

*Original Research Article***Energetic Factors and Seasonal Changes in Ovarian Function in Women From Rural Poland**GRAZYNA JASIENSKA^{1*} AND PETER T. ELLISON²¹*Institute of Public Health, Jagiellonian University, 31-351 Kraków, Poland*²*Department of Anthropology, Harvard University, Cambridge, Massachusetts 02138*

ABSTRACT Female mammals can optimize their fitness by temporal suppression of reproductive function in response to unfavorable environmental conditions. Since reproduction is energetically demanding for a human female, ovarian function is expected to be sensitive to factors influencing energy availability and metabolism. Dieting and exercise in women from industrial countries, and low-calorie diet and workload in women from developing countries, are often associated with ovarian suppression. This study shows that in Polish rural women seasonal changes in workload correlate with seasonal changes in indices of ovarian function (progesterone measured in saliva samples collected daily for six menstrual cycles for each subject). Mean levels of energy expenditure of the most work demanding weeks of the summer exceeded mean levels of energy expenditure during winter by 37%. Energy intake in this population was sufficient throughout the year. During the summer, when physical work was most intense, low values of progesterone levels were observed (178.2 pmol/L in July and 182.2 pmol/L in August), indicating ovarian suppression. Mean progesterone levels rose to 234.6 pmol/L in October when levels of energy expenditure were lower due to cessation of harvest-related activities. As indicated by several causal models tested through path analysis, energy expenditure was the only variable responsible for suppressed progesterone levels during the summer. Variables describing the nutritional status and energy balance did not correlate significantly with progesterone levels; neither body weight nor body fat or seasonal changes of these variables seem to influence ovarian function in this population. Thus work-related energy expenditure does not need to lead to negative energy balance in order to cause suppression of reproductive function in women. *Am. J. Hum. Biol.* 16:563–580, 2004. © 2004 Wiley-Liss, Inc.

The suppressive effect of energy expenditure on the ovarian function has been known for a long time. Numerous studies documented a negative relationship between energy expenditure resulting from sport activity and ovarian function in women (Elias and Wilson, 1993; Ellison, 1990; Henley and Vaitukaitis, 1988; Howlett, 1987; Loucks, 1990; Noakes and van Gend, 1988; Prior, 1985; Rosetta, 1993; Rosetta et al., 1998). Reproductive suppression was also described in populations where high levels of energy expenditure are necessitated by basic subsistence activities (Bailey et al., 1992; Bentley et al., 1990; Ellison et al., 1989; Panter-Brick and Ellison, 1994; Panter-Brick et al., 1993). Only a few studies, however, were able to point to energy expenditure as the dominant factor of ovarian suppression (Ellison and Lager, 1986; Jasienska and Ellison, 1998). In most of the studies of women involved in subsistence work (Bailey et al., 1992; Ellison et al., 1986, 1989; Panter-Brick et al., 1993), effects of energy expenditure were confounded with

the effects of inadequate energy intake, poor nutritional status, or negative energy balance. This study searched for answers to two questions: Does work-related energy expenditure have a suppressive effect on ovarian function? And if so, can this suppressive effect be direct or, alternatively, does it have to be mediated by the negative energy balance of a woman?

This study was conducted in a small village in southern Poland, which belongs to the Mogielica Human Ecology Study Site. Women were involved in agricultural work, which is

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labor-intense and highly seasonal. High energy expenditure of summer months, during which activities of harvest and haying were performed, remained in contrast to fall and winter months, when women were not involved in agricultural work. Even during periods of the most intense work women did not experience nutritional stress, since food availability is not limited in this population. Seasonal data (July, August, and January) were collected on the levels of energy expenditure, energy intake, and body composition of the study subjects. Daily samples of salivary progesterone were collected for 6 months (from July to October and in January and February).

INTERACTIONS AMONG VARIABLES: A CAUSAL APPROACH

The approach used here involves studying correlations among relevant variables as a means of detecting causal relationships, rather than analyzing differences in average properties of groups of subjects. The latter approach had several disadvantages. First, the basis on which such groups of subjects are distinguished is frequently arbitrary and not supported by any biological or statistical arguments. Second, variation among subjects and covariation among variables can be viewed as a source of valuable insight into functional relationships (Himmelstein et al., 1990) and should not be disregarded when the averages are computed.

The correlational approach involves presenting an explicit hypothesis describing what causal relationships among variables are postulated and which are considered unimportant or not plausible, based on current knowledge. Several applicable models are discussed and the choice of the model to be tested is determined by two considerations. First, the model should be comprehensive, i.e., should include as many relevant variables as possible. Second, the model should be testable by the use of the existing statistical methods, such as path analysis. This approach requires arranging variables into a model in which independent variables have both direct and indirect effects on the dependent variable. The models presented here address the question whether energy expenditure can be the sole factor influencing ovarian function, or whether it can only have an effect on the ovarian function if acting via changes in the energy balance of the individual. The potential effects of energy intake and age on ovarian function are also

included in the models. Types of models described below are presented in Figure 1 (the immediate-effect models), Figure 2 (the delayed-effect models), and Figure 3 (the cumulative-effect models).

Immediate-effect models

In the simplest model (Fig. 1), independent variables: age, total energy expenditure (TEE), total energy intake (TEI), and variables reflecting the energy balance (change in body fat percentage) directly or indirectly affect luteal progesterone levels. All variables involved are measured in Month 1, except the change in fat percentage, which represents differences in measurements between Month 1 and the following month.

In this model, TEI may affect progesterone levels either directly and/or indirectly (via energy balance). Similarly, TEE may have direct effects on ovarian function, or indirect effects via energy balance. There may also be a relationship between TEE and TEI, since women expending more energy may have greater caloric and nutritional requirements. On the other hand, the dependence of TEI on TEE is unlikely, since TEE of the individual results from demands of field- and housework and is not limited in this population by food availability.

Delayed-effect models

The assumption from the previous model (the immediate-effect model) that ovarian function should respond immediately to changes in independent variables may not be very realistic. If ovarian plasticity is an evolutionary, adaptive response to environmental stresses, there should exist a mechanism allowing for the distinction between a noise and the real signal (Ellison, 1990). Studies of short-term nutritional deprivation provide some evidence that ovarian function does not react to acute changes in the energy balance (Olson et al., 1995). Therefore, ovarian response to environmental factors which occurs with some time lag is more likely, or at least should be more pronounced, than the immediate response. Consequently, the delayed effect model (Fig. 2) uses the same set of variables, but tests the effects of TEE and TEI in a current month on progesterone levels of the following month.

