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P. Vats · A.K. Mukherjee · M.M.L. Kumria
S.N. Singh · S.K.B. Patil · S. Rangnathan
K. Sridharan

Changes in the activity levels of glutamine synthetase, glutaminase and glycogen synthetase in rats subjected to hypoxic stress

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Abstract Exposure to high altitude causes loss of body mass and alterations in metabolic processes, especially carbohydrate and protein metabolism. The present study was conducted to elucidate the role of glutamine synthetase, glutaminase and glycogen synthetase under conditions of chronic intermittent hypoxia. Four groups, each consisting of 12 male albino rats (Wistar strain), were exposed to a simulated altitude of 7620 m in a hypobaric chamber for 6 h per day for 1, 7, 14 and 21 days, respectively. Blood haemoglobin, blood glucose, protein levels in the liver, muscle and plasma, glycogen content, and glutaminase, glutamine synthetase and glycogen synthetase activities in liver and muscle were determined in all groups of exposed and in a group of unexposed animals. Food intake and changes in body mass were also monitored. There was a significant reduction in body mass (28–30%) in hypoxia-exposed groups as compared to controls, with a corresponding decrease in food intake. There was rise in blood haemoglobin and plasma protein in response to acclimatisation. Over a three-fold increase in liver glycogen content was observed following 1 day of hypoxic exposure (4.76±0.78 $mg \cdot g^{-1}$ wet tissue in normal unexposed rats; 15.82±2.30 $mg \cdot g^{-1}$ wet tissue in rats exposed to hypoxia for 1 day). This returned to normal in later stages of exposure. However, there was no change in glycogen synthetase activity except for a decrease in the 21-days hypoxia-exposed group. There was a slight increase in muscle glycogen content in the 1-day exposed group which declined significantly by 56.5, 50.6 and 42% following 7, 14, and 21 days of exposure, respectively. Muscle glycogen synthetase activity was also decreased following 21 days of exposure. There was an increase in glutaminase activity in the liver and muscle in the 7-, 14- and 21-day exposed groups. Glutamine synthetase activity was higher in the liver in 7- and 14-day exposed groups; this returned to normal following 21 days of exposure. Glutamine synthetase activity in muscle was significantly higher in the 14-day exposed group (4.32 µmol γ-glutamyl hydroxamate formed·g protein^{-1}·min^{-1}) in comparison to normal (1.53 µmol γ-glutamyl hydroxamate formed·g protein^{-1}·min^{-1}); this parameter had decreased by 40% following 21 days of exposure. These results suggest that since no dramatic changes in the levels of protein were observed in the muscle and liver, there is an alteration in glutaminase and glutamine synthetase activity in order to maintain nitrogen metabolism in the initial phase of hypoxic exposure.

Key words Hypoxia · Glutaminase · Glutamine synthetase · Glycogen synthetase · Glycogen

P. Vats · A.K. Mukherjee · M.M.L. Kumria · S.N. Singh
S.K.B. Patil · S. Rangnathan · K. Sridharan (✉)
Defence Institute of Physiology and Allied Sciences,
Lucknow Road, Timarpur, Delhi 110054, India

Introduction

Hypoxic stress at high altitude leads to changes in metabolic processes, especially in carbohydrate and protein metabolism, and changes in the endocrine system (Forbes 1936; Picon-Reategui et al. 1970; Brahmachari et al. 1973; Srivastava et al. 1975; Williams 1975). A reduced work capacity and increased creatine phosphokinase activity has been reported to occur in sea-level residents after a prolonged stay at high altitude (Knuttgen and Saltin 1973). Long-term exposure to high altitude results in body mass loss in man (Boyer and Blume 1984; Butterfield et al. 1992; Kayser 1994). Drastic reductions in lean body mass are observed in sea-level residents during their stay at a terrestrial height of 5000 m and above (Guilland and Klepping 1985). In addition, a diminished growth rate is seen in animals subjected to hypoxic stress, suggesting that changes in protein metabolism occur under such conditions (Klain and Hannon 1970; Schnakenberg et al. 1971; Purshottam et al. 1977). However, the causes for these changes are still not clear. Glutamine synthetase (EC 6.3.1.2), glutaminase (EC 3.5.1.2) and glycogen synthetase (EC 2.4.1.11) play significant roles in protein synthesis and energy metabolism (Smith et al. 1984; Jepson et al. 1988; Hoppeler and

