Metabolic profile and SHBG in different reproductive phases of Czech women and their relations to weight, body composition and fat distribution

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Summary

In our study, 213 healthy Czech women aged 20 to 65 years were examined and divided into fully reproductive, premenopausal, menopausal and postmenopausal group. In all subjects body composition was determined by classical anthropometry and metabolic profile was assessed. A total of 146 subjects completed 3-year longitudinal study. Results: total and LDL cholesterol increased and ratio HDL/total cholesterol decreased with age ($p < 0.001$), most significantly in menopause. Triacylglycerols increased only up to menopause. HDL had a very slight trend to decrease in menopause and postmenopause. Fasting blood glucose level increased progressively ($p < 0.001$), in postmenopause frequently exceeded normal range. Higher BMI, total fat mass and central fat indices were associated with higher total and LDL cholesterol, triacylglycerols, C-peptide, insulin and fasting blood glucose level ($p < 0.001$; fasting blood glucose level to WHR: $p < 0.01$) and lower HDL cholesterol ($p < 0.001$). Higher C-peptide and insulin were associated with lower HDL cholesterol and higher triacylglycerols ($p < 0.001$). Fasting glucose correlated with LDL cholesterol ($p < 0.01$). Higher SHBG was associated with higher HDL and lower LDL cholesterol ($p < 0.001$). HRT was related to lower fasting blood glucose level in postmenopausal women ($p < 0.01$). Oral contraception is suggestive of a positive influence on lipid spectrum by increasing the ratio HDL/total cholesterol. Markers of lipid and carbohydrate metabolism are not only age-related, but also BMI-, total fat mass- and central fat anthropometric indices-related. Therefore, preventive programmes should be focused above all on menopausal women.

Key words

lipid profile, SHBG, central adiposity, menopause

Introduction:

Overweight and obesity are well known risk factors for cardiovascular disease (CVD). Weight is related to blood pressure, total and low density lipoprotein (LDL) cholesterol, triacylglycerols and fasting insulin. Whilst weight increases progressively with age in both pre- and postmenopausal women, in postmenopausal women the increase in weight is associated with increased CVD risk, e. g. a drop in high density lipoprotein (HDL) and a rise in LDL (Owens et al. 1992). Fat distribution plays an important role; central obesity in European women is mostly defined as waist circumference ≥ 80 cm (Alberti et al. 2006) or

WHR value over 0.85 and is related to insulin resistance in both obese and non-obese women (Cnop et al. 2002), non-insulin dependent diabetes (Lundgren et al. 1989), plasma triacylglycerols, apolipoprotein B, LDL-apoB levels and total/HDL cholesterol ratio, as well as to plasma glucose, insulin, and C-peptide levels measured in the fasting state and after oral glucose load, and negatively related to HDL cholesterol levels. Age-related increase in visceral adipose tissue accumulation is a significant factor involved in the deterioration of the CVD risk profile noted in premenopausal women (Pascot et al. 1999). Metabolic syndrome X, well known correlate of CVD risk, is defined by central obesity (in European women: waist $\geq$ 80 cm; and if body mass index is > 30 kg/m^2 then central obesity can be assumed) plus any two of the following criteria: raised triacylglycerols $\geq$ 1.7 mmol/l, reduced HDL cholesterol < 1.29 mmol/l in females or specific treatment for this lipid abnormalities, raised blood pressure: systolic $\geq$ 130 mm Hg or diastolic $\geq$ 85 mm Hg or treatment of previously diagnosed hypertension, raised fasting plasma glucose $\geq$ 5.6 mmol/l or previously diagnosed type 2 diabetes (Alberti et al. 2006). Prevalence of metabolic syndrome X raises with age, especially in postmenopausal women (Park et al. 2003) and hyperandrogenemia and low sex hormone binding globulin (SHBG) are frequently found in such patients (Reinecke et al. 2002). Study on 26 Czech women with impaired glucose tolerance proved higher weight, thoracic mesosternal circumference and higher fat percentage assessed according to Pařízková. Women with WHR over 0.80 had higher diastolic blood pressure and higher body mass index (BMI) compared to women with WHR value equal or lower than 0.80 (Pobišová et al. 1990). Waist circumference and triacylglycerols were the strongest correlates of insulin resistance in a study on 347 American men and women, however, obese subject with BMI up to 46 participated in the study (Carr et al. 2004).