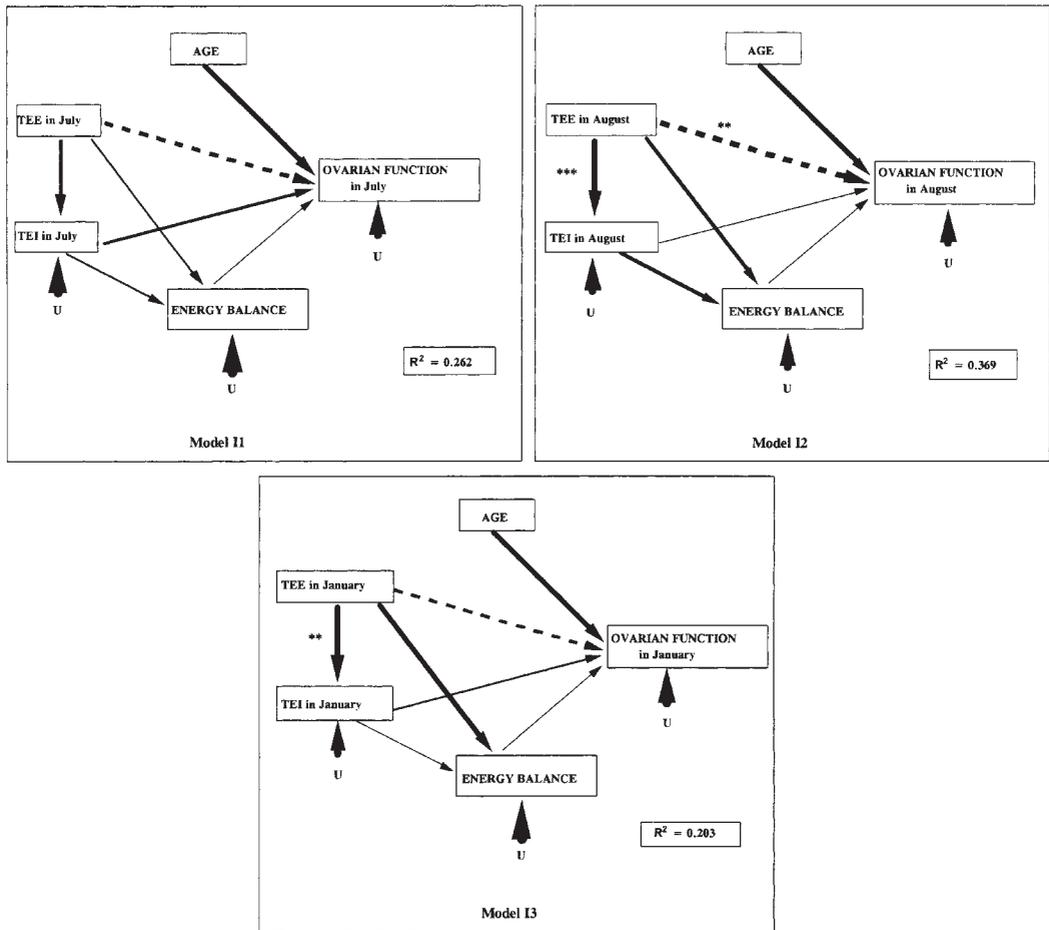


Fig. 1. The immediate effect models. Effects of independent variables in given month on the ovarian function in the same month. Arrow thickness is proportional to the magnitude of the path coefficients. ** $P < 0.01$, *** $P < 0.001$. Paths without signs are insignificant.

Cumulative-effect models

The assumption of this model is that longer-lasting stress will have more pronounced effects on the suppression of ovarian function than the stress which is present in a given month, but becomes relaxed in the following month. The model (Fig. 3) explores the effects of TEE and TEI in a given month (Month 1) on the progesterone levels in the following month (Month 2), but takes into account also whether energetic stresses continue or not in Month 2. For example, for the studied population average TEE is high in July and even higher in August. However, the harvest season ends in the third week of August and TEE in September is, therefore,

expected to be much lower. Consequently, ovarian suppression should be the most pronounced in August as a result of high TEE in both July (Month 1) and in August (Month 2). Only some level of ovarian suppression should be observed in September as ovarian function slowly returns to full function when the stress is relaxed. Even less ovarian suppression should be expected in October, when agricultural work for the year ends, and when the TEE should resemble more that of winter months. It is unlikely that the energetic stresses of July and August should still have an effect on ovarian function in October. However, it can be assumed that just as the suppression of ovarian function due to

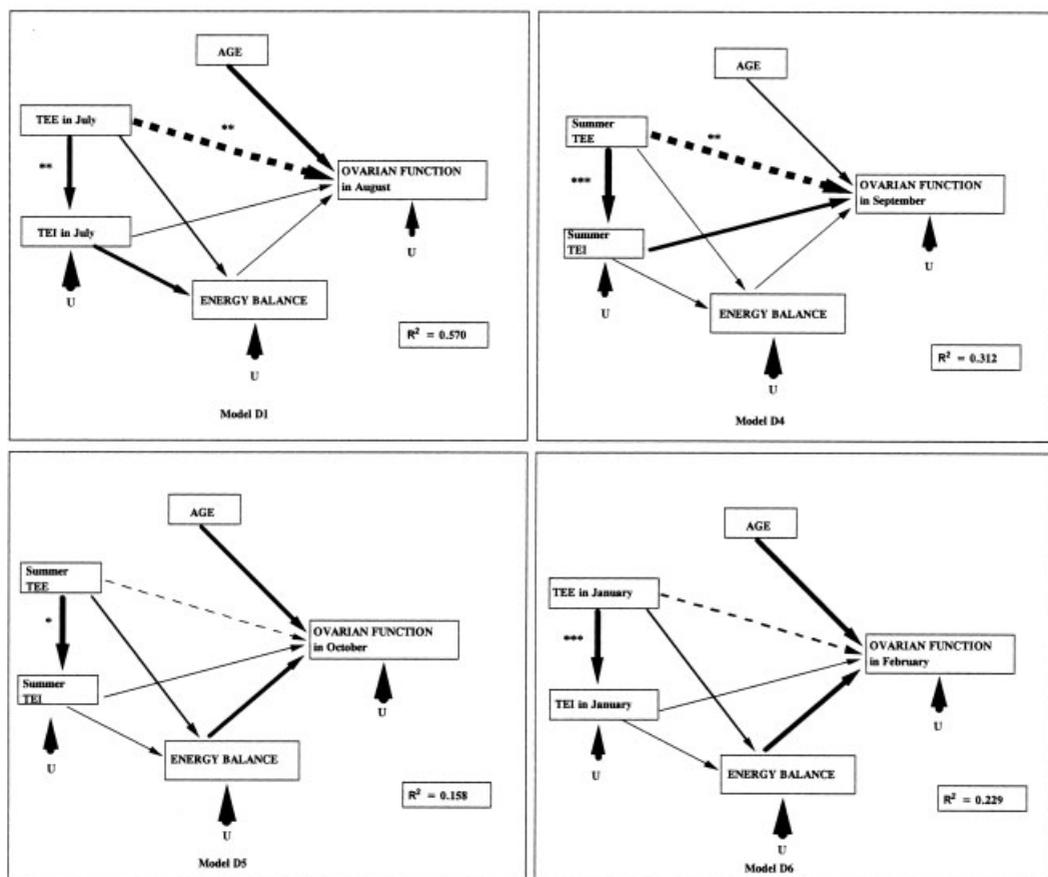


Fig. 2. The delayed effect models. Effects of independent variables in a given month or a season on the ovarian function in the following month. In models D4 and D5 TEE and TEI represent mean summer values. Arrow thickness is proportional to the magnitude of the path coefficients. ** $P < 0.01$, *** $P < 0.001$. Paths without signs are insignificant.

environmental stresses may occur with some time lag, the return to full functioning when stresses are relaxed may not be immediate.

In the analyses we used the adjusted cumulative effect model. Instead of using mean TEE in Month 1 and mean TEE in Month 2 as two separate variables, mean TEE in Month 1 and the Month 2 was used instead (Fig. 3, "summer TEE"). The same procedure was used for TEI (Fig. 3, "summer TEI").

