



EFFECT OF DIETARY FAT MANIPULATION AND CALORIE RESTRICTION ON COLLAGEN IN AGEING SKELETAL MUSCLE*

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ABSTRACT

The effect of short term dietary manipulation of fat with or without calorie restriction (at 40% level) on the collagen to minimize ageing process in skeletal muscle was studied. Twenty four male wistar albino rats were equally distributed as Group I, Group II, Group III and Group IV. Animals in the Group I and Group III were fed with diets containing linoleic acid low (coconut oil) and linoleic acid rich (corn oil) ad libitum, whereas those in Group II and IV were fed the same diets at 40 per cent calorie restriction respectively from 18 to 24 months of age with free access to water. The rats were sacrificed at the end of 24th month and skeletal muscle was collected for analysis collagen. Biochemical estimation of collagen was done. Collagen level was found to be significantly increased in both ad libitum fed groups. This increase in collagen level can be attributed to the premature ageing of skeletal muscle accelerated by free radicals in adlibitum fed rats. The collagen level in the skeletal muscle of senescent rats was found to be decreased in both the calorie restricted diets. Histological and histochemical studies on the section of gastrocnemius muscle were taken up to reveal the dietary effects on muscle collagen distribution.

KEYWORDS- Collagen, ageing, calorie restriction, skeletal muscle

INTRODUCTION

Collagen is the major insoluble fibrous protein in the extracellular matrix which helps the tissues to withstand stretching with great tensile strength. Collagen with other proteins of the muscle provides firmness, strength and resilience. As it is a macromolecule with complicated structure it is the target of ROS, due to this the structure become more complex with cross-linking of proteins hence it affects contractibility and excitability of the muscle.

MATERIALS AND METHODS

A trial was conducted in 24 wistar albino rats to study the effect of level of linoleic acid with or without calorie restriction on ageing skeletal muscle collagen architecture. The rats were divided into four equal groups, viz. group I to IV. Coconut oil (low linoleic acid content) was fed to groups I and II at adlibitum and 40% calorie restriction respectively. Similarly linoleic acid rich corn oil was fed to rats of group III and IV at adlibitum and 40% calorie restriction respectively. The trial was approved by the IAEC. The rats were fed with these diets from 18 to 24 months of age.

Group	Diet	No. of Animals
Group I	Low Linoleic acid diet	6
Group II	Low Linoleic acid diet+ Calorie restriction	6
Group III	Linoleic acid rich diet	6
Group IV	Linoleic acid rich diet + Calorie restriction	6
	Total	24

The rats were sacrificed at the age of 24 months. The gastrocnemius muscle was collected for histological and histochemical analysis of collagen as per the procedure of Neuman and Logan (1950). Collagen was estimated measuring hydroxyl proline which is the functional component of collagen. Histology of collagen was carried out from paraffin embedded 5-6 µm sections of gastrocnemius muscle using Masson's trichrome staining technique and histochemistry by per-iodic acid-Schiff method (PAS) (Luna, 1968).

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The data were subjected to the statistical analysis by completely randomized design, as per the method of Snedecor and Cochran (1989). Groups were compared by one way analysis of variance (ANOVA) and by the post hoc Duncan test, with the level of significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Biochemical analysis of collagen revealed feeding of coconut oil at 40% calorie restriction reduced collagen levels in ageing skeletal muscle compared to corn oil fed rats at both levels. This was evident in histological and histochemical analysis presented in plates 1 to 4.

Increased free radical production in senescent rat skeletal muscle causes increased accumulation of collagen due to the cross-linking of collagen molecules. The amount of collagen in skeletal muscle is related to its function. Increased amount of collagen cause more rigidity and toughness to skeletal muscle.

In the present study, the collagen level was found to be significantly increased in both *ad libitum* fed groups (Group II & IV). This increase in collagen level can be attributed to the premature ageing of skeletal muscle due to *ad libitum* feeding. Haseeb and Patnaik (1978) also observed a similar increase in

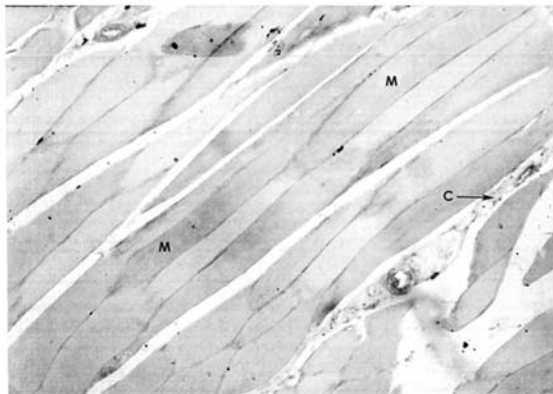


Plate 3: Photomicrograph of gastrocnemius muscle and collagen fibres in 24 month-old male rats fed *ad libitum*.
M-Gastrocnemius muscle
C-Collagen fibres.

