HHS Public Access

Author manuscript

Physiology (Bethesda). Author manuscript; available in PMC 2025 August 25.

Published in final edited form as:

Physiology (Bethesda). 2026 January 01; 41(1): 0. doi:10.1152/physiol.00002.2025.

What do we know of human fuel use during aerobic exercise, and how do we know it?

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Abstract

Aerobic exercise is arguably the most metabolically demanding challenge imposed on the human body. The metabolic adaptations to exercise are complex, involving most tissues, and differing substantially depending on the type, severity, and duration of exercise, as well as extent of prior training. Studies of these metabolic responses have been ongoing for decades, including the active NIH-supported consortium MotrPAC. Most studies have been carried out in model organisms, generally rodents or dogs. However, the metabolism of these model organisms substantially differs from humans. We therefore review here what is known specifically of human metabolism during exercise. For the sake of brevity, we focus on aerobic exercise, without extensive prior training. We review methods used to reach conclusions, highlight the many remaining unknowns, and discuss questions requiring future experimental attention.

Introduction

Acute exercise imposes significant challenges on the body's metabolic systems, triggering a wide array of physiological responses. Exercise induces many stimuli, from the release of circulating factors such as hormones (1), to local mediators like neurotransmitters(2). These stimuli then trigger downstream signaling pathways that converge and help sustain skeletal muscular work. The many exercise-induced signaling pathways, including well-known kinases such as Calcium/calmodulin-dependent protein kinase II (CAMKII), mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinases (PI3K), play crucial roles in coordinating the systemic response to exercise(3). Beyond skeletal muscle, other organs contribute to the broader metabolic adjustments required to support physical activity. These coordinated responses come at an energetic cost: whole-body oxygen consumption increases linearly with workload, reaching levels more than 20 times higher during maximal exercise compared to rest(4). To meet these demands, the body must adjust nutrient production and utilization across multiple systems.

Detailed studies of acute metabolic responses to exercise provide an important framework for understanding the beneficial effects of exercise on health. While both aerobic exercise and resistance training yield health benefits, aerobic exercise has garnered significant

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interest due to its beneficial effects on cardiorespiratory fitness and reducing CVD risk profiles (5). While many studies have examined how energy demands are met during acute aerobic exercise in humans, interesting questions remain. Currently, the field is missing comprehensive studies leveraging advanced techniques to quantify *in vivo* cell metabolism.

This review of the literature will aim to: i) examine how acute aerobic exercise changes whole-body oxygen consumption and systemic nutrient use, ii) investigate what is currently known about how specific organs use nutrients to meet exercise-induced energy demands, iii) assess available techniques to study metabolism and their relative strengths and limitations, and iv) identify meaningful gaps in our current understanding of acute exercise metabolism.

Exercise Increases Whole-Body Oxygen Consumption and Modulates RER

During submaximal aerobic exercise, whole-body oxygen consumption increases exponentially at the onset of exercise until reaching steady state(6, 7). If the intensity of exercise increases, oxygen consumption will increase linearly with workload until a maximum oxygen consumption is reached(6, 7). This rise in oxygen consumption must be supported by nutrient oxidation. Whole-body nutrient oxidation can be estimated using indirect calorimetry, which measures inhaled O_2 and exhaled CO_2(8, 9). Carbohydrates, proteins, and fats require different amounts of O_2 consumption and CO_2 production when they are burned (CO_2/O_2 is ~1 for carbohydrates, ~0.7 for fats, and in between for proteins). Therefore, the ratio of CO_2 produced and O_2 consumed can be used to estimate the relative oxidation of these substrates during exercise. This method is referred to as the respiratory exchange ratio (RER) and has been used to characterize whole-body substrate oxidation during exercise(9) (Figure 1). RER depends on a variety of factors, including exercise intensity and duration(8). Carbohydrate oxidation is predominantly used for high-intensity and short-duration exercise while fats are relied on for low-intensity and long-duration exercise(8, 10). While RER is a widely used technique, it has numerous limitations. Many methods of calculating RER assume plasma glucose is the sole source of carbohydrate oxidation, ignoring glycogen(9). RER calculations also assume CO_2 production is a direct result of substrate oxidation, but CO_2 can also be produced from HCO_3^- buffering the H^+ that accumulates from increased glycolytic flux(9). Thus, RER would overestimate carbohydrate oxidation at exercise intensities above the lactate threshold, or the point at which lactate accumulates in the arterial blood(11). Further, RER assumes oxidation is the sole metabolic process consuming O_2 and producing CO_2, yet many synthesis pathways such as gluconeogenesis and ketogenesis are active during exercise and could affect RER calculations(9). Additionally, while there are equations available to correct for protein oxidation, many exercise studies have assumed protein oxidation is negligible during exercise and ignored the role of protein in exercise metabolism[7].

