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Kinetics of Post-Exercise Excess CO_2 Production and Substrate Oxidation in Two Dysglycemic and Euglycemic Older Women A Case Study.

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Abstract

We examine the case of post-exercise excess CO_2 production and instantaneous substrate oxidation in two older women, one with impaired glucose tolerance and the other one is euglycemic. Both subjects stayed in the room-size calorimeter for 48 hours and performed three bouts of postprandial exercise on the second day. The instantaneous gas exchange rates have been estimated along with the instantaneous respiratory exchange ratio (RER) for the whole 48-hour experiment. The relative dynamics of O_2 consumption and RER showed a greater reliance on the carbohydrate as energy source in dysglycemic woman than in euglycemic woman. Also, the rate of post-exercise excessive CO_2 output, quantified as the time lag between peaks in O_2 consumption and peaks in RER was found to be higher in dysglycemic woman suggesting heavier reliance on anaerobic metabolism during exercise. For the first time, results relating the excess post-exercise CO_2 production and impaired glucose tolerance are presented.

Keywords: Post-exercise excess CO_2; Substrate utilization; Indirect calorimetry

Introduction

The post-exercise excess CO_2 production has been a subject of interest for clinicians for years [1-7]. The interest stems from the fact that excess CO_2 production is a hallmark of metabolic acidosis and was linked to a number of pathologies [4-7]. However, results linking type 2 diabetes and amount of excess post-exercise CO_2 are very limited and related to anaerobic threshold (AT) only [8-11]. Abnormal respiratory exchange has been reported in women with type 2 diabetes mellitus [8], specifically, their CO_2 kinetics was slower than controls. To date, there have been no attempts to analyze and contrast excess post-exercise CO_2 production between normoglycemic and dysglycemic subjects. It has been reported that while excess CO_2 with response to exercise is related to lactic acid production, its kinetics is delayed and is affected by hyperventilation [12]. Also, all results on determination of anaerobic threshold and gaseous exchange rates in type 2 diabetes patients were obtained with calorimeter carts and never in the whole-room calorimeter. We present novel data on two older women participating in a study designed to determine the effect of post-meal exercise on 24 h glycemic control in older people with impaired glucose tolerance (IGT). In contrast to other whole-room calorimeter studies, we estimated changes in RER on a minute-by-minute bases, which allowed unravelling the relative kinetics of substrate oxidation for a person with IGT and the normoglycemic person. The study performed over 48 h in a room calorimeter, was an efficient opportunity to also study instantaneous and continuous shifts in substrate utilization in response to multiple meals (with and without exercise) and to several bouts of exercise over various times of the day. In this way, we were able to determine prospectively not only the magnitude of the shift in substrate utilization, but also the temporal nature of the shift in response to the various challenges. We are not aware of any data such as these in older people.

Methods

Subjects

The first subject was 72 years old with class I obesity (BMI=32 $kg \cdot m^{-2}$; % body fat=44%, as determined by dual-energy x-ray absorptiometry (DXA)), and reported being inactive (<2 bouts of moderate physical activity per week), non-smoking, and not taking any medications. Impaired glucose tolerance was determined based on her 2 h post-challenge glucose concentration of 131.17 $mg.dl^{-1}$ following a 3 h screening Oral Glucose Tolerance Test (OGTT). The second subject was 72 years old, normoglycemic, overweight (BMI=27 $kg \cdot m^{-2}$; total body fat=40%). She was reported being inactive (<2 bouts of moderate physical activity per week), non-smoking, and not taking any medications. All studies took place at the Beltsville human nutrition research center (BHNRC) at the United States department of agriculture (USDA) in Beltsville, MD. The study protocol was approved by the Institutional Review Boards at the George Washington University Medical Center and at the USDA, and all subjects gave informed consent prior to their participation.

