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Review Article

LDH Isozymes- It's Relation with Infertility

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ABSTRACT:
Lactate Dehydrogenase is an enzyme found in all living cells. The conversion of lactate to pyruvate and vice versa is catalyse by Lactate Dehydrogenase enzyme. The two different sub-units are marked in LDH; notably LDH A is also known as M sub-unit and LDH B is known as H sub-unit. LDH isozymes are found in five common forms, two more mammalian LDH sub-units of tetramers is incorporated namely LDH C and LDH Bx.

Keywords: Lactate dehydrogenase, Isozymes, Sub-unit, Mammalian LDH

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LACTATE DEHYDROGENASE

Lactate Dehydrogenase (LDH or LD) is an enzyme found in all living cells (animals, plants and prokaryotes). The conversion of lactate to pyruvate and vice versa is catalyse by Lactate Dehydrogenase enzyme; as it converts NAD+ and NADH and back. Transfer of a hydride from one molecule to another is served by an enzyme dehydrogenase. In humans as a proton acceptor LDH uses His (193) and synchronously works with Arg 99 and Asn 138 as co-enzyme and Arg 106, Arg 169, Thr 248 as substrate binding residue. The convergent evolution of LDH is marked in different animals with humans with His (193) active site (Holmes R S et al., 2009).

The two different sub-units are marked in LDH; notably LDH A is also known as M sub-unit and LDH B is known as H sub-unit; both sub-units have same active site and same amino acid participate in the reaction. Replacement of alanine (M chain) with glutamine (H chain) in the LDH's tertiary structure makes a noticeable difference between two sub-units. This notable change allows H-sub-unit to bind faster and M- sub-units catalytic activity isn't reduced when subjected to same condition. Lactate dehyrogenase is a tetramer. The sub-unit of LDH (A and B) forms 5 tetramer (Isozymes) 4H, 4M and 3 different intermediates (3H1H, 2H2M, 1H3M). LDH 1 (4H) is present in heart, RBC, and brain, LDH 2 (3H1M) in reticuloendothelial system, LDH 3 (2H2M) in lungs, LDH 4 (1H3M) in kidneys, placenta and pancreas while LDH 5 (4M) is present in liver and straited muscles in abundant (Wikipedia.com). Two more mammalian LDH sub-units of tetramers is incorporated namely LDH C and LDH Bx. LDH C is encoded by LDH C gene and are specific protein of testis. LDH Bx is a peroxisome-specific LDH protein. LDH B mRNA generate the translation of LDH Bx (Schueren F et al., 2014).

SEMINAL LACTATE DEHYDROGENASE

Lactate dehydrogenase (L-Lactate: NAD+ oxido-reductase EC 1.1.1.27) is an enzyme present in most of the mammalian tissue and are responsible for the conversion of pyruvate into lactate and vice-versa. LDH isozymes are found in five common forms, which are tetramer built up from two parent subunits, M (Muscle type) and H (Heart type) subunit (Lojda and Frie, 1970; Cahn et al., 1962; Markert and Ursprung, 1962). Markert (1963) observed that each enzyme is tetramer of these two subunits, LDH1 (HHHH), LDH2 (HHHM), LDH3 (HHMM), LDH4 (HMMM) and LDH5 (MMMM). Among these five isozymes LDH1 and LDH2 are categorized as H-isozymes where as LDH4 and LDH5 as M-isozymes of LDH (Chan et al., 1964). The H-isozymes of LDH is responsible for conversion of lactate into pyruvate and M-isozymes converts pyruvate into lactate (Battellino et al., 1971; Clausen, 1970). Conversion of lactate into pyruvate leads to aerobic condition and in reverse, conversion of pyruvate into lactate leads to anaerobic condition.

Besides the above mentioned five isozymes of LDH, some additional isozymes have been reported in the semen of human (Wheat and Goldberg 1977; Skudev et al., 1984; Gavella et al., 1984; Gavella and Lipovac et al., 1987). Semen sample containing spermatozoa possess an additional LDH isozyme with electrophoretic mobility between LDH3 & LDH4 and have been named as LDH-X (Blanco and Zinkham, 1963) and have been reported to be present exclusively in germ cells (Blanco et al., 1975). In human, LDH-X is a homotetramer composed of four C-subunits hence the name LDH-C4. This isozyme is synthesized in primary spermatocytes to fulfill a very special function for the metabolic requirement of spermatozoa.

On electrophoretic separation of LDH isozymes, the bands moving towards the anode are regarded as H-isozymes and bands moving towards the cathode are called the M-isozymes. The fastest moving band towards the anode is LDH1 and above these LDH2, LDH3, LDH4 and LDH5 isozymes are separated in the polyacrylamide gel. In between LDH3 and LDH4 one extra band of LDH isozymes is present which is named as LDH-X (Blanco and Zinkham 1963). Chemically the H-subunit of LDH has high content of acidic amino acid (aspartic acid) while M-subunit has high content of basic amino acid (Lysine). Due to this reason, LDH1 migrate farthest towards the anode during electrophoresis as it carries the highest negative charge. This negative charge on LDH1 is mainly contributed by aspartic acid.