A decrease in estrogen/androgen ratio around menopause is related to fat centralization, insulin resistance (Seed 2002) and hyperinsulinemia (Golden et al. 2004). Increase in luteinizing/follicle-stimulating hormone ratio is a common finding in obese women, and together with hyperinsulinemia and a decrease in SHBG leads to hyperandrogenemia, which is even more evident in central obesity (Vondra and Stárka 1999). SHBG is negatively related to total body fat, upper-body adiposity, fasting serum glucose, insulin and triacylglycerols, positively with HDL cholesterol (Haffner et al. 1992), also in Czech women a negative correlation of SHBG to anthropometric central fat indices – waist and sagittal diameter at the L4 level – has been proved (Hainer et al. 2002). Low SHBG has also been identified as an independent CVD risk factor (Reinecke et al. 2002).

Physical activity decreases both BMI and central fat accumulation (Gilliat-Wimberly et al. 2001) and can partly counterbalance the negative age-related changes in lipid spectrum and increase in BMI by diminution of HDL decrease. The changes in lipids due to physical activity are largely independent of changes in body weight (Owens et al. 1992).

Studies are not always concordant in the dynamics of weight and fat distribution and their relation to metabolic parameters around menopause and may be population specific. Studies on large samples of Middle European women providing such data are not available.

A middle-sized sample of healthy Czech women was examined anthropometrically and the metabolic and endocrine characteristics were assessed. Weight increased progressively since fully reproductive years up to menopause with suggestive acceleration around menopause, the weight increase was probably greater than in other European populations. Fat tissue weight and fat percentage increased progressively up to early postmenopause. In postmenopause, only a relative decrease in lean components was evident. No fat centralization was proved in fully reproductive women, while it was suggestive in premenopausal women in cross-sectional data. Fat centralization was clearly proved in menopausal women, but still with significant gluteofemoral fat deposition, and a tendency was observed in postmenopausal women. The influence of hormone replacement therapy (HRT) on anthropometric parameters was not proved; oral contraception (OC) use was related to lower waist – to – hip ratio (WHR) in premenopausal women (Kosková et al. 2007a, 2007b).

The anthropometric indices are quite easy to assess without any risk for the patient and the relation to CVD is evident. It can be thus very helpful to use them in daily clinical practice and some of them also by educated population itself to identify candidates for further examinations and prevention. As some anthropometric characteristics can be population specific and there are no studies on large samples of Middle-European women, the aim of this study was to provide complex data on the relations of anthropometric characteristics to laboratory parameters in a middle-sized cohort of Middle-European women.

Methods

Subjects were outpatients of gynaecological consultations in Prague, healthy Czech women aged 20 - 65 years, with BMI below 35 who had not been treated for diabetes, CVD, dyslipidemia, endocrine disturbances or other serious diseases. Treatment modifying body composition was one of the excluding criteria, however, oral contraception (OC) and hormone replacement therapy (HRT) had no influence on the choice of subjects thus

representing the real situation of healthy Czech women living in Prague. OC/HRT users were defined as women who were taking such therapy at the time of examination; the length of the treatment was ignored. Age intervals representing each reproductive phase of average Middle-European women were established (Živný 1999) and women were accepted to have about 50 persons in each group: fully reproductive group aged 20-36 (n = 58, mean age 26.89 ± 4.69, 37 OC users), premenopausal women aged 38-45 (n = 48, mean age 42.54 ± 2.50, 11 OC users), menopausal women aged between 48-54 (n = 62, mean age 51.34 ± 2.51, 20 HRT users) and postmenopausal women aged between 55 and 65 (n = 45, mean age 59.53 ± 2.71, 14 HRT users). All subject were contacted again 3 years later, 146 out of 213 continued the study; baseline characteristics of the 146 subjects cohort was the following: fully reproductive group (n = 34, 26.96 ± 4.47, 23 OC users), premenopausal (n = 34, 42.23 ± 2.78, 10 OC users), menopausal (n = 45, 51.56 ± 2.61, 20 HRT users) and postmenopausal group (n = 33, 59.55 ± 2.82, 11 HRT users). OC/HRT users in the longitudinal study were defined as women who were taking OC/HRT at the time of the second examination in 2003; however, most subjects had been on the same or similar therapy before the beginning of the study in 2000. The study protocol was reviewed and accepted by the Ethics Committee of the General University Hospital in Prague 2; informed consent was given by all the subjects.