Table 1 Changes in body mass (g) of the rats (n=12 rats per group) during exposure to hypoxic conditions for either 1, 7, 14 or 21 days

Group	Initial weight		Weight on days							
			1		7		14		21	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Unexposed group	203.0	4.0	205.5	3.9	219.5*	3.8	236.5**	3.9	251.7**	4.0
Hypoxia-exposed	199.8	1.5	195.7***	1.6	183.2**#	1.6	168.0**#	1.7	174.6**#	1.5

* Significantly different from initial body weight; P<0.05
** Significantly different from initial body weight; P<0.001
*** Significantly different from the corresponding control; P<0.05
# Significantly different from the corresponding control; P<0.001

Table 2 Blood haemoglobin and glucose levels in hypoxia-exposed (1, 7, 14 or 21 days) and unexposed rats (n=12 rats per group)

Variable	Unexposed group		Hypoxia-exposed groups							
			1 day		7 days		14 days		21 days	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Blood haemoglobin ($g \cdot dl^{-1}$)	13.55	0.19	14.94*	0.13	15.11*	0.14	16.22*	0.12	17.01*	0.38
Blood glucose ($mg \cdot dl^{-1}$)	66.58	0.97	98.33*	1.47	84.67*	1.34	85.15*	1.92	77.97*	1.48

* Significantly different compared to unexposed group; P<0.001

Desplanches 1992). There is some controversy regarding the effects of hypoxia on carbohydrate metabolism, some pointing to hypoglycaemia and some indicating hyperglycaemia with increased levels of circulating insulin (Blume and Pace 1967; Johnson et al. 1974; Sawhney et al. 1986). Therefore, the present study was undertaken to elucidate the effects of hypoxia on the activity levels of glutamine synthetase, glutaminase and glycogen synthetase under hypoxic conditions using an animal model, as markers with which to follow the effects of hypoxia on protein metabolism.

Materials and methods

Studies were conducted on male albino rats (Wistar strain) weighing ≈200 g. The animals were maintained in the Institute's Animal House at a temperature of 22±2° C and with a 12-h light and dark cycle. Rats were fed ad libitum on a standard rat pellet diet supplied by Hindustan Lever (India). Groups of animals consisting of 12 rats each were exposed to hypoxia at a simulated altitude of 7620 m in a hypobaric chamber. Altitude was attained in 20 min at a uniform rate of ascent. Temperature was maintained at 22±2° C. To study the effects of hypoxia, animals were exposed to hypoxia for either 1, 7, 14 or 21 days (for 6 h daily). Before the last day of exposure the rats were fasted overnight. Animals were sacrificed after 6 h of exposure, as were unexposed animals, using ether anaesthesia. Blood was drawn from the heart, and the liver and thigh muscles were removed and processed for different biochemical assays. Another two groups of animals, one exposed to hypoxia for 21 days and the other, a normal group, were monitored for food intake and changes in body mass. Blood haemoglobin was estimated colorimetrically by the method of Dacie and Lewis (1984). Blood glucose levels and total protein levels in the plasma, liver and thigh muscles were estimated by the methods of Nelson (1957) and Lowry et al. (1951), respectively. The glycogen content of the liver and muscles was assayed by the method of Montgomery (1957). The activities of glutamine synthetase, glutaminase and glycogen synthetase in the liver and muscle homogenates were assayed using standard methods (Elliott 1955; Leloir and Goldenberg 1962; Kvamme et al. 1985). Statistical analysis was carried out using Student's t-test.