MATERIALS AND METHODS

Study site and study subjects

Subjects of this study were women from a small agricultural village, Chyszówki in

southern Poland. Chyszówki belongs to the *Mogielica* Human Ecology Study Site that includes five villages (Chyszówki, Jurków, Pólrzeczki, Słopnice, and Wilczyce), located in valleys around the mountain Mogielica in the Beskid Wyspowsy mountain range. In Chyszówki, families own small fields on the mountain slopes with land property often being highly fragmented and spread over a substantial area. Due to localization of most fields, mechanized equipment is rarely used. The majority of the work is done by hand and requires participation of the whole family. In these conditions, women's involvement in agricultural work is very high. Each house has adjoining stables for domestic animals, buildings for storage of hay and grains,

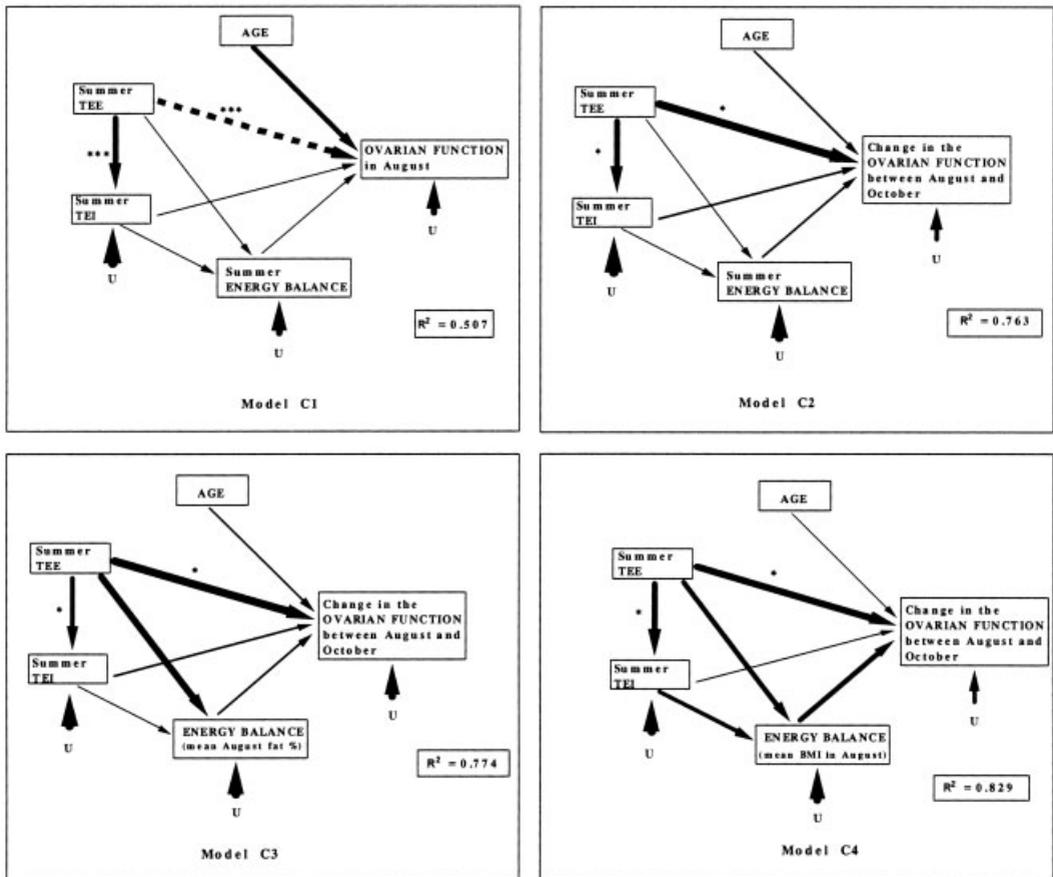


Fig. 3. The cumulative effect models. Effects of the cumulative TEE and TEI in the summer on ovarian function. Arrow thickness is proportional to the magnitude of the path coefficients. * $P < 0.05$, *** $P < 0.001$. Paths without signs are insignificant.

vegetable gardens, and small orchards. Most farms own 1–4 cows, chickens and ducks, usually one horse, several pigs, rabbits, and, occasionally, sheep. Pastures for the grazing animals are often located at a substantial distance from the house. Each farm grows rye, wheat, barley, oats, and potatoes. Most of the produce is used for the needs of the family and domestic animals. Fruits and vegetables are also grown, but only in quantities needed to support the family through the year. Dairy products are usually home-made, as often is bread and pasta. Meat comes from domestic animals, most often chicken and ducks, and may also be purchased, just as are additional food products not grown on the farm. Daily composition of diet varies seasonally, with more vegetables

and fruits being available in the summer and fall, and more homebaked products during the winter. During the time when fieldwork is most demanding a typical dinner may not be cooked: bread with cheese and cold meat will be eaten instead.

All 22 subjects lived within an area of $\sim 9 \text{ km}^2$. Subjects were recruited in the summer of 1990 when they took part in the pilot study (Jasienska and Ellison, 1993). Most of the women remained subjects of the study, which was conducted from July to October of 1992 (Jasienska and Ellison, 1998) and in January and February of 1993. Twenty subjects out of the original sample participated in the both summer/fall and winter parts of the study and data on these women were used in the analyses. Subjects met criteria

of having regular menstrual cycles of between 22–38 days and of not using oral contraceptives or other steroid medication, and not being pregnant or lactating for at least 6 months prior to the beginning of the study. Women in this population keep written records of menstrual dates, which allowed us to check the accuracy of reported menstrual onsets. A written informed consent was obtained from all study participants and the study protocol was approved by an institutional bioethical committee.

TABLE 1. Characteristics of the study subjects at the beginning of the study

	Mean	Standard deviation	Range	N
Age (yr)	31.4	4.55	24–39	20
Menarcheal age (yr)	14.5	0.66	13–16	20
Height (cm)	162.1	3.8	154–170	20
Predicted BMR (kJ/d)	5875.2	320.73	5291–6638	20
Number of children	2.7	1.34	0–5	20
Interbirth interval (months)	27.0	12.82	10–58	18
Age at birth of the first child (yr)	22.0	2.95	18–27	18

Data describing body composition and other characteristics of the study subjects (age, age at menarche, age at first reproduction, number of children, age of children) were collected by an interview at the beginning of the study (Table 1). Measurements of height were made once at the beginning of the study. Measurements of body mass were made every 2 weeks from the beginning of July to the end of August and twice in January. Measurements of body fat were taken at the same time with the "Futrex-1000" near-infrared reflectometer (Heyward et al., 1992). All anthropometric measurements were made by one observer in order to avoid interobserver error. Data on seasonal changes in subjects' weights and body fat percentage are presented in Table 2.