Metabolomics Identifies a Vast Network of Exercise-Responsive Metabolites

RER addresses basic questions about macronutrient oxidation but does not capture the complex system of small molecules that are produced from nutrient breakdown and

conversion to energy. In fact, there are thousands of metabolites constantly being produced by metabolic enzymes. Several studies in recent years have sought to investigate how exercise may alter this large network of metabolites using untargeted metabolomics. These studies use unbiased identification of metabolites in biological samples and make relative comparisons between exercise and resting conditions. A recent systematic review of metabolite concentration changes in human serum after acute exercise revealed common findings across 27 studies, including an increase in lactate, pyruvate, tricyclic acid (TCA) cycle intermediates, fatty acids, acylcarnitines, and ketone bodies during acute exercise(12). Bile acids decreased, while amino acid responses were extremely heterogenous across studies. Some tissue sources of these circulating metabolites can be surmised (e.g. the liver produces ketones, lipolysis produces fatty acids), but other metabolites likely come from multiple organs and their relative contributions have not been well quantified. It has been shown, however, that TCA cycle intermediates accumulate in skeletal muscle tissue(13) and extracellular fluid(14) during exercise; thus skeletal muscle is a likely source of these metabolites. In addition to characterizing changes in known metabolites, untargeted metabolomics can also identify novel metabolites. For example, it was recently discovered that exercise stimulates the production of *N*-lactoyl-phenylalanine (Lac-Phe) from immune cells and Lac-Phe suppresses feeding(15). Other ‘omics approaches, such as proteomics and phosphoproteomics, have provided valuable insight into the signaling networks that sustain tissue metabolic function during exercise. For example, analysis of the skeletal muscle phosphoproteome revealed a novel AMPK substrate (C18ORF25) that regulates fiber size, exercise capacity, and contractile function(16). As mass spectrometry techniques advance, more novel metabolites and pathways may be discovered. Follow-up studies should interrogate the physiological relevance of exercise-responsive metabolites and whether they can be leveraged as new potential therapeutic targets.

Isotope Tracing Probes Systemic Metabolic Activity

Metabolomics can provide novel insights into how the relative concentrations of metabolites change during exercise. These measurements, however, do not capture metabolic pathway activity, i.e. metabolic flux. If a given metabolite is increased in the circulation, for example, it is impossible to tell from metabolomics alone if the metabolite accumulated due to increased production of the metabolite or decreased consumption. For example, if glucose accumulates in the circulation, is there enhanced gluconeogenesis or impaired glucose uptake? Metabolic isotope tracing helps answer these questions by directly tracking a nutrient as it undergoes metabolic enzyme-mediated conversions(17, 18). Metabolic isotope tracers are chemically identical to the molecule of interest and have the same number of protons in their nuclei but a *different* number of neutrons and therefore a different mass. Metabolic isotope tracers are physiologically indistinguishable from endogenous metabolites but detectable via mass spectrometry or, if radioactive, by their emitted particles. While many different atoms can be labeled with isotope tracers, the most common in metabolic applications are carbon, nitrogen, and hydrogen(19). Carbon isotopes can include radioactive ^{14}C, which was commonly used in the past due to the high signal to noise ratio achieved by measuring emitted particles, but its potential radioactive danger to subjects has lessened its use in recent years. ^{13}C, not radioactive but detectable by mass spectrometry, is now much

more commonly used due to its stability and safety(17, 18). Stable isotope tracers can be introduced to systemic circulation via an intravenous infusion. Biological samples, such as blood and tissue, are sampled throughout the infusion (Figure 1). The isotope tracer can then be detected in these samples and its enrichment can be used to calculate various metabolic parameters.

The rate at which a metabolite is released from tissues into the bloodstream is defined as the rate of appearance (R_a)(17). The rate at which a metabolite is consumed from the bloodstream is the rate of disappearance (R_d)(17). In a steady-state infusion, the isotope tracer is infused slowly until its percent enrichment in the blood plateaus at a minimally perturbative, stable level (usually around 10%). At metabolic steady state, the concentrations of available metabolites (or pool sizes) do not change, and R_d=R_a + the rate of infusion, i.e. R_d~R_a. The steady-state flux of metabolites between the tissues and bloodstream, defined as R_a, is referred to as the circulatory flux (F_{circ}) of a given metabolite, and can be calculated from the observed percent enrichment of a given metabolite and the known rate of infusion of labeled metabolite (20). For example, if a rate of X of labeled glucose is infused, and a 10% enrichment is observed, then it can be assumed the endogenous R_a of unlabeled glucose must be 9X (i.e. the other 90% of glucose. In general, the term flux refers to the movement of a metabolite over time and uses the units of nmol metabolite/minute/gram (grams of body weight, a specific organ, etc.). In addition to providing information about metabolite kinetics, stable isotope tracers offer the advantage of being able to track the conversions of a given metabolite over time. For example, if 13-C labeled glucose is infused, converted to lactate, and released into the circulation there will be detectable 13-C labeled lactate in the bloodstream.

Glucose and Glucose-Producing Fluxes

Isotope tracing studies in humans have characterized systemic nutrient fluxes across a wide range of aerobic exercise intensities and durations (Tables 1–7) (Figure 2). Most human studies have assessed glucose flux during an acute bout of cycling (Tables 1–4). Glucose R_a generally scales with exercise intensity(21–30). Glucose R_a also tends to increase with exercise duration, often peaking at the end of exercise in humans. Glucose R_a is highest at exercise intensities >80% VO_{2max} and increases between 228–900% in human subjects(25, 31–33). The wide range in percent increase is an example of the marked variability often observed in human studies, when it can be difficult to maintain consistent conditions between trials or studies.

