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THE RELATIONSHIP BETWEEN CIRCULATING SEX HORMONES AND PLASMA CK-MM AND LDH ACTIVITY IN PHYSICALLY ACTIVE REGULARLY MENSTRUATING FEMALES

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ABSTRACT

Introduction: It has been noted that CK-MM response to physical stress is much less pronounced in females than in males probably due to lower striated muscle mass and protective estrogen action in the muscle. However, data concerning possible contribution of physiological fluctuation in ovarian hormones to enzyme leakage into blood are not available.

Aim of the study: This study aimed at evaluation of resting plasma CK-MM and total LDH activity in females in follicular and luteal phases of the menstrual cycle and comparison of data with these found in males.

Methods: A total of 46 subjects (25 males and 21 females) volunteered to participate in the study. Basal body temperature measurements 3 months before the study and assay of 17β-estradiol and progesterone plasma levels between days 5-8 and again between days 19-22 of the menstrual cycle allow to classify female subjects as ovulating. Male participants were tested twice, 2 week apart. Plasma CK-MM and LDH activities were determined at 340 nm using commercial kits (Alpha Diagnostics and Randox Laboratories, respectively). Ovarian hormones were determined using a standard radioimmunoassay commercial kits (POLATOM, Swierk).

Results: In females plasma CK-MM activity did not differ with respect to the menstrual cycle phase and there were no difference in enzyme activities between male and female subjects. In contrast, plasma LDH activity in the luteal phase was significantly higher than in the follicular phase of the menstrual cycle. Both in males in females weak, but significant correlations were found between plasma CK-MM and LDH activity indicating that only a minor part of LDH in plasma was muscle-originated. Multivariate regression analysis revealed that in females plasma LDH activity was more strongly associated with plasma 17β-estradiol levels than with plasma CK-MM activity.

Conclusion: Circulating ovarian hormones have no effect on plasma CK-MM activity in regularly menstruating women. On the contrary, physiological fluctuations in sex hormones due to menstrual cycle significantly contribute to the variability in plasma LDH activity.

Key words: females, menstrual cycle, creatine kinase MM, lactate dehydrogenase

Introduction

There is a wealth of studies suggesting that apart from well-known function in reproduction ovarian hormones contribute to the regulation of many metabolic processes. It is well documented that a close link exists between circulating ovarian hormones and whole-body insulin sensitivity (1,2,3). Additionally, 17β-estradiol receptors has been identified in the endocrine pancreas further supporting a special importance of female sex hormones in the regulation of islet function (4). Lower risk of coronary heart disease in premenopausal women as compared to men suggests a vasoprotective role of female sex hormones (5,6).

Numerous data indicated that the greater reliance of female working muscle on fat utilization is at least partially due to estrogen action (7,8). Moreover, estrogens are recognized to protect skeletal and cardiac muscle against oxidative damage and to diminish inflammatory response in the muscle induced by excessive physical stress (9).

Muscle damage and leakage of muscle-originated enzymes into the circulation in response to exercises of different intensity and duration is a common and normal event. One of the most valid and reliable methods for assessing muscular damage is to check plasma creatine kinase MM (CK-MM) activity following muscular work since the enzyme is located mainly in skeletal muscle and an increase in its plasma activity following exercise is correlated with muscular damage diagnosed using magnetic resonance images (10,11).

The elevation in plasma CK-MM activity following either a single exercise bout (marathon run, kayak marathon, prolonged bicycle race, repeated Wingate test) or training in different sports was reported by many authors (12,13,14,15,16).

The physical stress also induces the elevation in other marker enzymes in plasma (e.g. lactate dehydrogenase – LDH, aspartate and alanine aminotransferases – AST, ALT) (17,18).

It has been noted that CK-MM response to physical stress is much less pronounced in females than in males probably due to lower striated muscle mass (19,20,21). However, a protective effect of estrogens in the muscle has to be considered as a potential reason for less pronounced exercise-induced CK-MM leakage into plasma in female subjects (for review see 22). It seems feasible since repeated 17β-estradiol injections into male rats diminished exercise-induced CK-MM release to plasma (23). On the other hand, total muscle LDH activity seems to be sex-related and significantly lower in females versus male subjects both at rest and after exercise (24,25,26).

However, according to our best knowledge the effect of physiological variation in circulating ovarian hormones due to menstrual cycle on plasma CK-MM and LDH activity in physically active women has not been studied.

