Physical exercise and normobaric hypoxia: independent modulators of peripheral cholecystokinin metabolism in man

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Bailey, Damian M., Bruce Davies, Linda M. Castell, Eric A. Newsholme, and John Calam. Physical exercise and normobaric hypoxia: independent modulators of peripheral cholecystokinin metabolism in man. J Appl Physiol 90: 105–113, 2001.—The purpose of the present investigation was to determine the independent effects of hypoxia and physical exercise on peripheral cholecystokinin (CCK) metabolism in humans. Thirty-two physically active men were randomly assigned in a double-blind manner to either a normoxic (N; n = 14) or hypoxic (H; n = 18) group. During the acute study, subjects in the H group only participated in two tests, separated by 48 h, which involved a cycling test to exhaustion in normobaric normoxia and normobaric hypoxia (inspired O2 fraction = 0.21 and 0.16, respectively). In the intermittent study, N and H groups cycle-trained for 4 wk at the same relative exercise intensity in both normoxia and hypoxia. Acute normoxic exercise consistently raised plasma CCK during both studies by 290–723%, which correlated with increases in the plasma ratio of free tryptophan to branched chain amino acids (r = 0.58–0.71, P < 0.05). In contrast, acute hypoxic exercise decreased CCK by 7.0 ± 5.5 pmol/l, which correlated with the decrease in arterial oxygen saturation (r = 0.56, P < 0.05). In the intermittent study, plasma CCK response at rest and after normoxic exercise was not altered after physical training, despite a slight decrease in adiposity. We conclude that peripheral CCK metabolism 1) is more sensitive to acute changes than chronic changes in energy expenditure and 2) is potentially associated with acute changes in tissue Po2 and metabolic precursors of cerebral serotoninergic activity.

5-hydroxytryptamine; satiety; caloric intake; adipose tissue; aerobic capacity

Environmental hypoxia exerts potent anorectic effects and thus contributes to the hypophagia and cachexia frequently experienced during prolonged exposure to high altitude (2). Changes in long- and short-term signaling molecules that regulate food intake and energy homeostasis suggest a metabolic basis for these phenomena. Serum leptin has previously been shown to increase at 4,559 m (30), and, in a separate study, our laboratory recently demonstrated a marked increase in the plasma concentration of the satiety neuropeptide cholecystokinin (CCK) at 5,100 m, which was associated with a progressive and selective loss of torso adipose tissue (5). Furthermore, subjects with acute mountain sickness (AMS) presented markedly elevated resting plasma concentration ratios of free tryptophan to branched chain amino acids (BCAA) compared with apparently healthy controls without AMS (4). This may theoretically increase the formation of 5-hydroxytryptamine (5-HT) in the brain (6). The fact that resting plasma CCK was also elevated in these subjects may suggest a possible association between serotoninergic activity and peripheral CCK release.

Isolating the stimuli and potential mechanisms responsible for these metabolic changes has proved elusive because of the logistical limitations imposed by mountaineering expeditions, particularly the lack of normoxically exposed control groups. To our knowledge, the independent effects of acute/chronic changes in energy expenditure and whether peripheral CCK metabolism is altered by more modest reductions in ambient Po2, both important issues, have not been investigated.

Therefore, the present investigation was designed to systematically examine 1) the independent effects of acute, intermittent physical exercise and moderate normobaric hypoxia and 2) the potential association between selected amino acids that are known to influence serotoninergic activity and CCK turnover in a cohort of physically active men.

