tulated by Potts and Eddy[9]. However, the mechanism by which osmotic concentration influences the developing fish embryos remains obscure. When the early embryos were exposed to 10‰ S, the nuclei of the embryonic cells became either obscure or formed multipolar spindles with scattered chromosomes. This suggests that high osmotic concentration impaired the nuclear division of the embryonic cells.

The salinity tolerance of the zebrafish embryos improved with advancing developmental stage, i.e. the gastrulae are more tolerant to salinity change than the blastulae, and the blastulae more tolerant than the cleaving embryos. Holliday and Jones[10] showed that plaice eggs were able to osmoregulate immediately after fertilization. Our results suggest that developing zebrafish embryos gradually secure the osmoregulatory capacity during embryogenesis.

It is of interest to note that zebrafish blastulae and gastrulae exposed to higher salinities hatched markedly later than normal embryos. This is different from the observations that an increase in salinity shortened the hatching period in siganid[2], Pacific cod[11], winter flounder[12], yellowtail flounder[13] and Gulf killifish[14]. The difference may be due to different species. However, the possibility that the formation of hatching enzyme or its activity may be differently impaired by higher salinity, cannot be ruled out.

1. Van Derwal, E. J., *Aquaculture*, 1985, **47**, 239–244.
2. Young, P. S. and Duenas, C. E., *Aquaculture*, 1993, **112**, 363–377.
3. Hart, Piers, R. and Purser, G. J., *Aquaculture*, 1995, **136**, 221–230.
4. Haddy, J. A. and Pankhurst, N. W., *Aquaculture*, 2000, **188**, 115–131.
5. Eisen, J. S., *J. Neurosci.*, 1991, **11**, 311–317.
6. Westerfield, M., *The Zebrafish Book*, Univ. of Oregon Press, Eugene, OR, 1993.
7. Barnes, H., *Apparatus and Methods of Oceanography, Part-1: Chemical*, George Allen and Unwin Ltd, London, 1959, pp. 1–341.
8. Mc Manus, J. F. A. and Mowry, R. W., *Staining Methods: Histologic and Histochemical*, 1960, pp. 8–89.
9. Potts, W. T. W. and Eddy, F. B., *J. Comp. Physiol.*, 1973, **82**, 305–315.
10. Holliday, F. G. T. and Jones, M. P., *J. Mar. Biol. Assoc. UK*, 1967, **47**, 39–48.
11. Forrester, C. R. and Alderdice, D. F., *J. Fish. Res. Board Can.*, 1966, **23**, 319–340.
12. Rogers, C. A., *Fish. Bull.*, 1976, **74**, 52–58.
13. Lawrence, G. C. and Howell, W. H., *Mar. Ecol. Prog. Ser.*, 1981, **6**, 11–18.
14. Perschbacher, P. W., Aldrich, D. V. and Strawn, K., *Prog. Fish. Cult.*, 1990, **52**, 109–111.

ACKNOWLEDGEMENTS. We thank the staff of our college and all the Ph D scholars for their timely and valuable help. The work was partly supported by Ministry of Sciences and Technology (MOST) of China (G1999012006).

Received 18 August 2001; accepted 19 September 2001

^{31}P magnetic resonance spectroscopic study of healthy human calf muscle

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^{31}P magnetic resonance spectroscopy was performed on calf muscle of 25 healthy volunteers (during resting) to establish the baseline metabolic ratios on Indian population. The study reveals significantly low PCr/Pi ratio and the presence of phosphodiester (PDE) peak in all the healthy volunteers, in contrast to data reported in western literature. The spectra mimic the patterns obtained from patients of western population with mitochondrial impairment. The findings indicate muscular weakness in our population and may be attributed to sedentary lifestyle, poor nutrition (low protein intake) or due to ethnic differences. These novel findings are of importance and need in-depth investigations.

^{31}P Magnetic resonance spectroscopy (MRS) has been extensively used to investigate non-invasively the energy metabolism of the human muscle, since its first application using surface coils[1,2]. Various phospho metabolites such as phosphocreatine (PCr), inorganic phosphate (Pi), adenosine triphosphate (ATP), phosphomonoester (PME) and phosphodiester (PDE) can be measured *in vivo*, with ease, accuracy and high reproducibility. Hence, it has become a modality of choice to investigate energy metabolism in living tissue. Also, the intracellular pH from the spectra can be measured from the chemical shift of Pi resonance from internal reference peak of PCr[3,4]. PCr recovery rates after exercise are useful in evaluating oxidative phosphorylation[5,6] and the variations in the intracellular pH during exercise are related to lactate production. This technique thereby allows us to indirectly study the glycolysis and mitochondrial oxidative phosphorylation metabolism. There are numerous reports on the muscle bioenergetic impairment in various muscle myopathies, injuries and various other disorders involving muscle weakness[7–14] and a lower PCr/Pi ratio has been observed in comparison to controls. Decreased PCr/Pi ratio has also been reported in malnourished individuals in different age groups, namely children (age 10.5 ± 1.5 years)[15], middle-aged (age 25–62 years)[16] and elderly people