In the fasted state, glucose is produced either via hepatic glycogen breakdown or gluconeogenesis (GNG). In humans, glycogenolysis (GLY) is estimated to support the majority of glucose flux both at rest and across a variety of exercise intensities (Table 4). Methods for quantifying the relative contributions, as well as absolute rates, of GNG and GLY in humans vary. Arterial-venous (A-V) sampling across the splanchnic bed via catheterization of the brachial artery and hepatic vein enables direct measurements of gluconeogenic precursor uptake and splanchnic glucose production, which in the fasted state should reflect mostly hepatic glucose production (HGP)(34) (Figure 1). Using this method, HPG derivable from gluconeogenic precursor uptake averages 24% at exercise

onset but falls to between 6–16% by 40 minutes, with the lowest GNG contribution found at the highest exercise intensity(34). In lieu of invasive catheterization techniques, GNG can be estimated using the glucose recycling method, in which a carbon labeled glucose (^{13}C) is infused simultaneously with a deuterium labeled glucose (6,6-$^{2}H_2$). The ^{13}C of the carbon labeled tracer can recycle back to glucose via Cori cycling (glucose → lactate → GNG → glucose), while the deuterium label is lost in the Cori cycle(23) (Figure 1). The difference between the two rates can thus be used to approximate GNG. Glucose recycling methods show that while absolute rates of GNG increase during exercise and scale with intensity(23), GNG accounts for only 4–16% of glucose R_a(28, 35). Another tracer-based approach is to infuse a labeled precursor, such as lactate, and measure label incorporation into systemic circulating glucose. Labeled bicarbonate can also be used, which measures ^{13}C incorporation via pyruvate carboxylase and will account for contributions of all pyruvate-derived gluconeogenic substrates(36). After an overnight fast, precursor incorporation into systemic glucose measures 23–40% glucose flux from GNG during exercise(36–39). After a shorter fast (4.5–5 hours), GNG contribution to glucose decreases to 12–16%(28). An important consideration is that measuring precursor label incorporation into glucose can underestimate GNG due to label dilution in the TCA cycle, because the oxaloacetate pool is shared by both gluconeogenesis and the TCA cycle, and therefore label can be diluted by unlabeled carbons from the TCA cycle. Correction factors to account for label dilution have been suggested using carbon-labeled acetate as a proxy for measuring metabolic exchange reactions in the TCA cycle(40, 41). Rates of tracer dilution are likely specific to the metabolic environment of a given experiment and affected by factors such as exercise intensity, feeding status, or species, making the use of a fixed correction factor (as done in many studies in Table 4(22, 37–39, 42)) prone to error(22, 37–39, 42). Additionally, these correction factors do not account for potential dilution of the triose phosphate pool or of the contribution of glycerol as a gluconeogenic precursor(22, 37–39, 42). To better estimate the true isotopic labeling of precursor pools and improve estimates of GNG, Hellerstein and colleagues developed mass isotopomer distribution analysis (MIDA), which combines the relative abundance of mass isotopomers and combinatorial probability logistics(43). The MIDA technique applied in exercising humans measures the contribution of GNG to total glucose flux to be ~25% at rest and ~22–24% during exercise(29).

Lactate Flux

Once considered a metabolic waste product, lactate is now widely recognized as an important metabolic substrate. While not as well studied as glucose, a smaller body of literature led by George Brooks and colleagues(37, 38, 42, 44–46) have characterized lactate kinetics in exercising humans. Exercise dramatically increases lactate flux, and lactate R_a scales with exercise intensity (Table 5). At mid-intensities (60–70% VO_{2max}), fasted males increase their lactate R_a ~500–600% (Table 5). The majority of lactate appearing in the circulation during exercise comes from glycolytic activity in skeletal muscle(44). Most lactate is disposed of via oxidation, while conversion to glucose accounts for ~25% of lactate R_d(37). It is important to note that a nutrient's flux does not always correlate to plasma concentrations. For example, lactate flux may be high even as arterial lactate concentrations are not, due to the large capacity of skeletal muscle (and other organs) to take up lactate.

Fatty Acid Flux

Fatty acids also play an important role in supporting increased energy demands during exercise (Table 6). Contrary to carbohydrate flux, fatty acid flux peaks during low to mid-intensity exercise(24, 25, 32, 47–49). During low-intensity exercise (25% VO_{2max}), after exercise onset palmitate flux does not further increase with exercise duration(25). At mid-intensities (60–70% VO_{2max}), however, palmitate flux increases between 56–147% and peaks at the end of exercise(25, 32, 47–50). At exercise intensities 80% $VO_{2max,}$ fatty acid flux increases only minimally(32) or not at all(25, 33). Once free fatty acids (FFAs) enter the bloodstream, they may be taken up and oxidized by tissues or re-esterified and stored as triglycerides. At rest, oxidation accounts for only ~10% of fatty acid R_d(47, 49). During exercise, however, oxidation accounts for 50% or more of fatty acid R_d(47, 49).

Ketone Flux

Ketones bodies, produced when the liver breaks down fat, rise in the circulation during fasting or prolonged exercise(12). Studies led by Balasse & Fery(51–53) (Table 7) have established that at low plasma ketone concentrations (<1 mM) observed after a short-duration fast (~16–18 hours), exercise increases β-hydroxybutyrate (BHB) R_a 112–125%, peaking as exercise duration progresses(52, 53). At higher ketone plasma concentrations (>2.5 mM) achieved after more extreme fasting durations (2–5 days), BHB flux *decreases* during the first 10 minutes of exercise, before returning to baseline values for the remainder of the exercise bout(51–53). Thus, the effect of exercise on circulating ketone flux depends on initial ketone concentrations in plasma.