We hypothesized that, if an association exists between cerebral serotoninergic activity and the physiological regulation of peripheral CCK secretion, then the plasma CCK response would be sensitive to changes in the circulating venous concentration of nonesterified fatty acids (NEFA), free tryptophan, and...
BCAA. An increase in NEFA would be expected, via competitive inhibition, to decrease the proportion of tryptophan bound to albumin and thus increase the free concentration (13). A decrease in the plasma concentration of BCAA due to an increased oxidation rate would further increase the plasma concentration ratio of free tryptophan to BCAA. Because BCAA compete with tryptophan and other aromatic acids for transport across the blood-brain barrier on the same large neutral amino acid carrier, an increase in this ratio would favor the entry of free tryptophan into the brain, in which it is converted to 5-HT (6). We therefore anticipated a synergistic effect for acute hypoxia and physical exercise on CCK release due to a more pronounced mobilization of NEFA from adipose tissue and intramuscular stores, secondary to increased \( \beta_1 \) or \( \beta_2 \)-adrenoreceptor activation. Endurance training is associated with an overall reduction in plasma NEFA (20) and an increase in intramuscular glycolgen. The latter would effectively decrease the required amount of amino acid oxidation to maintain tricarboxylic acid intermediates, thus increasing plasma BCAA. In light of these findings, we also predicted a decrease in the plasma CCK response after intermittent hypoxic training, subsequent to a decrease in serotoninergic activity.

**METHODS**

**Subjects**

Subjects were excluded from the study if they had any history of digestive system disease, gastrointestinal distress, or evidence of any viral/bacterial infection or were smokers. Other exclusion criteria included prescribed antidepressants or any medication known to affect gastric motility or any other aspect of digestive function. After a detailed medical examination, written, informed consent was obtained from 32 physically active men who were university students [aged 22 ± 3 (SD) yr, body mass index (BMI) = 23.6 ± 1.6 kg/m\(^2\), and maximal aerobic capacity (\( \dot{V}O_2 \max \)) = 50 ± 9 ml·kg\(^{-1}\)·min\(^{-1}\)]. Ethical permission for the study was obtained from the Bro Taff Health Authority.

**Experimental Design**

Subjects were randomly assigned, in a double-blind manner, to either a normoxic \((n = 14)\) or a hypoxic \((n = 18)\) group that was matched for a variety of physical characteristics (Table 1). An overview of the experimental design is presented in Fig. 1.

**Table 1. Physical characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Normoxic Group</th>
<th>Hypoxic Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Age, yr</td>
<td>22 ± 1</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>23.9 ± 1.3</td>
<td>23.4 ± 1.8</td>
</tr>
<tr>
<td>Sum of skinfolds, mm</td>
<td>30.1 ± 7.3</td>
<td>30.9 ± 7.3</td>
</tr>
<tr>
<td>FVC, l/min</td>
<td>5.67 ± 1.08</td>
<td>5.63 ± 0.92</td>
</tr>
<tr>
<td>MVV, l/min</td>
<td>172 ± 45</td>
<td>181 ± 34</td>
</tr>
<tr>
<td>( \dot{V}O_2 \max ), l/min</td>
<td>3.97 ± 0.73</td>
<td>3.49 ± 0.47</td>
</tr>
</tbody>
</table>

Values are means ± SD. FVC, forced vital capacity (BTPS); MVV, maximum voluntary ventilation (BTPS); \( \dot{V}O_2 \max \), maximum oxygen uptake (STPD). No differences between group means were evident.

**Acute study.** The hypoxic group only participated in the acute study, which was designed to investigate the effects of acute normobaric hypoxia on the plasma CCK response at rest and during maximal exercise. Subjects were randomly assigned to perform a normobaric normoxic (inspired \( O_2 \) fraction (\( F_{I,O_2} \)) = 0.21) and normobaric hypoxic (\( F_{I,O_2} \) = 0.16) test. Each test involved 30 min of seated rest (passive state) that was preceded by a standardized cycling test to volitional exhaustion (active state). The tests were separated by 48 h to ensure full recovery, as previously established in our laboratory's pilot studies (DM Bailey, unpublished observations). The respective inspirates were regulated by continuously directing medical grade compressed gas mixtures (either 20.9% \( O_2 \)-balanced \( N_2 \) or 16.0% \( O_2 \)-balanced \( N_2 \); British Oxygen, Salford, England, UK) into a series of 1,000-liter plastic bags at the prevailing barometric pressure, which was determined using a mercury Fortin barometer (Cranleigh, Salford, UK). The bag system was connected to a four-way valve and a 2-m length of Falconia tubing (3.18 cm ID) attached to the inspiratory port of a two-way, non-rebreathing valve (2400 series, Hans Rudolph).