After a questionnaire and a medical interview the subjects underwent anthropometric examination done by the same investigator. The examination was carried out in morning hours; subjects were in underwear and without shoes. Weight was assessed with medical scales to the nearest 0.1 kg, heights with anthropometer in standing position to the nearest 0,1 cm - body height, height of suprasternal, iliocristal, iliospinal, symphysion points from floor, widths with pelvimeter and kefalometer to the nearest 0.1 cm: biacromial, transverse diameter of the chest, bicristal and bispinal width, sagittal diameter of the chest, width of the distal humeral and femoral epiphysis, ankle and wrist width. Circumferences with a flexible tape to the nearest 0.1 cm: mesosternal chest circumference, abdominal circumference at the level of umbilicus, waist at the minimal point between the xiphoid process and superior iliac crest, gluteal (hip) over the widest part of the hip region, relaxed arm, contracted arm, maximum circumference of the forearm, thigh circumference under gluteal muscle and median thigh circumference and calf maximum circumference. 14 skinfolds´ thickness with Best's callipers to the nearest 0.5 mm: facial, below the chin, on the chest I and II, suprailiacal, abdominal, over the patella, over the biceps, forearm I, over the triceps, subscapular, on the thigh over the quadriceps, on the calf I and II. Measured data were processed by the ANTROPO programme and the following quantities were used for further

statistical analysis: total fat percentage by the Pařízková method (Pařízková 1977), sum of 10 skinfolds: facial, below the chin, on the chest I and II, over the triceps, subscapular, abdominal, suprailiacal, over the patella and on the calf I and II, then BMI: weight/(height in m)^2, WHR: waist / hip circumference, absolute (in kg) and relative (in %) weight of bone, muscle, fat and residue by Matiegka. The absolute and relative values of the components were corrected by the difference between the real and calculated weight (Bláha et al. 1986).

Blood was taken after 12 hours fasting, in women having their menstrual cycle in the early follicular phase of the cycle, between day 1 and 7 since the beginning of period. The blood sample was taken with maximum delay of 1 month after the anthropometric examination.

Serum was examined for: glucose (only in 2000) and lipid profile – total cholesterol, LDL cholesterol, HDL cholesterol - by absorption spectrophotometry, HDL/total cholesterol ratio was calculated, triacylglycerols were assessed by enzymatic method. Insulin and C-peptide were assessed by radioimmunoassay (RIA), SHBG by radiometric immunoanalysis (IRMA).

Means and standard deviations were calculated for all the parameters in all groups. Pearson´s or Spearman´s correlation coefficient are used to define linear relationships between two parameters. T-test was used to test the differences between groups for parameters with normal distribution, Wilcoxon signed-rank test was used for comparison of two measurements of the same parameter in the longitudinal study. Two-sample t-test, resp. Mann-Whitney nonparametric test was used for comparison of two independent groups. Age groups comparison was done by one-way analysis of variance ANOVA1. Groups defined by both age and OC/HRT use were tested using two-way analysis of variance ANOVA2. Both tests were completed with Bonferroni method for multiple comparisons.

Baseline data, e. g. data collected in 2000 on 213 subjects were first evaluated as a transversal study. Data collected in 2003 on 146 subjects are compared to the baseline data of the same women as a longitudinal study.

Results

Baseline data were first analysed as cross-sectional study: values of laboratory parameters are shown in table 1. Total and LDL cholesterol increased significantly with age ($r = 0.44$ resp. $r = 0.42$, $p < 0.001$), the difference between the groups being significant only for LDL in the menopausal and postmenopausal women ($p < 0.05$). HDL level was not age-related, triacylglycerols increased slightly up to menopause and tended to decrease in postmenopausal women. OC/HRT influence on lipid profile was not proved in this cohort; postmenopausal

women on HRT had non-significantly lower total and LDL cholesterol, triacylglycerols and higher HDL.