Thus, this study was undertaken to evaluate resting plasma CK-MM and total LDH activity in females in follicular and luteal phases of the menstrual cycle. To explore further the possible effect of physiological variations in circulating ovarian hormones on enzyme leakage we included male subjects of matched physical activity into the study and performed the assays of plasma CK-MM and LDH according to a similar schedule as in females (i.e. twice, 14 days apart).

Materials and Methods

Subjects

The participants of the present study were recruited on a voluntary basis among male and female physical education students. None of the participants were engaged in high-performance sport, but their physical activity varied from 7 to 11 h/week due to an obligatory physical education program (games, swimming, track and field, and martial arts). The participants were asked to refrain from strenuous physical activity for 48 h before blood withdrawal and to follow their regular dietary habits throughout the study. The experimental protocol was approved by the Ethics Commission at the Academy of Physical Education.

Anthropometric characteristics

Three skinfolds (triceps, subscapular and abdominal) were measured twice using Harpenden caliper (United Kingdom) and mean values were taken for calculation of body fat (27).

Blood collection

Fasting blood samples were withdrawn from the antecubital vein using disposable needles and syringes. Prospective female subjects measured basal body temperature (BBT) for 3 month before the study (28). Exclusively females with biphasic BBT pattern were included into the study. Plasma 17β-estradiol and progesterone concentrations were determined twice per cycle – between days 5 and 8 and again between days 19-22 of the cycle. All females with plasma progesterone levels lower than 19 nmol/l between days 19 and 22 were excluded from the study (29). Finally we recruited 21 females with ovulatory menstrual cycles. Blood from male subjects were taken twice, 2 weeks apart.

Erythrocytes and plasma fractions were separated by centrifugation (15 min/4000 rpm, 4^0 C) and plasma was stored 7 days at – 70^0 C until analyses.

Biochemical analyses

Plasma lactate (La) was assayed colorimetrically at 550 nm, plasma LDH activity was determined spectrophotometrically at 340 nm using commercial kits (Randox Laboratories Ltd., United Kingdom). Plasma CK-MM activity was determined spectrophotometrically at 340 nm using commercial kits (Alpha Diagnostics, Poland). Both LDH and CK-MM activities were measured at 37^0C. Plasma zinc (Zn) concentrations were measured using atomic absorption spectrometry (30). Intra-and inter-assay coefficient of variations (CV) for zinc determinations were 3.4% and 5.6%, respectively. Plasma 17β-estradiol and progesterone levels were assayed using standard radioimmunoassay methods and commercial kits purchased from OBRI-ORION, Poland. Intra-and inter-assay coefficients of variation (CV) for 17β-estradiol were 3.8% and 7.9%, for progesterone 4.3% and 5.0%.

Statistical analysis

Data distribution was evaluated using the Shapiro-Wilk test. The comparison of normally distributed data was performed by use of Student t-test for paired and unpaired data. Mann-Whitney U test and Wilcoxon matched-pairs test were used for comparison of unpaired and paired data which were not normally distributed. Pearson product-moment and Spearman rang correlations were also calculated. Multivariate analysis were performed by use of forward stepwise regression, to determine the model which best predicts plasma LDH activity. All calculations were made using Statistica 6.0 (Statsoft, USA). All data are presented as means ± SD. Significance level was set at $p < 0.05$ for all statistical tests.

Results

Female subjects were slightly younger than males and were characterized by significantly higher fat mass, the percentage of body fat and lower lean body mass than their male counterparts (Table 1).

Table 1. *Physical characteristics of physically active male and female subjects*

	Males (n=25)	Females (n=21)
Age (years)	22.6 ±1.3	20.5 ±1.2[a]
Body height (cm)	180.8 ± 5.2	172.4 ±6.7
Body mass (kg)	79.0 ± 11.7	63.3 ±7.8
Fat (kg)	12.6 ± 5.2	15.4 ± 3.0 [b]
Fat (%)	15.9 ± 5.2	24.3 ± 2.0[a]
LBM*	66.4 ±7.6	47.8 ± 5.2[a]
Cycle length (days)	–	27.8 ± 1.9

Values are means ± SD.
*LBM – lean body mass
[a]$p < 0.0001$ vs. males; [b]$p < 0.006$ vs. males

Table 2. *Ovarian hormone plasma levels during follicular and luteal phases of the menstrual cycle*

	Days 5-8	Days 19-22
17β-estradiol (pmol·L^{-1})	206.5 ± 103.9	525.8 ± 122.3[a]
Progesterone (nmol·L^{-1})	2.2 ± 0.6	38.5 ± 13.1[a]

Values are means ± SD
[a]$p < 0.001$ - significantly higher vs. days 5-8.