**Intermittent study.** The intermittent study was designed to investigate the effects of 4 wk of intermittent hypoxic training on resting and exercise plasma CCK responses. Normoxic and hypoxic groups performed an exercise test in normobaric normoxia before (Pre) and after (Post) a supervised physical training program. Pre testing for the hypoxic group was established during the acute study, as previously described. Both groups exercised for the same duration, at the same relative exercise intensity, in either normoxia or hypoxia. The inspired partial pressure of oxygen (\( P_{O_2} \)) in hypoxia ranged between 111 and 118 Torr and between 147 and 152 Torr in normoxia. The hypoxic stimulus applied in the present study was substantially higher than that encountered in our laboratory's previous study (5), which was conducted at 5,100 m (\( P_{O_2} \) ~ 76 Torr).

**Physical training program.** A detailed description of the physical training program was previously published (3). Briefly, all subjects performed cycling exercise at precisely the same time of day every Monday, Wednesday, and Friday for a 4-wk training period. Each session was supervised by a physiologist, who had a 20-min duration for weeks 1–2, and was increased to 30 min for weeks 3–4 at 70, 75, 80, and 85% of the maximal heart rate (HR\(_\max\)), which was determined during either the normoxic or hypoxic Pre test (see Fig. 1). Pedalling frequency was maintained at 70 rpm, and the work load was adjusted continuously to achieve the desired HR. Desired HR was determined via an electrocardiographically calibrated, short-range telemetry system (Polar Vantage NV). Normoxic and hypoxic gas mixtures were presented double-blind to subjects in the normoxic and hypoxic groups, respectively.

**Dietary analysis and control.** A self-reporting dietary analysis (NutriCheck, Health Options) was completed days before the start and end of the study. Analysis of caloric intake and composition revealed no between-group differences, and thus subjects were subsequently advised to maintain their normal dietary habits for the duration of the experimental period. Behavioral compliance was established by conducting daily interviews with each subject, a physiologist, and a qualified nutritionist.

**Resting Measurements**

**Anthropometry.** After a 12-h overnight fast and after voiding and defecation, nude body mass and stature were determined using a balanced weighing scales and stadiometer.
Body fat percentage was calculated using the Durnin and Womersley equation (17) and the measurement of the sum of four skinfolds taken from the medial aspect of the biceps, triceps, subscapular, and suprailiac sites using a calibrated Harpenden skinfold caliper (British Indicators). A flexible metallic tape measure (Holtain, Crymych, UK) was used to assess waist (taken at the mid-umbilicus) and hip circumference (widest point).

Hematology. Subjects were instructed to refrain from physical exercise and alcohol consumption 48 h before arrival at the laboratory after a 12-h overnight fast. A venous sample was obtained at the same time of day for each subject after 30 min of supine rest to control for plasma volume shifts. An exercise venous sample was obtained immediately after a cycling test to volitional exhaustion and was subsequently corrected for plasma volume shifts (16).

NEFA and glycerol. Enzymatic assays were used to analyze plasma concentrations of NEFA (Behring Diagnostics, La Jolla, CA) and glycerol (Wako Chemicals, Neuss, Germany). The intra- and interassay coefficients of variation (CV) were (respectively) 1.6 and 5% for NEFA and 2 and 5% for glycerol.

Whole blood lactate. An arterialized capillary blood sample was obtained from a hyperaemic earlobe and analyzed for whole blood lactate concentration ([La]b) using an automated analyzer (Champion PLM-5, Analox). The intra- and interassay CVs were 1% and <5%, respectively.

Glucose. Plasma glucose was analyzed via the glucose/HK method (Boehringer Mannheim). The intra- and interassay CVs were 2 and 5%, respectively.

