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Metabolic Status of Patients with Hypoestrogenic Anovulation on the Long-Term Hormone Replacement Therapy

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Research Article

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ABSTRACT

27 patients with hypoestrogenic anovulation (11 - hypogonadotropic hypogonadism (HH) and 16 - premature ovarian failure (POF) have been observed for more than 5 (6.9 ± 1.8) years. The average age at the end of the study HH patients was 28.5 ± 4.9 years, with POF – 36.3 ± 6.4 years. Estradiol/dydrogesterone (E2/D) was used in 11 patients (n=4 – HH; n=7 – POF) Estradiol valerate/levonorgestrel (EV/LNG) – 16 patients (n=7 – HH; n=9 – POF). The prevalence of insulin resistance has increased significantly 26 (92.3%) vs. 20 (74.1%) χ2 A/D p<0.01). Stepwise regression method is carried out to find the most significant predictor of deterioration insulin sensitivity in patients with hypoestrogenism on a long-term hormone replacement therapy (HRT). Analysis of the cumulative effect of each other such factors as: the choice of medicine for HRT, age, BMI at the end of the study, triglyceride levels, family anamnesis of type 2 diabetes (T2D), has determined the leading predictor of increasing index HOMA only family anamnesis of T2D (R=0.92; p=0.00). The frequency of hypertriglyceridemia has also increased significantly (9 (33.3%) vs. 6(22.2%) χ^2 A/D p<0.05). The main predictor of growth hypertriglyceridemia recognized age of women at the end of the study (R=0.49; p<0.05), but the choice of the medicine. The number of women with impaired glucose tolerance increased to 14.8% (vs. 0%) χ2 A/D 21.0; p<0.001). Predictors of IGT (T2D) patients with hypoestrogenism on the long-term HRT called HOMA index (R=0.74; p<0.01) and anamnesis for T2D (R=0.76; p<0.001). HRT does not protect the deterioration of insulin sensitivity in patients with a family anamnesis of T2D and is not a means of prevention of this disease.

INTRODUCTION

It is known that patients with the syndrome hypoestrogenism have an increased risk of metabolic disorders [1]. This is due to the distortion of the steroid-mediated reactions in the synthesis and secretion of insulin [2,3] glucose utilization [4-6] and fat metabolism [7,8]. Use of hormone replacement therapy (HRT) per os is associated with intense participation of the liver in the biotransformation of exogenous steroids. Non-physiological estrogen-progestin impact on the body can lead to the development of insulin resistance [9], dyslipidemia [10], especially in individuals with a genetic predisposition to atherosclerosis [11] and type 2 diabetes (T2D) [12]. The present research is dedicated to the metabolic status of patients with hypogonadotropic hypogonadism (HH) and premature ovarian failure (POF) for long-term HRT.

PATIENTS AND METHODS

Patients were divided into groups based on the pathophysiology mechanism hypoestrogenic anovulation: 1 group – isolated HH (n=19), 19 (18; 21) years; 2 group – POF (n=44), 32 (29.5; 37.5) years.

Serum levels of LH, FSH, prolactin, testosterone, sex-binding globulin (SBG), dehydroepiandrosterone sulfate (DHEA-S) and other hormones were evaluated by solid chemiluminiscence immunoassay (IMMULITE I-DPC, Beckman Coulter DxI). For LH kit the variation intra-assay coefficient was 6.8%, the variation inter-assay coefficient was 7.2% and assay sensitivity was 0.2 ng/ml. For FSH kit variation intra-assay coefficient was 5.3%, the variation inter-assay coefficient was 5.5% and the assay sensitivity was 0.17

ng/ml. The other hormonal, biochemical and hematogical parameters were determined using commercially available automated chemiluminescence immunoassay and other systems. The intra- and inter-assay CV for all methods were <5.8% and <7.8%, respectively. Bioactive testosterone (bioTs) concentration estimated by on-line calculator ISSAM [13] using SBG level, constant SA [albumin concentration 4.3 g/dl]. Androgens levels compared to age-matched healthy women: Ts (50.8 (38.0; 60.9) ng/l); bioTs (13.2 (9.5; 19.6) ng/dl); DHEA-S (228 (180; 310) mg/dl).

Diagnosis was based on complaints, anamnesis, negative progesterone test, positive estrogen-progestin test, test with agonist of gonadotropin-releasing hormone (GnRH).