Table 3. *Biochemical characteristics of male and female subjects*

	Males (n=25)		Females (n=21)	
	I	II	Days 5-8	Days 19-22
CK-MM (U·L^{-1})	121.5 ± 76,5	138.7 ± 108.2	149.2 ±55.2	152.2 ±63.2
La (mmol·L^{-1})	1.8 ±0,4	1.7 ±0,4	1.7 ± 0,5	1.6 ± 0,6
Zinc (μmol·L^{-1})	13.9 ± 1.3	13.8 ±1.5	15.2 ± 1.2[c]	15.1 ± 1.2[c]

Values are means ± SD
[a]$p < 0.002$ - significantly higher vs. days 5-8; [b]$p< 0.0003$ - significantly higher vs. test II in males; [c]$p < 0.004$ -significantly higher vs. males

Plasma levels of 17β-estradiol and progesterone in the luteal phase were significantly higher than in the follicular phase of the menstrual cycle (Table 2). In male students no differences were found in plasma CK-MM activity, lactate and zinc levels tested 2 weeks apart (Table 3). In female participants the above-mentioned variables did not differ with respect to the menstrual cycle phase. Plasma zinc levels did not differ in the follicular and luteal phases of the menstrual cycle, however, in females were significantly higher than in males. On the contrary, in females plasma LDH activity in the luteal phase of the menstrual cycle was significantly (by 23.5%, $p<0.002$) higher than in the follicular phase of the menstrual cycle and markedly higher than in testing II in males (by 32,5 %, $p<0.0003$) (Fig. 1).

Neither in female nor in male students plasma LDH activity was correlated with plasma zinc levels. Both in male and female subjects plasma LDH activity was positively correlated with plasma CK-MM activity ($r=0.341$, $p <0.02$ and $r=0.322$, $p<0.04$ in males and females, respectively (Table 4). In female subjects significant correlation was also noted between plasma LDH activity and ovarian hormone plasma levels ($r=0.470$, $p< 0.002$ and $r=0.314$, $p<0.04$ for 17β-estradiol and progesterone, respectively).

However, multiple regression analysis revealed that the best model predicting plasma LDH activity in female students included CK-MM activity and 17β-estradiol levels (Table 5). Additionally, partial correlation coefficients showed that the effect of 17β-estradiol on resting LDH activity is more pronounced that of plasma CK-MM activity (Table 6).

Discussion

Our study showed that pronounced physiological elevation in plasma 17β-estradiol and progesterone levels in the luteal phase of the menstrual cycle had no effect on resting plasma CK-MM activity. This finding is further supported by the laCK-MM of dif-

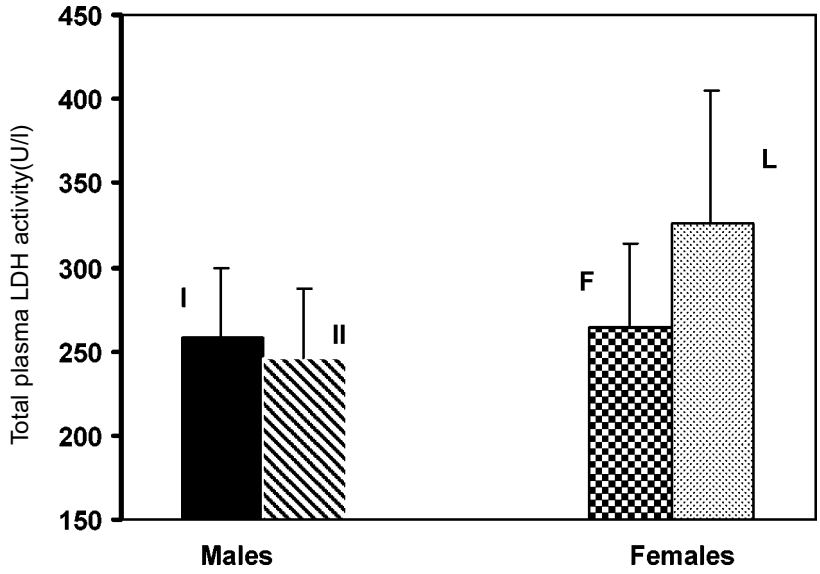

Fig. 1. *Total plasma LDH activity in males tested two weeks apart (I,II) and in females in the follicular (F) and luteal (L) phases of the menstrual cycle. Significantly higher vs. follicular phase (p<0.002) and vs. test II in males (p< 0.0003)*

