Endocr (2009) 35:262–268
DOI 10.1007/s12020-008-9137-z

ORIGINAL PAPER

Relationship between basal metabolic rate and cortisol secretion throughout pregnancy

Svetozar S. Damjanovic · Rada V. Stojic · Nebojsa M. Lalic · Aleksandra Z. Jotic · Djuro P. Macut · Sanja I. Ognjanovic · Milan S. Petakov · Bojana M. Popovic

Received: 9 October 2008 / Accepted: 24 November 2008 / Published online: 21 January 2009

Abstract The role of cortisol in mediating basal metabolic rate (BMR) changes that accompany the adjustment of maternal body weight (BW) and body composition during pregnancy is unknown. We tested whether increase in BMR during pregnancy is explained by variations in cortisol secretion. Longitudinal changes in BW, fat mass (FM), fat-free mass (FFM), BMR, hormonal, and metabolic parameters in 31 parous Caucasian women at gestational weeks 12, 26, and 36 were examined. Individual differences (Δ) between the last and the first measurement occasions for each variable were calculated. By gestational week 36, BW and BMR increased while both FFM/FM and BMR/BW ratio decreased ($P < 0.001$ for all) suggesting higher proportion of FM accretion. Cortisol, leptin, and insulin-like growth factor-1 (IGF-1) concentration rose, whereas non-placental growth hormone (GH) and thyroid hormones declined ($P < 0.001$ for all). Insulin resistance changed; basal glucose ($P < 0.001$) and ghrelin ($P < 0.014$) declined, whereas insulin ($P < 0.001$), homeostatic model index (HOMA-IR) ($P = 0.041$), and free fatty acid (FFA) concentration ($P = 0.007$) increased. The elevation in BMR showed inverse correlations with ΔBW ($r = 0.37$, $P = 0.047$) and Δcortisol ($r = -0.53$, $P = 0.004$). Significant portion (51.6%) of the variation in BMR change was explained by increases of cortisol (27.1%), FFA (13.4%), and free triiodothyronine (11.1%). In conclusion, the changes in maternal cortisol concentration are in relationship with changes in BMR and BW, further suggesting that increased cortisol secretion during pregnancy could be linked with the maintenance of maternal BW and body composition.

Keywords Basal metabolic rate · Cortisol · Insulin · Leptin · Ghrelin · GH–IGF-1 axis · Thyroid hormones

S. S. Damjanovic (✉) · N. M. Lalic · A. Z. Jotic · D. P. Macut · S. I. Ognjanovic · M. S. Petakov · B. M. Popovic
Institute of Endocrinology, Diabetes and Diseases of Metabolism, Belgrade University School of Medicine, Dr Subotica 13, 11000 Belgrade, Serbia
e-mail: sova@net.yu

R. V. Stojic
Department of Gynecology, Dom Zdravlja Vozdovac, Belgrade, Serbia

Introduction

Maternal adaptation to normal pregnancy is associated with cumulative rise in body weight (BW), fat mass (FM), fat-free mass (FFM), and basal metabolic rate (BMR) [1]. It has been shown that the magnitude of gestational weight gain is in correlation with the magnitude of the increase in cumulative BMR and with average prepregnancy body FM [2]. Metabolic changes in early pregnancy promote adipose tissue accretion with later onset of insulin resistance [3], while in late gestation, maternal adipose tissue depots decline, whereas free fatty acid (FFA) concentration and insulin resistance rise [4]. The recognition of changes in maternal body composition and energy balance during pregnancy extends back more than a decade [5, 6]. However, teleological meaning of cortisol rise during pregnancy is still incompletely known. We investigated, therefore, longitudinal changes in maternal serum cortisol, body composition, and other hormonal and metabolic parameters that are involved in the control of energy homeostasis, in relation to variability in the BMR changes during pregnancy.

Results

Body weight, body composition, and weight gain during pregnancy

Values of BW, BMI, FM, FFM, and FFM/FM ratio at 12, 26, and 36 weeks of gestation are shown in the Table 1 and Fig. 1. These variables significantly changed with advancing of pregnancy ($P < 0.001$ for all). In comparison to values at 12 weeks of gestation, FFM/FM ratio decreased significantly at week 26 and 36 of gestation, suggesting stronger contribution of FM in weight gain throughout the pregnancy. Increases in BW and FM were in positive relationship ($r = 0.56$, $P = 0.001$).

