INTRODUCTION

Carbohydrates play a crucial role as energy source for some tissues which are obligatory glucose users, such as nervous tissue, and as energy source for working muscle during intensive exercise. Depletion and subsequent restoration of body carbohydrate stores is one of the key elements of the training process and daily activity. There are few data concerning complex metabolic and hormonal changes occurring in subjects submitted to depletion of carbohydrate stores followed by various dietary treatments (1, 2). In these studies investigators were focused mostly on the high carbohydrate meals and glycogen supercompensation phenomenon, that is enhanced synthesis of glycogen in the muscles increasing its content above normal level (3). This phenomenon is particularly important in sport physiology. Metabolic changes following low carbohydrate meal given after glycogen depleting exercise have not been thoroughly examined so far (2). There are also only few data on hormonal and metabolic responses to carbohydrate stores depletion and meals in subjects of different training status. Our previous study published in this Journal was focused on phenomena occurring during exercise and demonstrated that it can markedly affect those responses (4). It was found that low carbohydrate availability caused by physical exercise and fasting resulted in elevated concentration of catecholamines only in untrained subjects. Apparently, training induced adaptation is able to prevent sympathetic activation and subsequent changes in plasma catecholamines. It is worth to note, that ingestion of a meal either high or low in carbohydrates diminished changes of plasma catecholamines in untrained subjects.

Sympathetic activation is only one element of the counterregulatory response directed at prevention of carbohydrate exhaustion. The contribution to this mechanism of several hormones acting in concert was evidenced under “emergency“ condition such as insulin-induced hypoglycemia or long term physical exercise. Less is known on the metabolic and hormonal responses to the depletion of glycogen – and its restoration cycle occurring in training athletes and incidentally in sedentary subjects. Most experiments investigated influence of the high carbohydrate meals, few dealt with protein-fatty meals and the complex studies with both of them are exceptional. Hormonal responses to ingestion of different meals in people with decreased glycogen stores are not fully explored, and the knowledge how those responses depend on subjects’ fitness is extremely limited. In contrast to the previous study (4) focused on physiological responses during exercise, the present one was designed to investigate the metabolic and hormonal responses to carbohydrate depletion followed by high or low...
carbohydrate meal under resting condition in sedentary and endurance trained subjects. For this purpose respiratory gas exchange (VO₂ and VCO₂), blood glucose and free fatty acids (FFA) were determined together with hormones controlling metabolism, including insulin (I), leptin, growth hormone (GH), testosterone (T), norepinephrine (NE) and epinephrine (E).

MATERIAL AND METHODS

Nineteen healthy male subjects volunteered to participate in the study after giving their informed consent. They were divided into two groups according to the level of their physical activity. Sedentary group (group S) consisted of 9 students who did not participate in any sport activities (VO₂max 37.2±2.6 ml/kg/min) and active group (group A) of 10 nonprofessional athletes of endurance sport disciplines (VO₂max 58.8±2.5 ml/kg/min). The study protocol was approved by the Ethics Committee at the Medical Research Centre, Polish Academy of Sciences in Warsaw.

Study protocol

The study consisted of four experimental sessions performed in at least one-week intervals. During the first session (control - C) the subjects reported to the laboratory after an overnight fast. The venous catheter was inserted to the antecubital vein and after 30 min rest blood samples for catecholamines (epinephrine - E and norepinephrine - NE), human growth hormone (hGH), testosterone (T), free fatty acids (FFA), leptin, glucose, ACTH (adrenocorticothropic hormone), cortisol and insulin were taken. Then respiratory gas exchange was determined and the subjects performed graded, incremental cycle-ergometer exercise till volitional exhaustion for determination of their aerobic capacity. During exercise oxygen uptake and heart rate were registered continuously.

The following three sessions with carbohydrate stores modifications were performed in random order, at least one week apart. Each of these sessions consisted of two parts. In the first part, in order to deplete body glycogen stores, in the late afternoon subjects exercised on the cycloergometer for 90 minutes at constant intensity of 70% of maximal heart rate obtained in the control session. Such an exercise, as reported by Hultman and Nilsson (5) results in almost complete depletion of glycogen in exercising muscles and significant (around 50%) decrease in liver glycogen store. Till the next morning subjects did not consume any meal in order to further reduce liver glycogen (only pure water was allowed). The second part of the session took place the following morning, after 16-18 hours since the last meal. The subjects were given breakfast of low (L-CHO – 35% protein, 64% fat, 1% carbohydrate) or high (H-CHO – 4% protein, 1% fat, 95% carbohydrate) carbohydrate content or remained fasted (N-CHO). Both meals had a similar energy value of approximately 1000 kcal and are presented in Table 1 (6). After meal ingestion subjects rested for two hours, what is a generally accepted minimal interval to achieve a postprandial steady state. Respiratory gases analyses and blood sampling for metabolite and hormone determinations as in the control session were performed before and 2 hours after meal ingestion.