Table 4. *Correlation coefficients between plasma LDH activity and other biochemical variables taken together for follicular and luteal phases in female subjects*

	Females (n=21)
LDH/CK-MM	0.322[b]
LDH/progesterone	0.314[b]
LDH/17β-estradiol	0.470[c]
LDH/Zn	- 0.040
LDH/La	0.110

[a]p < 0.02; [b]p < 0.04; [c]p < 0.002

Table 5. *The model used to predict plasma LDH activity in regularly menstruating female subjects*

Independent variables*	Model adjusted R^2	F	p
CK-MM ($U \cdot L^{-1}$)	0.106	5.85	0.02
17β-estradiol	0.355	12.29	0.00007
Progesterone	0.339	8.01	0.0003

*independent variables in order of introduction into the model

Table 6. *Regression and partial correlation coefficients of the best model used to determined predictors of plasma LDH* activity in female subjects*

Independent variables entered	Regression coefficient (B)	Standard error of B	Partial correlation coefficients	t	p
CK-MM ($U \cdot L^{-1}$)	0.0055	0.0018	0.437	3.035	0.004
17β-estradiol*	0.1970	0.0485	0.545	4.058	0.0002

*Plasma LDH activity and 17ß-estradiol levels were not normally distributed and logarithmically transformed data (natural logarithm) were introduced into the equation

ferences in plasma CK-MM in male and female subjects not engaged in sport and characterized by equal physical activity.

In all the participants of the current study plasma CK-MM activity was within physiological limits. This finding is in agreement with our previous data which indicated that the mean resting plasma CK-MM activity in male physical education students was 65.2 $U \cdot L^{-1}$ and significant (approximately 2-fold) elevation appeared after 1 week of intensive training (31).

In the present study we did not observe any fluctuation in plasma LDH activity in male students tested twice, 2 weeks apart. Additionally, correlation analysis revealed that only a minor part (11.6 %) of total LDH in males was probably muscle-originated, since there was a significant but weak relationship between plasma LDH and CK-MM activity (r=0,341). Similarly in females only 10.4% (r=0.322) possibly originated from the striated muscle.

The origin of total LDH activity in plasma in response to physical activity is difficult to define due to wide distribution of the enzyme in the body (32). Our previous study indicated that marked elevation in total plasma LDH activity in male sportsmen after the Ironman Triathlon was mostly due to hepatic isoenzyme release into circulation (33). On the contrary, others observed cardiac isoenzyme release in response to the triathlon competition (34). However, in the present study the plasma LDH isoenzyme profile was not determined.

The most important finding of the current study is significant increase in total LDH activity in the luteal in the comparison with in the follicular phase of the menstrual cycle. In addition, multivariate regression analysis revealed that 17β-estradiol is responsible for the most variability in plasma LDH activity in female subjects.

The reason for the above finding could be only speculated. There are a few data indicating that estrogen treatment depresses LDH leakage from muscle, heart and liver damaged due to ischemia, chemical treatment or pathological changes in muscle dystrophy (35-38).

On the other hand, it is well documented that LDH is highly expressed in the female reproductory system (uterus, uterine fluid and ovaries) (39-41). Moreover, ovary LDH activity is markedly elevated in response to hCH- induced ovulation (42). Additionally, LDH activity in follicular fluid is correlated with follicle size (43). Interestingly, salivary lactate dehydrogenase showed a peak at the ovulation with no changes in enzyme activity in females characterized by anovulatory menstrual cycle (44). The above data clearly indicate a close link between LDH activity and female sex hormones. Unfortunately, data concerning female sex hormone action on LDH activity in skeletal muscle, liver or cardiac muscle i.e. tissues which may possibly contribute to the elevation in plasma LDH are not available.

Summing up, physiological fluctuation in ovary hormone secretion during the menstrual cycle did not affect plasma CK-MM activity in female subjects. In contrast, plasma LDH activity is significantly elevated in the luteal phase of the menstrual cycle possibly due to 17β-estradiol action. However, the significance of this finding with respect to enzyme activity in the muscle and other tissues needs further studies.

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