

Full Length Research Paper

Unutilized energy reserves and mineral contents of fibroid tissues suggesting perturbed membrane transport processes

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Compositions of intramural uterine fibroid (IUF) and subserous uterine fibroid (SUF) tissues were studied. Results showed that IUF and SUF, respectively, contained (%) 77.26 ± 0.05 and 77.49 ± 0.02 moisture and 22.74 ± 0.05 and 22.52 ± 0.02 dry matter, on wet-weight basis. Their respective moisture, crude fat, crude protein, ash and total carbohydrates (%) were 4.67 ± 0.05 and 4.38 ± 0.17, 8.29 ± 0.41 and 5.84 ± 0.43, 72.66 ± 0.72 and 65.16 ± 0.91, 4.39 ± 0.08 and 4.22 ± 0.03 and 10.01 ± 1.18 and 20.40 ± 1.45 while their energy contents (kcal/ 100g) were 405.29 and 394.80, respectively, on dry-matter basis. Their crude fats contained fatty acids, triacylglycerols and cholesterol while their total carbohydrates contained glucose and glycogen. Ca²⁺, Fe²⁺, Zn²⁺, K⁺, Na⁺ and Mg²⁺ contents (mg/ 100g) showed that Na⁺ was the most abundant mineral amongst them unlike the norm in cells where K⁺ is the most abundant. The tissues contained high [Ca²⁺] and [Fe²⁺], low [Zn²⁺], [K⁺] and [Mg²⁺] and comparable [Na⁺] relative to cells. These suggested that Na⁺/ K⁺-ATPase activity may have been decreased or inhibited by either high [oestrogen] which is a pre-disposing factor to fibroid or low [Mg²⁺] which may have decreased or inhibited the activity of the electrochemical Na⁺ gradient-dependent Ca²⁺ transporter. Zn²⁺ transporter activity seemed to have been decreased or inhibited while cellular influx of Fe²⁺ seemed to have been increased. The results showed that the IUF and SUF had unutilized energy reserves and mineral contents suggestive of perturbed membrane transport activities.

Key words: Energy reserve, fibroid, minerals, membrane transport, proximate composition.

INTRODUCTION

The uterus, or womb, is a hollow organ with heavy, muscular walls. It is located between the urinary bladder in front and the rectum behind. It receives the oviduct on the right and on the left of its upper portion and opens into the vagina below through its cervix. It is where the fertilized egg is implanted and the foetus develops prior to its birth. Beyond its role in pregnancy, uterine diseases abound.

One of such uterine diseases is fibroid tumour of the uterus (leiomyomas or fibromyoma). Fibroid can grow in

different parts of the uterus and are named according to what part of the uterus they are found, for instance, subserous (grow in the outer uterine wall), intramural (grow inside or within the uterine wall), submucous (grow in the inner uterine wall), pedunculated (attached by stalk to the outer or inner uterine wall), interligamentous (grow sideways between ligaments which support the uterus in the abdominal region) and parasitic (rarest form which occurs when it attaches itself to another organ) (Cotran et al., 1999; Peddadah et al., 2008; Stewart, 2011). It is hormone dependent. One of such hormones implicated in the etiology of uterine fibroid is high oestrogen concentrations (Baird and Newbold, 2005). High progesterone concentration is also thought to play a role in fibroid

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growth. Histological studies of fibroid tissues from patients treated with progesterone showed more cellular growth than those from patients that were not treated with progesterone (DeCherney and Nathan, 2003). Fibroids are benign (non cancerous) neoplasm. The tumours are found in at least 25 percent of women in active reproductive life and are more common in blacks (Cotran et al., 1999). Tumours of this sort are rarely found elsewhere in the body. Indman (2010) and Layyous (2010) reported that surgery (myomectomy) is a treatment option. They reported that fibroid calcification of the fibroid was due to the deposition of calcium.

Fibroids are thought to be caused by environmental and genetic factors or a combination of them and the exact composition of their tissues are still debatable. Shryock and Swartout (1980) and Layyous (2010) reported that fibroid tumours were composed partly of muscle tissues growing from and resembling the muscle in the walls of the organ; with this special muscle tissue being intermingled with varying amounts of fibrous connective tissue.

The objective of this study was to evaluate some compositions of intramural uterine fibroid (IUF) and subserous uterine fibroid (SUF) tissues. Results of the study may give insights into possible reasons for such compositions.

MATERIALS AND METHODS

Procurement of tissues

The fibroid tissues used were procured with informed consent from ten patients who underwent myomectomy. Patients with IUF generally had more than one fibroid growth of varying sizes than their SUF counterparts.