Basal metabolic rate

Significant changes in BMR were detected during the pregnancy. As shown in Table 1, the average BMR increased from basal at gestational week 12, to higher values at gestational weeks 26 and 36. BMR was in stable and positive relationship with BW ($r = 0.63$, $P < 0.001$; $r = 0.64$, $P < 0.001$; and $r = 0.47$; $P = 0.007$), FM ($r = 0.53$, $P = 0.002$ and $r = 0.54$, $P = 0.002$) and FFM ($r = 0.38$, $P = 0.033$; $r = 0.57$, $P = 0.001$; and $r = 0.71$, $P < 0.001$) in gestational weeks 12, 26, and 36, respectively. However, the change in BMR varied considerably among women 661.2 ± 409.5 kJ/24 h (from −167.4 to 1757.3 kJ/24 h). Furthermore, ΔBMR was in positive correlation with ΔBMI ($r = 0.42$, $P = 0.023$) and ΔBW while it was in negative relationship with ΔCortisol (Fig. 2). The ratio of BMR to BW significantly decreased from 97.3 ± 7.3 kJ/day kg on the first, to 91.3 ± 6.4 kJ/day kg ($P < 0.001$) on the second and to 88.1 ± 7.5 kJ/day kg ($P < 0.001$) on the third measurement occasion.

Plasma glucose, insulin, and free fatty acid levels

We observed slight, but significant decrease in glucose concentration during pregnancy ($P < 0.001$). When compared with the value at gestational week 12, little change was observed in glucose concentration at gestational week 26, whereas it had decreased by the end of gestational week 36 (Table 1). During the entire pregnancy, insulin concentrations and homeostatic model index (HOMA-IR) gradually increased reaching statistical significance at week 36 (Table 1). In all women, significant fluctuations were observed in FFA concentrations throughout the study. After initial drop at gestational week 26, FFA concentration sharply rose at gestational week 36 (Table 1). The extent of basal insulin increase, was in positive association with ΔBMI ($r = 0.40$, $P = 0.034$), Δglucose ($r = 0.43$,

Table 1 Study variables in women in gestational weeks 12, 26, and 36

	Gestational week				
	12	26	36	Δ	*P* value
BMI (kg/m^2)	21.0 ± 1.9	23.7 ± 2.0*	25.9 ± 2.1*	4.8 ± 1.2	<0.001
FFM/FM ratio	2.8 ± 0.7	2.3 ± 0.4*	2.1 ± 0.5*	−0.6 ± 0.4	<0.001
BMR (kJ/24 h)	5870.9 ± 372.4	6180.8 ± 299.8*	6537.1 ± 479.6*	666.8 ± 403.7	<0.001
Glucose (mmol/l)	4.6 ± 0.5	4.5 ± 0.5	4.2 ± 0.4*	−0.4 ± 0.5	<0.001
Cholesterol (mmol/l)	4.7 ± 0.6	6.1 ± 1.0*	7.0 ± 1.1*	2.2 ± 0.9	<0.001
Triglycerides (mmol/l)	1.0 ± 0.3	1.6 ± 0.5*	2.6 ± 0.8*	1.5 ± 0.7	<0.001
FFA (mmol/l)	0.348 ± 0.149	0.329 ± 0.110	0.461 ± 0.181§	0.103 ± 0.215	0.003
Insulin (mU/l)	8.5 ± 3.4	9.5 ± 4.0	13.1 ± 5.8*	4.5 ± 6.6	0.001
HOMA-IR	1.8 ± 0.9	1.9 ± 0.9	2.4 ± 1.2¶	0.6 ± 1.4	0.031
Free T3 (pmol/l)	3.4 ± 0.3	3.1 ± 0.4‡	2.9 ± 0.3*	−0.5 ± 0.3	<0.001
Free T4 (pmol/l)	11.1 ± 2.5	7.8 ± 1.4*	7.7 ± 1.6*	−3.5 ± 1.9	<0.001
TSH (mIU/l)	1.95 ± 1.4	1.93 ± 1.0	2.12 ± 1.0	0.14 ± 1.4	0.525
GH (mcg/l)	8.2 ± 6.8	3.6 ± 4.4*	1.4 ± 1.4*	−6.7 ± 6.8	<0.001
IGF-1 (mcg/l)	158.9 ± 47.3	191.3 ± 55.9§	300.3 ± 104.6*	140.5 ± 94.3	<0.001
Leptin (mcg/l)	20.5 ± 11.2	29.9 ± 14.1*	33.2 ± 14.4*	12.7 ± 9.3	<0.001
Ghrelin (pg/ml)	845.3 ± 351.5	656.2 ± 366.8§	690.0 ± 287.7†	−149.4 ± 260.5	0.018
Cortisol (nmol/l)	525.1 ± 172.6	841.1 ± 392.3*	891.8 ± 321.0*	371.7 ± 288.6	<0.001

Values are means ± SD; Δ individual differences for studied variables, between the last and the first measurement occasions, at 36 and 12 weekS of gestation; *BW* body weight; *BMI* body mass index; *FM* fat mass; *FFM* fat free mass; *BMR* basal metabolic rate; *FFA* free fatty acids; *HOMA-IR* homeostatic model index. Significantly different from values at gestational week 12 (repeated measures ANOVA): * $P < 0.001$, † $P = 0.005$, ‡ $P = 0.006$, § $P = 0.013$, ¶ $P = 0.03$