Amino Acid Fluxes

A handful of studies have evaluated amino acid fluxes during exercise. Leucine has garnered particular interest for its known role as an activator of the mammalian target of rapamycin complex 1 (mTORC1) activity and muscle protein synthesis(54). Flux studies in humans, however, have not observed particularly notable changes in leucine flux with exercise (Table 7). It is unknown how flux of the other branched-chain amino acids, isoleucine and valine, change with exercise. Amino acids that serve as gluconeogenic precursors have also garnered interest. Glutamine can be a carbon source for glucose but also acts as an anaplerotic substrate and nitrogen transporter, both important processes during exercise. Studies in humans(55) have found no significant effect of exercise on glutamine R_a, despite a fall in arterial glutamine concentrations. Alanine is also a gluconeogenic precursor and nitrogen transporter. In fasted, untrained male humans, alanine R_a increased 136% during prolonged, low-intensity exercise(55). Due to the small number of studies, it is difficult to make generalized conclusions about amino acid fluxes during exercise.

Tissue-specific metabolism: Studying metabolic responses to exercise at the systemic level helps answer basic questions about energy expenditure, nutrient use, and metabolite responses. However, questions such as which organs contribute to the whole-body increase in energy expenditure, how organ-specific nutrient use and production changes, site of metabolite production, and how specific metabolic pathway activity changes, must all be answered using more advanced experimental approaches that probe metabolism on an organ

or cellular level. The following section will discuss what is currently known about how the skeletal muscles, pancreas, liver, kidneys, adipose tissue, heart, and respiratory system use nutrients to meet the physiologic and energetic demands of exercise as well as identify unanswered questions.

Skeletal Muscle Consumption of Carbohydrates and Lipids Depends on Exercise Intensity and Duration

Skeletal muscle makes up around 40% of body mass(3) and accounts for about 20–30% of resting metabolic rates(3, 56). During exercise, contracting skeletal muscle increases oxygen consumption and energy demands. ATP is required for enzymes involved in several key steps of skeletal muscle contraction, including membrane excitation, calcium handling, and cross-bridge cycling(57). Skeletal muscle ATP demand during exercise is coupled to mechanical work, i.e., as work increases ATP demand increases as well(58). Maximum exercise can increase skeletal muscle metabolic rates up to 20-fold and ATP turnover 100-fold(59, 60). ATP is rapidly broken down into ADP and Pi. Intramuscular stores of ATP, however, are small (~5mmol/kg/wet muscle)(57). ATP must be resynthesized, and this can be accomplished several ways, including anaerobically from phosphocreatine (PCr), via glycolysis, or aerobically via oxidative phosphorylation(57). The first few seconds of exercise are fueled by the limited amount of intramuscular fuel stores (Figure 3). Human muscle biopsies taken before and after 6 seconds of maximal effort exercise revealed equal contributions from PCr and glycogen(61). When the duration of exhaustive exercise increases to 30 seconds, glycolysis is the main source of ATP and provides three-times more ATP than PCr breakdown(62).

Skeletal Muscle Carbohydrate Uptake and Oxidation

Many quantifications of skeletal muscle fuel use during exercise rely on extrapolations from systemic measurements (Tables 8–11). If skeletal muscle is assumed to be the main metabolically active organ during exercise, then measuring systemic R_d can be used as a proxy for skeletal muscle nutrient uptake even in the absence of A-V sampling. Whole-body glucose R_d scales with exercise intensity, reflecting increased skeletal muscle glucose uptake (Table 9). The increase in glucose R_d and systemic carbohydrate oxidation with exercise intensity coincides with increased muscle pyruvate dehydrogenase (PDH) activity, which catalyzes the conversion of pyruvate to acetyl-CoA and thus promotes glycolytic flux into the tricyclic acid (TCA) cycle(24).

Studies have shown 90–100% of glucose tracer taken up by tissues during exercise (i.e. glucose R_d) is recovered in expired $^{13}CO_2$ (63). Glucose R_d, then, is considered a measure of skeletal muscle plasma glucose oxidation. The difference between RER-derived carbohydrate oxidation and plasma glucose oxidation (i.e. what carbohydrate oxidation is not accounted for by skeletal muscle glucose uptake) can then be used to estimate muscle glycogen oxidation. This method has been used by many studies to determine how the relative contributions of plasma glucose and muscle glycogen to carbohydrate oxidation change with exercise (Tables 8–11). The *absolute* rates of both plasma glucose and muscle glycogen oxidation increase with exercise intensity. The *relative* contribution

of muscle glycogen to total carbohydrate oxidation, however, outpaces plasma glucose at exercise intensities >25% VO_{2max} (24–26, 32, 35, 37, 64–67)(Table 9). At 60–70% VO_{2max}, glycogen is estimated to contribute between 35–68% of total energy expenditure (TEE)(23, 32, 37, 64) during exercise lasting less than 1 hour. Glycogen contribution falls to ~20% during prolonged exercise (120 min.)(25) and will eventually decrease further(Figure 3). At submaximal work intensities, type I skeletal muscle fibers are the first to break down glycogen, and as exercise duration increases or intensity reaches maximum workloads, type II fibers then break down glycogen stores as well(68). Interestingly, glycogen stores will remain intact in non-working skeletal muscle even as working skeletal muscle glycogen stores approach zero(69).