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(age 42.5 ± 19.5 years)[17]. Recovery of muscle PCr and Pi and returning to normal values after nourishment for a month has been observed[17]. We have a large population affected by thyroid disorders and these patients are known to have muscle weakness and exercise intolerance[18,19]. The present study was undertaken to establish the phosphorous metabolic ratios PCr/Pi, Pi/ATP, PDE/ATP and PCr/ATP in healthy volunteers of our population during resting state, so that the data can be used for future research on thyroid disorders. This is a detailed ^{31}P MRS report on the Indian population, hinting weakness in adult resting muscle.

We performed ^{31}P spectroscopy on 25 healthy volunteers (15 women and 10 men) in the age group of 20 to 40 years. The control subjects were recruited from our institute and had no previous history of muscle weakness or any other medical problem and were not under any medication for a minimum of six weeks preceding the study.

All the experiments were carried out on a 1.5 Tesla whole-body MRI system (Siemens, Magnetom Vision) at an operating frequency (Larmor frequency) of 63.6 MHz for proton and 25.7 MHz for phosphorous. A 15 cm double-tuned (^{1}H, ^{31}P) surface coil was used to acquire the spectrum. This coil has a linearly polarized receive-only segment for proton frequency and circularly polarized transmit/receive segment for phosphorous frequency. The magnetic field homogeneity was optimized on proton signal and after achieving the desired homogeneity, the frequency was switched to ^{31}P frequency. Free induction decay pulse sequence with a flip angle of 90° was used with repetition time of 1500 ms and 128 signal averages, resulting in a measurement time of approximately 3.2 min. After Fourier transformation and phase correction of the raw data, the metabolic concentrations of Pi, PCr, PDE and β-ATP were measured, by computing the areas under these peaks. For the purpose of computing the metabolic ratios, β-ATP peak was used because there is no contamination of other metabolites at this peak position. The intracellular pH of the muscle was directly calculated from the spectra by measuring the shift of Pi position (chemical shift) from the reference PCr peak and using the formula

$$pH = 6.75 + \log((\delta - 3.27)/(5.69 - \delta)),$$

where δ is observed chemical shift of Pi peak from PCr.

A typical phosphorous spectrum from the calf muscle of healthy volunteers is shown in Figure 1. In our study we have observed prominent PDE peak in the muscle spectra obtained from all the 25 healthy volunteers. The important metabolic ratios and intracellular pH during resting are shown in Table 1. The unique findings from our data are (i) much lower PCr/Pi ratio (6.08 ± 0.58), and (ii) presence of PDE peak in all the volunteers, in contrast to the literature on western population. The pH of the muscle on our population was calculated to be 7.07 ± 0.03, which correlates well with earlier reports[10,12].

Spectra measured on our population show an abnormally low PCr/Pi ratio and high Pi/β-ATP, PCr/β-ATP and PDE/β-ATP ratios compared to the western population (Table 1). Low PCr/Pi values are often observed in the muscle of patients with mitochondrial myopathies, because of decreased energy state at rest due to impaired mitochondrial function[10,12,20,21]. It was proposed that this ratio could be used as a sensitive diagnostic marker of mitochondrial myopathy[22], but abnormalities in resting energy state have also been reported in patients with hypothyroid myopathy[9,10,12], polymyositis, muscular dystrophy[8] and in cases of muscle injury[13]. These reports indicate that a low PCr/Pi ratio could reveal primary as well as secondary mitochondrial disorder. Our results show much decreased energy state (PCr/Pi) in healthy Indian volunteers. The high metabolic ratios like Pi/β-ATP, PCr/β-ATP and PDE/β-ATP in our volunteers also support some sort of muscle weakness in our population. The PDE peak has been observed on ^{31}P spectra of muscle in patients with muscle dystrophy[11], and also on intact heart muscle and slow twitch muscle[3]. Previous studies on patients with muscle myopathy and with thyroid disorders have shown PDE peak in the spectra, but this was found to be missing in muscles of healthy volunteers[9,10,12]. The PDE peak has been assigned to glycerol-3-phosphorylcholine