Fasting serum glucose increased with age ($r = 0.25$, $p < 0.001$), raised slightly up to menopause and the postmenopausal group showed a significant increase ($p < 0.001$) with frequent pathologic values; the differences between groups were non-significant. C-peptide increased slightly with age ($r = 0.16$, $p < 0.05$), insulin did not correlate to age, and its increase was evident up to menopause. OC influence on analysed carbohydrate metabolism markers was not proved, but the power of the test was lower than 80 %, HRT usage was associated with significantly lower fasting glucose in postmenopausal women ($p < 0.01$).

Levels of LH and FSH were adequate to age changes. As expected, SHBG was higher in the fully reproductive women on OC ($p < 0.001$), SHBG did not correlate significantly with age ($r = -0.13$, NS, OC/HRT users were excluded, $n = 131$). HRT use in the postmenopausal group was associated with lower fasting blood glucose level ($p < 0.01$). Values of LH, FSH, SHBG and fasting blood glucose level separately in OC/HRT users and non-users are showed in table 2.

Table 3 shows correlations of metabolic parameters to BMI, fat percentage by Pařízková and Matiegka, fat weight, abdominal, thoracic and waist circumference, WHR, abdominal skinfold and skinfold on the chin, representing the two strongest correlates of all skinfolds, and sum of 10 skinfolds by Pařízková. Higher BMI, fat mass weight and central fat indices were associated with higher total cholesterol, LDL, triacylglycerols, C-peptide, insulin and fasting blood glucose level and lower HDL ($p < 0.001$ for all, only fasting blood glucose level to WHR $p < 0.01$), insulin and C-peptide showed the strongest association with thorax circumference. CVD risk indicated by the inverse value of HDL/total cholesterol showed the strongest association with waist and thorax circumferences, followed downwards by BMI, WHR, abdominal circumference, sum of 10 skinfolds by Pařízková and total fat weight by Matiegka. LDL cholesterol was the second most significant correlate to the above listed anthropometric parameters ($p < 0.001$ all correlations).

Table 4 shows mutual correlations of laboratory markers in the entire cohort, correlation to SHBG does not comprise OC/HRT users: higher fasting glucose was significantly associated with higher total cholesterol ($p < 0.001$) and LDL cholesterol ($p < 0.01$) and HDL/total cholesterol ratio ($p < 0.001$), correlations of glucose to HDL and triacylglycerols were non-significant. C-peptide and insulin showed negative correlations to HDL cholesterol and a positive correlation to triacylglycerols ($p < 0.001$), insulin does not correlate to total and LDL cholesterol. Out of carbohydrate metabolism indices, C-peptide showed the strongest relation

to lipid spectrum, BMI and central fat. Higher SHBG was associated with higher HDL cholesterol and ratio HDL/total cholesterol ($p < 0.001$), lower total cholesterol ($p < 0.01$), LDL cholesterol ($p < 0.001$), triacylglycerols, fasting glucose, insulin (all $p < 0.05$) and C-peptide ($p < 0.01$).

Longitudinal study did not reveal any significant changes in lipid spectrum, insulin or C-peptide in any reproductive group, however, trend to lipid spectrum deterioration towards risk lipid pattern showed up in the menopausal group. The values are shown in table 5.

OC/HRT influence on C-peptide or insulin was not proved in the longitudinal study; however, the power of the test was lower than 80 %. OC use was associated with higher HDL/total cholesterol in the analysis of the fully reproductive and premenopausal groups together – in OC users the ratio increased of 2.15 %, in non-users decreased of 0.39 %, ($p < 0.05$), the same trend is seen in the premenopausal group.

Changes in FSH and LH are concordant with gradual extinction of sexual functions; some of them can be traced already in the premenopausal group. SHBG decreased non-significantly in all the groups with the exception of the postmenopausal one, where remains stable.