The failure to consider lactate as an oxidative fuel source reduces the validity of these methods. The discrepancy between total carbohydrate oxidation and glucose R_d could be accounted for not only by glycogen, but also by the oxidation of lactate. Some calculations acknowledge this possibility by using the term “other CHO” instead of specifically naming muscle glycogen(25, 64, 65) as the remaining fuel source. Lactate may be produced from several precursor nutrients, including glucose, glycogen, glycerol, alanine and glutamine(70). In a ^{13}C-glucose infusion, oxidized lactate labeled from circulating glucose would already be accounted for (although wrongly ascribed to plasma glucose) in expired $^{13}CO_2$. Unlabeled oxidized lactate, however, would not be accounted for and may affect estimates of muscle glycogen oxidation. Similarly, while 90–100% of infused glucose tracer is recovered in CO_2, this does not preclude glucose being converted to another metabolite prior to oxidation. Isotope tracing and A-V sampling studies in exercising dogs showed that at moderate exercise intensities a substantial amount of glucose taken up by the limb is released as lactate(71). Further, research in sedentary mice has shown the majority of glucose taken up by skeletal muscle is converted to lactate prior to entering the TCA cycle for oxidation(20, 72). Further research is needed to quantify the oxidative vs. non-oxidative partitioning of glucose taken up by skeletal muscle during exercise in humans.

A small subset of literature *has* recognized and quantified lactate as an oxidative substrate during exercise. Lactate oxidation is linearly correlated with oxygen consumption(38, 44, 73) and active limbs account for the majority of whole-body lactate oxidation(44). Oxidation is the predominant route of lactate disposal during exercise(37, 44, 73). Emhoff et al.(37) estimated that during exercise 21% of lactate R_d is accounted for by direct oxidation and 68% via indirect oxidation (conversion to glucose prior to oxidation). This calculation addresses the aforementioned issue of neglecting to account for the contribution of direct lactate oxidation. Using this method, Emhoff et al.(37) estimated glycogen accounts for 34% of TEE, which is close to the lower-range estimates of glycogen contribution in other studies using a similar exercise protocol(32).

Interestingly, the same muscle cell or tissue can concurrently take up lactate, oxidize, and release lactate to the circulation(74–76). It has been proposed that muscle cells are compartmentalized, with various parts of the muscle cell handling lactate differently. There could be a glycolytic compartment close to glycogen stores that breaks down glycogen and releases lactate into the circulation, and an oxidative compartment close to mitochondria that promotes lactate uptake and oxidation(76). The two compartments are not thought to be

mutually exclusive but may interact with each other to potentially promote energy transfer within the muscle. The high number of intramyofibrillar mitochondria are hypothesized to burn cytosolic lactate, in contrast to subsarcolemmal mitochondria that are hypothesized to burn plasma lactate (76–79). Further work is needed, however, to elucidate the details and demonstrate direct evidence of lactate compartmentalization.

Information about the spatial distribution of skeletal muscle glucose metabolism can be obtained via positron emission tomography (PET) with an ^{18}fluorine-labeled glucose analog tracer (FDG) that is taken up by cells and phosphorylated, but not metabolized further. The tracer is administered throughout exercise and accumulates in skeletal muscle. Imaging can be completed either during localized muscle movement (e.g. one leg knee extension) or post-exercise for more complex movements (e.g. running). The limited studies using PET-FDG to study complex movements have revealed in young male subjects, running exercise mainly stimulates glucose uptake in posterior lower limbs(80, 81). Cycling exercise, however, stimulates glucose uptake in the iliacus and anterior thigh(82). For a more detailed discussion of PET-FDG during acute exercise, including methodological considerations, advantages, and limitations see the extensive review by Rudroff and colleagues(83).

Skeletal Muscle Fat Oxidation

Like carbohydrates, most estimates of skeletal muscle fat oxidation during exercise come from systemic measures. Fat oxidation can increase over 500% during exercise(50, 84). Unlike carbohydrates, absolute and relative fat oxidation do not scale with exercise intensity (Table 9) (Figure 3). The *relative* contribution of fat to TEE is greatest during low-intensity exercise(24, 25, 32, 47, 48)[2]. *Absolute* rates of fat oxidation peak during mid-intensity exercise(24, 25, 32, 47, 48). At high workloads, however, FFA concentrations, total fat oxidation and the relative contribution of fat to TEE all decrease(24, 25, 33). The reduction in fatty acid oxidation at high exercise intensities, however, is not solely due to decreased plasma availability, as lipid infusions to restore FFA concentrations do not restore fat oxidation to the levels observed during mid-intensity exercise(33). The regulation of fat oxidation during exercise involves many different sites of control, including fatty acid uptake, transport in the cytoplasm, transport in the mitochondria, and more(57). Inhibition of CPT1 activity and CPT1 sensitivity to carnitine seems particularly important in limiting fatty acid mitochondrial transport during high intensity exercise(57, 85). Regulation of the balance between fat and glucose oxidation during exercise has long-been the subject of debate and is reviewed in more detail elsewhere(8, 57).

Fatty acids become an increasingly important oxidative fuel during prolonged periods of work (>90 minutes). As exercise duration progresses, carbohydrate contribution to TEE starts to fall as muscle glycogen stores are degraded and there is increased reliance on oxidation of plasma substrates, including FFAs(25, 50, 86, 87). As previously discussed, in addition to being oxidized, plasma FFAs can also be re-esterified into newly synthesized triglycerides (TGs). In humans, re-esterification accounts for 70–85% of total fat flux at rest(47, 87, 88). While exercise is typically thought of as a catabolic state, re-esterification does not fall to zero but instead modestly decreases to between 35–65% of total fat flux(47, 87, 88).

