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RELATIONSHIP BETWEEN FASTING INSULIN RESISTANCE INDEX (FIRI) AND PLASMA GLYCEROL AND FREE FATTY ACID LEVELS IN PHYSICALLY ACTIVE MALES AND FEMALES

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Abstract. It is well known that whole-body insulin sensitivity is greater in sedentary women than in men. However, data concerning insulin sensitivity in physically active males and females are not available. This study aimed at evaluation of insulin sensitivity expressed as fasting insulin resistance index (FIRI) and its relationship with plasma glycerol and free fatty acid levels in physical education students of both sexes not engaged in high-performance sport, but highly active (7 h/week) due to the obligatory study program. Blood was withdrawn from the antecubital vein after an overnight fast. Plasma glucose, glycerol and free fatty acids (FFA) were assayed colorimetrically using Randox commercial kits (Randox Laboratories, United Kingdom). Plasma insulin was determined by a standard radioimmunoassay method and Bio-Source commercial kits (Belgium) with monoclonal antibodies against insulin. Plasma glucose and insulin concentrations in females were found to be significantly lower than in males and in consequence the FIRI value in the former was markedly lower than in the latter indicating greater insulin sensitivity in females vs. males of matched physical activity. Plasma glycerol and FFA concentrations did not differ between the sexes. However, in women, but not in men there was a significant and positive correlation between plasma glycerol and FFA levels ($r=0.55$, $p<0.01$) indicating a balance between lipolytic activity and FFA uptake by peripheral tissue. In men, but not in women plasma FFA levels were significantly and inversely correlated with FIRI ($r=-0.59$, $p<0.01$). The above data suggest that FIRI values in males are more sensitive to the fluctuation in plasma FFA concentrations than in females. On the other hand, it could not be excluded that in physically active males both FFA release from adipose tissue and uptake by peripheral tissue is more sensitive to insulin action than in females.

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Key words: Male – Females – Fasting insulin resistance index (FIRI) – Glycerol – Free fatty acids

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Introduction

Since many years numerous studies have focused on sex-related differences in adipose tissue triacylglycerol metabolism at rest and during exercise. Greater reliance of working muscle on fat as an energy source in females is a consequence of greater fat stores in adipose tissue as well as greater adipose tissue sensitivity to adrenergic stimulation [3,11,19,22,24,32,45]. However, more recent studies have addressed the importance of insulin action in adipose tissue [1,21]. In both men and women insulin increases lipogenesis by stimulation of glucose transport into adipose tissue and in consequence exerts a potent antilipolytic effect [2,5,33], however, this action is more potent in female than in male subjects [26,34]. Thus, it seems that adipose tissue sensitivity to hormones responsible for the rate of lipolysis (both adrenalin and insulin) is greater in female than in male subjects.

Sex-related differences in insulin sensitivity were also noted in striated muscles. In women insulin-mediated glucose uptake by the muscles is markedly greater than in men, however the reason for this difference has not been fully elucidated [15]. However, it is well established that muscle sensitivity to insulin increases with the increasing percent of slow twitch fibres, capillary density in the muscle and insulin-induced vasodilation [4,18,43,44]. In females the percent of slow twitch fibers and muscle perfusion is greater than in males and both may contribute to greater insulin sensitivity in the former than in the latter [28].

In both sexes a close association between muscle insulin sensitivity and adipose tissue lipolysis has been noted [35]. It has been found that plasma glycerol and free fatty acid levels increase with decreasing insulin sensitivity suggesting parallel disturbances in adipose tissue and muscle insulin sensitivity.

It is well established that physical training increases adipose tissue and muscle insulin sensitivity and in consequence in trained subjects plasma insulin levels are lower than in sedentary counterparts [27,30,38,46]. However, it has to be underlined that the studies cited above were performed either with male or female participants. There is no data concerning the comparison of insulin sensitivity in subjects of both sexes enrolled in similar programs of physical activity.

This study was aimed at evaluation of plasma glycerol, free fatty acid, glucose and insulin levels as well as calculation of surrogate index of insulin resistance – FIRI – in physically active males and females. In addition, we looked for possible association between above-mentioned variables.

Materials and Methods

A total of 36 subjects volunteered to participate in the study (17 males and 19 females). All the subjects were physical education students not engaged in high-performance sport, however they were participating in different sports due to the obligatory study program (track and field, games, dancing, gymnastics). Their mean weekly physical activity equaled 7.5 h. All the subjects were healthy and they do not take any medication on a regular basis. All the females were regularly menstruating with cycle length 26-31 days. The participants were asked to follow their dietary habits throughout the study.