Seasonality of work

Farm work is very seasonal but varies little from year to year. Fieldwork starts in March with sowing of the "spring crops," rye and barley. In April potatoes and spring crops of wheat are planted. Most women are not involved in work outdoors until May, when they plant vegetable gardens. The grazing season starts in mid-May and cows need to be walked to and from the pastures on a daily basis. Work intensity increases in

TABLE 2. Characteristics of body composition, energy expenditure, and energy intake of the subjects for the 3 months of the study ($n = 20$ women in each month)

	July	August	January
	Mean, SD, range	Mean, SD, range	Mean, SD, range
Body weight (kg)	64.2 (8.42) 50–87	64.1 (8.85) 49–89	66.6 (9.15) 50–92
BMI (kg/m ²)	24.4 (2.89) 19.8–32.9	24.4 (3.06) 19.4–33.7	25.3 (3.17) 19.8–34.8
Body fat (%)	27.5 (6.60) 15.5–37.0	26.2 (6.67) 14.5–36.1	27.6 (6.16) 15.1–37.5
Total energy expenditure (MJ/day)	10.954 (1.876) 7.59–14.32	11.758 (2.437) 7.99–15.90	9.403 (0.880) 8.04–11.72
Total energy expenditure expressed as a multiple of BMR	1.877 (0.347) 1.33–2.43	2.019 (0.384) 1.51–2.47	1.562 (0.103) 1.40–1.78
Total energy expenditure per kg of body weight (kJ/kg/day)	170.82 (30.777) 116.5–214.4	184.56 (37.312) 124.7–232.0	140.85 (13.258) 113.7–160.9
Total energy intake (MJ/day)	11.591 (1.937) 8.087–14.730	12.599 (1.599) 10.829–15.239	11.841 (0.967) 10.148–13.920
% of energy intake from proteins	13.5 (3.94) 8.6–17.7	12.5 (3.30) 9.4–17.4	13.8 (4.11) 8.5–22.0
% of energy intake from fat	35.5 (7.37) 26.5–44.6	34.5 (5.55) 30.6–37.5	35.3 (6.74) 24.0–44.0
% of energy intake from carbohydrates	50.9 (8.57) 42.0–64.0	53.0 (5.88) 47.2–55.6	50.8 (7.72) 42.5–61.0

late June, when making and transportation of hay is carried out. The harvest season starts in the second half of July and haying may continue in that month. Also in July, fruits, mostly black and red currants, are picked from the gardens and wild mushroom and blueberries are gathered in the woods, often in substantial quantity. In August, harvest continues and cereals are transported and subsequently threshed with the use of threshing machines. This requires substantial work effort, since cereals must be loaded into the machine and later grains and the remaining thatch must be packed and transported for storage. In August also, the second hay of the season is collected, transported, and stored. Soon after the cereals are cleared from the fields, the ground is plowed using horses. In September, potatoes are harvested and "winter crops" of wheat and rye are sowed (to be harvested in July and August). In October and November, men cut and transport wood which will be used for heating and cooking throughout the year, since coal is used only in small quantities. During the winter months women spin wool, knit sweaters, and sew clothing and linens.

In addition to seasonal work, women are involved daily in housework, animal care, and child care. Time spent on these activities does not vary substantially across the year (Jasienska, 1996). Seasonal differences in mean daily energy expenditure are therefore due mainly to the seasonal nature of the fieldwork. Sexual division of labor is quite pronounced and women are almost never involved in work requiring the use of horsepower (e.g., plowing, sowing, fertilizing with manure). Men are generally not involved in housework and some forms of animal care, and only occasionally help with childcare.

Energy expenditure—data collection and analysis

Preliminary interviews showed that fieldwork is the most demanding for women during July and August and least demanding from October to March. Energy expenditure data in July, August, and January were collected by 24-hour recall interview (Brun, 1992). On average, three interviews in July, three in August, and two in January were conducted with each subject by trained assistants. Each interview lasted ~20 minutes. Subjects were asked to report approximate time of the day when the particular activity began, how long it

lasted, and if any breaks were taken and to provide additional information about a particular activity (e.g., when the activity was "walking," the subject was asked how fast she walked, down or up the slope, whether she was carrying any loads, etc.). Recall interviews of energy expenditure were conducted each time for the same 24-hour period as interviews of energy intake and included only data from working days (i.e., not from Sundays).

In addition to the 24-hour recall, direct observations of subjects' activities were conducted. Only activities carried out outside the house (e.g., fieldwork, picking wild fruits in the forest) could be observed. Each observation lasted 2–6 hours. The main purpose of direct observations was to determine whether subjects reliably recalled activities and their duration when asked during the interview the following day (Jasienska, 1996).

Data from 24-hour recall interviews were used to estimate subjects' energy expenditure patterns. Total daily energy expenditure (TEE) was defined as the sum of the energy expenditures of all activities performed during a 24-hour period. TEE was estimated by multiplying total time spent at each activity by the energy cost of that particular activity (expressed as the multiple of the predicted basal metabolic rate (BMR) [FAO/WHO/UNU, 1985]). When the activities performed by the study subjects were not listed in the published tables, they were assigned to the categories which were qualitatively closest in terms of energy expenditure. For example, care of domestic animals, which includes activities like hand-milking cows, feeding and giving water to domestic birds, pigs, cows, and horses, and cleaning stables was performed by most subjects on a daily basis (2–3 times a day). This type of work does not vary across the year and all these activities are performed in a similar sequence. In order to estimate energy expenditure of these activities the subjects estimated the total time spent on activities classified as animal care at a given time (separately for each time of day, i.e., morning, afternoon, evening). Half of the time period was given the value for light domestic activity (BMR factor of 2.7 from the published tables) and the second half the value of the moderate domestic activity (BMR factor of 3.7). Social activities were categorized as sitting and eating (BMR factor of 1.2) or sitting and sewing (BMR factor of 1.4, to substitute for card playing). The BMR factor for weeding (2.9) was used to estimate

energy expenditure associated with collecting wild fruits and mushrooms.

The BMR, expressed in kJ/day, was estimated for each woman from her body weight using the age-specific predictive equations developed by the FAO/WHO (1985). The equations were: $BMR = 14.7 W + 496$ for subjects younger than 30 years, and $BMR = 8.7 W + 829$ for subjects above 30 years of age, where W is body weight in kilograms.

Total daily energy expenditure (TEE) values were expressed either in absolute terms or relative to body mass (TEE/kg) and as multiples of the BMR (BMR factor, also referred to as the physical activity ratio [PAR]). TEE values were used in most analyses as recommended by Norgan (1996). According to the FAO/WHO/UNU (1985) recommendations, TEE can be graded from light ($1.56 \times BMR$ or less), light-moderate (1.56 to $1.64 \times BMR$), moderate-heavy (1.64 to $1.82 \times BMR$), to very heavy ($1.82 \times BMR$ or more) physical activity levels (PALs). The BMR factor is often interpreted as independent of body weight, but this assumption was shown to be true only for light activities. For more strenuous activities (including walking), energy expenditure expressed as the BMR factor increases with body weight (Norgan, 1996). In addition, the BMR factor as a ratio has unfavorable statistical properties (Jasienski and Bazzaz, 1999). Furthermore, the study presented here is concerned with the effects of the total energy expenditure of a woman. A heavier individual, with higher overall energetic costs of basal metabolism, may need the same amount of energy to support reproduction, as a lighter individual with lower absolute metabolic costs. However, a heavier individual may need more energy in general, since she needs to support both reproduction and her own, more costly, metabolism. Therefore, the use of TEE, instead of weight-independent estimates, seems to be more appropriate for the study investigating relationships between individual levels of energy expenditure and reproductive function.

Detailed energy expenditure data are not available for the fall months, since even though women collected saliva samples during this time, interviews and observations of work activities were not conducted. However, according to the data about the year-round work patterns in the village, all agricultural activities end for women by the third or fourth week of September. It was also directly observed that none of the cereals and hay

remained on the fields after the end of August. In September women are involved in the potato harvest, but that usually lasts only several days. After September only men are involved in the remaining agricultural activities, since those require the use of horses. It was specifically stated by questioned subjects and other individuals from the village that women never perform activities which require handling horses. Therefore, it can be assumed that energy expenditure in October resembles that in the winter months, since similar activities, including animal, house, and child care, are performed. The energy expenditure in September is probably higher than that in subsequent months, but lower than during the summer.