Intramuscular administration 1 ml of 1% solution of progesterone for 6-8 days or oral administration of dydrogesterone 10 mg daily for 7-10 days. The appearance menstrual-like reaction in 2-7 days after discontinuation of therapy indicates a mild of estrogen deficiency. A negative test shows a deep failure of estrogens.

Test with estrogen-progestin used after a negative progesterone test to assess the endometrial function. Method: use any drugs for cyclical HRT (we used the 17β-E2) 2 mg/day for 14 days and 17β-E2 2 mg plus Dydrogesterone (D) 10 mg/day for 14 days). A positive test (the appearance of menstrual-like reaction after the end of the cycle) confirms the functional safety of the endometrium. This is an indication for further examination on the subject of hypoestrogenism.

Test with agonist of GnRH for assessing the viability of gonadotropin cells. Patients with HH typically have subnormal gonadotropin responses after 1 and 4 h subcutaneus administration Triptorelin 100 mcg – LH less than 10 IU/L.

Women with POF have extremely high figures FSH and LH, typical for postmenopausal women age.

Fasting glucose and oral glucose tolerance test was determined in venous plasma (SUPER GL ambulance). Determination of cholesterol (CHO), triglycerides (TG), high density lipoproteins (HDL-C), low density lipoprotein (LDL-C) in serum was performed using photometric colorimetric test (OLIMPUS).

Criteria of insulin resistance (IR): basal hyperinsulinemia (HI) (>12.2 uU/ml) [14] and/or HOMA index (homeostasis model assessment)>1.8 [15]. Metabolic syndrome (MS) was verified on the basis of criteria International Diabetes Federation (IDF, 2005) [16]:

Statistical data analysis was run using software STATISTICA 6.0 and MedCalc Version 7.4.2.0.We used Shapiro-Wilk test to assess normality of distribution, and relevant t-test for comparison of two depending or non-depending samples. Descriptive non-parametrical statistics included Median (Me), Mean (M), standard deviation (SD), quartiles [25;75] and Min–Max range. To compare quantitative parameters of non-related samples, Mann-Whitney (U) test was applied. Wilcoxon test (W) and Friedman variance analysis (F) was used when comparing two or more related samples. Chi-squared test (χ^2) was done to analyze difference in distribution of categorical parameters in two groups. Correlation between numerical parameters was tested by Spearman (r). The method of the step-switching predictors for multiple regression analysis. All tests were two-tailed, and statistical significance was considered for $p<0.05$.

RESULTS AND DISCUSSION

In group 1 of 19 women HI detected in 10 (41.9%), HOMA index >1.8 - in 11(57.9%) cases. MS (abdominal obesity, increased triglycerides, decreased HDL-C) was diagnosed in 4 (21.1%) of patients.

In group 2 of 44 women showed 24 (54.5%) patients with HI. IR on the basis of HOMA >1.8 was diagnosed in 29 (65.9%) of patients. Dyslipidemia occurred in 35 (79.5%) cases. MS was verified in 15 (34.1%) of patients. Its components were represented by a combination of abdominal obesity with hypertriglyceridemia and low HDL-C in 11 (25%), with hypertriglyceridemia and low HDL-C and violation of carbohydrate metabolism – in 4(9.1%) (2 – impaired glucose tolerance (IGT), 2 – T2D) observations.

Monotherapy by estrogens (0.25 - 0.5 - 1.0 mg per day orally for 8-26 months to the first menstrual-like reaction) was applied only in women with HH (13 patients with the most severe estrogen deficiency). For 6 women in first group and all patients in second group was directly recommended cycled combined HRT. In both groups the choice of the medicine for combination HRT based on bioTs level (first quartile group of healthy women, age correlated=13 ng/dL). In women with androgen deficiency was appointied Estradiol Valerate (EV) 2 mg/day for 9 days and 2 mg EV plus Levonorgestrel (LNG) 150 mcg/day for 12 days. For patients with a level bioTs ≥ 13 ng/dl was assigned medication 17β-Estradiol (17β-E2) 2 mg/day for 14 days and 17β-E2 2 mg plus Dydrogesterone (D) 10 mg/day for 14 days).