Discussion

Transversal data show progressive shift of lipid profile towards more risky one across the groups: the rise in total cholesterol was caused by isolated LDL increase, and consequently increased CVD risk demonstrated by increase in the ratio HDL/total cholesterol. Triacylglycerols rise only up to menopause, there was even decreasing trend in postmenopause. HDL was quite stable, no decline was proved in menopause and postmenopause, only a very slight trend may be seen. HDL was the only age-independent lipid marker.

Hormonal changes associated with menopause were proved to raise CVD risk and postmenopause was proved to be an independent CVD risk factor (Lerner and Kannel 1986). Estrogens have a direct effect on vasodilatation and an indirect long-term effect consisting in modulation of a response to damage and arthrotic changes in vessels and in an influence on levels of serum lipoproteins and triacylglycerols. Postmenopausal women have higher levels of triacylglycerols, total and LDL cholesterol compared to premenopausal women (Stevenson et al. 1993; Gower et al. 1998), whilst in some studies those of HDL were significantly lower independently of age, BMI and other potentially confounding variables (Stevenson et al. 1993). Increases in LDL cholesterol and triacylglycerols and declines in HDL cholesterol are

greater during perimenopause than postmenopause, whereas increase in fasting glucose level is greater during postmenopause (Matthews et al. 2001). On the other hand, evidence of no influence of menopause on HDL cholesterol also exists. Besides that, that study proved relation of LDL cholesterol to estimated intraabdominal fat in pre- and postmenopausal women and to sum of central skinfolds in pre- but not in postmenopausal women. Triacylglycerols were related only to central adiposity, e. g. sum of central skinfolds and estimated intraabdominal fat. Adjustment for estimated intraabdominal fat decreased menopause-related differences in levels of total and LDL cholesterol by 44 % and 46 % resp., and by triacylglycerols levels by 73 %. This implies that menopause-related intraabdominal fat accumulation may adversely affect the plasma lipid profile (Gower et al. 1998).

The 3-year longitudinal follow-up did not prove any significant changes in lipid profile, only slight trend to a less risky profile in all groups except for the menopausal one where LDL cholesterol shows a tendency to rise and the ratio HDL/total cholesterol to decline. This tendency to lipid profile improvement in time in other groups in spite of weight/BMI increase and trend to fat centralisation could be explained by positive lifestyle changes of Czech women over the last years, as drop in total cholesterol in Czech population has been confirmed already in Countrywide Integrated Noncommunicable Diseases Intervention Program (CINDI) between the years 1991 and 2000 (Kazmarová and Kodl 2001) and our results support the continuity of this positive change.

We have proved significant positive correlations of total, LDL cholesterol and triacylglycerols to age, BMI, total body fat and indices of central adiposity and negative correlation of HDL cholesterol and ratio HDL/total cholesterol to BMI, total body fat and indices of central adiposity. Physical activity has a positive influence on lipid profile and a decrease in physical activity in most aging women is well-known.

Fasting blood glucose level increases significantly with age, most significant increase was proved in postmenopause, mean values of fasting glucose being in normal range in all groups, only in the postmenopausal group the mean value is close to upper limit and more women with impaired glucose tolerance were found in this group, these data are consistent with other authors (Matthews et al. 2001). C-peptide is less age-dependent and insulin was not age-dependent. Fasting blood glucose level, insulin and C-peptide correlate significantly to BMI, total body fat and central adiposity indices, only fasting blood glucose level shows a relatively weaker correlation to WHR, however, its correlation to waist and abdomen are very important and even more significant than to age. C-peptide and insulin show negative correlations to HDL and positive correlations to triacylglycerols and fasting glucose. On the other hand,

fasting blood glucose level does not correlate to HDL and triacylglycerols, but there is a significant correlation of fasting blood glucose level to LDL cholesterol, total cholesterol and the ratio HDL/total cholesterol. C-peptide shows the strongest relation to lipid metabolism, BMI and central adiposity indices. Evidence of relation between central fat and increased fasting glucose already in premenopausal women has been proved by other authors (Pascot et al. 1999). Previous data on Czech women show increased weight, thoracic mesosternal circumference and higher fat percentage by Pařízková in women with impaired glucose tolerance (Pobišová et al. 1990).

