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Effect of feeding *Treculia africana* seed protein on heart lipids of rats

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Feeding of cooked defatted *Treculia africana* seeds at 5, 10 and 15% protein levels to young rats for a period of 6 weeks significantly affected the heart lipids as compared to rats fed casein at 10% levels. There was a significant increase in heart total lipids, free and esterified cholesterol, total phospholipids, phosphatidyl choline, phosphatidyl ethanolamine and phosphatidic acid + polyglycerophosphatide fraction in rats fed the cooked seeds of *Treculia africana* at 10% protein level.

Feeding of cooked seed protein at 5 and 15% levels reduced free cholesterol and increased triglycerides, as compared to the control.

Total phospholipids, phosphatidyl choline and phosphatidic acid + polyglycerophosphatide were decreased in rats fed cooked seeds of *Treculia africana* at the 5% level.

An increased incorporation of palmitate-1-^{14}C glucose-U-^{14}C and $NaH_2{}^{32}PO_4$ was observed in heart lipids of rats fed cooked seed protein as compared to rats fed casein.

INTRODUCTION

In severely malnourished children, reduction in heart size and cardiac output, prolongation of systemic recirculation and bradycardia have been observed (Alledyne, 1966). There is serious electrolyte disturbance notably of potassium and magnesium (Montgomery, 1960; Garrow, 1965) and there is an uncoupling of oxidative phosphorylation in mitochondria in protein malnutrition (Vitale *et al.*, 1957).

The cooked seeds of *Treculia africana* are good sources of protein and certain essential nutrients, e.g. vitamins and minerals (Lawal & Bassir, 1987). The cooked seeds can be considered an important contributor to the dietary requirements of the people of Nigeria.

Adequate information on the effect of certain plant dietary proteins (the cooked seeds of *T. africana*) on heart lipid is lacking. In the experiment reported here, the effect of feeding defatted cooked seeds of *Treculia africana* at 5, 10 and 15% protein levels has been studied and compared with rats fed casein at the 10% level.

MATERIALS AND METHODS

The fruit of *Treculia africana*

The fruits of *Treculia africana* were obtained from Ishara-Remo in Ogun State of Nigeria where they are commonly found.

Ten large fresh fruits, each about 9 kg in weight and 0·45 m in diameter, were allowed to soften by placing them in water in large containers. After 5 days, the seeds, which were embedded in the spongy pulp of the fruits, were extracted. These raw seeds were washed with water and then boiled for 15 min. The boiling water was discarded and the seeds were allowed to cool. Then the boiled seeds were dried in the sun for 2 days, after which the seed-coats were removed with the aid of small stones, thus exposing the white cotyledons.

The cooked seeds of *Treculia africana* were obtained by boiling the 'testa-free' seeds (white cotyledons) for about 1 h until the cotyledons became softened. The soft cotyledon material was then broken up into small pieces and made into a paste.

The procedures carried out for the determination of

the moisture and protein contents were based on the standard technique adopted by the Association of Official Analytical Chemists (AOAC, 1980).

The cooked seeds of *Treculia africana* were defatted using the Soxhlet extraction method and chloroform as the solvent.

Male rats (Wistar Strain from the University of Ibadan, Faculty of Veterinary Medicine Colony) were used in this investigation. They were divided into four groups of 24 rats each. Three groups of rats were fed defatted cooked seeds at 5, 10 and 15% protein levels respectively for 6 weeks. To the fourth group, casein was fed at 10% level for 6 weeks and this group served as the control.

At the end of 6 weeks, rats in each group were further subdivided into four groups of six rats each. Rats of each group were intraperitoneally injected with acetate-1-^{14}C (10 μCi/100 g body weight), palmitate-1-^{14}C (10 μCi/100 g body weight), glucose-U-^{14}C (10 μCi/100 g body weight) and $NaH_2{}^{32}PO_4$ (10 μCi/100 g body weight) respectively.