In group 1, estrogen monotherapy was characterized by significant decrease basal insulin (9.2 (6.9; 14.6) vs. 8.6 (5.6; 12.0) mU/ml, p=0.006); HOMA (2.22 ± 1.08 vs. 1.8 ± 0.74; p=0.007), total cholesterol (5.3 (4.9; 6.0) vs. 4.8 (4.3; 5.1) mmol/L, p<0.001); LDL-C (3.5 (2.7; 4.0) vs. 3.0 (2.4; 3.4) mmol/L, p=0.001), increased HDL-C (1.3 (1.2; 1.5) vs. 1.4 (1.3; 1.5) mmol/L; p=0.29). The average value of the body mass index (BMI) has not changed (22.2 ± 3.8 vs. 22.6 ± 5.45; p=0.21).

Combined HRT for 2 years accompanied by weight gain, but the median did not exceed normal levels. At 15(78.9%) of patients with HH on the 17β-E2/D sensitivity to insulin remained on the positions achieved by estrogen monotherapy. There was a trend to lower atherogenic potential of blood **(Table 1)**.

However, 4 (21.1%) of observed patients noted increase in the basal insulin and HOMA index, TG, LDL-C (1 patient on 17β-E2/D and 3 – EV/LNG). Undesirable effects were most marked with EV/LNG **(Table 1)**, probably due to the adverse effects on hepatic metabolism nor testosterone derivative – LNG.

Table 1. Dynamics of metabolic indicators on the combined therapy estrogen-progestin in women HH.

	Estrogen monotherapy (n=13), before treatment (n=6) Me (25; 75)	After 27 (18;30) months 17β-E2/D(n=9) / EV/LNG (n=10) Me (25; 75)	W-тест (p) * before treatment vs. 17β-E2/D after treatment ** before treatment vs. EV/LNG after treatment
BMI (kg/m²)	21 (20; 22)	22.0 (22; 23)	***0.020863**
	22.5 (20; 25)	23 (21; 24)	**0.059337
Insulin (mU/ml)	9.6 (8.1; 14.1)	9.8 (8.2; 12.1)	*0.812704
	8.9 (6.9; 10.1)	9.5 (7.1; 13.3)	**0.075062
Glucose 0 (mmol/Ll)	4.5 (4.1; 4.8)	4.3 (4.0; 4.4)	*0.090970
	4.5 (4.4; 4.7)	4.1 (3.9; 4.4)	****0.005062**
Glucose 120 (mmol/L)	5.8(5.0; 6.3)	5.9 (5.2; 6.4)	*0.065254
	5.3 (4.9; 5.9)	6.1 (5.4;6.7)	**0.056784
HOMA	1.9 (1.6; 2.6)	1.8 (1.7; 2.1)	*0.858955
	1.6 (1.4; 2.2)	1.9 (1.5; 2.4)	**0.076659
CHO (mmol/L)	4.6 (4.5; 4.9)	4.5 (4.3; 4.9)	*0.400815
	5.0 (4.8; 5.1)	4.9 (4.6; 5.1)	**0.779435
TG (mmol/L)	1.2 (0.9; 1.2)	1.0 (1.0; 1.2)	*0.123486
	1.1 (0.8; 1.7)	1.2 (1.0; 1.4)	**0.069337
LDL-C (mmol/L)	2.6 (2.4; 3.0)	2.4 (2.3; 3.0)	*0.400815
	3.2 (2.9; 3.4)	2.9 (2.6; 3.1)	**0.202623
HDL-C (mmol/L)	1.5 (1.3; 1.8)	1.6 (1.5; 1.7)	*0.092893
	1.35 (1.3; 1.9)	1.5 (1.2; 1.7)	**0.575403

In group 2, after 6 cycles of hormone therapy reported an improvement in insulin sensitivity, serum lipid. However, after 2 years of combined HRT on a number of indicators observed patients the negative dynamics of metabolic status. In particular, POF women by using 17β-E2/D previous tendency to decrease in BMI (p=0.09), a significant reduction an insulin level (p=0.047), HOMA index (p=0.046) was lost.

After 6 cycles using 17β-E2/D was a significant decrease in the concentration of atherogenic lipids – TG (p=0.04), LDL-C (p=0.04), increased HDL-C (p=0.009). The values obtained after 2 years of HRT did not differ significantly from earlier **(Table 2)**. But, deterioration in measures of pro-atherogenic dyslipidaemia occurred in 3 (25%) from 12 patients.

Table 2. Metabolic parameters in women with POF application 17β-E2/D and EV/LNG.