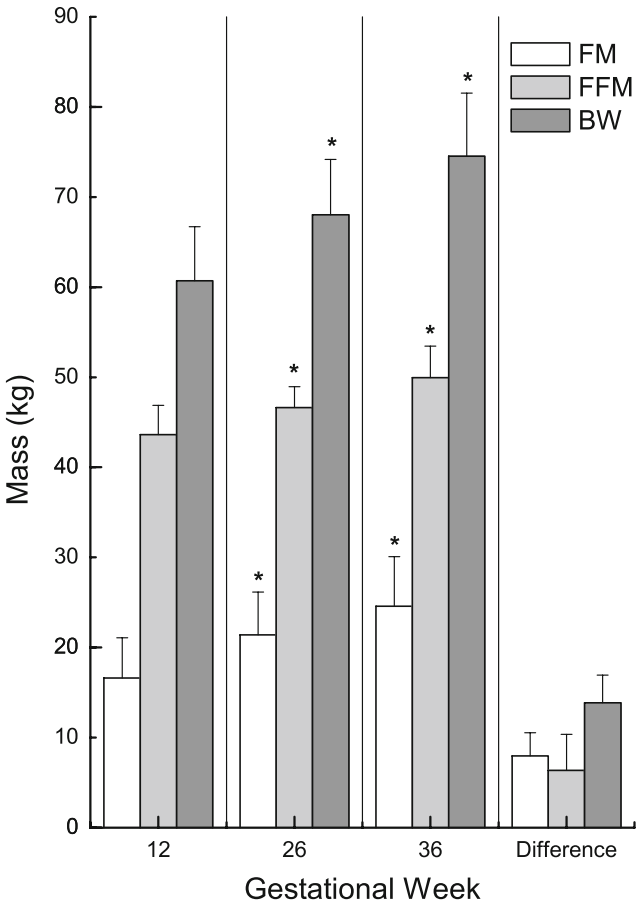

Fig. 1 Longitudinal changes in body weight (BW), fat mass (FM), and fat free mass (FFM) during pregnancy. Values are presented as means ± SD. Individual differences for studied variables, between the last and the first measurement occasions, at 36 and 12 weeks of gestation. * $P < 0.001$ versus gestational week 12 (repeated measures ANOVA)

$P = 0.024$) and Δleptin ($r = 0.44$, $P = 0.018$). Similarly, ΔHOMA-IR correlated positively with ΔBMI ($r = 0.44$, $P = 0.017$), Δglucose ($r = 0.59$, $P = 0.001$) and Δleptin ($r = 0.40$, $P = 0.036$).

Thyroid hormones

The rise in TSH concentration did not reach statistical significance. However, both, FT_4 and FT_3 significantly decreased (Table 1). Positive relationship was found between ΔFT_4 and Δghrelin ($r = 0.42$, $P = 0.026$) and between ΔFT_3 and Δglucose ($r = 0.39$, $P = 0.045$).

GH–IGF-1 axis

Significant changes in growth hormone (GH)–insulin-like growth factor-1 (IGF-1) axis were observed with advancing of pregnancy in all women (Table 1). While IGF-1 concentrations increased in gestational week 26 and 36, non-placental GH levels changed in the opposite direction. Positive correlation between the ΔGH and Δtriglcerides ($r = 0.50$, $P = 0.007$) was found.

Ghrelin and leptin secretion during pregnancy

Ghrelin and leptin concentrations at gestational weeks 12, 26, and 36 are given in the Table 1. Ghrelin declined during pregnancy. After initial drop of ghrelin at gestational week 26, an increase in concentration at gestational week 36 was recorded, but it remained significantly lower than at the end of the first trimester. On the contrary, leptin levels increased throughout pregnancy. The increase in