Rats were sacrificed 3 h after the injection of the isotopes except for the palmitate-1-^{14}C group, which were sacrificed 20 min after the injection of the isotope. The rats in each group were sacrificed by cervical dislocation and hearts were quickly removed, immersed in ice-cold saline, cleaned, weighed, and processed for lipid isolation (Misra, 1964).

The lipid extracts were freed of non-lipid impurities and proteolipids. The final solutions of heart lipids were made in a known volume of chloroform and stored at −20°C until used for analysis. All the solvents used were freshly distilled.

Analysis

Heart lipid extracts were assayed for total lipids (gravimetrically), total cholesterol (Hanel & Dam, 1955), total phospholipids (Barlett, 1959) and total glycerides by difference (total lipids − total cholesterol + phospholipids) and by glyceride–glyceride estimation (Van Handel & Zilversmit, 1957).

The ^{14}C and ^{32}P radioactivities of heart lipid extracts were determined in an end-window GM Counter (Tracer Laboratories, Waltham, MA, USA).

Heart lipids were further fractionated into individual neutral lipid and phospholipid components by means of TLC (Abramson & Blecher, 1964).

The neutral lipid components—free and esterified cholesterol and mono-, di- and triglycerides—were quantitated after elution from the gel by their respective chemical assays and phospholipids by the estimate of phosphate (Misra, 1969).

The recoveries of neutral lipids from the TLC plate were between 85 and 90% and of phospholipids between 95 and 98% respectively.

RESULTS AND DISCUSSION

Body weights of rats fed the cooked seeds of *Treculia africana* proteins were significantly lower than those fed casein (Table 1). The results of total lipids have been expressed both on a unit tissue weight and a unit body weight basis.

Heart weight of rats fed the cooked seeds of *Treculia africana* at 5 and 10% protein levels was significantly reduced as compared to the control (Table 1).

Feeding of the cooked seeds at 5, 10 and 15% protein levels resulted in profoundly retarded growth of rats, as compared to the control.

On a unit tissue weight basis, heart total lipids, cholesterol and phospholipids showed significant increases in rats fed cooked seeds of *Treculia africana* at 10% protein levels as compared to the control. These lipids either remained unchanged or were reduced in rats fed the cooked seeds of *Treculia africana* at 5% and 15% levels respectively (Table 2).

Total glycerides, on the other hand, remained unchanged at 10% protein levels, but showed a significant increase in rats fed cooked seeds of *Treculia africana* at 5 and 15% protein levels respectively as compared to the control (Table 3).

Significant alterations were observed in the quantities

Table 1. Body weight and heart weight of rats fed the cooked seeds of *Treculia africana*. Values are mean ± SE for 24 rats in each group. Values significantly different from controls are shown clearly.

	Control	5%	10%	15%
Body weights (g)				
Initial	94·0 ± 2·5	95·0 ± 3·0	95·3 ± 2·4	96·0 ± 3·6
Final	155·5 ± 5·5	113·5 ± 2·2	136·8 ± 4·2	152·0 ± 3·5
Difference (g)	+61·5	+18·5	+41·5	+56·0
Total heart weight	0·40 ± 0·001	0·30 ± 0·01 ($P < 0·01$)	0·35 ± 0·01 ($P < 0·02$)	0·39 ± 0·02
Heart weight per 100 g body weight	0·27 ± 0·07	0·37 ± 0·01 ($P < 0·001$)	0·32 ± 0·01 ($P < 0·001$)	0·36 ± 0·01 ($P < 0·001$)

Table 2. Heart lipids of rats fed the cooked seeds of *Treculia africana*. Values are mean ± SE from 24 rats in each group. Values significantly different from controls are indicated in brackets