Free tryptophan. Plasma free tryptophan was separated from albumin-bound tryptophan by using the ultrafiltration method according to Bloxam et al. (8). The plasma concentration of tryptophan in the ultrafiltrate (free tryptophan) was subsequently measured by the fluorimetric method of Denckla and Dewey (15), as modified by Bloxam and Warren (9). The intra- and interassay CVs were <5%.

BCAA. The plasma concentration of BCAA was measured by using the enzymatic method described by Livesey and Lund (23) with modifications. The intra- and interassay CVs were 6.2 and 5%, respectively.

CCK radioimmunoassay. CCK was measured by a specific radioimmunoassay, as previously described (5). The intra- and interassay CVs were 6.2 and 12.1%, respectively.

Physical Exercise Test

After a 2-wk habituation period, each subject performed a continuous incremental cycling test (Monark 824E Ergomedic) to volitional exhaustion, as previously described (3). Each subject started unloaded cycling exercise at 70 rpm for the first 4 min and power output was increased by 50 W every 4 min for the first 5 stages and thereafter by 25 W every minute until volitional exhaustion. Each subject was in-
structed to signal clearly to the investigators when they considered they could continue at the specified power output for no longer than 60 s. Pilot study data prove this is an accurate method for the determination of Vo2 max using established physiological criteria. Rating of perceived exertion (RPE), arterial hemoglobin oxygen saturation (SaO2), and HR were measured continuously. SaO2 was determined by using an earlobe pulse oximeter (model 8800, Nonin) and validated in hypoxia, with a reported accuracy of ±1%. [La−]b was assayed from arterialized capillary blood that was taken from a hyperemic earlobe during the last 30 s of each 4-min stage and 10 s before completion of the test. Expiratory gases were collected during the last 60 s of the 4-min stages and during the last 60 s of the test and were analyzed using a semi-automated Douglas Bag system. Gas fractions were assessed from arterialized capillary blood that was taken from a hyperemic earlobe during the last 30 s of each 4-min stage and 10 s before completion of the test. Expiratory gases were collected during the last 60 s of the 4-min stages and during the last 60 s of the test and were analyzed using a semi-automated Douglas Bag system. Gas fractions were measured using paramagnetic O2 and infrared CO2 analyzers (1400 series Analyzer, Servomex, Crowborough, UK), which were calibrated with precision-analyzed quality gas mixtures containing pure nitrogen and 17% O2-5% CO2. The volume of expired gas was measured using a dry gas meter (Harvard, Crowborough, UK), which was calibrated with a 600-liter Tissot spirometer (Collins).

Statistics

Repeated Kolmogorov-Smirnov and Shapiro-Wilk W tests confirmed that all dependent variables were normally distributed. Between-group comparisons of baseline physical characteristics (Table 1) were compared using an independent samples t-test. Physiological responses to acute normobaric hypoxia (Table 2) were assessed using a paired samples t-test. Anthropometric and dietary data (Table 3) were analyzed using a two-way split plot [A × B] mixed ANOVA that incorporated one between-subjects (group: normoxic vs. hypoxic) and one within-subjects (time: Pre vs. Post) factor. Acute responses (Fig. 2.) were analyzed using a two-factor repeated measures ANOVA(state: rest vs. exercise × condition: normoxic vs. hypoxic). With the exception of the plasma ratio of free tryptophan to BCAA, intermittent training responses (Table 4, Fig. 3) were analyzed using a three-way [A × (B × C)] mixed ANOVA with one between-subjects factor (group) and two within-subjects factors (state and time). After simple main effects and interaction effects, Bonferroni-corrected, paired-sample t-tests were applied to make a posteriori comparisons at each level of the between-subjects factor. Between-subjects differences were analyzed using an one-way ANOVA with an a posteriori Tukey honestly significant difference test at selected levels of the within-subjects factors. Comparisons of the plasma concentration ratios of free tryptophan to BCAA were assessed using nonparametric statistics. Within-subjects effects were analyzed using a Friedman test and Bonferroni-corrected Wilcoxon matched pairs signed-rank tests. Between-subjects effects were analyzed using a Kruskal-Wallis test and Bonferroni-corrected Mann-Whitney U tests. We used a Pearson product moment correlation to analyze the relationship between selected dependent variables (Fig. 4). Significance for all two-tailed tests was established at an alpha level of P ≤ 0.05, and data are expressed as means ± SD.