Study protocol

On the first day of the calorimeter study (control day), the subjects reported to the BHNRC at 7:30 AM in the fasted state. Height and weight were measured, as well as blood pressure and heart rate. Next, the sensor portion of a continuous glucose monitoring system (CGMS; iPro, Medtronics, Northridge, CA) was placed subcutaneously in the abdomen. The subjects then entered the calorimeter where they would spend the next 48 h and were instructed to remain inactive for the rest of the control day. For both days, the subjects were fed a diet comprising 50% carbohydrate, 20% protein, and 30% fat provided by the Beltsville human study facility (BHSF). The first day was a control day when subject was engaged into her daily activities, such as having meals, watching TV, reading, napping, and browsing the Internet; however no exercise was permitted. Standardized meals were provided at 08:00, 12:00, and 18:00 h.

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On the second day of the study (experimental day) the subjects were awakened in the calorimeter at approximately 06:00 h and were was instructed to lie quietly for 30 min to obtain measurements of resting metabolic rate. At 08:00, they were given a standardized breakfast to be consumed within 30 min. Following breakfast, the subject sat quietly for 30 min and then performed a single bout of post-meal exercise in the calorimeter (15 min of treadmill walking at 3.0 METs or ~60% VO_{2peak}, as determined by a prior graded exercise challenge). This post-meal exercise protocol was repeated 30 min following lunch and 30 min following dinner. The subjects remained inactive in the calorimeter for the rest of the day and night. The next morning, the subject left the calorimeter at 07:00, the CGMS sensor was removed, and following breakfast, they returned to their homes.

Measurement of substrate oxidation

Energy expenditure over the 48-h study period was measured continuously by respiratory exchange using a room-sized calorimeter. The BHNRC indirect, open-circuit, room-size chamber is a "push" type calorimeter with total physical volume of 21,000 L designed to comfortably house subjects for ≥ 24 h while measuring the subject's gas exchange rates, energy expenditure, and respiratory quotient. Currently, BHNRC has three identical chambers each equipped with furniture, a personal computer, TV, treadmill, and a "Murphy Style" folding bed. The chamber has a window and an air lock within the chamber's door used to pass meals and other essential materials into and out of the chamber. The air is thoroughly mixed inside the chamber by a ceiling fan. Oxygen consumption (VO_2) and CO_2 production (VCO_2) were determined by analyzing the mass and composition of air flow into and out of the calorimeter by standard mass spectrometry procedures [13,14]. The mole fractional concentrations of nitrogen, oxygen, argon, helium, methane, and carbon dioxide in the air entering and exiting the calorimeter are measured every 80 sec using a multiple-gas analyzer (model MGA-1200, Perkin-Elmer Industrial Instruments, Pomona, CA). This device is a multiple collector mass spectrometer designed to measure the partial pressure of helium [2% full scale (FS)], methane (1% FS), nitrogen (100% FS), oxygen (22% FS), carbon dioxide (2% FS), and argon (2% FS) in air within 0.1% FS. The multiple-gas analyzer can accurately measure differences in nitrogen concentration (60.003 %FS; as determined from changes in inlet air composition over 24 h and differences in inlet and outlet air composition during equilibrium), FO_2 (60.02%FS),and FCO_2 (60.03%FS) in chamber air so that oxygen depletion and carbon dioxide accumulation can be determined. The chamber air flow rate i.e., inlet dry air flow rate at standard temperature and pressure, is measured every 5 s using a laminar flow element (CME Vol-O-Flow11–25–300A, Aero space Control Products, Davenport, IA), and a 1-min average (typically1.5 L/sec) is determined. The volumetric flow rate is a function of the pressure drop across the laminar flow element (Pd; electronic manometer, Datametrics, Wilmington, MA), the inlet air temperature (Ti; model RTDPR-14–2–100, Omega Engineering, Stamford, CT), the absolute air pressure (Pi; model PX623-020A10CV, Omega Engineering), and the inlet water vapor fraction (FH2Oi; model 1200APS dew point hygrometer, General Eastern Instrumentation, Watertown, MA). A complete description of the calorimeter system is available in [13] with only major change being rewriting of the software using LabViewTM (National Instruments Corp, Austin, TX) and relocating to a new facility). All urine was collected during the calorimeter measurements. Urine samples were weighed and subsamples were stored at -80° C until analyses. The weight of the urine voided was calculated as the difference between the full weight and the pre-tare empty weight. Urine was analyzed for combustible energy by adiabatic bomb calorimetry (Parr Instrument Company, Moline, IL) and for nitrogen content by combustion (Leco Corp, St. Joseph, MI).