Concordantly with other sources, the relation of SHBG to LDL and HDL cholesterol (women on OC/HRT had been excluded) was proved also in our sample: higher SHBG is associated with lower LDL and higher HDL, both on the same significance level, SHBG is also associated with carbohydrate metabolism parameters (Haffner et al. 1992). SHBG has been identified as independent CVD factor in postmenopausal women (Reinecke et al. 2002). Significant correlations between carbohydrate and lipid metabolism and BMI, total fat and central adiposity indices were proved in this sample and are consistent with metabolic changes brought about by changes of weight, body composition and fat distribution: risk metabolic profile and even metabolic syndrome X with all health consequences. Estrogens contained in OC increase SHBG and as a consequence of that the free testosterone increases, OC users further have lower dehydroepiandrostendione sulphate (DHEAS), prolactin, higher corticosteroid binding globulin and therefore total cortisol, higher thyroxin binding globulin whilst free thyroxin (fT4) increases very slightly and so thyroid gland metabolism remains unchanged (Wiegratz et al. 2003). In the longitudinal follow-up of this study a positive influence of OC on the ratio HDL/total cholesterol was shown: premenopausal OC users show an increase of 2.15 %, whilst non-users showed a decrease of 0.39 % ($p < 0.05$), the same trend was found in the fully reproductive group, these changes being consistent with previous findings (Wiegratz et al. 1998). No significant HRT effect on lipid profile was proved. We would like to point out that as the study was not primarily planned to proof OC/HRT effects, the power of the tests is lower than 80 %. However, postmenopausal HRT users showed non-significantly lower total and HDL cholesterol and triacylglycerols and higher HDL cholesterol and fasting glucose, these findings are consistent with other studies (Salpeter et al. 2006). The opinion that women receiving HRT for the relief of menopausal symptoms might also benefit from the cardioprotective effects of restored estrogen levels has been challenged by the results of the first randomized trial of HRT for primary CVD prevention: results of this study suggest that HRT may further contribute to the increased risk

of CVD in postmenopausal women (Rossouw et al. 2002). Recent controlled trials have failed to show a beneficial effect of HRT on the incidence of coronary events and the discrepancy with some previous studies may be explained by the failure to adjust for baseline differences such as socioeconomic status or education. However, actually the over-all benefits of HRT have been recognised to outbalance potential risks for postmenopausal women (Clearfeld 2004).

There is evidence of age-related CVD risk, menopause being the most critical period, the unfavourable changes in anthropometric and metabolic parameters associated with CVD risk can be at least partly counterbalanced by healthy initiatives and preventive programmes focused on healthier diet and increase in physical activity. It is therefore necessary for the menopausal women to be more involved in such activities.

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Table 1: Lipid and carbohydrate profile in the different reproductive phases and correlation to age for the whole sample.

	FR	PM	M	PM	Correlation to age
T-Ch (mmol/l)	5.19±0.01	5.71±1.01	6.00±1.0	6.55±1.13	0.44***
HDL (mmol/l)	1.58±0.37	1.58±0.39	1.53±0.36	1.55±0.36	–0.06
LDL (mmol/l)	3.09±0.83	3.48±0.72	3.81±0.946	4.39±1.15*	0.42***
HDL/T-Ch (%)	30.90±6.60	28.20±7.20	26.37±8.24	24.60±7.40	–0.33***
TG (g/l)	1.15±0.49	1.25±0.74	1.59±1.19	1.53±0.60	0.22**
FBG (mmol/l)	4.67±0.54	4.76±0.58	4.77±0.57	5.19±0.63***	0.25***
C-peptide (pmol/l)	0.69±0.27	0.71±0.42	0.78±0.42	0.86±0.34	0.16*
Insulin (mUI/l)	7.32±3.75	7.79±6.08	8.32±10.34	7.48±4.17	0.01

Mean±SD of parameters are shown, significance level of the difference in the mean values between an * marked group and the previous "younger" one is shown. The last column shows the correlation of each parameter to age for the whole sample and the significance level is marked: *** P<0.001, ** P<0.01, * P<0.05.