RESULTS

Acute Responses

Arterial desaturation was more marked during maximal exercise in normobaric hypoxia, resulting in a 10 ± 4% decrease in absolute VO2 (Table 2). Figure 2 illustrates the marked increase in plasma CCK relative to the resting value after normoxic exercise (from 10.7 ± 4.5 to 50.6 ± 34.7 pmol/l, P < 0.05), whereas a decrease was observed after hypoxic exercise (from 9.9 ± 4.8 to 3.7 ± 2.5 pmol/l, P < 0.05). The suppressive effects of hypoxia were clearly not evident during passive exposure. The decrease in CCK during hypoxic

| Table 2. Effects of acute normobaric hypoxia on physiological responses to maximal exercise |
|--------------------------------------|------------------|------------------|
|                                    | Normoxia (F02 = 0.21) | Hypoxia (F02 = 0.16) |
| SaO2, %                             | 95 ± 2            | 85 ± 6*          |
| VO2, l/min                          | 3.50 ± 0.49       | 3.15 ± 0.65*     |
| [La−]b, mmol/l                     | 8.2 ± 1.4         | 8.6 ± 1.5        |
| Power output, W                     | 321 ± 42          | 291 ± 47*        |
| Time to exhaustion, s              | 1,169 ± 101       | 1,123 ± 114*     |

Values are means ± SD; n = 18 subjects in each condition. F02, inspired O2 fraction; SaO2, arterial hemoglobin O2 saturation; VO2, absolute O2 uptake; [La−]b, corrected whole blood lactate. Time to exhaustion excludes warm-up time. *P < 0.05 vs. normoxia.
Exercise (exercise minus rest value) was correlated with the decrease in \( \text{S}_\text{a} \text{O}_2 \) \((r = 0.56, P < 0.05)\). The increase in CCK during the normoxic test was related to absolute \( \text{V}_\text{O}_2 \text{max} \) \((r = 0.56, P < 0.05)\).

**Intermittent Training Responses**

**Physical training.** All subjects successfully completed the training program, and only 5 out of 384 individual sessions were missed due to injury. There were no between-group differences for weeks 1–4 for power output, rate pressure product, or RPE, whereas \( \text{S}_\text{a} \text{O}_2 \) was lower and \([\text{La}^-]_w\) response was higher during hypoxic training. Thus both relative and absolute training intensities were comparable between groups.

**Plasma CCK response.** Four weeks of intermittent normoxic or hypoxic training did not affect the resting or exercise plasma CCK response in normoxia (Fig. 3).

**Caloric intake, dietary composition, and anthropometric characteristics.** Total caloric intake and dietary composition did not change during the experimental period and was not different between groups (Table 3). Physical training decreased the sum of skinfolds, fat mass, and waist-to-hip ratio, but no changes were observed for body mass and fat-free mass. However, these variables did not differ between groups either before or after training.

**\( \text{V}_\text{O}_2 \text{max} \).** Intermittent hypoxic training increased \( \text{V}_\text{O}_2 \text{max} \) from 3.49 ± 0.47 to 3.96 ± 0.60 l/min \((P < 0.05\) vs. Pre), whereas no changes were observed after a comparable program of normoxic exercise. Changes in \( \text{V}_\text{O}_2 \text{max} \) (Post − Pre) were not related to changes in resting or exercise CCK in either group.

**Metabolic Responses**

**NEFA.** Acute exercise increased the plasma concentration of NEFA during both Pre and Post tests (Table 4). Physical training had no effect on either the resting or exercise NEFA response.