UTERINE LACTATE DEHYDROGENASE

Singh et al., (1995) reported an additional sixth LDH-isozyme, which was designated provisionally, LDH-Y. This isozyme is more cathodic than LDH5. This new LDH-isozyme is estrogen dependent and appears during early pregnancy. It was observed that LDH-Y acts as fertility factor during pre-implantation in the uterine tissue of mice. Heimback and Prezyna (1960) found increased level of serum LDH during pregnancy. LDH synthesis in rat uterus can be influenced by estradiol and progesterone. However, estradiol has a selective influence on synthesis of M-subunits. Goodfriend and Kaplan (1964) observed that progesterone induced two LDH forms in the uterus. Brinster (1965) found high LDH activity during preimplantation stages. Auerbach and Brinster (1967) noticed predominance of LDH-isozyme during the blastocyst stage while LDH5 is associated with the process of implantation into the uterus.

LDH-ISOZYMES IN RELATION TO INFERTILITY

In the rat embryo there are three fold fall in LDH activity during pre-implantation period (Brinster et al., 1967). In mammals, uterine-LDH activity is influenced by six steroids during preimplantation stages (Georgiev et al., 1970). In rat uterus, ovarian steroids mainly induce endometrium (Clark and Yochim, 1971) rather than myometrium. When ovariectomised rats are treated with estrogen and progesteron, these hormones stimulate both M and H-isozymes of LDH. Progesterone suppressed the action of estrogen hence modify LDH activity by affecting intrauterine oxygen tension. Thus, it is clear that during progestation, both estrogen and progesterone are secreted and affect uterine metabolism. During pseudopregnancy the decline in enzyme activity was due to M-isozymes rather than H-subunits.

Galbraith et al., (1970) found increase in percentage of M-subunits of LDH in rat uterus at proestrus and estrus under the influence of estrogen. Yochim and Clark (1971) reported peak LDH activity in the rat endometrium during estrus, which declined during diestrus. The M-isozymes also show high activity during estrus, metestrus and low at diestrus. During proestrus, a stage of maximum estrogen level, the M-isozymes show high activity in uterine horns of rat. This isozyme level is low at diestrus that enables the uterus to maintain TCA cycle in full functional state. In this way, uterus is able to derive the maximum energy from glucose metabolism and assists the catabolism of uterine protein.

Singh and Roy (1980) observed that LDH subunits are dependent chiefly on hormonal responses resulting in metabolic adaptation in the rat uterus to meet energy requirement. Singh and Singh (1990) reported that LDH and its isyzomes particularly of H-types in uterine luminal fluid of rat undergo maximum increase at proestrus and estrus followed by a decrease at metestrus reaching a minimum at diestrus. This rise and fall of H-isozymes of LDH in rat uterine fluid may be due to changing titers of endogenous estrogen level during different phases of estrus cycle.

Singh (1994) found predominance of M-isozymes throughout the menstrual cycle in the uterine luminal fluid of both parous and infertile women. In parous women M-isozymes (LDH4 and LDH5) maintain higher activity during early follicular phase to ovulatory phase than infertile women. During the post-ovulatory phase M-isozymes show lowest level in parous women. However, in the uterine fluid milieu of infertile women, M-iszoymes show high activity during this phase. This suggests that significantly high activity of M-isozymes in uterine fluid of parous women from early follicular phase to ovulatory phase compared to that of infertiles, enables transformation of pyruvate into lactate which is the important source of energy for the spermatozoa at this stage. Thus, estrogen induced luminal M-isozymes enable the uterus to derive maximum energy from lactate in parous women which may help in vital reproductive process like nutrition, capacitation, transportation and metabolism of spermatozoa. That is not the case in the decreased level of M-LDH in the uterine fluid of infertile women.

Such difference in LDH-isozymes (M-type) in uterine fluid of both parous and infertile women during early proliferative phase to ovulatory phase may be due to difference in sensitivity of endometrium to estrogen that results in low M-LDH activity in infertile women causing uterine dysfunction and less availability of lactate which is the only source of energy at this stage. Hence it may be concluded that the post-ovulatory rise in M-LDH among infertile women makes a change in the uterine luminal fluid from aerobic to anaerobic condition that inhibits implantation thus causing sterility among them.

According to Goodfriend and Kaplan (1964), ovarian estrogen has selective and directional influence on M-isozymes of LDH. Rani et al., (2009) observed that the neem oil maintains throughout higher level of total LDH activity from first day after mating to seventh day after mating in the uterine fluid of mice and M-isozymes of LDH also show higher level than the control. However, M/H ratio of LDH-isozymes in control rapidly decline from first to seventh day after mating. In the similar study Rani et al., (2009) suggested that neem oil has selective and directional influence on M-isozymes of LDH which leads to predominance of M-LDH (LDH4 and LDH5) from first to seventh day after mating which causes the anaerobic condition by converting more pyruvate into lactate in the uterine lumen of neem treated mice. These M-isozymes provide low oxygen tension in the uterus which leads to various uterine dysfunctions and promote uterine contractility thus reducing the receptivity of uterus for uterine attachment of growing blastocyst in the uterus.