Methods

Catecholamines concentrations were determined using radioenzymatic test (Immunotech, Czech Republic). Human growth hormone, testosterone, cortisol and insulin were determined using radioimmunological tests (Polatom Swierk, Poland). Glucose concentration was measured with glucometer (Lifescan, USA). Free fatty acids were determined using spectrophotometric microenzymatic method of Shimizu (7). Radioimmunological commercial kits were used to determine concentrations of ACTH (CIS bio International, France) and leptin (Linco Research, USA). Oxygen uptake (VO₂), carbon dioxide production (VCO₂) and heart rate (HR) were measured using Vmax 29 analyzer (Sensormedics, USA).

Statistics

All data are presented as means±SE. Values’ normal distribution was examined with Kolmogorow-Smirnow test. Two values were compared appropriately with Student’s t test for dependent or undependent measures. For more complex analyses two-way ANOVA for repeated measures was used. Accepted level of significance was p<0.05. All analyses were performed with Statistica 6.0 software.

Table 1. Composition of the meals.

<table>
<thead>
<tr>
<th>Meal</th>
<th>Foodstuff</th>
<th>Quantity [g]</th>
<th>Energy value [kcal]</th>
</tr>
</thead>
<tbody>
<tr>
<td>High carbohydrate (H-CHO)</td>
<td>Glucose</td>
<td>100</td>
<td>401</td>
</tr>
<tr>
<td></td>
<td>Honey</td>
<td>75</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>2 rolls</td>
<td>200</td>
<td>450</td>
</tr>
<tr>
<td>Low carbohydrate (L-CHO)</td>
<td>Hard cheese</td>
<td>100</td>
<td>284</td>
</tr>
<tr>
<td></td>
<td>Processed cheese</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>Sausage</td>
<td>200</td>
<td>406</td>
</tr>
<tr>
<td></td>
<td>Mayonnaise</td>
<td>15</td>
<td>93</td>
</tr>
</tbody>
</table>

* p<0.05.
RESULTS

Metabolic and hormonal responses to depletion of body carbohydrate stores

Depletion of subjects’ body carbohydrate stores resulted in significant (p<0.05) decrease of respiratory exchange ratio in comparison with control session (Fig. 1). Blood glucose and FFA concentrations did not change in any group. Plasma concentration of leptin decreased significantly in both groups (p<0.05) and it was higher in group S than in A both before (p<0.01) and after (p<0.001) glycogen depletion (Fig. 2). Insulin concentration decreased significantly (p<0.001) only in group A and was lower then in group S (p<0.01) (Fig. 2). Lower body

Fig. 2 Hormonal responses to glycogen depletion in sedentary (S) and active (A) subjects; * p<0.05, *** p<0.001, crosses denote difference between groups S and A; ++ p<0.01, +++ p<0.001.
carbohydrate stores did not affect plasma concentrations of ACTH and cortisol (Fig. 2). Plasma norepinephrine concentration decreased only in group A (p<0.001), whereas epinephrine remained unchanged (Fig. 2). Concentrations of growth hormone and testosterone did not change after body carbohydrate stores depletion (Fig. 2).

Metabolic and hormonal responses to meal ingestion in glycogen depleted subjects

After the high carbohydrate meal in both groups respiratory ratio (RQ) and blood glucose significantly increased while the plasma concentration of FFA decreased (p<0.001). After L-CHO meal FFA increased only in group S (p<0.01).

Concentration of leptin did not change after the meals, but was higher in group S then in A (p<0.05). Insulin concentration increased after both kind of meals and was higher after H-CHO then after L-CHO (p<0.05). After L-CHO meal higher insulin concentration was found in group S then in group A (p<0.05). Concentration of growth hormone (Fig. 6) decreased after the meals in both groups (p<0.05). Ingestion of the meals resulted in decreased testosterone concentration in group A (H-CHO p<0.001; L-CHO p<0.01) and in group S only tendency (p=0.07) occurred after H-CHO meal (Fig. 6).