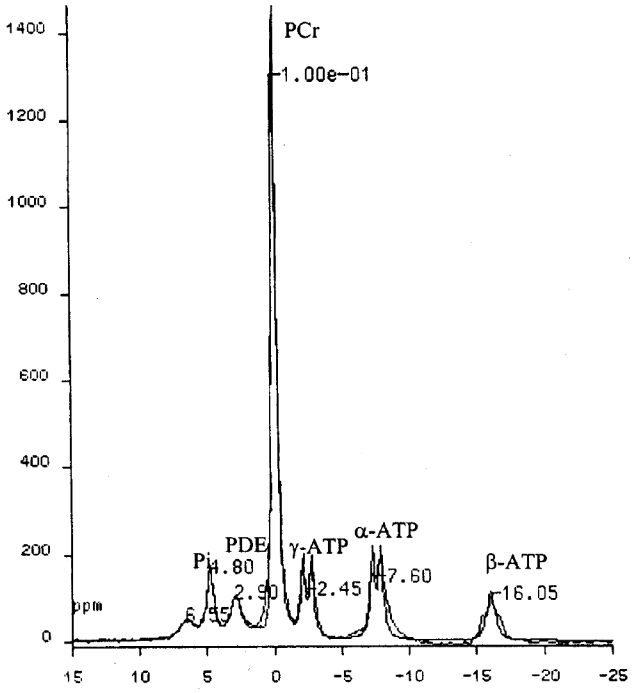

Figure 1. Typical ^{31}P MR spectra from calf muscle of a healthy volunteer. (Values indicate the chemical shift position in ppm with respect to PCr.)

Table 1. Metabolic ratios and intracellular pH in healthy controls (in comparison with the literature)

Metabolite parameter	Control			
	Present result ($n = 25$)	Taylor *et al.*[12] ($n = 26$)	Keminsky *et al.*[10] ($n = 9$)	Argov *et al.*[9] ($n = 20$)
PCr/Pi	6.08 ± 0.58	9.49 ± 1.93	–	8.5 ± 2.1
Pi/ATP	1.04 ± 0.13	0.34 ± 0.06	0.78 ± 0.13	0.4–0.85
PDE/ATP	0.84 ± 0.31	0.02 ± 0.04	–	No PDE peak
PCr/ATP	6.27 ± 0.78	3.08 ± 0.15	5.24 ± 0.56	5.5 ± 0.7
PCr/(PCr + Pi)	0.85 ± 0.01	–	–	–
pH	7.07 ± 0.03	7.03 ± 0.03	7.04 ± 0.04	–

Table 2. Metabolic ratio in healthy men and women

Metabolic ratio	Male ($n = 10$)	Female ($n = 15$)
PCr/Pi	5.91 ± 0.60	6.11 ± 0.56
Pi/ATP	1.09 ± 0.12	1.00 ± 0.13
PDE/ATP	0.96 ± 0.28	0.78 ± 0.23
PCr/ATP	6.47 ± 0.64	6.10 ± 0.91
PCr/(PCr + Pi)	0.85 ± 0.01	0.85 ± 0.01

and to glycerol-3-phosphorylethanolamine (PDEs)[23]. Correlation between the PDE content measured on resting gastrocnemius muscle of healthy volunteers and age has been observed[24,25], suggesting that the increase in PDEs is probably linked to aging due to muscle fibre degradation, and not to disease. But in our study we have observed a PDE peak in all the volunteers (age group of 20–40 years) irrespective of age. Though a minimal PDE peak is seen in the earlier spectra[24,26], the authors have not taken note of this peak in normal volunteers. In a recent article[27], though there is a mention of detectable PDE peak in controls, there is no quantification of the peak or inference. PDE peak and low PCr/Pi ratio have been observed in malnourished individuals, which improves with nutrition within a month[17]. Conditioning the muscle by physical activity is found to increase the PCr/Pi ratio in the healthy volunteers[28]. PCr/Pi in our population is found to be in a lower range (4.8–6.7) and overlapping only in the lower limit of the PCr/Pi ratio (6–12) from the western population[14]. The presence of a prominent PDE peak in our population together with low PCr/Pi and high PCr/ATP ratio, may be due to fibre degradation and could be attributed to low protein intake, sedentary lifestyle in our population or due to racial differences. The small variations in the metabolic ratios in our study compared to previous reports may be due to the selection of volunteers from a more homogeneous group in respect to economic condition and lifestyle. The large variations in PCr/Pi ratio (9.49 ± 1.93 and 8.5 ± 2.1)) observed in previous reports on healthy volunteers[9,12,14] illustrate the diversity of a normal population. The mean metabolic ratios in men and women do not significantly differ ($P > 0.05$) in healthy population, showing no dependence of sex on metabolic rates in resting muscle (Table 2). Homogeneity in the group of subjects (sedentary vs trained, male vs female) and sufficiently large population of volunteers will be required to reach a biostatistical significance in the description of normal muscle in our population, compared to the western literature. Our findings are of importance in the improvement of physical endurance in our population, particularly, in sports persons and soldiers. Keeping this in view, more detailed studies to assess muscle function by various other established techniques would be required, to establish the cause of muscle weakness in our population.