Similar to the previously discussed method of estimating glycogen oxidation, discrepancies between RER-measured fat oxidation and plasma FFA R_d have been used to estimate non-plasma sources of fat oxidation. Muscle stores fat as intramuscular triglycerides (IMTGs). Efforts to quantify IMTG oxidation have been hampered by technical limitations and inconsistent results between studies(89). While there is net IMTAG utilization during prolonged exercise, the large majority of IMTAGs are not oxidized, even after 12 hours of exercise(90, 91). In general, it is thought IMTGs become a more important fuel source in endurance-trained athletes(92).

While there have been considerable studies focusing on skeletal muscle nutrient use during acute exercise, experiments leveraging modern advances in stable isotope tracing and mass spectrometer techniques could offer valuable insight. Many studies have relied on extrapolations about skeletal muscle fuel use based on systemic measurements. These extrapolations ignore fuel consumption by other organs. Additionally, even studies using A-V catheterization across the muscle bed cannot query specific skeletal muscles or fiber types within a muscle. Femoral vein catheterization will also capture blood drained from tissue outside of skeletal muscle, including subcutaneous adipose depots(93). It is now possible to identify stable isotopes in downstream tissue metabolites. Future studies could directly measure nutrient use in a specific muscle, fiber type, or cell type by detecting labeled carbons in metabolic pathways. Pathways outside of oxidation such as the pentose phosphate pathway, nitrogen-handling pathways, and glycogen synthesis and disposal pathways could be interrogated as well. Also, much of the work to date has focused on carbohydrates and long-chain fatty acids. However, there are thousands of metabolites present in muscle. Future studies could investigate previously underappreciated exercise-responsive metabolites, such as the branched-chain amino acids (BCAAs), which have been implicated in many chronic metabolic diseases(54).

The Pancreas Releases Key Glucoregulatory Hormones During Exercise

Other organs support skeletal muscle work by coordinating systemic metabolic adjustments. Hormones are important stimuli regulating these metabolic adjustments. The endocrine pancreas controls the secretion of two key hormones, insulin and glucagon, that regulate blood glucose homeostasis. The alpha-cells of the pancreas secrete glucagon, which promotes HGP. Glucagon concentrations in the portal vein, which delivers blood from the pancreas to the liver, are 10-fold higher during exercise compared to arterial blood in exercising dogs(94). It is not known, however, if the high concentrations of glucagon in the portal vein are due to increased secretion from the pancreas or reduced blood flow to the liver(95). The beta-cells of the pancreas secrete insulin[89]. Insulin secretion falls during acute exercise, a response possibly mediated by alpha adrenergic inhibition of beta cells(96–98). Functions of the endocrine pancreas during exercise have received the most attention yet the exocrine portion comprises most of the pancreatic mass. The exocrine pancreas regulates the synthesis, storage, and release of digestive enzymes. Serum lipase significantly increases during high-intensity exercise in rats(99), while pancreatic bicarbonate release decreases in exercising dogs(100). The physiological function and cross-species relevance of these changes is unclear. The energetic costs in the pancreas of both endocrine and exocrine

responses to exercise are also unknown, and neither is pancreatic nutrient consumption during exercise (Figure 3).

The Liver Plays a Key Role in Meeting the Energetic Demands of Exercise

The liver has several vital roles during exercise, including taking up, converting, and releasing potential energy as well as removing toxic compounds from the circulation and helping to mitigate oxidative stress(101, 102). Several converging signals help activate and maintain hepatic GLY and GNG during exercise. Exercise stimulates ATP breakdown which leads to an accumulation of ADP and AMP in the liver that go on to activate GNG(103, 104). The exercise-induced fall in insulin primarily stimulates liver GLY(98, 105). In conjunction, the exercise-induced rise in glucagon stimulates hepatic GNG and further augments GLY(95, 105). Other circulating factors, including the myokines IL-6 and apelin, are thought to also stimulate HGP, highlighting the multifactorial regulation of glucose homeostasis during exercise(102, 106, 107).

GNG allows the liver to recycle carbon molecules from glycerol, lactate, and certain amino acids and release them as glucose for exercising skeletal muscle. The increase in amino acids being recycled leaves nitrogen leftover that must be converted to a non-toxic form and excreted(102). Additionally, increased AMP breakdown means AMP must be deaminated to IMP and nitrogen, which further increases the demand to convert and excrete nitrogen(102). The liver handles this nitrogen burden via the urea cycle, which converts toxic ammonia to urea which can then be released into the bloodstream and excreted via the kidneys(102). Strikingly, during exercise the liver becomes a net consumer of glutamine and conversion of glutamine to urea increases 80%, highlighting the importance of nitrogen removal(108).

The increases in GLY, GNG, and urea production place an energetic burden on the liver. This demand is hypothesized to be met via increased fatty acid oxidation(71, 102) (Figure 3). Catheterization of the brachial artery and liver vein revealed increased liver uptake up of certain medium (6:0 and 8:0) and long chain (14:0, 14:1, 16:1) fatty acids during exercise(109). However, direct evidence of liver nutrient oxidation during exercise is lacking. Further, attempts to estimate or measure hepatic oxygen consumption during exercise in humans have produced conflicting results. Recent work by Hu et al.(109) observed an exercise-induced 2-fold increase in hepatic oxygen consumption with no change in hepatic blood flow, while others have observed significant decreases in hepatic blood flow during exercise(110, 111). However, Hu et al. controlled for fasting status of the subjects and used modern blood gas analysis methods, rendering their measurements likely more accurate. Future experiments could help resolve these questions by directly measuring liver nutrient oxidation.