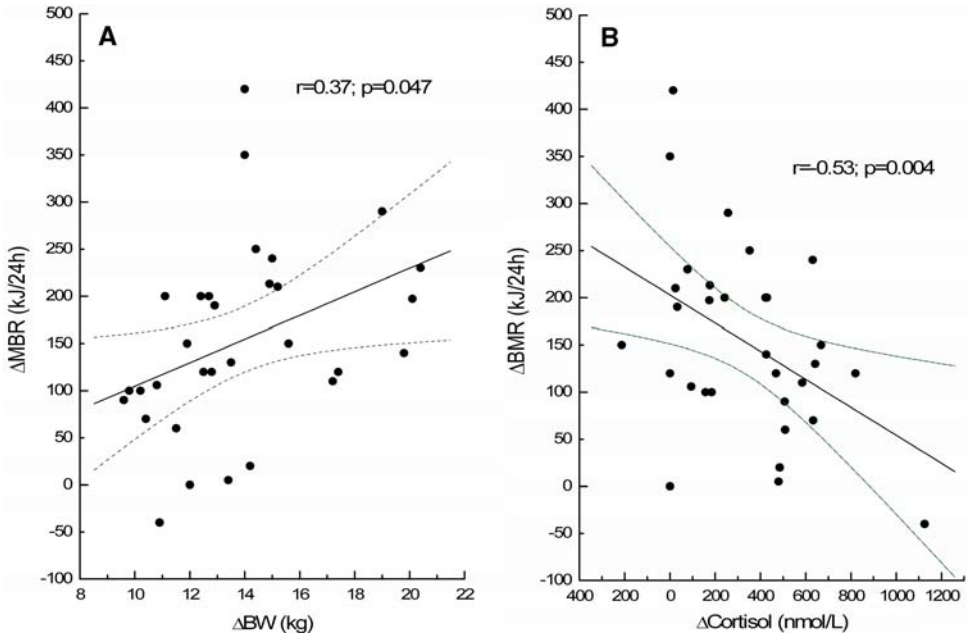

Fig. 2 Changes in basal metabolic rate (ΔBMR) were in correlation with weight gain (**a**) and cortisol alterations (**b**) in late pregnancy. *BW* body weight; Δ represents individual difference between the last and the first measurement occasions, at 36 and 12 weeks of gestation. *Full line* denotes linear regression; *dashed lines* denote 95% confidence interval

Table 2 Stepwise multiple linear regression analysis

Dependent variable	Variables in equation	Coefficient	SE	*P* value	Model R^2
BMR (kJ/24 h)	BW (kg)	10.07	1.88	<0.001	0.398
Gestational week 12	Length of gestation (days)	−199.3	62.8	0.004	**0.557**
BMR (kJ/24 h)	BW (kg)	5.22	1.46	0.001	0.409
Gestational week 26	Total cholesterol (mmol/l)	−29.28	8.6	0.002	0.510
	Parity (number)	−57.8	19.13	0.005	**0.633**
BMR (kJ/24 h)	FFM (kg)	23.38	4.31	<0.001	**0.503**
Gestational week 36					
ΔBMR (kJ/24 h)	Δcortisol (nmol/l)	−0.211	0.049	<0.001	0.271
	ΔFFA (mmol/l)	167.58	63.36	0.014	0.405
	ΔFT_3	117.33	51.21	0.031	**0.516**

BMR basal metabolic rate, Δ, individual differences between values at 36 and 12 week of gestation, *BW* body weight, *FFM* fat free mass, *FFA* free fatty acids, FT_3 free triiodothyronine

Bold figures represent total cumulative R^2 value

leptin concentration was in correlation with ΔBW ($r = 0.50$, $P = 0.007$) and Δtriglycerides ($r = -0.45$, $P = 0.016$). Relationships of ghrelin and leptin with other hormones and metabolic parameters are presented in previous sections.

Cortisol secretion in pregnancy

In all women cortisol concentration increased significantly (Table 1). As described in the text before, cortisol and BMR changes were in correlation during pregnancy (Fig. 2).

Investigated factors in relation to variability in BMR during pregnancy

Body weight and length of gestation explained 55.7% of the variability in BMR in gestational week 12, while in gestational week 26, 63.3% of the variance in BMR can be justified by the variations in BW, total cholesterol concentration, and parity. In the last trimester, in gestational week 36, FFM contributed 50.3% of the variance in BMR (Table 2). Another stepwise regression analysis model was tested, with ΔBMR as a dependent variable (Table 2). In this model, changes in cortisol, FFA, and FT_3 concentration explained 51.6% of the variance in ΔBMR independently of other variables.

Discussion

In the current study, we have shown that increasing of body mass during pregnancy is accompanied by increase of oxygen consumption and by expansion of both, FM and FFM. As the body size increases, a lower ratio of FFM to FM and BMR to BW were deposited. In the whole cohort, Δcortisol explained 27.1% of the variation in BMR change during pregnancy, followed by ΔFFA and ΔFT_3, adding further 13.1% and 11.1% to the predictability of BMR increase.

The extent of change in BMR varied significantly among women during pregnancy. ΔBMR was in correlation with ΔBW, but there was no relationship with ΔFFM or ΔFM. On the other hand BMR was in close relationship with BW, FFM and FM values throughout pregnancy as shown in previous reports [1, 3, 7], suggesting that bioelectric impedance analysis (BIA) can be used for measurement of body composition in pregnant women [5, 8, 9].