**Glycerol.** Acute exercise increased the plasma concentration of glycerol during both Pre and Post tests (Table 4). Physical training resulted in a greater increase in glycerol during acute exercise (pooled normoxic + hypoxic group values, \(P < 0.05\) vs. Pre), but no effects were observed at rest. Maximal exercise resulted in a greater increase in glycerol after hypoxic training.
Decrease in exercise $\text{[La}^-\text{]}_B$ and glucose. The increase in exercise $\text{[La}^-\text{]}_B$ was more marked in the normoxic group during the Pre test (Table 4). Both normoxic and hypoxic training decreased resting $\text{[La}^-\text{]}_B$; however, no effects were observed for maximal exercise. Plasma glucose decreased after training and was greater in the normoxic group throughout the study (Table 4). However, there were no between-group differences as a function of either time or state.

Free tryptophan and BCAA. Maximal exercise increased the plasma concentration ratio of free tryptophan to BCAA due to an increase in plasma free tryptophan and a decrease in BCAA (Table 4). The increase in free tryptophan (pooled maximal exercise – rest values) correlated with the increase in NEFA ($r = 0.61$, $P < 0.05$). Pooled values for free tryptophan and plasma concentration ratio of free tryptophan to BCAA (rest + maximal exercise) increased in the hypoxic group only during the Post test ($P < 0.05$ vs. Pre). Physical training had no effect on the plasma BCAA. The increase in the plasma concentration ratio of free tryptophan to BCAA correlated with the increase in CCK ($r = 0.58–0.71$, $P < 0.05$) during all acute normoxic exercise tests (Fig. 4).

DISCUSSION

The present study was undertaken to examine the independent effects of physical exercise and moderate normobaric hypoxia on peripheral CCK metabolism in humans. The potential relationship between plasma CCK and changes in selected amino acids that could potentially affect cerebral serotoninergic activity was also investigated.

Our results highlight three important findings. First, relative to resting conditions, plasma CCK increased markedly after acute normoxic exercise, whereas a decrease was observed during acute hypoxic exercise. Second, the plasma CCK response appeared to be more sensitive to acute, rather than chronic, changes in energy expenditure; 4 wk of intermittent normoxic/hypoxic training did not alter the CCK response at rest or immediately after normoxic exercise. Third, we have described potential relationships between peripheral CCK secretion and acute changes in tissue $\text{PO}_2$ and metabolic precursors of cerebral serotoninergic activity.

Physical Exercise and Hypoxia

Acute exercise and hypoxia. In support of our previous observations (5), acute normoxic exercise was a potent CCK releaser, a response that was independent of either local or systemic changes in adiposity and was therefore related to changes in the metabolic milieu invoked by physical exercise per se. In marked contrast to our original hypothesis, acute hypoxic exercise resulted in a decrease in plasma CCK. Assuming that the physical exercise component of the acute hypoxic test induced an increase in CCK similar to that observed in normoxia ($+473\%$ relative to resting control), the inhibitory effect of hypoxia on CCK release ($−536\%$) appeared more marked. Although it is possible that differences in the exercise load between tests could have accounted for the differential effects on CCK metabolism, the lack of correlation between absolute CCK values and exercise duration or maximal power output tends to challenge this contention.

A decrease in $\text{SaO}_2$ was associated with the acute hypoxic depression of CCK, and this more marked arterial desaturation observed during physical exercise might explain why CCK homeostasis was affected by active and not by passive exposure. Although this observation does not unequivocally establish cause and effect, it raises the attractive possibility that periph-

![Fig. 3. Changes in plasma CCK at rest and during maximal exercise in normobaric normoxia after intermittent normobaric hypoxic ($\text{FIO}_2 = 0.16$) training. Main effects are shown for state (rest vs. exercise, $P < 0.05$) and time (pre- vs. posttraining) × state interaction ($P < 0.05$); $n = 14$ for the normoxic group; $n = 18$ for the hypoxic group.](http://jap.physiology.org/)