Energy intake—data collection and analysis

Energy intake data were collected by 24-hour recall interviews (Burgess and Burgess, 1975; Ulijaszek, 1992), conducted immediately preceding the 24-hour activity recall interview. On average, three interviews in July, three in August, and two in January were conducted with each subject by trained assistants. Subjects were asked to list all food items eaten during and between meals and to provide information about quantities of each food item (estimated in cups, tablespoons, etc.). Recipes, according to which each dish was prepared, were also collected.

Energy intake data were analyzed using the Nutritionist III computer software for Macintosh. Numerous food items specific for the Polish diet were added to the Nutritionist III database, based on the Polish published sources (Los-Kuczera, 1990). In addition, percentages of kJ in the diet from protein, fat, and carbohydrates were calculated. Data were analyzed by comparing mean total energy intake (TEI) between summer (July and August) and winter (January), and by comparing 3 months (July, August, and January) of the study (Table 2).

Ovarian function—data collection and laboratory procedures

Subjects collected daily saliva samples for a total of 6 months, from June to October 1992 and in January and February 1993. Each woman was provided with a set of polystyrene collection tubes pretreated with sodium azide as a preservative, a calendar

for keeping records of sample collection and marking menstrual dates, and pretested chewing gum to be used as the stimulant of saliva flow. Subjects were requested to collect samples daily, in the evening, at least 30 minutes after the last meal. Very few omissions occurred. Samples were stored at room temperature until the end of the collection period and then transported to the laboratory and frozen at -20°C until assayed.

Samples belonging to each menstrual cycle were arranged in order starting from the first day of the menstrual bleeding. The day before the onset of the next menstruation was identified as day -1 , with the previous days identified correspondingly. The last 18 daily samples of each cycle (days -1 to -18) were assayed for progesterone. A total of 111 menstrual cycles were assayed, with less than 10% daily samples missing due to missed or improper collection or loss during the laboratory procedure. Samples belonging to a particular cycle were analyzed in the same assay, with cycles from two different subjects run in each assay.

Progesterone was measured in each subject's samples by the radioimmunoassay (RIA) according to published protocols (Ellison, 1988; Ellison and Lager, 1986). Quality control was maintained through monitoring values of saliva pools at low (follicular), medium (luteal), and high (pregnancy) levels. Assay sensitivity, i.e., the smallest amount distinguishable from 0 with 95% confidence, averaged 22.5 pmol/L. Intraassay variability (CV) at the 50% binding point of the standard curve was 6.3%. Interassay variability estimated from pools containing various levels of progesterone averaged 20.2% for low (late follicular/early luteal) pools, 10.7% for medium (midluteal) pools, and 13.9% for high (pregnancy) pools.

Two ovarian indices were used in statistical analyses: progesterone concentration between cycle days -14 to -1 representing mean luteal phase progesterone levels and progesterone concentration between cycle days -11 to -7 representing mean midluteal phase progesterone levels. Values of midluteal progesterone characterize part of luteal phase with highest progesterone production.

Statistical analyses

Seasonal differences in ovarian function and independent variables. Comparisons among months and between seasons in mean levels

of progesterone, total energy expenditure, total energy intake, and body composition variables were performed in one- or two-way analyses of variance (ANOVA). Tests of main effects were followed by multiple comparisons of means through the Tukey-Kramer post-hoc comparisons, with the overall level of significance kept at 0.05.

Models of interactions among variables—path analysis. Path analysis allows for tests of relationships with more than one dependent variable and for tests of the effects of dependent variables on one another (Mitchell, 2001). Path analysis techniques are used here to estimate the direct and indirect effects of age, total energy expenditure, total energy intake, and energy balance on ovarian function. A path coefficient is a standardized partial regression coefficient and represents the magnitude of the direct effect of the independent variable X on the dependent variable Y, with all other independent variables held constant (Schemske and Horvitz, 1988). The residual variable U includes all unmeasured variables that affect dependent variables and reflects variation in Y that is left unexplained by a given model. The path diagrams show relationships among independent and dependent variables, path coefficients between variables, and residual variables U for all dependent variables. Indirect influences of independent variables on the Y variable may be expressed via correlations with other independent variables X.

The "immediate effect" (I1–I3, Table 4, Fig. 1), the "delayed effect" (D1–D6, Table 4, Fig. 2), and the "cumulative effect" (C1–C4, Table 4, Fig. 3) models were tested separately for the summer/fall season and for the winter season. All models have the following independent variables: age, total daily energy expenditure (TEE), and total daily energy intake (TEI). As the fourth independent variable, models use energy balance, calculated usually as the change in body fat percentage for each individual. Additional models were also tested with body fat percentage per se, or body mass index per se, either variable providing a quantitative index of the energy balance.

The dependent variable in all models is ovarian function, expressed as mean luteal progesterone for individuals in a given month or as change in progesterone levels between 2 months (calculated for every

subject as mean log-transformed luteal progesterone in October minus mean log-transformed luteal progesterone in August). The latter variable represented the extent to which mean luteal progesterone increased in October from the suppressed August values. This variable is less affected by interindividual variation in the overall levels of luteal progesterone and shows how mean progesterone concentration changes for each individual. Biological (genetic and developmental) variation in ovarian function, independent of the influence of environmental factors operating at adulthood, is likely to be present among women (Ellison, 1996; Feigelson, 1998). Therefore, a change in ovarian indices may be a more interesting variable for investigation than the absolute levels of hormones during any given menstrual cycle.

The statistical significance of the path coefficients and the estimates of indirect effects were evaluated by a randomization method, with 2,000 permutations of the original data. Each full path analysis model was recalculated for each of the randomized ("null") dataset, yielding a set of path coefficients and indirect effects. The distributions of such "null" estimates were used to obtain probability values for the actual coefficients and effects. All computations were performed using the Resampling Stats program (www.resample.com).

RESULTS

Body composition

Mean body weight showed significant variation among months (two-way ANOVA, $F_{2,25} = 25.121$, $P < 0.0001$). While mean body weight did not change between July and August, it increased between both summer months and January (Tukey-Kramer tests, $P < 0.05$). Mean body mass index (BMI) varied in a fashion similar to body weight (two-way ANOVA, $F_{2,38} = 24.793$, $P < 0.0001$). In addition, there was significant variation among months in mean body fat percentage (two-way ANOVA, $F_{2,38} = 5.012$, $P < 0.05$). Mean fat percentage decreased between July and August and increased between August and January, but did not differ between July and January (Tukey-Kramer tests, $P < 0.05$) (Table 2).

Energy expenditure

The seasonality of daily work patterns was reflected in the collected data: mean daily

total energy expenditure (TEE) in the summer (averaged over July and August) was 22% higher than that in the winter (based on the January data). Moreover, mean TEE in the first 2 weeks of August, the most demanding time of harvest, exceeded that of winter by 37%. TEE varied significantly among months (two-way ANOVA, $F_{2,38} = 36.268$, $P < 0.0001$); increased between July (10.9 MJ) and August (11.8 MJ), and decreased between both summer months and January (9.4 MJ, Tukey-Kramer tests, $P < 0.05$). Similarly, there was a significant variation among months in mean daily total energy expenditure per kilogram of body mass (TEE/kg, two-way ANOVA, $F_{2,38} = 48.194$, $P < 0.0001$). Mean TEE/kg increased between July (170.82 kJ/kg) and August (184.56 kJ/kg), and decreased between both summer months and January (140.85 kJ/kg) (Tukey-Kramer tests, $P < 0.05$, Table 2).