Fujii and Villee (1969) and Vanithakumari (1976) observed that testosterone propionate treatment increases the organ weight, total protein and LDH activity suggesting its growth promoting influence, as has been observed in the seminal vesicle and ventral prostase. However, the stimulatory influence of androstenedione and oestradiol could not be ruled out. Testosterone and its metabolites are well known for their anabolic effects in other accessory sex glands (Williams-Ashman and Reddi et al., 1972). The LDH-isozyme

patterns of the coagulating gland in normal adult rats revealed that the metabolic pattern is oriented towards aerobic respiration, since the total H-subunits are predominant over the total M-subunits. Furthermore, it indirectly suggests that the gene locus 'B', responsible for the synthesis of H-subunits, is more active than the gene locus 'A', responsible for the synthesis of M-subunits (Sarkar et al., 1978).

Castration reduces the total LDH activity and also altered the total M and H subunits in a manner which made these two subunits more or less equal in activity. A similarity between testosterone propionate and androstenedione was evident with respect to the LDH5 isozyme, which is exclusively formed of anaerobic subunits (M-subunits). Both these hormones have specifically raised this subunit, favouring anaerobic metabolism in the tissue. This may be due to the fact that the coagulating gland is dependent on, and responding to, the androgenic hormones to rejuvenate the tissue from the castration loss. This naturally involves extra metabolic demands, which are aptly met with, from the anaerobic source also. As evidence of these ultrastructural studies, which revealed a complete restructure of the epithelial layers and rejuvenation of the rough endoplasmic reticulum and an increased number of secretory granules due to androgenic influence on the castrated coagulating gland (Dahl et al., 1973).

In addition to the increase in LDH5, testosterone propionate was able to bring about a significant increase in LDH3 and LDH4 in such a manner as to restore the castration induced decrease in M-subunit back to the Sham operated control level. However, androstenedione was not able to increase LDH3 and LDH4 isozymes. This perhaps confirms that the testosterone propionate is the most potent hormone which increases the M-isozymes of LDH (Pereira et al., 1981). This oestradiol like testosterone propionate triggered a rise in LDH3 and LDH4 isozymes, unlike androstenedione. It is likely that the events associated with an increase in LDH3 and LDH4 alone may be due to the specific oestrogenic stimulation of fibromuscular layer in the coagulating gland (Tisell et.al. 1971).

In human testis, there is appearance of an additional isozyme of LDH which is differs from H and M-subunits found in other five isozymes. Though being the major isozymes of human spermatozoa its presence is also reported in rabbit as well as other animals. In the mouse, LDH-X activity becomes apparent when the animals are about 22 day old and reaches a maximum about three weeks later (Wilkinson et al., 1970). Goldberg and Howtrey (1967) noticed the presence of LDH-X in detectable amount when there is first appearance of primary spermatocyte in mice. Being sperm specific this isozyme is used as an antigen to induce infertility in female mice. The reduced number of pregnancies was marked significantly after the injection of anti-mouse LDH-X serum to rabbit on days 1-4 after coitus in these animals. When LDH-X was injected in mice, pregnancy was significantly decreased with 6-8 week after the primary immunization.

Goldberg (1971) demonstrated that LDH-X is immunologically distinct from LDH1 and LDH5. Goldberg (1972) reported that this LDH-X is different from other LDH-isozymes in chemical and enzymatic properties. Rabbit antiserum to mouse LDH-X can suppress pregnancy in mice (Goldberg and Lerum, 1972). Goldberg (1973) found that immunization of female rabbits with mouse LDH-X reduces fertility. Lerum and Goldberg (1974) reported the immunological impairment of pregnancy in mice by LDH-X. Goldberg (1963) noticed that out of six isozymes found in human seminal plasma, 1-5 originate from the prostate whileLDH-X is associated with the presence of spermatozoa.

Hintz and Goldberg (1977) and Storey and Kayne (1977) reported that LDH-C4 is a cytosolic enzyme. Its extra cellular occurrence therefore represents leakage from germ cells. Blanco et al., (1976) found that LDHx isozyme must be integrated in metabolic pathway that provides energy for motility and survival of spermatozoa. Gerez-de- Bergos et al., (1979) reported that this isozyme of LDH is bound to the surface of spermatozoa, so it can be found in seminal plasma due to an outward diffusion of the isozyme from the spermatozoa or to spontaneous destruction of cells. In oligospermia, high value of LDH-X was reported by Gavella et al., (1982). This isozyme also shows higher activity in patients having genital tract infections (Virji et al., 1985). The activity of this isozyme did not change after

ejaculation. Verma et. al 2017 also observed that alteration in LDH after the administration of Piper betel leaf stalk, the alteration in LDH isozymes increases anaerobic condition in seminal plasma by increasing M sub-unit of LDH which relates infertility in male mice.

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