![Fig. 4. Change in plasma CCK relative to the plasma ratio of free tryptophan to branched chain amino acids (BCAA) during acute normoxic exercise before (Pre) and after (Post) 4 wk of intermittent normoxic/hypoxic training. Significance values are as follows: $r = 0.67$, $P < 0.05$, Pre hypoxically trained group; $r = 0.58$, $P < 0.05$, Pre normoxically trained group; $r = 0.60$, $P < 0.05$, Post hypoxically trained group; $r = 0.71$, $P < 0.05$, Post normoxically trained group.](http://jap.physiology.org/)
eral CCK secretion is regulated by changes in tissue Po2 and that a decrease in satiety and a subsequent increase in caloric intake may prove to be an adaptive mechanism that serves to counter the generally catabolic effects of hypoxia. The inhibitory effect of hypoxia on the normal exercise-induced increase in CCK has also been observed during chronic exposure to a substantially lower Po2 (5) than that found in the present study (86 Torr vs 5,100 m vs 111–118 Torr at ~2,000 m). The changes in plasma CCK are considered physiologically significant and in excess of those previously associated with changes in satiety, hunger, and caloric intake (22).

Intermittent exercise and hypoxia. A previous study demonstrated that the resting serum concentration of CCK was not different between long-distance women runners and age-weight matched sedentary controls (19), suggesting that baseline CCK is relatively resistant to chronic changes in energy expenditure. Our data tend to support these findings, as 4 wk of physical training did not effect the plasma CCK response either at rest or during maximal exercise, which may have implications for individuals attempting to lose body mass. However, the earlier study identified an attenuation of the CCK and insulin response to a standardized meal, and subjective ratings of hunger were elevated in the athletes (19), which may reflect an adaptation to physical training.

A selective increase in absolute Vo2 max was observed after intermittent hypoxic training only, which was the subject of a recent study (3). This increase was independent of changes in resting hemoglobin concentration and was possibly associated with other unquantified central and/or peripheral adaptations in the ultrastructural and biochemical properties of skeletal muscle.

Caloric intake did not change, despite a moderate reduction in adiposity, which, according to the most recent theory of energy homeostasis (26), would be expected to activate the neuronal activity of anabolic effector pathways that initiate metabolic responses to increase caloric intake. Assuming that an energy expenditure of 14.6 MJ is required to metabolize 0.45 kg of adipose tissue (1), the generalized decrease in fat mass after training (~1.3 ± 1.0 kg) would equate to a cumulative energy deficit of ~42 MJ. Whether perturbations in peripheral CCK metabolism contributed to this relative hypophagia remains to be established, and future studies need to more directly examine changes in CCK dynamics and acute satiety/hunger ratings in response to a test meal. It is also important to emphasize that changes in long-term adiposity signals such as leptin and/or insulin can effectively modulate the satiogenic sensitivity of CCK; satiety induced by intraperitoneal CCK administration has been shown to increase with intracerebroventricular infusion of insulin (18) or a systemic injection of leptin (24). Therefore, the clinical implications of changes in absolute concentrations of plasma CCK need to be considered with tentative caution in the absence of other long-term signaling molecules.

Cerebral Serotonergic Activity

Our findings demonstrate a potential association between peripheral CCK and 5-HT during acute normoxic exercise in humans. Recent neuropharmacological studies have also demonstrated an intimate relationship between 5-HT, CCK, and nutritional intake. In one study, the inhibitory effect of CCK on food intake was antagonized by 5-HT blockers (28), most likely mediated by 5-HT2C receptor inactivation (25). In a separate study, the effect of an antiobesity serotonergic agonist (dex-fenfluramine) was blocked by the CCK-A receptor antagonist devazepide (12).