Results

Changes in the body mass of rats exposed to hypoxia for different lengths of time (1, 7, 14 or 21 days), along with that of the control group are given in Table 1. Animals exposed to hypoxia were unable to maintain their initial body mass, whereas in the control group a gradual increase over their initial weights was observed throughout the study period. The weight loss in the hypoxia-exposed groups was always significant in comparison to that of the normal group irrespective of the time spent under hypoxic conditions. There was a 16.5, 28.9 and 30.6% decrease in the mean body mass of the exposed groups following 7, 14 and 21 days of exposure, respectively, as compared with the unexposed group on the same day. The 1-, 7- and 14-day exposed groups showed a decrease (≈36.3%) in food intake, and following 14 and 21-days of exposure the reduction was only around 25%, thereby indicating an improvement.

A comparison of blood haemoglobin and blood glucose levels of the various groups is given in Table 2. Blood haemoglobin exhibited a gradual increase from the normal levels. Blood sugar levels were significantly higher in all hypoxia-exposed groups as compared to the unexposed group. The highest level of blood glucose was seen in the 1-day hypoxia-exposed group (47.6% increase over the unexposed control). There was no signifi-

Table 3 Plasma and tissue protein levels in hypoxia-exposed (1, 7, 14 or 21 days) and unexposed rats (n=12 rats per group)

Variable	Unexposed group		Hypoxia-exposed groups							
			1 day		7 days		14 days		21 days	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma protein ($g \cdot dl^{-1}$)	6.99	1.04	7.29	0.99	8.58*	0.86	8.87*	0.80	9.20*	0.58
Liver protein ($g \cdot 100\ g^{-1}$)	19.77	0.33	20.00	0.48	19.40	0.79	20.12	0.32	20.21	0.44
Muscle protein ($g \cdot 100\ g^{-1}$)	17.17	0.53	17.15	0.49	17.11	0.62	17.40	0.48	18.04	0.79

Significantly different compared to unexposed group; P<0.001

Table 4 Liver and muscle glycogen levels in hypoxia-exposed (1, 7, 14 or 21 days) and unexposed rats (n=12 rats per group)

Variable	Unexposed group		Hypoxia exposed group							
			1 day		7 days		14 days		21 days	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Liver glycogen ($mg \cdot g\ tissue^{-1}$)	4.76	0.78	15.82**	2.30	4.96	0.69	4.98	0.81	3.55	0.26
Muscle glycogen ($mg \cdot g\ tissue^{-1}$)	6.71	0.87	7.96	0.95	2.92**	0.29	3.31**	0.30	3.88*	0.68

* Significantly different compared to the unexposed group; P<0.05
** Significantly different compared to the unexposed group; P<0.001

Table 5 Enzyme activity levels in the liver of hypoxia-exposed (1, 7, 14 or 21 days) and unexposed rats (n=12 rats per group)

Variable	Unexposed group		Hypoxia exposed groups							
			1 day		7 days		14 days		21 days	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Glutaminase (μmol NADH oxidised $\cdot$ min^{-1} $\cdot$ mg protein $^{-1}$)	3.06	0.16	2.75	0.15	4.47	0.33	4.51	0.29	4.71*	0.41
Glutamine synthetase (μmol γ-glutamyl hydroxamate formed $\cdot$ min^{-1} $\cdot$ g $protein^{-1}$)	1.69	0.33	1.95	0.14	3.23*	0.25	1.90	0.09	1.57	0.13
Glycogen synthetase (μmol UDP formed $\cdot$ min^{-1} $\cdot$ mg $protein^{-1}$)	63.28	5.69	61.12	5.83	69.21	6.91	58.40	5.40	28.22*	1.24

* Significantly different compared to the unexposed group; P<0.001

cant difference in blood glucose levels between the 7-day and 14-day exposed groups, whereas the 21-day hypoxia-exposed group showed a decreasing trend, but the values were still higher than those of the unexposed group.