Months also differed significantly when total energy expenditure was expressed as the multiple of predicted basal metabolic rate (BMR factors, two-way ANOVA, $F_{2,38} = 42.251$, $P < 0.0001$). Mean BMR factor increased between July (1.88) and August (2.02), and decreased between both summer months and January (1.57) (Tukey-Kramer tests, $P < 0.05$, Table 2). Therefore, mean physical activity levels (PALs) of the summer months can be characterized as very heavy, while mean PALs of January as light/moderate (Table 2).

Individual differences in daily total energy expenditures are substantial, especially in the summer, ranging from 7.59 MJ/day to 14.32 MJ/day in July, and from 7.99 MJ/day to 15.90 MJ/day in August. Even during the winter, when overall workloads are lower, individual differences still are observable, ranging from 8.04 MJ/day to 11.72 MJ/day.

Energy intake

Mean total daily energy intake (TEI) did not differ between seasons (two-way ANOVA, $F_{1,19} = 1.7906$, $P > 0.05$, Table 2). Mean TEI in the summer was 12.2 MJ/day and in winter 11.8 MJ/day. When months were analyzed separately, however, there was a significant variation among months in mean TEI (two-way ANOVA, $F_{2,38} = 13.5078$, $P = 0.0001$), with higher mean TEI in August than either in July or January (Tukey-Kramer tests, $P < 0.05$).

Mean TEI did not differ between July and January (Tukey-Kramer test, $P > 0.05$).

Percentages of energy in diet from protein, fat, and carbohydrates did not differ between summer and winter (two-way ANOVA, protein: $F_{1,19} = 2.2589$, $P > 0.05$; fat: $F_{1,19} = 0.1859$, $P > 0.05$; carbohydrates: $F_{1,19} = 1.3679$, $P > 0.05$). No differences in diet composition among individual months (July, August, and January) were detected (Table 2) (two-way ANOVA, protein: $F_{2,38} = 2.2497$, $P > 0.05$; fat: $F_{2,38} = 0.6612$, $P > 0.05$; carbohydrates: $F_{2,38} = 2.1683$, $P > 0.05$).

Ovarian function

During the study all subjects had regular menstrual cycles. Mean length of menstrual cycle ranged from 26.0 days to 27.5 days (Table 3) and did not vary significantly among months (one-way ANOVA, $F_{5,97} = 1.094$, $P = 0.369$).

There was significant variation among months in the mean progesterone concentration between cycle days -14 to -1 (mean luteal-phase progesterone, two-way ANOVA, $F_{5,88} = 7.4872$, $P < 0.0001$, Table 3). Mean luteal-phase progesterone level was higher in October than in any other analyzed month and in September it was higher than in July (Tukey-Kramer tests, $P < 0.05$). Similarly, mean progesterone concentration between cycle days -11 to -7 (mean midluteal phase) varied significantly among months (two-way ANOVA, $F_{5,88} = 4.337$, $P = 0.0007$). Both summer months (July and August) had lower midluteal progesterone than October and January (Tukey-Kramer tests, $P < 0.05$, Table 3).

Causal models: summary of the results

During the summer, when levels of energy expenditure were the highest, low values of the indices of ovarian function were observed, indicating ovarian suppression, as shown by a robust negative relationship between both variables (Table 4). Women with the highest levels of energy expenditure had the lowest levels of ovarian progesterone. At the same time, variables related to the energy balance did not correlate significantly with the indices of ovarian function (Table 4). As indicated by the tested models, neither total energy intake, nor body weight and body fat, nor seasonal changes in these variables seemed to influence ovarian function. Therefore, the main findings of this study support the working hypothesis that suppression of ovarian function can be caused by energy expenditure, even in the absence of negative energy balance.

In general, models for the summer season explained a higher percentage of variation in ovarian function than did models for winter months (Table 4). None of the winter models showed significant effects of TEE on ovarian function. Only low fraction of variation in ovarian function was explained by models investigating effects of summer independent variables on late fall (October) indices of ovarian function and levels of TEE during the summer did not have significant effect on progesterone levels in October.

The highest percentage of variation in ovarian function was explained by the cumulative effects models, in which cumulative values of independent variables measured in the summer had an effect on the change in ovarian function between the summer and fall (Table 4, Fig. 3). Each of the three models (Model C2, C3, and C4) explained about

TABLE 3. Characteristics of the menstrual cycles and the luteal progesterone levels calculated for mean luteal phase (last 14 days of the menstrual cycles) and mean midluteal phase (days -11 to -7)

	July	August	September	October	January	February
Mean luteal progesterone concentration (pmol/L)	178.2 (100.70)	182.2 (111.31)	204.0 (131.54)	234.6 (142.36)	202.0 (116.01)	195.3 (124.39)
Range of mean luteal progesterone (pmol/L)	83.9–361.2	29.6–359.0	111.3–426.0	143.6–414.3	121.4–348.5	82.8–285.2
Mean midluteal progesterone concentration (pmol/L)	221.5 (117.22)	237.5 (124.52)	259.7 (154.37)	292.1 (139.05)	268.3 (127.60)	268.6 (139.51)
Number of daily samples analyzed for progesterone	252	271	280	207	262	222
Number of menstrual cycles	18	20	20	16	20	17
Mean cycle length (days)	26.7 (2.60)	26.3 (2.64)	27.5 (2.76)	26.2 (2.58)	26.0 (2.42)	27.1 (2.46)
Range of cycle lengths (days)	22–31	19–31	24–33	22–30	23–32	23–32

Values in brackets are standard deviations.

TABLE 4. Results of path analyses of causal models

Type of model/effect of impact variables in:	Ovarian function in:				TEE		TEI		Energy balance		Age		Unexplained causes (residual variable U for ovarian function)
	direct effect	indirect effect	total effect	total effect	direct effect	indirect effect	total effect	total effect	direct (= total) effect	direct (= total) effect	direct (= total) effect		
Immediate effect 1/July	-0.411	0.101	-0.353	0.266	-0.006	0.260	-0.044	-0.353	0.858				
Immediate effect 2/August	-0.508**	0.006	-0.502	-0.024	-0.025	-0.049	0.106	-0.383	0.794				
Immediate effect 3/January	-0.312	0.154	-0.158	0.209	0.005	0.214	0.072	-0.380	0.893				
Delayed effect 1/July	-0.726**	0.047	-0.679	0.096	0.011	0.085	-0.044	-0.406	0.655				
Delayed effect 2/August	-0.710**	0.325	-0.385	0.512	-0.031	0.481	0.129	-0.207	0.818				
Delayed effect 3/August	0.078	0.055	0.133	0.093	0.057	0.150	-0.339	-0.334	0.911				
Delayed effect 4/summer	-0.726**	0.280	-0.446	0.424	-0.001	0.423	-0.011	-0.223	0.829				
Delayed effect 5/summer	0.101	0.061	0.161	0.006	-0.006	-0.000	-0.354	-0.354	0.918				
Delayed effect 6/January	0.248	-0.147	0.101	-0.105	-0.027	-0.133	-0.359	-0.458*	0.878				
Cumulative effect 1/summer	-0.731***	0.107	-0.642	0.158	0.004	0.162	0.043	-0.407	0.702				
Cumulative effect 2/summer	0.734*	0.085	0.819	0.117	-0.004	0.113	-0.130	0.147	0.487				
Cumulative effect 3/summer	0.664*	0.156	0.820	0.108	0.005	0.113	0.199	0.133	0.475				
Cumulative effect 4/summer	0.599*	0.231	0.830	-0.005	0.122	0.117	0.403	0.061	0.414				

Impact variables are TEE, TEI, energy balance, and age. All models show effects of impact variables on ovarian function. See Jasienska, 1996, for the effects of impact variables on TEI and energy balance. Direct and indirect effects are indicated separately and summed as the total effect. For models investigating effects of independent variables in summer months energy balance was calculated as the change in body fat % for each individual between the beginning of July and the end of August. For "cumulative effect" models C2, C3 and C4 energy balance was expressed as "change in body fat % for each individual between beginning of July and the end of August; "mean body fat percentage in August; and "mean body mass index (BMI) in August. For models investigating effects of independent variables in winter months energy balance was calculated for each individual as the change in body fat % between August and January. Ovarian function is expressed as mean luteal progesterone in a given month or as change in progesterone levels between 2 months (calculated for every subject as mean log-transformed luteal progesterone in October minus mean log-transformed luteal progesterone in August). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Paths without signs are not significant at $P = 0.05$. The value of U is the square root of $1 - R^2$ (where R^2 is the coefficient of determination of the "ovarian function", variable by the four impact variables).