Histological examination of tissues

Histological study of each representative tissue was carried out using the method of Okoro (2002). The procured and fixed tissues were dehydrated through different grades of alcohol, cleared in xylene, infiltrated with melted paraffin wax and picked on albumenized slides. Staining was done using Haematoxylin-Eosin (H and E), dried and mounted using distrene tricresyl phosphate xylene (DPX). The stained and mounted slides were examined using a light microscope and photographed at a magnification of x400.

Assay for proximate composition

The tissues were analysed for their proximate compositions using the methods of AOAC (1990). The protocol was that tissues were dried at 90°C for 24 h, their fats were exhaustively extracted with petroleum ether (crude fat contents) and the defatted samples used for crude protein and ash determinations. Total carbohydrates were estimated by difference and energy contents (kcal/ 100 g) determined by summing up the products got by multiplying the crude protein, crude fat and total carbohydrates contents by the factors of 4, 9 and 4, respectively, as described by Wardlaw and Kessel (2002).

Detection of some lipid constituents

Their crude fat extracts got above were tested for the presence of fatty acids, triacylglycerols and cholesterol using the soap formation, acrolein and Salkowski tests, respectively, as described by Plummer (1971) and Mathotra (1989).

Detection of some carbohydrate constituents

A quantity, 1.0 g of the respective tissue, was homogenized in 50 ml of ice-cold 5% trichloroacetic acid (TCA) and their filtrates tested for the presence of glucose and glycogen, respectively, using the Barfoed and iodine tests, as described by Plummer (1971).

Assay for some mineral contents

Analyses for some of their mineral contents (mg/100 g) were determined using the mixed-acid digestion and atomic absorption spectrophotometer of 1.0 g wet tissue as described by Allen et al. (1983) and AOAC (1990).

Statistical analysis

The results were compared using the students' t-test of significance at 95 percent confidence limit.

RESULTS

Figure 1A shows that the IUF had larger fibrous stroma than the SUF which appeared muscular. The epithelium was columnar because of the observed numerous long or rectangular nuclei. Tiny papillae were observed. Bundle of smooth muscle was present; appearing white on sectioning before staining.

Figure 1B shows that the SUF had numerous fibrous cysts within the stroma. No papillary projection was observed. Some of the fibrous stroma cells were large. The nuclei were oval shaped. Bundle of smooth muscle was present; appearing white on sectioning before staining.

Table 1 shows the moisture and dry matter contents of IUF and SUF (on wet-weight basis). The results showed that their compositions in the tissues did not vary significantly ($p > 0.05$).

Table 2 shows the proximate composition of the fibroid tissues (on dry-weight basis). Results showed that the IUF contained more ($p < 0.05$) crude fats and proteins than SUF, while the SUF contained more ($p < 0.05$) total carbohydrates than the IUF. Their ash and moisture contents did not vary significantly ($p > 0.05$).

Table 3 shows the qualitative detection of some the tissues' crude fats and total carbohydrate constituents. Results showed that crude fats contained fatty acids, triacylglycerols and cholesterol while the total carbohydrates contained glucose and glycogen.

Table 4 shows some types of minerals that were contained in the tissues. Results indicated that the mineral of highest concentration was Na^+ while the