	Control	5%	10%	15%
Total lipids[a]	32·40 ± 0·20	33·60 ± 0·6	40·6 ± 0·90 ($P < 0·001$)	32·4 ± 1·7
Total cholesterol[a]	2·20 ± 0·04	1·81 ± 0·05 ($P < 0·001$)	3·3 ± 0·09 ($P < 0·001$)	0·9 ± 0·05 ($P < 0·001$)
Total phospholipids[a]	23·6 ± 0·3	22·2 ± 0·4 ($P < 0·05$)	30·1 ± 0·6 ($P < 0·001$)	22·4 ± 1·4
Total glycerides[a]	6·6 ± 0·3	9·8 ± 0·2 ($P < 0·01$)	7·2 ± 1·6	9·1 ± 0·6 ($P < 0·001$)
Total lipids[b]	8·6 ± 0·09	12·2 ± 0·2 ($P < 0·001$)	12·2 ± 0·3 ($P < 0·001$)	10·5 ± 0·2
Total cholesterol[b]	0·6 ± 0·01	0·66 ± 0·02	1·0 ± 0·04 ($P < 0·001$)	0·3 ± 0·13
Total phospholipids[b]	6·2 ± 0·08	8·0 ± 0·1 ($P < 0·001$)	9·2 ± 0·2 ($P < 0·001$)	7·2 ± 0·2 ($P < 0·01$)
Total glycerides[b]	1·8 ± 0·07	3·5 ± 0·01 ($P < 0·001$)	1·90 ± 0·4	2·9 ± 0·1 ($P < 0·001$)

[a] Values are expressed in mg/g wet wt.
[b] Values are expressed in mg/100 g body weight.

Table 3. Heart neutral lipids[a] of rats fed the cooked seeds of *Treculia africana* protein. Values are expressed as mean ± SE from 24 rats in each group. Values that are significantly different from control are indicated by $P < 0·05$, or $P < 0·02$ or $P < 0·01$ or $P < 0·001$

Neutral lipids	Control	5%	10%	15%
Free cholesterol	1·9 ± 0·04	1·2 ± 0·04 ($P < 0·01$)	2·9 ± 0·9 ($P < 0·001$)	0·7 ± 0·04 ($P < 0·001$)
Esterified cholesterol	0·3 ± 0·02	0·6 ± 0·01 ($P < 0·001$)	0·4 ± 0·02 ($P < 0·05$)	0·20 ± 0·08 ($P < 0·001$)
Monoglycerides	1·4 ± 0·09	2·5 ± 0·1 ($P < 0·001$)	1·36 ± 0·2	2·2 ± 0·14 ($P < 0·001$)
Diglycerides	1·4 ± 0·06	2·17 ± 0·05 ($P < 0·001$)	1·38 ± 0·2	2·4 ± 0·14 ($P < 0·001$)
Triglycerides	3·70 ± 0·17	5·7 ± 0·3 ($P < 0·001$)	3·6 ± 1·1	4·6 ± 0·27 ($P < 0·02$)

[a] Results expressed as mg/g wet tissue weight.

Table 4. Heart phospholipids[a] of rats fed the cooked seeds of *Treculia africana* protein. Values are expressed as mean ± SE from 24 rats in each group. Values significantly different from control are shown by $P < 0·05$, or $P < 0·02$ or $P < 0·01$ or $P < 0·001$

Phospholipids	Control	5%	10%	15%
Monophosphatidylinositol	14·4 ± 0·6	31·4 ± 2·0 ($P < 0·001$)	20·3 ± 4·4	26·1 ± 1·8 ($P < 0·001$)
Phosphatidylserine + lysophosphatidyl choline	33·0 ± 1·1	72·0 ± 4·0 ($P < 0·001$)	68·0 ± 6·0 ($P < 0·001$)	29·0 ± 3·0
Lysophosphatidyl ethanolamine + sphingomyelin	65·4 ± 4·0	80·3 ± 1·8 ($P < 0·01$)	135 ± 9·0 ($P < 0·001$)	97·0 ± 8·0 ($P < 0·01$)
Phosphatidyl choline	348·0 ± 13·0	281·0 ± 4·0 ($P < 0·001$)	607 ± 51 ($P < 0·001$)	324 ± 19
Phosphatidyl ethanolamine	347 ± 6·0	324·0 ± 13	510 ± 40 ($P < 0·01$)	306 ± 22
Phosphatidic acid + polyglycerophosphatide	135·0 ± 9·0	101 ± 6·0	199 ± 20 ($P < 0·01$)	118 ± 8·0

[a] Results expressed as μg phospholipid/g wet tissue weight.