The minute-by-minute values of oxygen consumption (VO_2) and CO_2 production (VCO_2) were then calculated using the technique developed at BHNRC [15]. The RER was calculated as the ratio of VCO_2/VO_2.

Calculation of instantaneous gas exchange rate and RER

In indirect calorimetry five major measurable quantities are of interest: air flow rate F, inlet mole fractional concentrations of O_2 and CO_2, and outlet mole fractional concentrations of O_2 and CO_2. Having obtained these five variables and the volume of the chamber, the oxygen consumption (VO_2) and CO_2 production (VCO_2) can be calculated [14] provided derivatives of the outlet mole fraction concentrations can be reliably estimated. Having obtained the gas production rates, the RER can be estimated as their ratio and energy expenditure can be evaluated using the Weir equation. The RER values are of primary interest in nutrition studies as they reflect which oxidation energy source is used by the subject (RER is accepted as 1 for carbohydrate oxidation, 0.7 for fat, and 0.83 for protein oxidation). The RER values calculated in this study have not been corrected for protein oxidation.

To obtain reliable estimation of the derivatives of gaseous concentrations, we applied the regularization technique [16], which is the standard method of dealing with the problem of differentiation of noisy data.

Finally, the relative dynamics of the simultaneous signals (VO_2 and RER) were investigated by centering them to a zero-level mean and then estimating the cross-correlation function between them. The maximum of the cross-correlation function indicates a delay between these two signals, and therefore provides an indication of the time lag between metabolic demand (VO_2) and the substrate utilization (RER) shift.

Results

Figure 1 displays the relative dynamics of the instantaneous VO_2 and RER signals over about 14 hours during the second day of the study. The three peaks in VO_2 consumption indicate three post-prandial exercise periods. Figure 2 shows only the last exercise period from Figure 1a, to elucidate details of post-exercise gaseous exchange rates and substrate oxidation for dysglycemic subject. As expected, the RER increased following the meals, indicating a shift toward more glucose oxidation with greater substrate availability. The RER also increased with the metabolic demands of the exercise performed in the post-prandial state. The dynamics of exercise-induced substrate oxidation is characterized by two values: the height of the RER peaks in the post-exercise periods, denoted by h in Figures 1 and 2 and the time lag between the peak of O_2 consumption and RER peak, denoted by τ on the graphs. The average height of the RER peaks for the dysglycemic subject calculated as a difference between resting baseline RER (0.85) for this subject and the average RER post-exercise peak values was 0.33 (SE 0.06), while the average time lag over three exercise sessions was found to be 20 (SE 1) min. Data from this subject can be contrasted with those from the other, euglycemic subject, in Figure 1b, for whom the increase in RER in response to exercise was smaller with average height of RER peaks being 0.25 (SE 0.02) and the lag of 16 (SE 2) min. The differences in lag times and RER peak values are statistically significant with p=0.05. Figure 2 shows a detailed picture of gaseous exchange rates and substrate oxidation for the one of the exercise periods for dysglycemic subject. Notice, after exercise had stopped,