	Before treatment 17β-E2/D (n=15) Me (25; 75) / EV/LNG (n=24) Me (25; 75) for glucose M ± SD	After 6 months (n=14) Me (25; 75) / (n=24) Me (25; 75) for glucose M ± SD	After 26 (24; 31) months (n=12) Me (25; 75) / (n=21) Me (25; 75)	F (p<0.05)
BMI (kg/m²)	24.1 (20.45; 26.3)	23.5 (22.4; 25.7)	24.2 (22.5; 27.3)	p<0.54452
	23.0 (21.5; 26.3)	22.4 (21.9; 30.6)	24 (23.7; 27.0)	***p<0.01401***
Glucose 0 (mmol/L)	4.6 ± 0.44	4.45 ± 0.29	4.9 ± 0.29	***p<0.00219***
	4.4 (4.2; 5.0)	4.3 (4.0; 5.0)	4.9 (4.6; 5.0)	***p<0.03542***
Glucose 120 (mmol/L)	5.1 ± 1.0	6.2 ± 0.8	6.1 ± 0.9	***p<0.01609***
	4.6 (4.2; 5.3)	5.8 (4.8; 6.3)	6.03 (5.2; 6.9)	p<0.22313
Insulin (mU/ml)	9.8 (8.4; 12.4)	9.4 (6.2; 16.7)	10.9 (9.69; 14.9)	p<0.11790
	11.9 (8.7; 17.4)	10.9 (9.4; 12.5)	12.3 (9.6; 19.2)	p<0.43080
HOMA	2.0 (1.5; 3.4)	1.7 (1.4; 2.3)	2.0(1.8; 3.1)	p<0.07428
	2.5 (1.6; 3.7)	2.4 (2.0; 2.8)	3.2 (2.2; 4.3)	***p<0.00018***
CHO (mmol/L)	5.3 (4.8; 5.8)	5.1 (4.9; 5.2)	5.0 (4.9; 5.2)	p<0.31997
	5.3 (5.0; 5.72)	5.0 (4.9; 5.2)	5.2 (4.9; 5.6)	***p<0.00536***
TG (mmol/L)	1.3 (1.0; 1.6)	1.1 (1.0; 1.3)	1.0 (0.9; 1.1)	p<0.19746
	1.5 (1.1; 2.0)	1.2 (1.0; 1.4)	1.3 (1.2; 1.6)	***p<0.00694***
LDL-C (mmol/L)	3.6 (2.6; 3.7)	3.1 (2.9; 3.2)	3.3 (2.8; 3.5)	p<0.49659
	3.7 (3.1; 3.9)	3.0 (2.8; 3.3)	3.6 (3.1; 3.8)	p<0.09223
HDL-C (mmol/L)	1.1 (0.85; 1.0)	1.3 (1.0; 1.3)	1.35 (1.2; 1. 5)	***p<0.00010***
	0.9 (0.7; 1.05)	1.2 (0.7; 1.4)	1.2 (0.9; 1.6)	***p<0.00003***

During 6 cycles EV/LNG applications have not undergone significant dynamics of basal insulin levels (p=0.06), HOMA index (p=0.29). Reduced serum atherogenic potential was significant (p=0.02 – for total cholesterol, p<0.001 - for TG, p=0.01 – for LDL-C, p<0.001 – for HDL-C **(Table 2)**. After 2 years, a statistically significant deterioration was registered for BMI, fasting glucose. HOMA index exceeded the corresponding figure before treatment. TG, HDL-C remained at the level reached after 6 cycles of the EV/LNG therapy. After 2 years of HRT lipidogramm negative changes reported in 5(23.8%) of 21 women.

As a result of 2 years cyclical HRT was performed stepwise regression analysis to study the effect on the index HOMA in complex of these independent factors such as BMI, age, family anamnesis of T2D, the choice of medicine. The leading predictor of increasing HOMA index in women 2 groups identified factor "family anamnesis of T2D» (R=0.66; p=0.02). It became obvious dependence hypertriglyceridemia by BMI (R=0.39; p=0.029), patient age (R=0.73; p=0.04), and the choice of medicine EV/LNG (R=0.66; p=0.004).

Therefore, for women with POF choice of progestin as part of hormone replacement therapy should be considered in improving the atherogenic lipid profile parameters. Patients with genetically determined development of T2D can be expected manifestation an IR and dyslipidemia, independently of the combination of estrogen-progestin.