Renal Contributions to Exercise-Induced Glucose Production are Unknown

The kidneys perform several critical functions during exercise, including maintaining peripheral vascular resistance, secreting endocrine factors, and regulating fluid & electrolyte balance. The kidneys release various circulating molecules that help coordinate systemic responses. Plasma renin, an enzyme in the hormonal system that regulates blood pressure

and fluid balance, increases with exercise intensity(112–114). The kidneys also release norepinephrine, a hormone that helps mediate cardiovascular and metabolic responses to exercise, and plasma norepinephrine levels increase with exercise intensity(112–114). How much the kidneys are responsible for the exercise-induced increase in circulating norepinephrine, however, is not known.

The kidneys are also capable of performing GNG. The role of renal GNG during exercise has not been investigated. However, a suite of elegant studies from Christopher Meyer's group using renal A-V balance and isotope infusions in human subjects exposed to epinephrine offer valuable insight that can be used to generate hypotheses about renal GNG during exercise(115–118) (Figure 3). There is evidence for organ-specific selectivity of gluconeogenic precursors. The kidney is the primary site for glutamine conversion to glucose, accounting for ~70% of glutamine's overall incorporation into systemic glucose in sedentary, postabsorptive humans(117, 118). At rest, glutamine and alanine are responsible for 19 and 1.4% of total renal glucose release, respectively(118). Epinephrine increases systemic glucose R_a and renal glucose R_a more than 2-fold(116, 118). Epinephrine also specifically increases renal glucose production from glutamine, and the kidneys account for 90% of overall glutamine incorporation into glucose after epinephrine exposure(118).

Despite being a major site of glutamine conversion to glucose, the kidneys still use lactate as a major carbon source for GNG. Epinephrine increases renal glucose production from lactate, and lactate accounts for 85% of renal epinephrine-stimulated glucose release(116). Lactate is therefore the predominant precursor for both the kidneys and the liver during epinephrine-stimulated GNG(116). Renal and hepatic glucose release from glycerol increase more modestly in response to epinephrine, and glycerol accounts for only 7–9% of total renal glucose release(116). Epinephrine-stimulated renal uptake of lactate and glycerol increased without any change in their fractional extraction, highlighting the importance of substrate delivery to renal fuel selection. Given that the calculated relative contributions of gluconeogenic precursors to glucose add up to over 100% across studies, however, there is obviously some degree of technical error between trials(116, 118).

Overall, the liver is estimated to account for ~60% and the kidneys ~40% of epinephrine-induced GNG(116). These studies could lead to interesting hypotheses about the kidneys' role in glucose homeostasis. To date, however, there have been no studies directly measuring renal glucose production during exercise. Stable isotope tracing experiments alone (without A-V catheterization) would not answer this question. However, they could reveal if there are changes in glucose fractional labeling from gluconeogenic precursors, both in circulation and in the kidney itself. For example, a large increase in glucose derived from glutamine could indicate an increase in kidney contribution to GNG. More experiments are needed to directly test these hypotheses, however.

WAT Serves as an Important Fuel Reservoir, While BAT Remains Unexplored During Acute Exercise

White adipose tissue (WAT) is an important source of energy stores. Exercise stimulates WAT lipolysis and FFAs can then be used as fuel by other organs. A variety of signals are

thought to stimulate WAT lipolysis during exercise, including rising plasma catecholamines, cortisol, glucagon, and natriuretic peptides, as well as falling insulin(119). While lipolysis itself does not require ATP, re-esterification of fatty acids and glyceroneogenesis both do(120) and could contribute to WAT energy expenditure during exercise. Re-esterification accounts for the majority of basal energy expenditure in WAT(120, 121). The *relative* proportion of fatty acids re-esterified decreases during prolonged exercise(87), but re-esterification is still active and may contribute to energy expenditure in WAT during exercise.

Subcutaneous adipose tissue blood flow increases during moderate intensity exercise(122). Determining oxygen and nutrient uptake in adipose tissue, however, has proved challenging. A-V catheterization of the anterior abdominal wall revealed no significant changes in oxygen uptake during exercise at 40% VO_{2max} or 60% VO_{2max} in human subjects(123). However, this could be confounded by the very small A-V differences measured and high blood flow rates during exercise. Additionally, there could be large variability between different fat depots. A lack of rigorous studies makes it difficult to determine if and how exercise changes WAT energy expenditure and nutrient consumption (Figure 3).

While traditionally thought of in the context of chronic exercise training and adipocyte browning, brown adipose tissue (BAT) could have a role in acute bouts of aerobic exercise as well. Exercise induces a number of factors that could stimulate BAT(124), including circulating norepinephrine, ANP(125), irisin(126, 127), and IL-6(128, 129). However, the role of BAT in acute exercise is currently unclear and there is scarce research about BAT metabolism during acute bouts of exercise. Norepinephrine promotes lipolysis and glucose uptake into BAT(124, 130) and there is significant norepinephrine release during exercise. It is unknown, however, if acute exercise causes changes in BAT nutrient use. Future isotope tracing experiments could quantify exercise-induced nutrient consumption in both WAT and BAT.