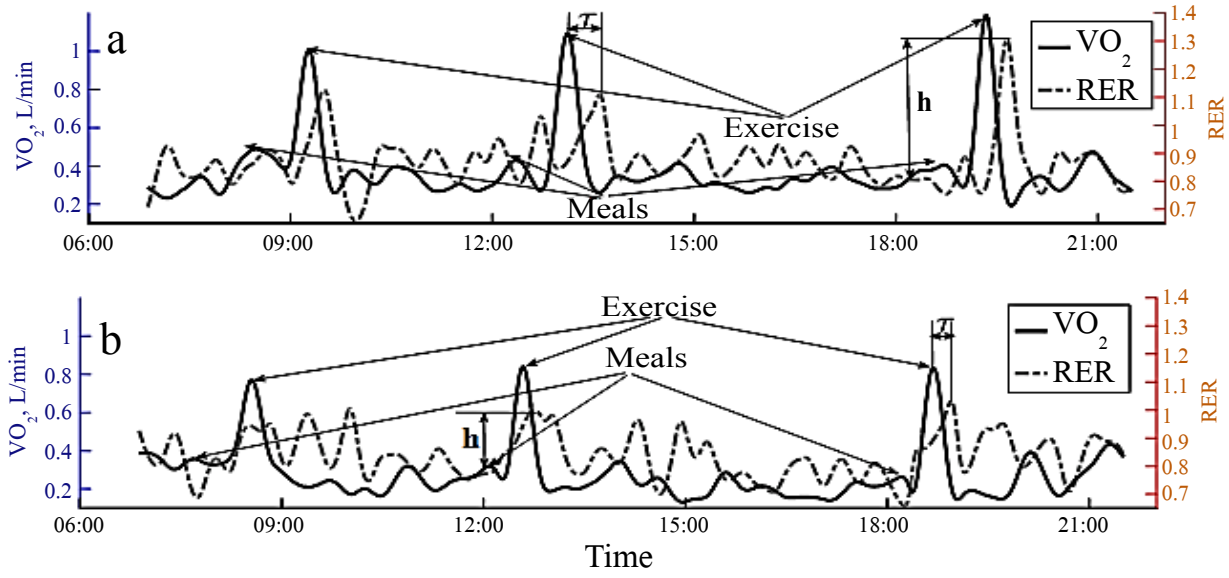

Figure 1: The relative 24-hour kinetics of O_2 consumption and the respiratory exchange ratio for the older woman with obesity and impaired glucose tolerance (a) and in an older woman who was overweight without impaired glucose tolerance (b) in response to three 15 min bouts of post-prandial treadmill walking (3 METs).

the VO_2 and VCO_2 reached the excursions and started to decline, the VCO_2 overtook VO_2 at some point of time causing the RER values to reach superunity. The shaded area in Figure 2 shows the difference between post-exercise O_2 consumption and CO_2 production. The RER peak reaches its maximal value when this difference is the largest. Also, the time while VCO_2 supersedes VO_2 is the post-exercise excess CO_2 removal time which is very close to the lag time (τ).

Discussion

During exercise the muscle produces lactic acid which is quickly converted into its conjugate base - lactate by releasing a hydrogen ion. Four major mechanisms were proposed to explain the sudden rise in blood lactic acid concentrations in the exercising muscle. Historically, the first hypothesis presented to explain the phenomenon was the increased reliance on anaerobic metabolism during incremental exercise [17]. Because of the low levels of O_2, the muscle relies more on anaerobic pathways, thus producing more lactic acid as a byproduct. However, this explanation was challenged by research supporting the idea of failure of hydrogen shuttle system in contracting muscle to keep up with the increased rate of NADH production by glycolysis [18] which would cause pyruvic acid to absorb excess hydrogens via lactate dehydrogenase (LDH) reaction to form lactic acid regardless of the oxygen availability. The third theory explaining the existence of lactate threshold is related to the recruitment of fast-twitch muscle fibers during intense exercise [19]. It is hypothesized that the enzyme promoting the LDH reaction in "fast" fibers has more propensity in converting pyruvic acid to lactic acid than the enzyme in "slow" fibers [20]. Thus, as "fast" fibers are recruited during intense exercise, more lactic acid is produced. The final hypothesis for lactic accumulation in working muscle is related to the idea of reduced rate of lactic acid removal in comparison to the rate of lactic acid production [21]. The LDH reaction is reversible and under appropriate conditions and lactic acid can be converted back to pyruvic acid and used as a substrate in skeletal muscle. By this process, lactic acid is removed from blood and if the rate of removal is slower than the production rate, the lactic accumulation will follow. However, regardless of the proposed mechanism, the central idea to all of them is the conversion of pyruvic acid into lactic acid in the exercising muscle.