During from 5 to 10 (6.9 ± 1.8) years, we observed 27 women (11 - with HH 16 - with POF). Some patients withdrew from the study because they are needed in the application not only HRT but medicines for the treatment of metabolic disorders – metformin, orlistat. The average age of the end of the study women with HH was 28.5 ± 4.9 years, POF – 36.3 ± 6.4 years. 17β-E2/D took 11 patients. EV/LNG – 16.

In the intermediate and final results, weight gain was registered in all women without exception (from 4 to 36 kg; 12 Me (5.0; 15.1) kg). At the same time BMI<26 kg /m^2 was in 3(27.3%) of 11 patients with HH, 4(25%) of 16 – with POF, the rest – overweight or obese. Increasing BMI values are significant **(Table 3)**.

Table 3. Intra-group differences in metabolic rates depending on the selected HRT.

	After 2 years HRT	After 6,9 ± 1,8 years	(p) W-тест
	17β-E2/D (n=11)		* 1(17β-E2/D) vs. 2(17β-E2/D) **1(EV/LNG) vs. 2(EV/LNG)
	EV/LNG (n=16)		
BMI (kg/m^2)	24,5±5,47	28,7±5,4	*0,003346
	24,3±4,5	27,4±4,2	**0,001474
Glucose 0 (mmol/l)	4,9 (4,3; 5,0)	4,9 (4,5; 5,3)	*0,308064
	4,3 (4,2; 5,0)	4,3 (3,9; 5,0)	**0,284504
Glucose 120 (mmol/L)	5,7 (5.3; 6.5)	5,7 (5,2; 5,9)	*0,858863
	6,4 (5,2; 6,7)	6,6 (6,2; 6,8)	** 0,749691
Insulin (mU/ml)	10,9 (8,3; 16,2)	13,5 (10,8; 19,3,3)	*0,005234
	12,1(8,30;14,8)	14.3 (10,4; 26.9)	**0,016369
HOMA	2,2 (1,6; 3,3)	2,8 (2,2; 4,1)	*0,040861
	2,3 (2,0; 3,5)	3,2 (2,1; 6,5)	**0,014597
IGT (T2D)	no	9,1% (1/11)	
		6,25% (1/16)	
family history of T2D	18,2% (4/11)		
	12,5% (2/16)		
CHO (mmol/L)	5,0 (4,9; 6,2)	4,96 (4,8; 5,6)	*0,504880
	5,6 (5,0; 6,5)	5,3 (4,9; 6,2)	**0,084285
TG (mmol/L)	1,0 (0,9; 1,3)	1,1 (0,9; 1,3)	*0,878482
	1,3 (1,2; 1,4)	1,3 (1,2; 1,6)	**0,776425
LDL-C (mmol/L)	3,3 (2,9; 3,5)	3,2 (2,9; 4,0)	*0,230025
	4,1 (3,4; 4,8)	3,6 (3,0; 4,5)	**0,125154
HDL-C (mmol/L)	1,3 (1,2; 1,6)	1,2 (1,1; 1,4)	*0,093493
	1,2 (0,9; 1,5)	1,1 (0,9; 1,35)	**0,504880

Carbohydrate metabolism, in particular, basal insulinemia and HOMA index recorded after 2 years of HRT, have significant undesirable dynamics 5-10 years of treatment. At the same time named indicators do not depend on the choice of 17β-E2/D, or EV/LNG (p>0.05). Basal HI occurred in 15 (55.6%) of 27 and HOMA index >1.8 – in 23 (85.2%) cases. Stepwise regression method is carried out to find the most significant predictor of deterioration insulin sensitivity in patients with hypoestrogenism on a long-term therapy HRT. Analysis of the cumulative effect of each other such factors as: the choice of medicine for HRT, age, BMI at the end of the study, TG levels, family anamnesis of T2D, has determined the leading predictor of increasing index HOMA only family anamnesis of T2D (R=0.92; p=0.00).

Hypertriglyceridemia (≥ 1.7 mmol/L) occurred in 4(14.8%), ≥ 1.3 mmol/L – 12(44.4%) of 27 patients. In this case, the comparison of lipid spectrum at the end of 2 years, and the final results of long-term HRT showed a progressive increase of TG

only in 1 (9.1%) of 11 women using 17β-E2/D, in 3 (18.75%) of 16 – EV/LNG. However, the difference is insignificant (χ^2 p=0.49). Multiple regression analysis confirmed that the choice of the medicine does not determine the deterioration of the lipidogramm (R=0.15; p=0.06), in contrast to the statistical results after 2 years of HRT. The main predictor of growth hypertriglyceridemia declared age of the patients at the end of the study (R=0.49; p=0.047).