The relationship between oxygen consumption and body composition can be affected by increased metabolic activity of FM [10] and by different ability of fat oxidation [11–14]; however, decrease in ratio of FFM to FM and the lack of correlation between the changes in body composition and BMR may indicate a lower proportion of high-metabolic rate tissue accretion during pregnancy. Observed decrease in ratio of BMR to BW is in line with data obtained from studies on common obesity where the oxygen consumption decreases with greater BW [15]. The lack of correlation between leptin and BMR increases suggests that the adjustment of oxygen consumption during gestation might not be mediated by leptin-induced activation of hypothalamic melanocortin system. This finding is consistent with animal studies confirming increased expression of the orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP) mRNA and decreased expression of α-MSH and leptin receptor in rat medial basal hypothalamus during pregnancy [16–19]. Similarly, increased insulin concentrations in non-pregnant women with normal insulin sensitivity should be catabolic (i.e., reducing food intake and decrease in BW) [20], the opposite of what we have found. Possible interpretation of these data is that central

and peripheral insulin resistance travel together during gestation, further supporting the existence of cross-talk between leptin and insulin signaling in the hypothalamus.

There was no relationship between ghrelin and oxygen consumption; fall of ghrelin concentration with advancing of pregnancy may result from increased BW, development of insulin resistance, and high-serum levels of placental GH, although the role of insulin resistance is still controversial issue [21, 22]. Measured GH and IGF-1 concentrations changed inversely during the study as placental GH is released into maternal circulation where it suppresses pituitary GH secretion and stimulates IGF-1 production, which has the role in fetal and postnatal growth [23–26].

The decrease of serum concentrations of free thyroxine (FT_4), and triiodothyronine (FT_3) with insignificant increase of TSH during pregnancy is in good agreement with earlier findings [1]. Since leptin regulates TSH secretion through hypothalamic melanocortin system and its administration to humans raises the concentration of bioactive thyroid hormones, decline of hypothalamo–pituitary–thyroid axis activity in pregnancy could be the result of relative leptin insufficiency at the central and resistance at the peripheral level [27, 28]. This could be part of a regulatory mechanism that counteracts stimulatory effect of maternal weight gain and fetal growth on energy metabolism during pregnancy.

Glucose concentration slightly decreased with advancing of pregnancy. In view of the apparent importance of neuroendocrine neurons that respond to changes in glucose concentrations [29] and of fact that placenta can extract 40–60% of the total glucose and oxygen supplied by the uterine circulation [23–26], mother's brain should recognize fetus as novel physiological competitor for glucose. Although speculative, neuroendocrine hypothalamic responses in glucose-responsive and glucose-sensitive neurons to physiological changes in glucose concentration [29–32] might be of importance in different aspects of food intake regulation, from feeding-related behaviors to motor activity during pregnancy.

We found negative relationship between cortisol and BMR increases during pregnancy. Basal cortisol levels may not represent 24-h cortisol secretion, but it should be valuable marker of cortisol secretion throughout gestation. Total and free plasma cortisol and 24-h urinary free cortisol concentrations rise in parallel through gestation with reported elevations of 2- to 4-fold across several studies [33–35]. Besides, genetic factors play a role in the regulation of basal cortisol levels and significant individual stability of baseline cortisol concentrations in both sexes has been demonstrated [36, 37]. Thus, we cannot exclude that observed cortisol and BMR rise might be in causal relation as cortisol should be involved in regulation of glucose allocation between mother and fetus [38]. It challenges the view that in addition to increased production of placental corticotropin-releasing hormone [39], central mechanisms could play a role in the pregnancy-induced adjustment of maternal hypothalamo–pituitary–adrenal axis. Our data do not support direct effect of cortisol in development of insulin resistance during pregnancy, however, augmented lipolysis, evidenced by the elevation of triglycerides and FFAs in late pregnancy, could be due to coordinate effect of cortisol [40] and leptin [4, 41–44].

In pregnancy, there may be too many interactions and effects between several different factors. Besides, we cannot exclude biological effects without significant change in hormone concentrations. Although the methodology we used here does not provide causation, parallel changes in maternal cortisol concentration, oxygen consumption, and BW suggest that increased cortisol secretion during pregnancy could be connected with the maintenance of maternal BW and body composition.

Materials and methods

Subjects

This prospective study was conducted in the Clinical Center of Serbia (KCS), at the Institute for Endocrinology Diabetes and Metabolic Diseases, Medical School of Belgrade from 2005 to 2006. The Institutional Ethical Committee approved the study, and written informed consent was obtained from each subject before enrollment. Thirty-one Caucasian women with early pregnancy living in the Vracar area of Belgrade were recruited through the health care system for the study. Gestational age was assessed on the basis of an ultrasound scanning measurement, which was taken in gestational week 12. All women remained healthy during pregnancy and delivered healthy full-term singleton infants. Characteristics of women and their infants are given in the Table 3 and Fig. 1.