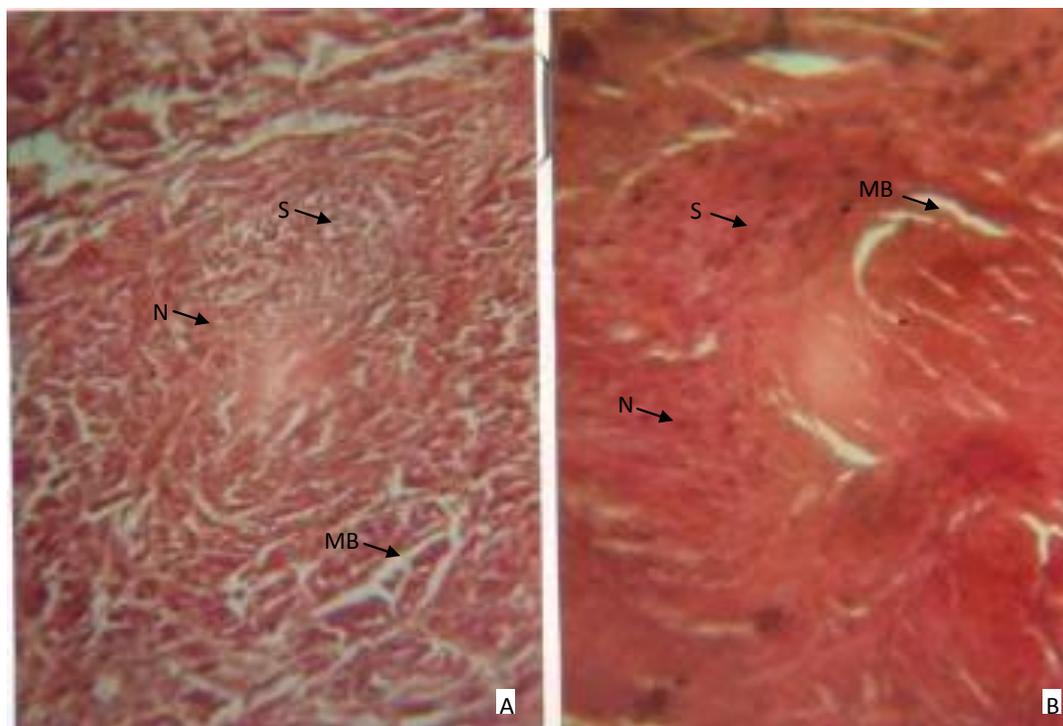


Figure 1. Histomorphology of SUF (A) and IUF (B) S = stroma; N = nucleus; MB = muscle bundle.

Table 1. Moisture and dry matter contents of the fibroid tissues (% wet-weight)*.

| Sample | Moisture | Dry matter |
|--------|--------------|--------------|
| IUF | 77.26 ± 0.05 | 22.74 ± 0.05 |
| SUF | 77.49 ± 0.02 | 22.52 ± 0.02 |

*Values are means ± S.D of duplicate determinations. IUF = intramural uterine fibroid. SUF = subserosal uterine fibroid.

mineral of least concentrations was Zn^{2+} . It also showed that the IUF was less ($p < 0.05$) mineralized than the SUF.

DISCUSSION

The exact compositions of fibroid tissues have been a source of debate among researchers. However, a general assumption is that they may have the same basic compositions of uterine muscle tissues.

The tissues used in the study were confirmed to be fibroids (Figures 1A and 1B). The tissues showed that there may have been changes in hormonal activities because each stroma was composed of somewhat plump fibroblasts resembling the theca.

The moisture contents of the tissues (Table 1) compared favourably with that of the average adult human body (60%) as reported by Tomlinson et al. (1997). Their proximate compositions (Table 2) indicated that high proportions of their dry weights were proteins.

Carbohydrates (Tables 2 and 3) formed lower parts of their compositions. Tomlinson et al. (1997) reported that a small amount of glucose was available to satisfy immediate energy needs and that fat formed a greater part of the body composition of a 65 kg woman. The ash contents of the tissues were the lowest. Major minerals occur in the body in molar quantities; the minor minerals occur in millimolar quantities while the trace elements occur in micromolar quantities but are essential for life (Tomlinson et al., 1997). The study also showed that the tissues contained good energy reserves which the body could have put to good use. Their energy contents were high and should have been very useful to the body if they had not been sequestered in the fibroids. However, these stores of energy can only be made available to the body after menopause when the myomas are expected to regress, diminish or disappear; if surgeries are not performed.

The presence of fatty acids (normally stored in triacylglycerols) and glucose (normally stored as glycogen) (Table 3) indicated that the tissues were

Table 2. Proximate composition (%) and energy contents (kcal/ 100g) of the fibroid tissues (dry matter basis)*.

| Sample | Moisture | Crude fat | Crude protein | Ash | Total carbohydrates | Energy content (kcal/ 100g) |
|--------|-------------|-------------|---------------|-------------|---------------------|-----------------------------|
| IUF | 4.67 ± 0.05 | 8.29 ± 0.41 | 72.66 ± 0.72 | 4.39 ± 0.08 | 10.01 ± 1.19 | 405.29 |
| SUF | 4.38 ± 0.17 | 5.84 ± 0.43 | 65.16 ± 0.91 | 4.22 ± 0.03 | 20.40 ± 1.45 | 394.8 |

*Values are means ± S.D of duplicate determinations.

Table 3. Qualitative detection of some crude fat and total carbohydrate constituents.

| Sample | Fatty acids | Triacylglycerols | Cholesterol | Glucose | Glycogen |
|--------|-------------|------------------|-------------|---------|----------|
| IUF | + | + | + | + | + |
| SUF | + | + | + | + | + |

+ = present.