1. Ackerman, J. H., Groove, T. H., Wong, G., Gadian, D. G. and Radda, G. K., *Nature*, 1980, **283**, 167–170.
2. Hoult, D. I., Busby, J. W., Gadian, D. G., Radda, J. K., Richards, R. E. and Seeley, P. J., *Nature*, 1974, **252**, 285–287.
3. Chance, B., Clarkes, B. J. and Nioka, H., *Circulation*, 1985, **72**, 103–110.
4. Burt, C. T., Gloneck, T. and Barany, M., *J. Biol. Chem.*, 1976, **251**, 2584–2591.
5. Arnold, D. L., Matthews, P. M. and Radda, G., *Magn. Reson. Med.*, 1984, **1**, 307–315.
6. Taylor, D. J., Bore, P. J., Styles, P., Gadian, D. G. and Radda, G., *Mol. Biol. Med.*, 1983, **1**, 77–94.
7. Arnold, D. L., Taylor, D. J. and Radda, G. K., *Ann. Neurol.*, 1985, **18**, 189–196.
8. Bank, W., Argov, Z., Leigh, J. S. and Chance, B., *Ann. NY Acad. Sci.*, 1987, **508**, 448–450.
9. Argov, Z., Renshaw, P. F., Boden, B., Winokur, A. and Bank, J., *J. Clin. Invest.*, 1988, **81**, 1695–1701.
10. Keminsky, P. *et al.*, *J. Clin. Endocrinol. Metab.*, 1991, **74**, 124–129.
11. Newman, R. J., Bore, P. J., Chan, L., Gadian, D., Styles, P., Taylor, D. and Radda, G. K., *Br. Med. J.*, 1982, **284**, 1072–1074.
12. Taylor, D. J., Rajagopalan, B. and Radda, G. K., *Eur. J. Clin. Invest.*, 1992, **22**, 358–365.
13. McCully, K., Argov, Z., Boden, B. P., Brown, R. L., Bank, W. J. and Chance, B., *Muscle Nerve*, 1988, **11**, 212–216.
14. Argov, Z., Lofberg, M. and Arnold, D. L., *Muscle Nerve*, 2000, **23**, 1316–1334.
15. Gupta, R. K., Mittal, R. D., Agarwal, K. N. and Agarwal, D. K., *Acta Paediatr.*, 1994, **83**, 327–331.
16. Thompson, A., Damyanovich, A., Madapallimattam, A., Mikalus, D., Allard, J. and Jeejeebhoy, K. N., *Am. J. Clin. Nutr.*, 1998, **67**, 39–43.

17. Bourdel-Marchasson, I. *et al.*, *Am. J. Clin. Nutr.*, 2001, **73**, 832–838.
18. Layzer, R. B., *Neuromuscular Manifestation of Systematic Disease* (ed. Davis, F. A.), Philadelphia, 1985, pp. 79–98.
19. Ruff, R., in *Myology: Basic and Clinical* (eds Engel, A. G. and Banker, B. Q.), McGraw-Hill Book Co, New York, 1986, pp. 1871–1906.
20. Argov, Z., Bank, W. J., Maris, J., Peterson, P. and Chance, B., *Neurology*, 1987, **37**, 257–262.
21. Chance, B., Leigh, J. S., Smith, D., Nioka, S. and Clark, B. J., *Ann. NY Acad. Sci.*, 1986, **488**, 140–153.
22. Matthews, P. M., Allaire, C., Shoubridge, E. A., Karpati, G., Carpentar, S. P. and Arnold, D. A., *Neurology*, 1991, **41**, 114–120.
23. Burt, C. T., Gloneck, T. and Barany, M., *Biochemistry*, 1976, **15**, 4850–4853.
24. Satrustegui, J. *et al.*, *Mech. Age. Dev.*, 1988, **42**, 105–114.
25. Mattei, J. P., Bendahan, D., Roussel, M., Lefur, Y. and Cozzone, P. J., *FEBS Lett.*, 1999, **450**, 173–177.
26. Jagannathan, N. R., Govindraju, V. and Raghunathan, P., *Proc. Natl. Acad. Sci. India*, 1996, **A66**, 9–16.
27. Park, J. H., Phothimat, P., Oates, C. T., Hernanz-Schulman, M. and Olsen, N. J., *Arthritis Rheuma.*, 1998, **41**, 406–413.
28. Tartaglia, M. C., Chen, J. T., Caramanos, Z., Taivassalo, T., Arnold, D. L. and Argov, Z., *Muscle Nerve*, 2000, **23**, 175–181.