Table 3 Characteristics of the women included in the study and their infants

Before pregnancy	Value (range)
Age (years)	28.8 ± 4.8 (20–41)
BMI (kg/m^2)	24 ± 1.9 (17.5–24.6)
%FM	29.0 ± 4.5 (18.4–38.1)
FFM (kg)	43.6 ± 3.2 (30–47.5)
Length of gestation (days)	275.5 ± 9.8 (254–290)
Birth weight of baby (g)	3485.2 ± 498.9 (2,700–4,800)
Head circumference of baby (cm)	34.8 ± 1.4 (32–39)
Apgar score	8.7 ± 0.7 (7–10)

Values are means ± SD; *BMI* body mass index; *%FM* percent of fat mass; *FFM* fat free mass

Protocol

The women were examined during gestational weeks 12, 26, and 36. They were advised to refrain from strenuous physical activity the day before tests. After an overnight fast, the women arrived at the hospital metabolic unit and the venous blood samples were drawn at 09:00 h in supine position. The BW and height were recorded and body composition was assessed by BIA. After the 30 min rest in supine position, heart rate, blood pressure, and BMR were measured. The birth weight and length of the infants was obtained from hospital records.

Body weight, height, and body composition

All measurements were done following the admission to the hospital with the subjects wearing light clothes and no shoes. Stature was measured in triplicate to the nearest 0.1 cm using a SHORR stadiometer with a reading made after the maximal inspiration. BW was measured three times to the nearest 0.2 kg using an electronic scale (TBF-305, Tanita Corp., Tokyo, Japan). BMI was calculated as weight (kg) divided by height (m) squared (kg/m^2).

To measure FM, %FM, and FFM, we used the foot-to-foot pressure contact electrode BIA system (TBF-305, Tanita Corp., Tokyo, Japan) that incorporates two stainless-steel foot pad electrodes mounted on a platform scale. The scale consists of a load cell that transforms platform weight into an electrical signal. Each foot-pad is divided in half so that the anterior and posterior portions form two separate electrodes. Current is applied through the anterior footpad electrodes and the voltage drop is measured in the posterior portion of the footpad electrodes. Impedance (50 kHz–500 μA) of the lower extremities and BW are measured simultaneously while the subject is standing on the scale. The computer software in this BIA system uses the programmed height and the measured impedance and weight to calculate the body composition estimates based on equations obtained from regression analyses with dual energy X-ray absorptiometry as the reference method. It has been shown previously that within- and between-day coefficient of variations (CV) for % of body fat was 0.9 (SD 0.5)% and 2.1 (SD 1.0)%, respectively [45]. All bioelectrical impedance measurements were performed after 10 min of standing to reduce possible errors from acute changes in body fluid distribution.

Basal metabolic rate

After the rest, we used ventilated hooded system (Deltatrac Metabolic Monitor, Datex Instrumentation Corp Helsinki) to measure carbon dioxide production and oxygen consumption during a 25-min period. BMR was calculated according to de Weir [46].

Biochemical and hormonal analyses

Plasma glucose was determined by the glucose oxidase method (Beckman Glucose Autoanalyser, Fullerton, CA). Commercial enzymatic methods were used to determine serum cholesterol and triglycerides and plasma concentrations of FFAs [47–49].

Serum concentration of thyrotropin was determined by using electrochemiluminescence immunoassay (Roche Diagnostics GmBH, Mannheim, Germany) with lower detection limit of 0.005 mIU/l and total precision of less than 8.7%. Free triiodothyronine (FT_3) and tetraiodothyronine (FT_4) measurements were performed with RIA, using antibody-coated test tubes (RIA-gnost, Cis bio international, Gif-Sur-Yvette Cedex, France). Lower detection limits were 0.6 and 0.5 pg/ml for FT_3 and FT_4, respectively. Both, inter- and intra-assay CV were less than 7.6 pg/ml. Plasma GH was determined by solid phase two-site fluorometric assay based on direct sandwich technique with two monoclonal antibodies directed against two different epitopes of hGH molecule (Delfia, Wallac Oy, Turku, Finland). Minimal detection limit was 0.011 μg/l and intra- and inter-assay CVs were less than 5.0% and 6.3%. Concentrations of serum IGF-I was measured by means of monoclonal immunoradiometric assay (CIS bio international, Gif-Sur-Yvette Cedex, France) with lower detection limit of 1.0 ng/ml. Intra- and inter-assay CVs were less than 3.8% and 5.9%, respectively. Insulin concentration was determined by RIA (INEP, Zemun, Serbia); lower limit of sensitivity was 3.0 mU/l while intra- and inter-assay CVs were less than 10.0%. Total leptin and ghrelin concentrations were measured by using commercially available RIAs (Human RIA Kits, Linco Research, Inc., St. Charles, MO, USA). The lowest levels of leptin and ghrelin that could be detected by these assays were 0.5 μg/l and 93.0 pg/ml, respectively. The intra- and inter-assay CVs were less than 10.0% for leptin, while for ghrelin intra- and inter-assay variations were less than 10.0% and 16.0%, respectively. Cortisol concentration was measured by means of RIA (CORT-CT2, CIS bio international, Gif-Sur-Yvette Cedex, France). Minimum detectible concentration was 4.6 nmol/l; intra- and inter-assay variations were below 5.4% and 7.3%, respectively.