There was a continuous increase in plasma proteins in the hypoxia-exposed groups. Protein levels in the liver and muscle of the unexposed control group and all hypoxia-exposed groups were similar (Table 3).

A comparison of the muscle and liver glycogen contents of the hypoxia-exposed groups and the unexposed group are given in Table 4. There was more than a three-fold increase in liver glycogen in the 1-day hypoxia-exposed group; the levels returned to near-normal levels on day 7 and there was a slight decrease in glycogen content on day 21. Muscle glycogen levels also increased 13%) although not significantly, following 1 day of exposure. There was, however, a significant reduction of 56.5, 50.6 and 42.0% in 7-, 14- and 21-day hypoxia-exposed groups, respectively, in comparison with the unexposed group.

Table 6 Enzyme activity levels in the muscles of hypoxia-exposed (1, 7, 14 and 21 days) and unexposed rats (n=12 rats per group)

Variable	Unexposed group		Hypoxia exposed groups							
			1 day		7 days		14 days		21 days	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Glutaminase (μmol NADH oxidised · min^{-1} · mg $protein^{-1}$)	0.38	0.05	0.34	0.03	0.89*	0.12	0.78*	0.05	2.17*	0.38
Glutamine synthetase (μmol γ-glutamyl hydroxamate formed · min^{-1} · g $protein^{-1}$)	1.53	0.28	1.58	0.11	4.32*	0.50	1.23	0.13	0.91	0.09
Glycogen synthetase (μmol UDPformed · min^{-1} · mg $protein^{-1)}$)	115.2	12.2	100.5	6.1	104.5	6.2	104.7	8.3	55.5*	2.9

* Significantly different compared to the unexposed group; $P<0.001$

There was an increase in liver glutaminase activity in 7-, 14- and 21-day hypoxia-exposed rats, although there was an initial decrease in the 1-day exposed group over the unexposed group. Glutaminase activity in muscle also followed a similar trend, however the intensity of this increase was more pronounced (Tables 5 and 6). Liver glutamine synthetase activity was higher in the 1-day, 7-day and 14-day hypoxia-exposed groups, and reduced in the 21-day exposed group in comparison with the unexposed group. Muscle glutamine synthetase activity was significantly higher in the 7-day exposed group (182%), and reduced (40.5%) in the 21-day exposed group (Tables 5 and 6). There was almost no change in glycogen synthetase activity in the liver and muscle, except in the 21-day exposed group where a decrease of 55.4 and 51.8%, respectively, was observed (Tables 5 and 6).

Discussion

The weight loss that was observed following exposure to hypoxia in the present study is due to decreased food intake and is in agreement with the results of an earlier study by Singh et al. (1996). It is well-known that anorexia occurs at high altitude during the initial stages of acclimatisation, and this causes a drastic reduction in lean body mass (Guilland and Klepping 1985; Butterfield et al. 1992; Kayser 1994). A decreased growth rate in animals subjected to high altitude exposure in comparison to sea level exposure is also well documented (Klain and Hannon 1970).