Acute normoxic exercise altered the venous concentration of key amino acids previously shown to alter 5-HT synthesis in the animal brain (7). A slight decrease in the plasma concentration of BCAs was observed and was presumably due to an increase in the rate of oxidation by skeletal muscle. The increase in free tryptophan was associated with an increase in NEFA, which, via competitive inhibition, decreases the binding affinity of tryptophan to albumin, thus increasing the free (i.e., non-albumin-bound) concentration (12). Animal studies have demonstrated that an exercise-induced increase in the plasma ratio of free tryptophan to BCAs (+36 vs. +40% in the present study) accelerates tryptophan flux across the blood-brain barrier, thus increasing the synthesis of 5-HT (+28%) in the hypothalamus (7). This is an area of the brain in which changes in CCK have also been observed during stress procedures (27). The circulating plasma concentrations of NEFA, free tryptophan, and BCAs may therefore serve as putative metabolic signals associated with the peripheral release of satiety neuropeptides.

We can only speculate as to whether the changes in the plasma amino acid ratio were sufficient to alter cerebral 5-HT, clarification of which, in the human, represents a formidable analytical challenge. However, metabolic control logic would suggest that any increase in this plasma ratio would theoretically increase cerebral 5-HT synthesis, as none of the metabolic reactions in the brain approaches saturation with pathway substrates. Systemic 5-HT synthesis has been shown, in previous studies, to increase by ~50% during 60 min of hypoxic exercise at FIO2 = 0.14 (29). However, we elected not to perform this measurement because of the analytical deficiencies of this assay. The plasma concentration of 5-HT depends on the number of damaged platelets in the circulation, which, to our knowledge, cannot be accurately quantified (D. Perrett, personal communication).

Acute hypoxia was previously shown to decrease the synthesis of 5-HT in the whole brain of rats (14), and a decrease in the venous concentration of prolactin, an indirect neuroendocrine marker of serotonergic activity, was also demonstrated in humans acutely exposed to an FIO2 of 0.145 (10). These observations may lend further support to the proposed mechanism and provide a possible explanation for the acute hypoxic depression of plasma CCK seen in the present study.
However, it was unfortunate that we did not establish whether hypoxic exercise decreased NEFA mobilization and the subsequent plasma amino acid ratio to further consolidate our original hypothesis. Alternatively, intravenous infusion of sulfated CCK octapeptide (CCK-8) was shown to increase the secretion rates of prolactin, cortisol, and growth hormone in humans (11), which also suggests a potential regulatory role of hypothalamic CCK per se in the activity of the hypothalamo-hypophyseal-adrenal axis.

The plasma ratio of free tryptophan to BCAA and CCK appeared to be more sensitive to acute compared with chronic changes in energy expenditure. The plasma ratio increased after intermittent hypoxic training only (pooled rest + exercise values), whereas a time × group interaction effect was not observed for plasma CCK. This apparent disassociation between the plasma ratio and CCK may be explained by changes in the sensitivity of brain serotoninergic function. A 16-wk endurance training program was shown to downregulate serotoninergic activity by ~30% in response to a selective 5-HT agonist (21).

In conclusion, the present findings demonstrated a consistent increase in plasma CCK after acute normoxic exercise, whereas a decrease was observed after acute, normobaric hypoxic exercise. The suppressive effects of acute hypoxia were only apparent during active as opposed to passive exposure and may be associated with changes in tissue PO₂. In contrast, 4 wk of intermittent hypoxic training only (pooled rest + exercise values), whereas a time × group interaction effect was not observed for plasma CCK. This apparent disassociation between the plasma ratio and CCK may be explained by changes in the sensitivity of brain serotoninergic function. A 16-wk endurance training program was shown to downregulate serotoninergic activity by ~30% in response to a selective 5-HT agonist (21).

We remember the late Simon Humphrey and the late Dr. Gail Butterfield, who remain constant sources of inspiration. We also extend our thanks to the undergraduate students of the University of Glamorgan that volunteered for the study; to the doctoral students who assisted in data collection, particularly M. Richards; to N. Hiscock, J. McGuire, S. Lucas, and M. Jordinson for kind efforts with the analysis of blood samples; and to Professor K. Frayn for informative discussions.

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