80% of variation in the change of the ovarian function, with TEE being the only variable with significant effect on ovarian function.

In almost all analyzed models summer TEE had a significant negative effect on ovarian function during the summer (Table 4). Direct effects of age, energy intake, and energy balance as well as indirect effects of energy expenditure and energy intake on ovarian function were insignificant in all models. Apart from the relationship between energy expenditure and ovarian function, the only other significant positive relationship existed between TEE and TEI.

For the summer season, cumulative effect models (relationships between mean summer TEE, mean summer TEI, age, summer energy balance, and ovarian indices in August) (Fig. 3) and delayed effect model (relationships between independent variables in July and ovarian function in August) (Fig. 2, model D1) explained between 50% and 65% of variation in ovarian progesterone. These models showed a significant negative relationship between TEE and ovarian indices.

Immediate effect models for the summer (effects of independent variables in July on ovarian function in July, and effects of independent variables in August on ovarian function in August) (Fig. 1, Models I1, I2) did not explain the high percentages of variation. The August model explained a higher percentage of variation (37%) and effects of TEE on ovarian function were significant in this model. The July model explained only 26% and none of its path coefficients showed significance.

In order to see how long-lasting the effects of intense TEE are during the summer, the relationship between summer independent variables and ovarian function in two fall months was tested (Table 4, Models D2, D3, D4, D5). Models exploring impact of independent variables on the ovarian function in September (D2, D4) explained a higher percentage of variation than did models for October. Effects of independent variables in August and mean summer variables on the ovarian function in September explained about 30% of variation. Both showed significant effects of TEE on ovarian function. In contrast, the models exploring effects of either independent variables in August (D3) or mean summer independent variables (D5) on October progesterone levels explained only slightly more than 15% of variation. None of the effects of independent variables were significant in these models.

DISCUSSION

Effects of energy expenditure

Results of this study support two main predictions: 1) high energy expenditure has a negative effect on ovarian function, and 2) this effect does not have to be mediated by negative energy balance, but can be direct. Most of the analyzed summer models not only explained high percentages of variation in the ovarian function, but also had high, statistically significant path coefficients between total energy expenditure and indices of ovarian function (Table 4). Ovarian function responded negatively to high levels of energy expenditure in the summer. Several patterns of the relationships between energy expenditure and ovarian function emerge from the tested models (Fig. 4). First, models assuming some delay in the effects of the TEE explained a higher portion of variation in the ovarian function than did models assuming immediate effects. Second, models using the change in ovarian progesterone as the dependent variable, instead of the absolute progesterone levels in a given month, explained the highest percentage of variation. Third, the suppressing effects of summer TEE are not very long-lasting. While mean summer TEE had a significant negative effect on the ovarian function in September, but by October this impact was statistically absent.

The variable expressing the change in progesterone levels between the menstrual cycles (between August and October), rather than the absolute progesterone levels in a given menstrual cycle, reacts more strongly to variation in energy expenditure: during August, when work is the most demanding, the hardest-working women experienced the most severe ovarian suppression. Therefore, these women were the ones showing the greatest change in ovarian function from August to October, when the environmental stresses were no longer present.

Lack of significant effects of energy intake

None of the models tested showed significant effects of energy intake on the variation in ovarian function (Table 4). This finding is not surprising in a population which does not experience food shortages and where dieting in order to lose weight is not common. In all models, both for summer and winter seasons, a strong positive relationship was observed

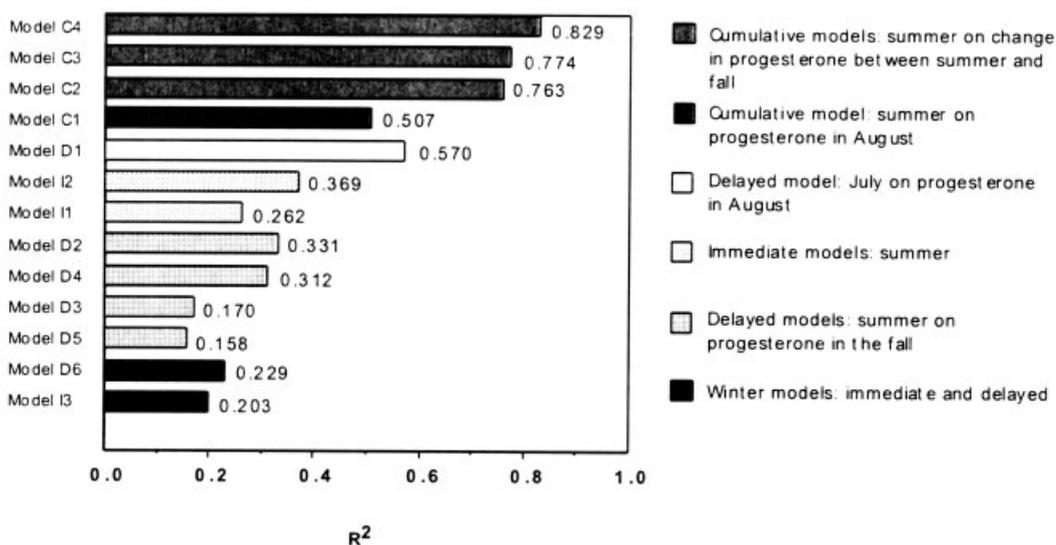


Fig. 4. Fractions of variation in indices of ovarian function explained by different types of path analyses models. Description of model types is presented in the text.

between energy expenditure and energy intake. Clearly, women with higher levels of physical activity also had higher levels of energy consumption. The lack of influence of energy intake on ovarian function in this population does not, of course, contradict results from the studies reporting suppressive effects of low energy intake in dieting Western women or women from populations experiencing seasonal food shortages (Lager and Ellison, 1990; Panter-Brick et al., 1993; Pirke et al., 1985; Schweiger et al., 1989).

Lack of significant effects of energy balance

Energy balance is a difficult variable to measure and the choice of anthropometric variables used to represent energy balance has often been criticized in the literature (Barr, 1987; Lunn, 1994). However, in this study tests of models using different variables as representations of energy balance indicate that the choice of variables did not influence the outcome of statistical analyses. Women in this study were characterized, on average, by rather high body weight, BMI, and body fat percentage (Table 2). Although they experienced on average small seasonal changes in these characteristics (Table 2), statistical analyses did not show any significant relationships between these changes and ovarian function. Not only did energy

balance not have any significant effect on ovarian function, but indirect effects of either TEE or TEI via energy balance also did not show significant relationships with ovarian progesterone levels.