FR – fully reproductive, FBG – fasting blood glucose level, HDL – high desitity lipoprotein cholesterol, LDL – low desitity lipoprotein cholesterol, M – menopausal, PostM – postmenopausal, PreM – premenopausal, T-Ch – total cholesterol, TG – triacylglycerols.

Table 2: LH, FSH, SHBG and fasting blood glucose level in the different reproductive phases and influence of OC/HRT.

	FR		PreM	
	OC, n=37	no OC, n=21	OC, n=11	no OC, n=37
LH (IU/l)	4.7±2.6	7.0±3.9	7.8±5.1	8.2±7.4
FSH (IU/l)	7.0±3.1	6.3±2.4	9.5±8.6	12.2±12.6
SHBG (nmol/l)	140.0±68.0	72.3±50.5***	103.4±53.6	73.1±41.4
FBG (mmol/l)	4.65±0.52	4.69±0.59	4.83±0.67	4.74±0.54

	M		PostM	
	HRT, n=20	no HRT, n=42	HRT, n=14	no HRT, n=31
LH (IU/l)	28.7±23.8	28.9±24.3	37.1±20.0	33.2±13.5
FSH (IU/l)	40.2±28.9	40.7±34.6	49.0±18.8	65.7±27.8
SHBG (nmol/l)	75.0±43.5	75.3±45.8	51.6±23.1	55.0±24.9
FBG (mmol/l)	4.68±0.51	4.81±0.60	4.77±0.24	5.44±0.88**

Mean±SD of parameters are shown , significance level of the difference in the mean values in OC/HRT users and non users are marked: *** P<0.001, ** P<0.01, * P<0.05.

FR – fully reproductive, FSH - follicle stimulating hormone, FBG – fasting blood glucose level, HRT - hormonal replacement treatment, LH - luteinizing hormone, M – menopausal, OC - oral contraception, PostM – postmenopausal, PreM – premenopausal, SHBG - sex hormone binding globulin.

Table 3: Significant correlations of carbohydrate and lipid metabolism parameters to anthropometric characteristics: BMI, fat %, total fat, abdominal, thoracic and waist circumferences, abdominal and chin skinfold and sum of 10 skinfolds.

	Insulin	C-peptide	FBG	HDL/T-Ch	TG	HDL	LDL	T-Ch	SHBG
BMI	0.36 ***	0.43 ***	0.33 ***	-0.50 ***	0.35 ***	-0.39 ***	0.38 ***	0.29 ***	-0.29 ***
Fat % P	0.28 ***	0.33 ***	0.32 ***	-0.45 ***	0.31 ***	-0.32 ***	0.36 ***	0.30 ***	-0.42 ***
Fat % M	0.26 ***	0.30 ***	0.31 ***	-0.43 ***	0.25 ***	-0.31 ***	0.37 ***	0.31 ***	-0.39 ***
Total fat	0.38 ***	0.40 ***	0.31 ***	-0.47 ***	0.33 ***	-0.37 ***	0.36 ***	0.29 ***	-0.38 ***
Abdomen	0.29 ***	0.38 ***	0.28 ***	-0.47 ***	0.34 ***	-0.32 ***	0.39 ***	0.32 ***	-0.29 ***
Thorax	0.41 ***	0.45 ***	0.35 ***	-0.51 ***	0.39 ***	-0.42 ***	0.36 ***	0.27 ***	-0.34 ***
Waist	0.35 ***	0.29 ***	0.29 ***	-0.51 ***	0.43 ***	-0.39 ***	0.40 ***	0.31 ***	-0.30 ***
WHR	0.24 ***	0.31 ***	0.19 **	-0.48 ***	0.42 ***	-0.37 ***	0.40 ***	0.30 ***	-0.25 ***
Abd SF	0.21 ***	0.33 ***	0.36 ***	-0.44 ***	0.32 ***	-0.28 ***	0.39 ***	0.35 ***	-0.40 ***
Chin SF	0.26 ***	0.32 ***	0.29 ***	-0.42 ***	0.32 ***	-0.26 ***	0.39 ***	0.31 ***	-0.45 ***

Significance level of the correlations is marked: *** P<0.001, ** P<0.01, * P<0.05.