PAS - 100x

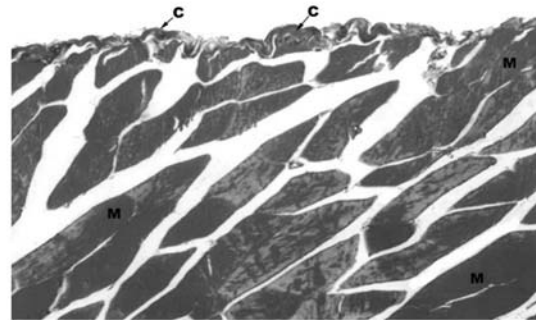


Plate 1: Photomicrograph of gastrocnemius muscle and collagen fibres in 24 month-old male rats fed *ad libitum*.
M-Gastrocnemius muscle
C-Collagen fibres.

Masson's trichrome 200x

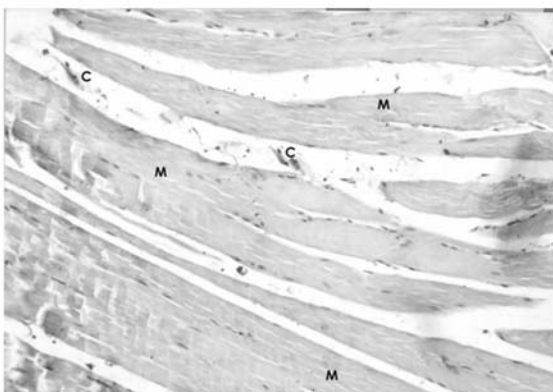


Plate 4: Photomicrograph of gastrocnemius muscle and collagen fibres in 24 month-old male rats fed with calorie restricted diet.
M-Gastrocnemius muscle
C-Collagen fibres.

PAS - 100x

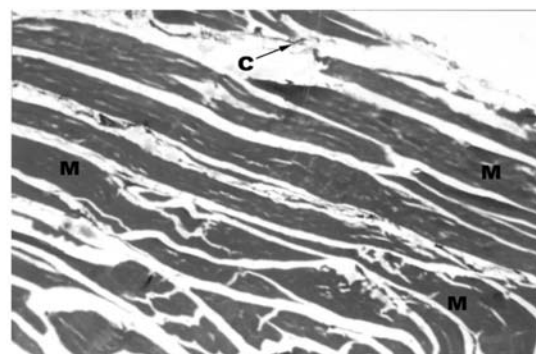


Plate 2: Photomicrograph of gastrocnemius muscle and collagen fibres in 24 month-old male rats fed with calorie restricted diet.
M-Gastrocnemius muscle
C-Collagen fibres.

Masson's trichrome 200x



collagen level in male garden lizard and Alnaqeeb *et al.* (1984) reported an increase in collagen accumulation within skeletal muscle associated with ageing skeletal muscle. The cross-linking of collagen molecules makes it more resistant to degradative enzymes. (Mohan and Radha, 1980).

The collagen level in the skeletal muscle of senescent rats was found to be decreased in both the calorie restricted diets as reported by Boreham *et al.* (1988) in calorie restricted diets. This may be due to the effect of calorie restriction in retarding protein oxidation and cross-linking as reported by Zainal *et al.* (2000). Among the calorie restricted rats the collagen level was found to be lower in the rats fed with coconut oil compared to the corn oil. It was postulated that the composition of fatty acids (medium chain) in coconut oil preserves the membrane from oxidative damage and discourages free radical production.

Histological studies of gastrocnemius muscle in 24 month-old rats with *ad libitum* feeding of both corn oil and coconut oil confirmed higher collagen level by Masson's trichrome staining (Luna, 1968). Histochemical techniques using periodic acid-Schiff (PAS) staining further confirmed the collagen levels in *ad libitum* groups as PAS positive than calorie restricted group which recorded PAS negative, as reported by Bancroft and Gamble (2008) that the type IV collagen would be strongly positive to PAS method. Further it was stated by Ahtikoski *et al.* (2003) that type IV collagen is a major protein in the basement membranes surrounding and supporting skeletal muscle cells of gastrocnemius. Type IV collagen is closely associated with significant amount of carbohydrate complexes which gives strong reaction with PAS method. It strengthens the observation of this study that ageing profoundly affects cross-linking of proteins in skeletal muscle by increasing the collagen sheets which accounts for

poor contractibility and excitability. Both the calorie restricted groups revealed reduced collagen deposition.

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