chicks fed diets containing LER were significantly lower than chicks fed diets containing SFO but greater than when HER was fed.

Oxidative activity of mitochondria isolated from cardiac muscle of chicks fed diets containing 20 parts SFO, HER or LER ad libitum is shown in Table 3. Rates of oxygen uptake and ATP synthesis observed are similar in magnitude to those previously reported for rat cardiac mitochondria isolated in the presence of heparin (6, 8). Results utilizing cardiac mitochondria isolated from chicks fed diets containing 20 parts of either HER or LER for 24 days show significantly reduced ADP/O ratios and reduced rates of ATP synthesis when compared with mitochondria isolated from chicks fed diets containing SFO. This reduction in the efficiency of metabolic conservation of energy when pyruvate was provided as the respiratory substrate was similar irrespective of whether the diets fed contained HER or LER.

TABLE 2

WEIGHT GAIN, ENERGY CONSUMPTION AND ENERGY UTILIZATION OF CHICKS PAIR-FED THE EXPERIMENTAL DIETS FOR 23 DAYS

Diet	Weight gain (g)	Energy consumption[1] (kcal)	Fat gain (g)	Protein gain (g)	Energy utilization[2] (kcal gained/kcal consumed)
HER	713[b,3]	3545[b]	72.8[a]	125.9	0.395[a]
LER	686[a]	3360[a]	78.0[b]	123.2	0.426[b]
SFO	663[a]	3285[a]	82.5[c]	125.8	0.454[c]

[1]Calculated using determined metabolizable energy values for the diets

[2]Kilocalories of energy gained/kilocalorie of metabolizable energy consumed

[3]Values are averages of duplicate experiments. Values without a common superscript are significantly different ($P < 0.05$).

TABLE 3

OXIDATIVE ACTIVITY OF CARDIAC MITOCHONDRIA ISOLATED FROM CHICKS FED HER, LER OR SFO CONTAINING RATIONS

Diet	State 3 (ng atoms O/mg protein/min)	State 4 (ng atoms O/mg protein/min)	ADP/O	ATP Synthesized (nmole/mg protein/min)
HER	253	88	2.06[a]	517[a]
LER	298	103	1.93[b]	575[a]
SFO	282	90	2.53[b]	691[b]
GRAND MEAN	278	93.5	2.17	594
STANDARD ERROR	17.1	3.9	0.09	25.1

Comparison of treatment means was conducted using Duncan's new multiple range test(14). Within a column values without a common superscript are significantly different ($p < 0.05$) n = 24.

Decreased efficiency of caloric utilization observed for chicks fed HER and LER containing diets is related to the efficiency of mitochondrial oxidative phosphorylation. Preliminary investigations of oxidative respiration by skeletal muscle mitochondria isolated from the perioneus longus and tibialis anterior muscles further supports this concept.

Concomitant with the dietary fat induced changes in metabolic conservation of energy, results illustrating analogous transitions in the fatty acid composition of mitochondrial membrane phospholipids further supports the concept of modification of mitochondrial functions by dietary fat. Feeding of HER or LER containing diets to growing chicks relative to SFO-fed controls, resulted in changes in the fatty acid composition of some of the membrane phospholipids. Rapeseed oil induced changes in fatty acid composition of total lipid extracts were characterized by decreased $C_{18:2}$ content and increased membrane n-9 fatty acid level by virtue of increased concentrations of $C_{18:1}$ and $C_{22:1}$. Significant changes in the n-9 fatty acid content, level of saturates of $C_{18:2}$content of phosphatidyl choline or phosphatidyl ethanolamine were not observed. Erucic acid was not incorporated in significant levels into chick mitochondrial phosphatidyl choline or phosphatidyl ethanolamine. The fatty acid composition of diphosphatidyl-glycerol was markedly affected by dietary rapeseed oil feeding. Notably a decreased level of $C_{18:2}$ with corresponding increases in $C_{20:1}$ and $C_{22:1}$ content were observed for cardiolipin of chicks fed diets containing HER or LER when compared to chicks fed SFO containing diets. These basic changes in the molecular components of diphosphatidyl glycerol, when combined with observations concerning efficiency of energy utilization in the growing chick are indicative of a complex dynamic mechanism associating mitochondrial structural-functional transitions to dietary fatty acid balance. Similar modification of mitochondrial structure and function by dietary fat has recently been indicated for the rat (7, 8, 18, 19).

ACKNOWLEDGEMENTS

The support of this work by the National Research Council of Canada, the Rapeseed Association of Canada and the Alberta Agricultural Research Trust is gratefully acknowledged.

REFERENCES

1) Clement, H. and R. Renner, 1977. J. Nutr. 107: 251-260.

2) Vogtmann, H., D.R. Clandinin and A.R. Robblee, 1973. Nutr. Metab. 15: 252-266.

3) Hornstra, G., 1972. Nutr. Metab. 14: 282-297.

4) Houtsmuller, U.M.T., C.B. Struijk and A. Van der Beek, 1970. Biochim. Biophys. Acta. 218: 564-566.

5) Hornstra, G, 1972. Nutr. Metabol. 14: 282-297

6) Dow-Walsh, D.S., S. Mahadevan, J.K.G. Kramer and F.D. Sauer, 1975. Biochim. Biophys. Acta. 396: 125-132.

7) Hsu, Chi Ming Lee and F.A. Kummerow, 1977. Lipids 12: 486-494.

8) Clandinin,M.T., 1978. J. Nutr. 108: 273-281.

9) Rocquelin, G., 1977. Med. et Nut. 13: 269-275.

10) Kramer, J.K.G. and H.W. Hulan, 1977. Can.J. Anim. Sci. 57: 305-312.

11) Dow, D.S., 1967. Biochemistry 6, 2915-2922.

12) Dow, D.S., 1967. Biochemistry 6, 3350-3355.

13) Chance, B. and G.R. Williams, 1956. Adv. Enzymol. 17: 65-134.

14) Steel,R.G.D. and J.H. Torrie, 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc., New York.

15) Schnaitman, C. and J.W. Greenawalt, 1968. J. Cell Biol. 38: 158-175.

16) Awasthi, Y.C., T.F. Chuang, T.W. Keenan and F.L. Crane. 1970. Biochem. Biophys. Res.Commun. 39: 822-832.

17) Metcalfe, L.D. and A.A. Schmitz, 1961. Anal. Chem. 33: 363-364.

18) Blomstrand, R. and L. Svensson, 1975. Acta Medica Scandinavia Supp. 585, 51-73.

19) Clandinin, M.T., 1976. FEBS Letters 68, 41-44.