Abd SF – abdominal skinfold, BMI – body mass index, Fat % P - FBG – fasting blood glucose level, HDL – high desitity lipoprotein cholesterol , HRT - hormonal replacement treatment, LDL – low desitity lipoprotein

cholesterol, OC - oral contraception, SHBG - sex hormone binding globulin, T-Ch – total cholesterol, TG – triacylglycerols, WHR – waist to hip ratio.

Table 4: Mutual correlations of lipid and carbohydrate metabolism and SHBG.

	T-Ch	HDL	LDL	HDL/T-Ch	TG	FBG	C-peptide	Insulin
HDL	0.12							
LDL	0.95***	0.95***						
HDL/T-Ch	-0.57***	0.72***	-0.69***					
TG	0.29***	-0.43***	0.25***	-0.60***				
FBG	0.24***	-0.11	0.23**	-0.24***	0.11			
C-peptide	0.21**	-0.41***	0.20**	-0.46***	0.48***	0.22**		
Insulin	0.09	-0.30***	0.10	-0.30***	0.36***	0.21**	0.67***	
SHBG	-0.20**	0.29***	-0.29***	0.38***	-0.15*	-0.17*	-0.19**	-0.14*

Correlations of SHBG only in OC/HRT non users, mutual correlations of other parameters for the entire cohort. Significance level of the difference in the mean values in OC/HRT users and non users are marked: *** P<0.001, ** P<0.01, * P<0.05.

BMI – body mass index, FBG – fasting blood glucose level, HDL – high density lipoprotein cholesterol , HRT - hormonal replacement treatment, LDL – low density lipoprotein cholesterol, OC - oral contraception, SHBG - sex hormone binding globulin, T-Ch– total cholesterol, TG – triacylglycerols, WHR – waist to hip ratio.

Table 5: Lipid profile, C-peptide and insulin and their changes over 3-year period in the different reproductive phases.

	FR		PreM	
	n = 34		n = 34	
Baseline age	26.96±4.47		42.23±2.78	
Year of measurement	2000	2003	2000	2003
T-Ch (mmol/l)	5.40±1.14	5.11±0.91	5.46±0.91	5.51±0.99
HDL (mmol/l)	1.68±0.36	1.65±0.30	1.48±0.36	1.53±0.35
LDL (mmol/l)	3.20±0.92	2.98±0.83	3.37±0.71	3.33±0.64
HDL/T-Ch (%)	31.50±6.96	32.79±6.11	27.64±7.54	28.05±5.79
TG (g/l)	1.22±0.55	1.08±0.44	1.27±0.83	1.20±0.59
C-peptide (pmol/l)	0.69±0.24	0.60±0.26	0.80±0.51	0.77±0.42
Insulin (mUI/l)	7.22±2.71	6.34±3.73	8.90±7.46	8.15±6.52
	M		PostM	
	n = 45		n = 33	
Baseline age	51.56±2.61		59.55±2.82	
Year of measurement	2000	2003	2000	2003
T-Ch (mmol/l)	5.78±1.08	5.96±0.79	6.48±1.23	6.21±0.99
HDL (mmol/l)	1.53±0.35	1.54±0.71	1.58±0.35	1.54±0.42

LDL (mmol/l)	3.66±0.93	3.78±0.75	4.36±1.21	4.01±0.98
HDL/T-Ch (%)	27.23±8.29	25.84±6.87	25.22±7.29	25.57±7.95
TG (g/l)	1.37±0.79	1.44±0.71	1.62±0.78	1.62±0.78
C-peptide (pmol/l)	0.74±0.42	0.83±0.93	0.88±0.36	0.91±0.50
Insulin (mUI/l)	8.51±12.23	8.40±12.26	7.54±3.97	10.46±11.34

Mean±SD of parameters are shown, significance level of the difference in the mean values between the two measurements are non significant.

FR – fully reproductive, FBG – fasting blood glucose level, HDL – high desitity lipoprotein cholesterol, LDL – low desitity lipoprotein cholesterol, M – menopausal, PostM – postmenopausal, PreM – premenopausal, T-Ch – total cholesterol, TG – triacylglycerols.