Of the 27 women passed the entire period of observation, the violation of carbohydrate metabolism first detected in 4(14.8%) of patients: 1 woman with HH – impaired fasting glucose, 3 – with POF – IGT (n=2) and T2D (n=1). The difference in frequency increase the IGT from the start of the study until its completion highly significant (A/D χ^2=21.04; p=0.0000). Predictors of IGT or/and T2D in patients with hypoestrogenism on a long-term HRT are increasing HOMA index (R=0.74; p=0.002), BMI (R=0.88); p=0.000) and family anamnesis of T2D (R=0.76; p=0.00). But the main of predictors is a family history of T2D.

CONCLUSION

1. Estrogen monotherapy in small doses, as the first stage of HRT with hypogonadotropic hypogonadism, characterized by the significant positive changes in the metabolism of carbohydrates and fats.

2. On a cyclical combined HRT patients with hypogonadotropic hypogonadism syndrome and premature ovarian failure, insulin sensitivity and lipid profile can be changed in the direction of improvement, maintain stability, or indicate a negative tendency. It depends on the individual metabolism of carbohydrates and fats.

3. HRT does not protect the deterioration of insulin sensitivity in patients with a family anamnesis of type 2 diabetes and is not a means of prevention of this disease.

REFERENCES

1. Manukhin IB, et al. Anovulation and insulin resistance. Monograph. Moscow. Geotar-Media (in Russ). 2006;4:16.
2. Nadal A, et al. Nongenomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor α and estrogen receptor β. Proc Natl Acad Sci U S A. 2000;97:11603-11608.
3. Grillo ML, et al. Testosterone rapidly stimulates insulin release from isolated pancreatic islets through a non-genomic dependent mechanism. Horm Metab Res. 2005;37:662-665.
4. Kumagai S, et al. The effects of oestrogen and progesterone on insulin sensitivity in female rats. Acta Physiologica. 1993;149:91-97.
5. Beckett T, et al. Effect of ovariectomy and estradiol replacement on skeletal muscle enzyme activity in female rats. Metabolism. 2002;51:1397-1401.
6. Campbell SE, Febbraio MA. Effect of the ovarian hormones on GLUT4 expression and contraction-stimulated glucose uptake. Am J Physiol Endocrinol Metab. 2002;282:1139-1146.
7. Pedersen SB, et al. Estrogen controls lipolysis by up-regulating alpha2A-adrenergic receptors directly in human adipose tissue through the estrogen receptor alpha. Implications for the female fat distribution. The J Clin Endocrinol Metab. 2004;89:1869-1878.
8. Monjo M, et al. Alpha2- to beta3-Adrenoceptor switch in 3T3-L1 preadipocytes and adipocytes: modulation by testosterone, 17beta-estradiol, and progesterone. Am J Physiol Endocrinol Metab. 2005;289:145-150.
9. Sullivan AJ, Ho KK. A comparison of the effects of oral and transdermal estrogen replacement on insulin sensitivity in post-menopausal women. J Clin Endocrinol Metab. 1995;80:1783-1788.
10. Naaz A, et al. Effect of ovariectomy on adipose tissue of mice in the absence of estrogen receptor alpha (ERalpha): A potential role for estrogen receptor beta (ERbeta). Horm Metab Res. 2002;34:758-763.
11. Fan YM, et al. Hepatic lipase C-480T genotype-dependent benefit from long-term hormone replacement therapy for atherosclerosis progression in postmenopausal women. J Clin Endocrinol Metab. 2005;90:3786-9792.
12. Wei M, et al. Association of the hepatic lipase gene -250G/A promoter polymorphism with the susceptibility to type 2 diabetes mellitus combining with coronary heart disease. Chinese Journal of Medical Genetics. 2009;26:219-222.
13. http://www.issam.ch/freetesto.htm
14. McAuley KA, et al. Diagnosing insulin resistance in the general population. Diabetes Care. 2001;24:460-464.
15. Esteghamati A, et al. Optimal threshold of homeostasis model assessment for insulin resistance in an Iranian population: The implication of metabolic syndrome to detect insulin resistance. Diabetes Res Clin Pract. 2009;84:279-287.
16. Alberti KG, et al. IDF Epidemiology Task Force Consensus Group. The metabolic syndrome-a new worldwide definition. Lancet. 2005;366:1059-1062.