It is worth emphasizing that, although both energy intake and energy expenditure of the subjects were estimated in this study, no attempt was made to use these values to construct an index of energy balance. None of the conclusions regarding energy balance is based on a comparison between estimated energy intake and estimated energy expenditure. The 24-hour recall survey is recommended in the literature as a reliable method for collecting nutritional and energy expenditure data in field conditions (Ulijaszek, 1992). Data gathered by this method are valuable for detecting seasonal and among-subject differences. This method, however, has limitations which invalidate attempts at comparing energy intake and energy expenditure. In addition, results of the studies which evaluated data obtained from 24-hour recalls with data from measurements of energy intake and expenditure (e.g., doubly labeled water, indirect and direct calorimetry) suggest that energy intake surveys tend to underestimate actual caloric intake, while energy expenditure surveys usually tend to overestimate actual energetic costs (Black et al., 1991, 1993; Borel et al., 1984; Martin et al., 1996; Pearson, 1990; Sawaya

et al., 1996; Ulijaszek, 1992; Webb, 1991). It should be noted that while the methods used for estimating energy expenditure are not very precise, the values for total daily energy expenditures obtained by this study are comparable with those reported for women from other subsistence populations (Alemu and Lindtjorn, 1995; Ategbo et al., 1995; Benefice et al., 1996; Bleiburg et al., 1981; Dufour, 1984; Edmundson and Edmundson, 1988; Katzmarzyk et al., 1994; Leonard et al., 1996; Norgan, 1996; Panter-Brick, 1992; Singh et al., 1989).

Lack of significant effects of age

In addition, results from causal models showed that age is not a significant factor affecting ovarian function in study subjects. This proves that the age range criterion used for the selection of subjects was suitable for this kind of study, but does not question earlier findings of the strong influence of age on ovarian function in premenopausal women described for several human populations (Lipson and Ellison, 1992).

How long-lasting are the effects of energy expenditure?

The energy expenditure in the summer showed a significant negative relationship with ovarian function in August (Fig. 3, Model C1) and September (Fig. 2, Model D4). No significant effect, however, was discovered of the summer TEE on progesterone levels in October (Fig. 2, model D5). Agricultural work, very intense in July and especially in August, relaxes during September and ceases by October. Ovarian function is still affected by high levels of summer physical activity in September, but does not show this response in October. The time-lagged response observed in September may be explained by the sequence of physiological events during the menstrual cycle. In this study, luteal phase progesterone was used as an index of ovarian function. Luteal progesterone production may be influenced by events occurring earlier, during the follicular phase of the cycle (DiZerega and Hodgen, 1981). It is during this phase that the dominant follicle develops and the future size of the corpus luteum may be determined (McNeely and Soules, 1988). No study has hitherto shown conclusive results about the influence of duration and timing of energetic stress on menstrual function. It is not known

for how long an energetic stress needs to persist in order to affect ovarian function. Total fasting lasting for 3 days did not suppress ovarian function (Olson et al., 1995). The hypothesis of a "graded continuum of ovarian response" (Ellison, 1990) proposes that ovarian function responds with an increasing degree of suppression as environmental stress continues in duration or becomes more severe. While mild stress may result in the production of an ovum of decreased fertilizability, a more severe stress may result in the total absence of ovulation. Therefore, while mild stresses may lower the probability of conception during a particular menstrual cycle, severe stresses may result in that probability declining to zero (Ellison, 1990).

Another question can be asked about the relative importance of the occurrence of energetic stresses in either follicular or luteal phases of the menstrual cycle. While the evidence shows that both follicular estradiol levels and luteal progesterone levels (Ellison, 1990) may be low due to environmental stresses, low progesterone production may simply be a consequence of follicular phase disturbances. It is less well understood whether energetic stresses which begin during the luteal phase of the cycle would also be able to negatively affect progesterone production.

In this study, while physical activity was the most intense in August, the demands of agricultural work require a high level of energy expenditure also in July. However, ovarian function in July was not affected by energy expenditure during that month (Table 4, Fig. 1, model I1). A few possibilities can be raised as potential explanations for the lack of a relationship between July TEE and progesterone levels in that month. First, levels of TEE in July may not be high enough to affect ovarian function. Second, physical activity in July may lack the appropriate accumulated duration for ovarian function to react. Third, ovarian function does not respond immediately to high energy expenditure, but with some considerable time lag, due possibly to the sequence of events occurring during the menstrual cycle. A time-lag response may also be understood as an adaptive phenomenon, suggesting that suppression of reproductive function occurs in response to the "real" signal about deteriorating conditions, as opposed to random environmental "noise." These hypotheses cannot be evaluated, however, with the data collected in this study.

Why energy expenditure has negative effects on ovarian function—two evolutionary hypotheses

This study of Polish rural women is the first to show that high energy expenditure associated with subsistence work can cause ovarian suppression in women, even in the absence of negative energy balance (Jasienska and Ellison, 1998). These results point to energy expenditure as an important energetic factor shaping adaptive responses of female reproductive function during the course of human evolution.

Two evolutionary hypotheses aimed at explaining why intense physical work may cause ovarian suppression in women who maintained energy balance have been proposed (Jasienska, 2001, 2003; Jasienska and Ellison, 1998). The "preemptive ovarian suppression" hypothesis assumes that intense workload commonly led in our ancestors to a state of negative energy balance. This scenario is based on the premise that when an individual was sustaining high levels of energy expenditure, a compensatory increase in energy intake was not likely to occur. Energy intake might have been limited by food availability and by physiological constraints to energy assimilation and production (e.g., metabolic ceilings to energy budgets [Peterson et al., 1990; Weiner, 1992]). Consequently, an increase in workload was a reliable predictor for human ancestors of an imminent energetic hardship (negative energy budget), and therefore functioned as a cue for the preemptive suppression of ovarian activity.

An alternative view (the "constrained downregulation" hypothesis) is based on the assumption that intense workload compromises women's ability to allocate sufficient energy to reproduction. Women, who as a result of an increase in workload remain in the state of high energy flux (high energy expenditure compensated by high energy intake), may have an impaired ability to downregulate their own metabolism when faced with the increasing energetic needs of pregnancy and lactation. It is well documented that lowering of the basal metabolism serves as one of the mechanisms allowing women from traditional subsistence populations to allocate more energy to reproduction (Poppitt et al., 1993). On the other hand, an increase in basal metabolism is a well-known phenomenon observed in individuals who experience increases in physical activity

(Sjodin et al., 1996). It is possible, then, that when hard-working women have elevated basal metabolism, their ability to manipulate it in order to redirect energy for reproductive processes is constrained. In this situation, a temporal suppression of ovarian function may be adaptive even in individuals who are still sustaining energy balance.

The results of this study may be relevant in terms of public health, especially for areas concerned with women's fertility, contraception, and reproductive cancers (Ellison, 1999; Jasienska and Thune, 2001a, b). Growing evidence shows that physical activity lowers the risk of breast cancer (Friedenreich and Rohan, 1995; Matthews et al., 2001; Thune et al., 1997; Wyshak and Frisch, 2000), possibly via reducing levels of ovarian steroid hormones (Bernstein, 2002; Jasienska et al., 2000; Key and Pike, 1988; Pike et al., 1993). Our results suggest that such activity does not need to lead to negative energy balance, and even in women of good nutritional status it may reduce circulating concentrations of ovarian hormones.

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