DISCUSSION

Metabolic and hormonal responses to depletion of body carbohydrate stores

Decreased respiratory quotient in both groups reflects the increased contribution of fatty energy substrates in covering energy demands after the depletion of body carbohydrate stores by 90 min of exercise at 70% of maximal heart rate followed by over 12 hours of fasting. Slight tendency to elevation of blood free fatty acid concentration indicates the balance in the processes of fatty acid mobilization and utilization. Glucose concentration remained unchanged likely due to increased liver gluconeogenesis and decreased glucose utilization by peripheral tissues. Thus, the carbohydrates are spared for central nervous system. Described above metabolic responses are controlled by changes in the secretion of several hormones and sensitivity of target tissues to these hormone action.

Decreased secretion of insulin is a key factor in the adjustment to starvation. It results in enhanced lipolysis, protein breakdown and gluconeogenesis and in attenuation of peripheral...
glucose uptake. The present data showed that concentration of insulin decreased only in active subjects and reached significantly lower values than in sedentary group. This difference may result from the fact that active subjects had greater exercise capacity and thus workloads resulting in 70% of maximal heart rate were approximately twice higher than in sedentary ones. Although relative workloads were the same, the total energy expenditure was higher in active group than in sedentary subjects and energy deficit was more profound. In addition, it is known that in the endurance trained subjects tissue sensitivity to insulin is enhanced, what results in increased tissue uptake of glucose. However, blood glucose level did not decrease after glycogen depletion. It can not be excluded, therefore, that in endurance athletes regularly depleting glycogen stores the glucostatic mechanism is more efficient.

Concentration of norepinephrine decreased after glycogen depletion also only in active subjects. Inhibition of sympathetic nervous system activity, reflected by drop of norepinephrine, was described in hunger (8, 9). Decreased sympathetic activity results in lower resting metabolic rate which is important under condition of energy deficit. Moreover, catecholamines increase sensitivity of skeletal muscle to insulin that promotes muscle glucose uptake (10). On the other hand, sympathetic nerves stimulate lipolysis in adipose tissue, so decrease in the sympathetic activity may be not beneficial. However, it must be considered, that blood norepinephrine concentration correlates mostly with activity of sympathetic fibers innervating skeletal muscles, mainly blood vessels spread out all over the muscle cells (11). In other tissues, including adipose tissue, sympathetic activity could be unaffected or even elevated. Such an interrelationship of hormonal regulation of glucostatic mechanism seems to be better developed in physically active people. Epinephrine concentration did not change in any group, indicating that total energy deficit induced by applied glycogen depletion protocol was not sufficient to change epinephrine synthesis by the adrenal medulla (9).

Among several hormones involved in maintenance of body energy balance is leptin (12). The main source of leptin is adipose tissue, but it was also found in different organs like skeletal muscles, brain (13) and in stomach (14). Concentration of leptin is partly dependent on insulin which stimulates expression of the ob gene (15), but it is also influenced by cortisol, catecholamines and growth hormone. Growth hormone and cortisol enhance leptin synthesis (16, 17), whereas catecholamines cause an inhibition in the hormone release (18). After glycogen depletion in both groups the plasma concentration of leptin decreased. Majority of papers concerning influence of exercise exceeding one hour on leptin concentration report no changes (19, 20) or decrease (21, 22). Measurements performed 24 and 48 hours after exercise (23, 24) showed decrease only after 48 hours. Van Aggel-Leijssen et al. (25) followed plasma leptin for 24 h and concluded, that in energy balanced state leptin is lower if exercise was performed. Positive energy balance resulted in elevated leptin even after exercise.
Those observations were confirmed by Hilton and Loucks (26) and indicate, that leptin decreases at certain threshold of negative energy balance, which could be induced either by insufficient feeding or exhaustive exercise. Compensation of energy balance does not return leptin to normal values within 24 hours (27). Decreased leptin observed in both groups in present study reflects negative energy balance caused by 90 min exercise and 12-16 hrs of fasting. This change appeared to be independent on the initial leptin concentration. Subjects of the active group had significantly lower BMI and corresponding leptin than sedentary ones throughout the whole study. It is consistent with other studies stating decreased leptin in endurance training accompanied with decline in a content of fat tissue (28, 29).

Depletion of carbohydrate stores did not influence concentrations of ACTH and cortisol, which is in agreement with previous findings (30). There were no differences between groups S and A in concentrations of growth hormone and testosterone. Therefore, we failed to confirm the findings showing an increase in plasma testosterone and decrease of growth hormone concentration with endurance training (31). The difference could result from larger testosterone receptors content in muscles of endurance trained athletes with several years experience as in our study, in contrast to subjects submitted occasionally to 5 weeks of training (32). Thus, the physiological effect is achieved without the necessity to secrete high concentrations of acting hormones. Neither plasma levels of testosterone nor growth hormone changed significantly after glycogen depletion. Growth hormone concentration increases with decreasing glucose and constitutes hormonal response to starvation, especially in its initial stage. In present study average concentrations of growth hormone increased threefold in sedentary subjects and almost twofold in active group, without hitting the level of significance due to huge intersubject variations and relatively small experimental groups.