ACKNOWLEDGEMENTS. We thank Dr T. Lazar Mathew, INMAS for technical discussions and support.

Received 27 July 2001; revised accepted 1 October 2001

Re-examination of quartz–sillimanite–hypersthene–cordierite gneisses from the Vijayanagaram district: Does surinamite occur in the Eastern Ghats Belt?

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Ramesh Kumar *et al.*[1] reported a boron- and gallium-bearing variety of the rare beryllosilicate surinamite in quartzofeldspathic gneisses from 7 localities near Vizianagaram, the first report of surinamite in India. These surinamite-bearing gneisses are unusually enriched in beryllium compared to other pelitic gneisses, and few non-pegmatitic silicate minerals simultaneously contain significant amounts of beryllium and boron, so the report of surinamite in the Vizianagaram gneisses is of special geochemical and mineralogical interest. In samples from two of the localities studied by Ramesh Kumar *et al.*[1], including four chips of their original specimens, no surinamite was found. Instead, we identified abundant hypersthene. Petrographic study and electron microprobe analyses of minerals in the original samples show these to contain quartz, K-feldspar, aluminum-rich hypersthene (7.92–10.18 wt% Al_2O_3), garnet (on average $Alm_{0.58}Prp_{0.39}Sps_{0.01}Grs_{0.01}$), zinc- and chromium-poor hercynite, sillimanite (1.47–1.75 wt% Fe_2O_3), magnetite and ilmenite–hematite intergrowths. Secondary cordierite is poor in alkalis and calcium, whereas biotite is rich in titanium and fluorine. Mineral compositions indicate that these gneisses crystallized under relatively oxidizing and very dry conditions near 1000°C and 7 kbar. No evidence was found for beryllium, boron or gallium enrichments, but a new occurrence of the borosilicate prismatine indicates the presence of a boron-enriched layer in the gneisses.

SURINAMITE $(Mg,Fe^{2+})_3(Al,Fe^{3+})_3O[AlBeSi_3O_{15}]$, is found in high-grade metapelites and metapegmatites at seven localities worldwide, most recently from South Harris, Scotland[2]. In addition, Ramesh Kumar *et al.* [1] reported a B- and Ga-bearing surinamite in relative abundance from seven localities in the Vizianagaram district. This unusual surinamite composition and abundance attracted our interest for several reasons. Surinamite is a very sparse accessory in metapelites at the other localities, where only a few grains are found in a thin section. Ramesh Kumar *et al.*[1] reported 30–40 grains in each of two thin sections, i.e. possibly 0.1 or more modal per cent. The contribution to the whole-rock Be budget by this amount of surinamite (1.6 wt% Be) would be 15–20 ppm, leading to whole-rock Be contents of at least this amount, i.e. Be contents well above the normal range of 1–5 ppm Be for pelitic sediments and their metamorphosed equivalents[3]. The Eastern Ghats surinamite-bearing metapelites would be unique in their relatively elevated Be contents over a large area. In addition, Ramesh Kumar *et al.*[1] reported 2.01–3.17 wt% B_2O_3 in the surinamite, a very high content given the absence of associated boron minerals. In general, minerals simultaneously enriched in Be and B are rare in metamorphic rocks. Thus, the B-bearing surinamite[1] could provide insight into the crystal chemistry of Be and B in silicate minerals, while the surinamite-bearing host rocks could shed light on Be enrichment in metamorphic environments.

With these objectives in mind, we examined samples of gneisses and associated rocks from the area studied by Ramesh Kumar *et al.*[1], including their four original sample chips (Table 1). No mineral resembling surinamite was found in thin sections of gneisses similar to the surinamite-bearing gneisses described by Ramesh

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