Calculations and statistical analyses

Values given are means ± SDs. We used homeostatic model (HOMA-IR) to assess insulin resistance [50]. FFM/FM and BMR/BW ratios were estimated. Individual differences (Δ) for studied variables, between the last and the first measurement occasions, at 36 and 12 weeks of gestation, were calculated. Significant changes in measurements throughout gestation were identified by repeated-measures analysis of

variance. Linear relationships between variables were tested by correlational analysis (Pearson Product Moment). To identify independent effects of variables associated with variation in BMR and ΔBMR during pregnancy, only variables that were significant in univariate regression analyses entered the equations in stepwise multivariate linear regression analyses. *P*-value less than 0.05 was considered as statistically significant.

Acknowledgment We thank the subjects who participated in this study. This research was supported by Clinical Center of Serbia Belgrade and by Serbian Ministry of Science Grant M145019.

References

1. M. Lof, H. Olausson, K. Bostrom, B. Janerot-Sjöberg, A. Sohlstrom, E. Forsum, Am. J. Clin. Nutr. **81**, 678–685 (2005)
2. A.M. Prentice, C.J. Spaaij, G.R. Goldberg, S.D. Poppitt, J.M. van Raaij, M. Totton, D. Swann, A.E. Black, Eur. J. Clin. Nutr. **50**(Suppl 1), S82–S111 (1996)
3. M.N. Bronstein, R.P. Mak, J.C. King, Br. J. Nutr. **75**, 659–668 (1996)
4. P.M. Catalano, S.E. Nizielski, J. Shao, L. Preston, L. Qiao, J.E. Friedman, Am. J. Physiol. Endocrinol. Metab. **282**, E522–E533 (2001)
5. P.M. Catalano, N.M. Roman-Drago, S.B. Amini, E.A.H. Sims, Am. J. Obstet. Gynecol. **179**, 156–165 (1998)
6. J.V.G.A. Durnin, Lancet **2**, 895–896 (1987)
7. A.M. Johnstone, S.D. Murison, J.S. Duncan, K.A. Rance, J.R. Speakman, Am. J. Clin. Nutr. **82**, 941–948 (2005)
8. M. Bolanowski, B.E. Nilsson, Med. Sci. Monit. **7**, 1029–1033 (2001)
9. G. Larciprete, H. Valensise, B. Vasapollo, F. Altomare, R. Sorge, B. Casalino, A. De Lorenzo, D. Arduini, Acta Diabetol. **40**((Suppl 1)), S225–S232 (2003)
10. M. Rebuffe-Scrive, L. Enk, N. Crona, P. Lönnroth, L. Abrahamsson, U. Smith, P. Björntorp, J. Clin. Invest. **75**, 1973–1976 (1985)
11. A. Astrup, P.C. Gøtzsche, K. de Werken, C. Ranneries, S. Toubro, A. Raben, B. Buemann, Am. J. Clin. Nutr. **69**, 1117–1122 (1999)
12. C. Bogardus, S. Lillioja, E. Ravussin, W. Abbott, J.K. Zawadzki, A. Young, W.C. Knowler, R. Jacobowitz, P.P. Moll, N. Engl. J. Med. **315**, 96–100 (1986)
13. C. Bouchard, A. Tremblay, A. Nadeau, J.P. Després, G. Thériault, M.R. Boulay, G. Lortie, C. Leblanc, G. Fournier, Metabolism **38**, 364–370 (1989)
14. E. Ravussin, C. Bogardus, Am. J. Clin. Nutr. **49**(Suppl 5), S968–S975 (1989)
15. M. Elia, in *Energy metabolism: tissue determinants and cellular corollaries: energy expenditure in the whole body*, ed. by J.M. Kinney, H.N. Tucker (Raven, New York, 1992)
16. N. Hoggard, D.V. Rayner, S.L. Johnston, J.R. Speakman, J. Mol. Endocrinol. **33**, 693–703 (2004)
17. T.M. Mizuno, H. Makimura, J. Silverstein, J.L. Roberts, T. Lopingco, C.V. Mobbs, Endocrinology **140**, 4551–4557 (1999)
18. D. Spanswick, M.A. Smith, V.E. Groppi, S.D. Logan, M.L. Ashford, Nature **390**, 521–525 (1997)
19. D.R. Grattan, S.R. Ladyman, R.A. Augustine, Physiol. Behav. **91**, 366–374 (2007)
20. K.D. Niswender, D.B. Baskin, M.W. Schwartz, Trends Endocrinol. Metab. **15**, 362–369 (2004)