The reaction of pyruvic acid conversion into lactic acid and further into lactate can be written as:

$$C_3H_4O_3 + NADH + H^+ \leftrightarrow C_3H_6O_3 + NAD^+ \rightarrow La^-Na^+ + NAD^+ + H^+ \quad (1)$$

The first LDH reaction is reversible and the second reaction produces sodium lactate and hydrogen ions. The sodium lactate is the major form of lactate in physiological systems [22]. The ionization of lactic acid and its conversion to sodium lactate is accompanied by release of free hydrogen ions:

$$La^-H^+ + Na^+ \rightarrow Na^+La^- + H^+ \quad (2)$$

The dissociated H^+ are subsequently buffered according to the following equation:

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2 \quad (3)$$

The carbonic acid (H_2CO_3)-bicarbonate (HCO_3^-) system is a buffer which shifts the balance in equation (3) either to the left or to the right depending on proton concentrations thus maintaining normal homeostasis. If there is an excessive amount of free hydrogen ions, the balance in the equation (3) is shifted to the right thus producing more carbon dioxide to avoid metabolic acidosis, however under alkalosis, the balance is shifted to the left and more free hydrogen ions are released lowering blood pH. The bicarbonate buffering pair is a very fast chemical buffer, which is aided if necessary by a slower respiratory compensation to remove access CO_2.

The rise of hydrogen ions' (protons) concentration in bodily fluids causes drop in muscle and blood pH and hence metabolic acidosis of exercise ensues. Because the hydrogen release is caused by ionization of lactic acid, this specific type of metabolic exercise-induced acidosis is termed lactic acidosis. As explained above, the removal of excess hydrogen ions is thought to be performed through body bicarbonate buffering system, which lowers blood's pH by accepting the protons and producing water and excess carbon dioxide. This theory is supported

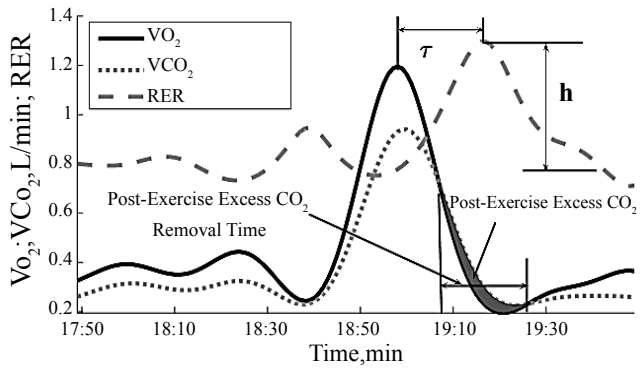

Figure 2: The relative kinetics for the third post-prandial exercise period for dysclycemic subject. The heights of the RER peak and thier relative lag demonstrate the dynamics of exercise-induced gaseous exchange and substrate oxidation.

by experimental evidence such as negative correlation between blood lactate and bicarbonate concentrations and negative correlation between blood lactate and blood pH [23]. If the bicarbonate's buffering capacity is exceeded, the respiratory control center in the brain increases breathing rate thus removing more excess CO_2. In contrast to chemical buffering, the pulmonary regulation is not immediate and lags the bicarbonate buffering. Due to a much larger time constant, the pulmonary buffering is the major remover of excess CO_2 after the stop of the exercise. Lactate concentration (La) is increasing for few minutes after the exercise [12] reaching its maximum concentration several minutes later after the cessation of the exercise. The lactate is produced in the working muscle and it takes some time (several minutes) for it to reach other organs, this is the reason for the time lag between end of the exercise and maximal value of blood lactate [12]. While excess post-exercise CO_2 is related to increase in La, it is delayed relative to lactate production [12]. The post-exercise gaseous exchange has been the subject of extensive research in relation to different pathological conditions [6]. However, its relation to diabetes type 2 has been overlooked. To the best of our knowledge, in this case study we present the first experimental results demonstrating the differences in post exercise gaseous exchange between euglycemic and dysglycemic subjects.