Table 4. Some mineral contents of the fibroid tissues, wet weight basis (mg/ 100g tissue)*.

| Mineral | IUF | SUF |
|------------------|--------------|--------------|
| Ca ²⁺ | 6.04 ± 0.02 | 9.71 ± 0.01 |
| Fe ²⁺ | 2.67 ± 0.01 | 4.50 ± 0.01 |
| Zn ²⁺ | 2.29 ± 0.00 | 2.91 ± 0.02 |
| K ⁺ | 11.14 ± 0.03 | 36.78 ± 0.01 |
| Na ⁺ | 30.28 ± 0.02 | 52.60 ± 0.02 |
| Mg ²⁺ | 2.84 ± 0.01 | 7.91 ± 0.04 |

*Values are means ± S.D of duplicate determinations.

metabolically active. The oils may explain the oily feel of the tissues. Garrett and Grisham (1999) reported that sugars (a disaccharide of glucose and galactose) were found covalently attached to 5-hydroxylysine residues in the hole regions of collagen. The cholesterol may have been stored, or served as precursors for synthesis of sex hormones. Further research on these constituents is suggested as their detection was exploratory.

The most abundant mineral in the tissues was Na⁺ (Table 4) unlike the norm in cells where the

most abundant mineral is K⁺ (Cooper, 2000; Nelson and Cox, 2000; Devlin, 2006). Cells and tissues maintain the order: [K⁺] > [Na⁺] > [Mg²⁺] > [Zn²⁺] > [Ca²⁺] > [Fe²⁺] as confirmed in the reports of Garrett and Grisham (1999), Cooper (2000), Nelson and Cox (2000), Huerta-Leidenz et al. (2003) and Devlin (2006). The fibroid tissues rather maintained the order: Na⁺ > K⁺ > Ca²⁺ > Mg²⁺ > Fe²⁺ > Zn²⁺. This increased the concentrations of Na⁺, Ca²⁺ and Fe²⁺ and reduced the concentrations of K⁺, Mg²⁺ and Zn²⁺. These

showed that the activities of their Na⁺/ K⁺-ATPases may have been decreased or inhibited by either high oestrogen or low Mg²⁺ concentrations. Davis et al. (1978) reported that synthetic ethinyl oestrogen decreased hepatic Na⁺/ K⁺-ATPase activity and bile flow to 50 percent and altered the composition and structure of surface membrane lipids in rats. Mg²⁺ on its own is required as a cofactor for the phosphorylation of a specific aspartic acid residue on the α - subunit of the Na⁺/ K⁺-ATPase to form a β- aspartylphosphate,

during ion translocation (Garrett and Grisham, 1999; Devlin, 2006). Increasing Mg^{2+} concentrations resulted in a significant activation of Na^+/K^+ -ATPase, which related to Mg^{2+} concentration (Romanini et al., 1991). Mg^{2+} and Ca^{2+} have been reported as universal regulators of the cell and effectively influence the functional activity and conformational states of Na^+/K^+ -ATPase (Kratvsov and Kratsova, 2001). Many membrane transporters depend on the electrochemical Na^+ gradient established when it (Na^+) is transported to the extracellular space by the Mg^{2+} -dependent Na^+/K^+ -ATPase (Cooper, 2000; Devlin, 2006). One of such transporters is the Na^+/Ca^{2+} antiporter. If the activity of the Na^+/K^+ -ATPase is decreased, it would mean that there shall be no Na^+ gradient to drive Ca^{2+} translocation to the cell's exterior milieu. This would mean an increase in cytosolic concentration of calcium, leading to calcification of the cell and tissue in general. Our results (Table 4) showed calcification of the tissues, especially the SUF. Garrett and Grisham (1999) reported that hypertensive patients that had the sodium pump of the cells lining the blood vessel wall inhibited by cardiac glycosides or cardiotoxic steroids accumulated sodium and calcium in those cells. Calcification of fibroid tissue is thought to interfere with enzymes that dissolve the fibrin within, but such calcification disappeared on administration of magnesium supplements (Indman, 2010; Layyous, 2010) which may be due to the restoration of Na^+/K^+ -ATPase activity (since Mg^{2+} is a cofactor) leading to the re-establishment of the electrochemical Na^+ gradient and restoration of Na^+/Ca^{2+} antiporter activity. The tissues' Zn^{2+} and Fe^{2+} contents (Table 4) indicated that cellular influx of Zn^{2+} via the Zn^{2+} transporter may also have been decreased while cellular influx of Fe^{2+} via increased synthesis of transferrin receptors and decreased synthesis of apoferritin may have been increased, especially in SUF. Murray and Stein (1968) though reported that injected oestrogen did not change the absorption of iron by mature female rats. The mineralization of the tissues by calcium, or some other minerals that were not assayed for, may be responsible for the firm texture of the fibroid tissues.

Conclusion

The study showed that the tissues sequestered high amount of energy and contained minerals whose concentrations indicated that the normal activities of their transporters may have been perturbed, either by being decreased, inhibited or increased.

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