Blood samples: Blood was withdrawn from the antecubital vein after an overnight fast using disposable needles and syringes into polypropylene tubes containing EDTA for free fatty acid determination and lithium heparin for other biochemical variables. To separate plasma blood was centrifuged (15 min, 3000 rpm). Plasma was stored at -20^0 C until analysis. Blood from female subjects was taken between days 5 and 9 of the menstrual cycle.

Biochemical analyses: Plasma glucose, glycerol and FFA concentrations were measured colorimetrically using Randox commercial kits (Randox Laboratories, United Kingdom). Plasma insulin was determined using a standard radioimmunoassay method and monoclonal antibodies against insulin (BioSource, Belgium). Intra- and inter-assay coefficients of variations were 6.8% and 9.3%, respectively. Coefficient of variations for other variables did nor exceed 6%.

Calculations and statistical analysis: Fasting insulin resistance index (FIRI) was calculated according to Duncan (16) and following formula; FIRI = [fasting glucose (mmol/l) x fasting insulin (mIU/l)]/25.

Data distribution was evaluated using Shapiro-Wilk W test. Student t- test was used for comparison of normally distributed data (glucose, glycerol and FFA). Mann-Whitney U test was used for comparison of data with non-Gaussian distribution (insulin, FIRI). Pearson product moment and Spearman rank correlation coefficients were calculated to evaluate the associations between variables. Data are presented as means ±SD. Significance was set at $p<0.05$. All calculations were done using Statistica v.6 (StatSoft, USA).

Results

Anthropometric characteristics of the subjects are presented in Table 1. Female subjects differed significantly with respect to body mass, body height, lean body mass, percent of body fat ($p<0.001$) and maximal oxygen uptake ($p<0.03$) in comparison with their male counterparts.

Table 1
Subject characteristics (mean ±SD**)**

	Females (n=19)	Males (n=17)
Age (years)	22.1±0.8	22.5±1.7
Body mass (kg)	58.8±6.0*	77.2±7.4
Body height (cm)	166.4±5.2*	181.4±5.5
Fat (kg)	11.8±3.6	10.9±1.9
Fat (%)	20.0±4.3*	14.2±2.1
Lean body mass (kg)	46.9±3.7*	66.2±6.4
VO_2max (ml/kg/min)	43.9±7.9^	50.6±9.3

*significantly higher vs. males ($p<0.001$);
^determined during incremental cycling exercise, significantly lower vs. males ($p<0.03$)

Plasma glucose and insulin levels in males were significantly higher than in females ($p<0.01$ and $p<0.04$, respectively) (Table 2). In consequence FIRI in men was markedly higher than in women ($p<0.02$). Plasma glycerol and FFA concentrations did not differ in male and female subjects. In females, but not in males there was a significant ($p<0.01$) and positive association between plasma glycerol and FFA levels (Table 3). In male, but not in female participants FIRI was significantly and inversely related to plasma FFA concentrations ($r=-0.59$, $p<0.01$).

Table 2
Plasma glucose, insulin, glycerol and free fatty acid levels and fasting insulin resistance index (FIRI) in physically active males and females (mean ±SD)

	Females (n=19)	Males (n=17)
Glucose (mmol/l)	4.5±0.6	5.0±0.5[a]
Glycerol (µmol/l)	58.8±17.7	49.4±14.5
FFA (µmol/l)	237.9±119.7	247.6±129.8
Insulin (mU/l)	7.5±3.5	9.9±2.9[b]
FIRI	1.41±0.76	1.68±0.77[c]

*FFA – free fatty acids;
[a]significantly higher vs. females ($p<0.01$);
[b]significantly higher vs. females ($p<0.04$);
[c]significantly higher vs. females ($p<0.02$)

Table 3
Correlation coefficients between tested variables in physically active males and females

	Women (n=19)	Men (n=17)
Glycerol-FFA	0.55[a]	0.22
FIRI Glycerol	-0.25	-0.23
FIRI -FFA	-0.17	-0.59[b]

[a]Pearson correlation coefficient ($p<0.01$);
[b]Spearman rank correlation coefficient ($p<0.01$)

Discussion

Higher plasma insulin levels and higher FIRI in males than in females is in agreement with the data of Fernández - Real *et al.* [17]. These authors have also demonstrated that in males FIRI increases with increasing fat free mass, but no such relationship has been noted in females. Thus it could be presumed that higher fat free mass in males of the present study partially contributes to higher FIRI i.e. lower insulin sensitivity. It is worthy to note that in high performance male athletes

FIRI varied from 0.82 to 1.30 [38,39]. On the other hand, in trained women FIRI was markedly lower than in male athletes and equaled 0.44 [21]. In sedentary men and women aged approximately 30 years FIRI values of 1.6 and 1.3 have been found and they are similar to those noted in the present study.