Instantaneous gas measurements from the calorimeter over 48 h allowed us to unmask previously undetected phenomena relating the effects of post-meal walking on the temporal dynamics of post-exercise excess CO_2 production in older women with IGT and without IGT. Indeed, for the IGT subject, we observed a longer delay in the shift in RER in response to exercise than for non-IGT subject. This rather marked delay in RER response provides novel evidence of both the presence and the dynamics of excess post exercise CO_2 production as it relates to IGT. The height of the RER peaks in both persons are indicative of the effectiveness of their fat mobilization to match energy demands. The dysglycemic person has higher RER peaks indicating that to meet the exercise energy requirements she needs to tap into her glycogen resources since her fat mobilization is impaired. On the other hand, the euglycemic women has significantly lower post-exercise RER peaks, indicating that her energy demands are met more by fat oxidation.

Metabolic inflexibility in response to substrate availability has been described extensively under resting conditions [24] and less so under exercise [25] conditions; however, we are not aware of any data describing the temporal dynamics of this highly coordinated regulation of fuel supply to meet oxidative demand. The RER increased following meals and with 15 min of post-prandial exercise performed at 3 METs, indicating a greater reliance on carbohydrates relative to fats for fuel. It has been observed [26] that carbohydrate oxidation was significantly greater, while fat oxidation was lower during moderate intensity exercise in older compared with younger study subjects matched on sex and lean body mass. The authors attributed this difference in substrate oxidation to age-related changes in skeletal muscle respiratory capacity, however, rather than to lower lipolytic rates and FFA availability in the older people. That the magnitude of the exercise-related increase in RER observed in the older woman with IGT was greater than what was observed in the euglycemic woman is more than likely explained by a greater VO_{2peak} in the euglycemic subject, thus allowing her to exercise at a lower relative intensity with less reliance on glucose oxidation. This can also be possibly due to low local storage of muscle glycogen, a sluggish recruitment of liver glycogen, or a slow fractional turnover of stored triglycerides. While multi-tissue insulin resistance certainly modulates this impairment, the underlying cellular mechanisms are more than likely related to defects in the bioenergetic capacity of the mitochondria that often are observed with aging [27], disuse [28], and obesity [29,30].

What was very striking in our data was the delay in the response between metabolic demand (exercise) and the shift in the RER necessary to fuel that demand. This lag in response was consistent over three episodes of exercise and was as long as 20 min in duration for the dysglycemic subject and about 10 mins for the euglycemic subject. The authors attribute this difference to a larger volume of post-exercise CO_2 which dysglycemic subject had to remove by respiratory buffering following the exercise. Since the CO_2 is produced in the process of free hydrogen chemical buffering, this points to a significantly more acute exercise-induced metabolic acidosis in dysglycemic subject. The post-exercise rise in RER is explained by the fact that $RER=VCO_2/VO_2$ is a ratio and after the exercise is terminated, the VO_2 values are sharply decreasing while the CO_2 are either stay the same or even increases due to respiratory buffering of post-exercise CO_2. This causes post-exercise elevation in RER until majority of post-exercise CO_2 is removed and normal homeostasis is restored. The larger the volume of post-exercise CO_2 that needs to be removed, the longer the time delay between termination of the exercise and maximum RER value. Since the bulk of lactic acid is produced during anaerobic metabolism, this results suggest larger reliance on anaerobic ATP production in dysglycemic subject.

While our observations are intriguing, further longitudinal studies are needed to connect post-exercise CO_2 production with glucose tolerance. Our results provide a novel view of the association between exercise-induced CO_2 production, substrate utilization, and glucose tolerance. Excess post-exercise CO_2 production may therefore constitute an early indicator of the progression toward frank type 2 diabetes.

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