The increase in blood haemoglobin and plasma protein observed during high altitude exposure may be attributed to changes in haematocrit and haemoconcentration that are a result of acclimatisation to altitude stress (Picon-Reategui et al. 1970). In the present study on acute exposure to altitude there is a highly significant increase in blood glucose levels, but these tended to recover to normal values following longer-term exposure (e.g. our 21-day exposed group). This is in agreement with the findings of Forbes (1936). The alteration in fasting glucose levels could be due to altered glucocorticoid levels as a result of hypoxic stress. Similar changes in glucose tolerance at altitude in humans has been reported by Srivastava et al. (1975). There was a significant increase in liver and muscle glycogen following 1 day of hypoxic exposure. In the liver, glycogen levels returned to normal in the later phase, whereas in muscle glycogen levels were significantly decreased in the later stages of hypoxia exposure. Van Liere and Stickney (1963) have reported a marked elevation in the liver glycogen of fasted animals exposed to acute hypoxia over unexposed fasted rats, raising the possibility of increased interconversion of either protein or fat to carbohydrate in excess of oxidative requirements. Increased levels of blood sugar and liver glycogen during the initial days of hypoxic exposure may be due to increased interconversion from fats and a decrease in glycogen breakdown in the liver, respectively. In our present study, glycogen synthetase activity remained unchanged in 1-day, 7-day and 14-day exposed rats, but decreased in the 21-day exposed group, indicating decreased glycogen synthesis in the later stages. The basis for an increase in glycogen content can not yet be explained and requires detailed investigations on glycogen turnover and glycogen phosphorylase activity during exposure to hypoxia.

In the present study the protein content of the liver and muscle remained unchanged during hypoxic exposure, although increase in the catabolism of protein during an acute (2 days) exposure to hypoxia has been reported to occur by Klain and Hannon (1970). The 6 h of exposure instigated in the present study may not have been sufficient to produce changes in the protein content of the muscle and liver. The enzymes glutaminase and glutamine synthetase, which regulate glutamine levels, were studied because they are key enzymes in the process of protein metabolism. Glutamine also acts as a respiratory substrate since it is substantially oxidised to provide energy (the end products of its metabolism are CO_2, gluta-

mate and aspartate). Wagenmakers (1992) has reported that the rate of glutamine synthesis is reduced during a long-term stay at altitude, with alterations in energy metabolism and reduced muscle glycogen levels. The changes in glutamine synthetase and glutaminase activity observed in the present study are similar to those achieved in the above-mentioned study. Pettersen et al. (1986) have shown, using human cell lines, a reduction in protein synthesis during hypoxia, along with increased catabolism. In the present study, no change in glutaminase and glutaminase synthetase activity was observed, thereby pointing initially, to no change in protein metabolism. However, these two studies are not comparable due to differences in the experimental conditions used (i.e. in the present study whole organisms were investigated). The increase in glutamine synthetase activity observed in liver and muscle following up to 7 days of exposure to hypoxia is similar to the findings of Dao et al. (1991) who have demonstrated increased enzyme activity in the cerebellar tissue of children who died of acute or chronic hypoxic ischaemic insult. From the changes seen in the enzyme activity in the present study, it may be stated that there is an alteration in glutaminase and glutamine synthetase activity. In order to maintain the nitrogen balance during the initial stages of hypoxic exposure, without dramatic changes in the levels of protein in muscle and liver.

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References

Blume FD, Pace N (1967) Effect of translocation to 3800 m altitude on glycolysis in mice. J Appl Physiol 23:75–79

Boyer SJ, Blume FD (1984) Weight loss and change in body composition at high altitude. J Appl Physiol 57:1580–1585

Brahamchari HD, Malhotra MS, Ramachandran K, Radhakrishnan U (1973) Progressive change in plasma cortisol, antidiuretic hormones and urinary volumes of normal low landers during short stay at altitude. Int J Exp Biol 11:454–455

Butterfield GE, Gates J, Fleming S, Broosk GA, Sutton JR, Reeves JT (1992) Increased energy intake minimizes weight loss in men at high altitude. J Appl Physiol 72:1741–1748

Dacie JV, Lewis SM (1984) Practical haematology, 6th ed. Churchil Livingstone, London, p 34

Dao DN, Ahdab-Barmada M, Schor NF (1991) Cerebellar glutamine synthetase in children after hypoxia or ischemia. Stroke 22:1312–1316

Elliott WH (1955) Glutamine synthesis. In: Colowick SP, Kaplan NO (eds) Methods in enzymology, vol II. Academic Press, New York, pp 337–339