21. J. Fuglsang, C. Skjaerbaek, U. Espelund, J. Frystyk, S. Fisker, A. Flyvbjerg, P. Ovesen, Clin. Endocrinol. **62**, 554–559 (2005)
22. M. Riedl, C. Maier, A. Handisurya, A. Luger, A. Kautzky-Willer, J. Intern. Med. **262**, 458–465 (2007)
23. M.K. Bauer, J.E. Harding, N.S. Bassett, B.H. Breier, M.H. Oliver, B.H. Gallaher, P.C. Evans, S.M. Woodall, P.D. Gluckman, Mol. Cell. Endocrinol. **140**, 115–120 (1998)
24. M. Hernandez-Valencia, A. Zarate, R. Ochoa, M.E. Fonseca, D. Amato, O.M. de Jesus, Diabetes Obes. Metab. **3**, 457–462 (2001)
25. N.M. Gude, C.T. Roberts, B. Kalionis, R.G. King, Thromb. Res. **114**, 397–407 (2004)
26. V.E. Murphy, R. Smith, W.B. Giles, V.L. Clifton, Endocr. Rev. **27**, 141–169 (2006)
27. R. Ahima, J. Kelly, J. Elmquist, J. Flier, Endocrinology **140**, 4923–4931 (1999)
28. M. Rosenbaum, R. Goldsmith, D. Bloomfield, A. Magnano, L. Weimer, S. Heymsfield, D. Gallagher, L. Mayer, E. Murphy, R.L. Leibel, J. Clin. Invest. **115**, 3579–3586 (2005)
29. M.A. Borg, R.S. Sherwin, W.P. Borg, W.V. Tamborlane, G.I. Shulman, J. Clin. Invest. **99**, 361–365 (1997)
30. B.E. Levin, A.A. Dunn-Meynell, V.H. Routh, Am. J. Physiol. Regul. Integr. Comp. Physiol. **276**, R1223–R1231 (1999)
31. C.V. Mobbs, L.M. Kow, X.J. Yang, Am. J. Physiol. Endocrinol. Metab. **281**, E649–E654 (2001)
32. I.A. Silver, M. Erecinska, J. Neurophysiol. **79**, 1733–1745 (1998)
33. C.W. Burke, F. Roulet, Br. Med. J. **1**, 657–659 (1970)
34. B. Allolio, J. Hoffmann, E.A. Linton, W. Winkelmann, M. Kusche, H.M. Schulte, Clin. Endocrinol. **33**, 279–289 (1990)
35. J.R. Lindsay, L.K. Nieman, Endocr. Rev. **26**, 775–799 (2005)
36. N.A.T.M. Huizenga, J.W. Koper, P. de Lange, H.A.P. Pols, R.P. Stolk, D.E. Grobbee, F.H. de Jong, S.W.J. Lamberts, J. Clin. Endocrinol. Metab. **83**, 47–54 (1998)
37. M. Bartels, E.J.C. de Geus, C. Kirschbaum, F. Sluyter, D.I. Boomsma, Behav. Genet. **33**, 421–433 (2003)
38. A. Peters, U. Schweiger, L. Pellerin, C. Hubold, K.M. Oltmanns, M. Conrad, B. Schultes, J. Born, H.L. Fehm, Neurosci. Biobehav. Rev. **28**, 143–180 (2004)
39. G. Mastorakos, I. Ilias, Ann. N. Y. Acad. Sci. **997**, 136–149 (2003)
40. A. Vegiopoulos, S. Herzig, Mol. Cell. Endocrinol. **275**, 43–61 (2007)
41. Y. Minokoshi, K. Young-Bum, O.D. Peroni, L.G.D. Fryer, C. Muller, D. Carling, B.B. Kahn, Nature **415**, 339–343 (2002)
42. N.C. Okereke, L. Huston-Presley, S.B. Amini, S. Kalhan, P.M. Catalano, Am. J. Physiol. Endocrinol. Metab. **287**, E472–E479 (2004)
43. C. Verdich, S. Toubro, B. Buemann, J.J. Holst, J. Bulow, L. Simonsen, S.B. Sondergaards, N.J. Christensen, A. Astrup, Obes. Res. **9**, 452–461 (2001)
44. V. White, E. González, E. Capobianco, C. Pustovrh, N. Martínez, R. Higa, M. Baier, A. Jawerbaum, Reprod. Fertil. Dev. **18**, 425–432 (2006)
45. C. Nunez, D. Gallagher, M. Visser, F.X. Pi-Sunyer, Z.M. Wang, S.B. Heymsfield, Med. Sci. Sports Exerc. **29**, 524–531 (1997)
46. J. de Weir, J. Physiol. **109**, 1–9 (1949)
47. J. Siedel, E.O. Hagele, J. Ziegenhorn, A.W. Wahlefeld, Clin. Chem. **29**, 1075–1080 (1983)
48. K. Itaya, U. Michio, J. Lipid Res. **6**, 16–20 (1965)
49. A.N. Wahlefeld, in *Methods of enzymatic analysis: triglycerides determination after enzymatic hydrolysis*, ed. by H.V. Bergmayer (Academic Press, New York, 1974)
50. D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Diabetologia **28**, 412–419 (1985)