Metabolic and hormonal responses to meal ingestion in glycogen depleted subjects

Ingestion of high carbohydrate meal increased glucose concentration in blood and contribution of carbohydrate substrates to covering energy demand; as it is indicated by elevated respiratory quotient. Simultaneously concentration of free fatty acids decreased. Similar responses were described by Okano et al. (33) and Kirwan et al. (34) who administered meals of medium and high glycemic indexes. Low carbohydrate meal did not influence blood glucose concentration. It elevated concentration of free fatty acids in blood but only in sedentary subjects. According to previous publications the same response should be expected in trained individuals (35). However, it must be emphasized that in present study time interval between the meal and blood sampling was relatively short – 2 hours. During that time income of chylomicrones, source of release of free fatty acids, does not reach maximal value. In papers mentioned above concentrations of free fatty acids were measured in 3.5 and 4 hours interval. In physically active subjects the lack of increase of free fatty acids after low carbohydrate meal probably results from enhanced and more efficient tissue uptake, which is due to endurance training.

Increased insulin concentration observed after both meals and considerably higher after carbohydrates is commonly known and described. Lower fasting and post low carbohydrate meal insulin observed in active group reflects higher tissue sensitivity to insulin present in endurance trained individuals (36, 37). In this way sufficient metabolic effects of insulin occurs at its lower concentration.

Concentration of leptin was not changed by any meal. It could be due to short interval of blood sampling after the meal – 2 hours. Described postprandial elevations of leptin were observed 4 to 24 hours after the meal (38). Moreover, 1000 kcal energy value of the meals did not produce positive energy balance, it only covered the deficit obtained during glycogen depletion protocol. In energy balanced conditions no change in leptin concentration was described (39), only overfeeding leads to its elevation regardless of carbohydrate to fat ratio of the meal (40).

Norepinephrine concentration increased after both meals in sedentary group, in trained subjects it raised only after high carbohydrate meal and reached higher values. Increased activity of sympathetic nervous system in response to glucose administration (41, 42) or ingestion of a meal constitutes a physiological mechanism preventing the fall in blood pressure due to enhanced visceral flow. It also allows to dissipate some energy, what is important in case of rapid or excessive feeding. Among the factors directly stimulating postprandial sympathetic activity partial role is played by baroreceptor reflex, released by vasodilatation in visceral circulation. The key role, however, is played by insulin acting directly on central nervous system (43). Higher concentration of norepinephrine in physically active subjects observed after high carbohydrate meal could result from the greater insulin sensitivity of neurons in endurance trained group (44).

Resting epinephrine concentration two hours after the meals did not vary from the fasting values. The only described elevation of epinephrine after glucose administration occurred between 3 and 4 hours of the test (44). In recent work (45) the rapid postprandial drop of epinephrine was described with consecutive slow restoration to the baseline values.

Results obtained in the present study did not confirm previously described (46) postprandial increase in ACTH and cortisol. However, it was observed one hour after a meal, so another hour later values could have approached the baseline again. Concentration of growth hormone decreased after meals in both groups. Growth hormone secretion is inhibited by glucose and stimulated by amino acids. Nevertheless, in some papers the increase in growth hormone concentration after ingestion of high protein meal was not confirmed (47). Results obtained in the present study are consistent with findings reporting decrease of growth hormone after high carbohydrate meals. However, after the low carbohydrate meal, including 35% of protein, the opposing effect to the previously described elevation was found. It could result from the glycogen depletion procedure applied during the study protocol and before the meals.

Testosterone concentration decreased in physically active group after both meals, whereas in sedentary subjects it only tended to decrease after high carbohydrate meal. Mechanism of this process is most probably linked with increased hepatic clearance of testosterone. After the meal splanchnic blood flow is significantly enhanced and testosterone is degraded in hepatocytes. With constant secretion it results in decreased testosterone concentration in blood. Such changes were previously described after high fat meals (48, 49) and data obtained in the present study shows, that similar effect occurs after the high carbohydrate meal, too. More pronounced changes in trained subjects could result from the greater redistribution of circulating blood to the splanchnic region than in sedentary ones.

Summarizing, hormonal and metabolic changes observed after depleting carbohydrate stores resemble those occurring during starvation. Composition of the ingested meal affects postprandial metabolism, which is additionally dependent on the training status of the subjects.

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