The reason for lower FIRI in females than in males participating in our study could be only speculated. However, the contribution of female sex hormones in the regulation of insulin sensitivity has to be taken into consideration. It is well documented that disturbed ovarian hormone secretion and subsequent hyperandrogenism brings about insulin resistance [10,42]. Moreover, insulin sensitivity in females decrease with age and HRT during menopause is known to improve whole-body insulin action [6].

However, lifestyle factors are also known to affect insulin sensitivity. It is well known that a fat-rich diet depresses insulin sensitivity [7,47]. However, our unpublished data have shown that both males and females consumed equal percentages of energy from fat (approximately 30%). On the other hand, it has been found that fluctuation in insulin sensitivity may be due to the last meal consumed on the evening preceding blood sampling [36]. Unfortunately, this factor was not controlled in the present study.

The results of the present study have shown that sex-related differences in insulin sensitivity persist even in highly active subjects since FIRI values in females were lower than in males as has been noted in sedentary subjects [15]. This may be explained by faster glycogen replenishment after exercise in women than in men [29]. On the other hand, greater insulin sensitivity may contribute to greater blood glucose uptake by working muscles in women than in men [11,37].

It is well known that after 72 h fasting blood glucose levels are lower in women than in men [14]. This is due to depressed neural and hormonal response to hypoglycemia in females than in males [11-13]. On the other hand, even after 6 h fasting the tendency to lower blood glucose in women has been noted [31]. Our results have indicated that after overnight fast plasma glucose in women was slightly, but significantly lower than in men (by 10%).

Plasma glycerol levels are a reliable index of subcutaneous adipose tissue lipolysis [23]. The contribution of glycerol released from intramuscular triacylglycerols to total plasma glycerol is low and does not exceed 15% [33]. Literature data concerning sex-related differences in plasma glycerol concentrations are equivocal and indicate either higher or similar metabolite levels in women vs. [9,13,20]. It could not be excluded that these discrepancies are due to the differences in subjects' fitness level and/or dietary habits. This seems feasible, since elevated lipolysis may be a consequence of restricted diet or endurance

training [8,25]. In our study plasma glycerol levels were similar in both sexes suggesting that adipose tissue lipolysis does not differ in female and male participants.

Plasma FFA levels reflect both their release from the adipose tissue and peripheral uptake, mostly by striated muscle. After overnight fast FFA are the main energy source for the muscle [40]. In the present study there were no sex-related differences in plasma FFA concentrations suggesting that their release and uptake were similar in both sexes.

Surprisingly, exclusively in females plasma FFA concentrations were significantly and positively correlated with plasma glycerol levels. It could be tentatively speculated that in females adipose tissue lipolysis matched FFA peripheral uptake. At present it is unclear why no such correlation was observed in males.

Significant and inverse correlation between fasting plasma FFA levels and FIRI in male but not in female participants suggests that there is sex-related difference either in FFA effect on insulin sensitivity or in insulin action on FFA uptake by peripheral tissues, since no effect of FIRI on adipose lipolysis has been noted. The reason for this could be only speculated. Under hyperinsulinemic conditions plasma FFA levels were found to increase with decreasing insulin sensitivity [21,26]. According to our best knowledge there is no data concerning the relationship between insulin sensitivity and plasma FFA concentrations under fasting conditions. However, it could be presumed that striated muscle less sensitive to insulin rely more on FFA than on glucose uptake and in consequence plasma FFA levels decrease with increasing FIRI value.

Summing up, our study has revealed that even in physically active subjects insulin sensitivity expressed as fasting insulin sensitivity index (FIRI) is greater in physically active females than in males. Neither plasma glycerol nor FFA plasma concentrations differed with respect to sex. However, exclusively in males a significant and inverse correlation between FIRI and plasma FFA levels has been found possibly indicating a greater contribution of insulin sensitivity to FFA fate in the body and/or more pronounced action of FFA on insulin sensitivity in men vs. women.

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