Table 5. Incorporation of acetate-1-^{14}C, palmitate-1-^{14}C. glucose-U-^{14}C and $NaH_2{}^{32}PO_4$ into heart total lipids (counts/min/heart/100 g body weight) of casein fed and the cooked seeds of *Treculia africana* protein-fed rats

Incorporation of:	Control	5%	10%	15%
Acetate-1-^{14}C	$0{\cdot}5 \times 10^3$	$0{\cdot}5 \times 10^3$	$0{\cdot}25 \times 10^3$	$0{\cdot}45 \times 10^3$
Palmitate-1-^{14}C	$0{\cdot}1 \times 10^3$	$7{\cdot}5 \times 10^3$	$0{\cdot}75 \times 10^3$	$0{\cdot}46 \times 10^3$
Glucose-U-^{14}C	$0{\cdot}25 \times 10^3$	$1{\cdot}5 \times 10^3$	$0{\cdot}25 \times 10^3$	$1{\cdot}5 \times 10^3$
$NaH_2{}^{32}PO_4$	$2{\cdot}2 \times 10^3$	$5{\cdot}0 \times 10^3$	$3{\cdot}75 \times 10^3$	$4{\cdot}0 \times 10^3$

of all phospholipid components of the heart as a result of feeding cooked seeds of *Treculia africana* at different protein levels as compared to controls (Table 4).

The major phospholipids of heart phosphatidyl choline and phosphatidyl ethanolamine were significantly increased in the 10% group and the former were reduced in the 5% group as compared to the control.

Other heart phospholipids showed an increase in the 5% and 10% groups but on increasing the dietary protein level their amounts were reduced to control values (Table 4).

The heart is known to utilize free fatty acids as a source of energy (Fritz, 1961) and to synthesize lipids from suitable precursors (Stein & Stein, 1966) and also has a lipoprotein lipase activity (Schnatz *et al.*, 1963). However, under normal conditions carbohydrate is the preferred source of energy.

Witteles & Bresler (1964) suggested that the heart takes up free fatty acids from plasma and uses them for energy. During fasting, increases in myocardial triglycerides and plasma free fatty acids have been demonstrated in guinea pigs (Wittles & Bresler, 1964).

Chronic administration of alcohol has been shown to increase heart glycerides without any changes in lipogenic enzymes (Regan *et al.*, 1966) suggesting an esterification process to be responsible for glyceride increase.

The alterations noted in heart neutral lipid and phospholipid components of rats fed cooked seeds of *Treculia africana* at different protein levels, can arise from either an increased synthesis and/or decreased breakdown.

Martinetti *et al.* (1958) pointed out that the synthesis of triglycerides and phospholipids in heart takes place by pathways similar to liver.

Table 5 shows that the incorporation of acetate-1-^{14}C into heart lipids of rats fed cooked seeds of *Treculia africana* protein was lower than the control, indicating that the lipogenesis is not contributing a great deal towards the increased levels of triglycerides. However, the incorporation of palmitate-1-^{14}C and glucose-U-^{14}C was markedly increased in rats fed cooked seeds of *Treculia africana* protein. These results indicate an enhanced esterification of long chain fatty acids with α-glycerophosphate made available by the enhanced utilization of glucose (Misra *et al.*, 1974). Similar results obtained for the incorporation of $NaH_2{}^{32}PO_4$ may indicate an increased turnover of heart phospholipids.

The increases observed in heart triglycerides and phospholipids are perhaps due to their increased synthesis.

SUMMARY

1. The metabolism of heart lipids in relation to dietary protein obtained from the cooked seeds of *Treculia africana* has been shown from this experiment.
2. The feeding of defatted cooked seeds of *Treculia africana* at 5, 10 and 15% protein levels to young rats for a period of 6 weeks significantly affected the heart lipids as compared to the control.
3. Increases observed in heart triglycerides and phospholipids might be due to their increased synthesis in the heart.

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