Forbes WH (1936) Blood sugar and glucose tolerance at high altitudes. Am J Physiol 116:309–316

Guilland JC, Klepping J (1985) Nutritional alterations at high altitude in man. Eur J Appl Physiol 54:517–523

Hoppeler H, Desplanches D (1992) Muscle structural modifications in hypoxia. Int J Sports Med 13 [Suppl]1:S 166–168

Jepson MM, Bates PC, Broadbent P, Pell JM, Millward DJ (1988) Relationship between glutamine concentration and proteins synthesis in rat skeletal muscle. Am J Physiol 225:166–172

Johnson HL, Consolazio CF, Burk RF, Daws TA (1974) Glucose 14C-UL metabolism in man after abrupt altitude exposure at 4300 m. Aerosp Med 45:849–854

Kayser B (1994) Nutrition of Energetics of exercise at altitude. Theory and possible practical implications. Sports Med 17: 309–323

Klain GJ, Hannon JP (1970) High altitude and protein metabolism in the rat. Proc Soc Exp Biol Med 134:1000–1004

Knuttgen HG, Saltin B (1973) Oxygen uptake, muscle high energy phosphates and lactate in exercise under acute hypoxic conditions in man. Acta Physiol Scand 87:368–376

Kvamme E, Torgner IAA, Svenneby G (1985) Glutaminase from mammalian tissues. In: Meister A (ed) Methods in enzymology, vol 113. Academic Press, New York, pp 241–244

Leloir LF, Goldenberg SH (1962) Glycogen synthetase from rat liver. In: Colowick SP, Kaplan NO (eds) Methods in enzymology, vol V. Academic Press, New York, pp 145–146

Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Montgomery R (1957) Determination of glycogen. Arch Biochem Biophys 67:378–386

Nelson N (1957) Colorimetric analysis of sugars. In: Colowick SP, Kaplan NO (eds) Methods in enzymology, vol III. Academic Press, New York, pp 85–86

Pettersen EO, Juul NO, Ronning OW (1986) Regulation of protein metabolism of human cell during and after acute hypoxia. Cancer Res 46:4346–4351

Picon-Reategui E, Buskirk ER, Baker PT (1970) Blood glucose in high-altitude natives and during acclimatization to altitude. J Appl Physiol 29:560–563

Purshottam T, Kaveeshwar U, Brahmachari HD (1977) Altitude tolerance in rats in relation to carbohydrates and fats in their diet. Aviat Space Environ Med 48:438–442

Sawhney RC, Malhotra AS, Singh T, Rai RM, Sinha KC (1986) Insulin secretion at high altitude in man. Int J Biometeorol 30:231–238

Schnakenberg DD, Krabill LF, Weiser PC (1971) The anorexic effect of high altitude on weight gain, nitrogen retention and body composition of rats. J Nutr 101:787–796

Singh SB, Sharma A, Sharma KN, Selvamurthy W (1996) Effect of high-altitude hypoxia on feeding response and hedonic matrix in rats. J Appl Physiol 80:1133–1137

Smith RJ, Larson S, Stred SE, Durschlag RP (1984) Regulation of glutamine synthetase and glutaminase activities in cultured skeletal muscle cells. J Cell Physiol 120:197–201

Srivastava KK, Kumria MML, Grover SK, Sridharan K, Malhotra MS (1975) Glucose tolerance of lowlanders during prolonged stay at high altitude and among high altitude natives. Aviat Space Environ Med 46:144–146

Van Liere EJ, Stickney JC (1963) Hypoxia. The University of Chicago press, Chicago, pp 261–262

Wagenmakers AJ (1992) Amino acid metabolism, muscular fatigue and muscle wasting. Speculation on adaptations at high altitude. Int J Sports Med 13 [Suppl 1]S 110–113

Williams ES (1975) Mountaineering and the endocrine system. In: Clarke C, Ward M, William E (eds) Mountain medicine and physiology. Alpine Club, London, pp 38–44