Exercise Increases Myocardial Workload and Circulating Fuel Oxidation

A principal role of the cardiovascular system during exercise is increasing cardiac output (CO) to help augment blood flow and oxygen delivery to skeletal muscle. During exercise, CO increases with workload until the maximum oxygen consumption is reached(131). Myocardial work, oxygen demand, and fuel consumption are tightly coupled(132). Studies have leveraged catheterization of the coronary sinus (CS), which drains blood from the heart, and an artery (A/CS sampling) to measure myocardial fuel use and metabolite secretion(132) (Figure 1). Exercise increases myocardial oxygen consumption(133–136), up to 400% during heavy exercise in humans(137). Almost all the heart's oxygen consumption is dedicated to ATP generation(132). Unlike skeletal muscle, which uses PCr as an immediate fuel for ATP regeneration, the myocardial PCr/ATP ratio does not change with exercise(138–140), reflecting the remarkable dynamic range of cardiac oxidative capacity. Cardiac glycogen content has been shown to decrease with exercise in rats(141, 142), but it is unknown if and how the heart relies on glycogen during exercise in humans.

In sedentary, fasted subjects, 20% of myocardial-extracted glucose is immediately oxidized, 13% is released as lactate, and the remaining ~65% is hypothesized to contribute to glycogen synthesis(143). Moderate exercise more than doubles myocardial glucose uptake and increases the percent of myocardial-extracted glucose that is immediately oxidized to ~50%(133). Interestingly, though, there is no correlation between arterial glucose concentrations and myocardial glucose extraction(135). At higher exercise intensities, myocardial glucose uptake actually decreases to levels observed at rest(144) Instead, rises in arterial lactate concentrations during exercise become correlated to linear increases in myocardial lactate extraction(135, 137) (Figure 3). Unlike skeletal muscle, there is no evidence of a plateau in myocardial lactate uptake(137). Further, during heavy exercise with high arterial lactate levels, there is no evidence of myocardial lactate release(137). Studies have also shown that heart uptake of lactate correlates to arterial lactate concentrations, but release does not(133), suggesting the heart compartmentalizes lactate metabolism(76, 133).

Exercise also increases myocardial uptake and extraction of FFAs(133, 135, 145), seemingly regardless of plasma FFA concentrations(133, 135). Despite these changes in uptake and extraction, there is no significant difference in the relative contributions of total carbohydrate and lipids to myocardial oxidation during prolonged exercise(135). These measures, however, do not account for ketone bodies, amino acids, or stored metabolites such as glycogen or TAGs. Future isotope tracing studies could address these unaccounted-for metabolites. Tracing experiments could also test if there is any direct glucose oxidation during exercise, or if the heart solely relies on lactate as its carbohydrate fuel source.

Respiratory System Metabolic Demands Are Unknown During Exercise

Key functions of the respiratory system during exercise include preserving the ratio of alveolar ventilation to carbon dioxide production (Va/VCO2) and the arterial partial pressure of carbon dioxide (PCO2)(146, 147). There are several respiratory responses that help maintain Va/VCO2 and PCO2 close to resting values (i.e., maintain respiratory system homeostasis). One method is increasing ventilation via hyperpnea, or even hyperventilation during intense exercise(146). This increase in breathing frequency is coupled with changes in respiratory muscle function. The larger respiratory muscles, including the diaphragm, abdominal expiratory muscles, and accessory inspiratory and expiratory muscles all help meet ventilatory requirements during exercise. Many of these muscles have high mitochondrial and capillary density(146, 148). Despite their large capacity, however, respiratory muscle oxygen consumption is low compared to limb muscles during exercise. Respiratory muscle oxygen consumption is thought to account for only 8–10% of VO_{2max} during exercise(146, 149, 150), but more research is needed to better characterize respiratory muscle energy demands and nutrient handling.

Fuel use and nutrient consumption in the lung tissue itself are not well understood. Studies in isolated perfused lung tissue have shown ~22% of glucose taken up is oxidized while ~40% is converted to lactate(151), but it is unclear how nutrient partitioning changes when more fuel sources, such as fatty acids, are available. Further, these *ex vivo* methods of assessing tissue metabolism often do not accurately recapitulate *in vivo* physiology. Surfactant-producing cell types in the lung have a high demand for lipid synthesis, an

ATP-costly cellular process(151). There is limited evidence that acute exercise increases production of pulmonary surfactant(152) and changes its composition(153). How energy-costly these processes may be during exercise, however, is not known. Intriguingly, the lung expresses AMP-protein kinase (AMPK), a master regulator and energy sensor, at relatively high levels compared to other tissues(151). It would be interesting to see if exercise changes lung AMPK phosphorylation status, as it does in other tissues such as skeletal muscle and the liver(154), and whether AMPK helps regulate lung nutrient handling during exercise.

Other organs that are not mentioned here, such as the brain, gut, skin, and bone, contribute to the body's response to acute exercise. These demands on organs likely require fine-tuned metabolic changes. Experiments are needed to characterize and quantify how organ-specific energy demands and nutrient consumption change with exercise.

Influence of Sex on Fuel Use During Exercise

Sex and sex hormones are variables that potentially regulate nutrient use during exercise (Table 2, Table 10). Most investigations characterizing substrate oxidation have used male subjects. There is some evidence that human female subjects have a lower RER during exercise compared to males(155–160). However, the effect of sex on RER has not been consistent, with some studies finding no sex difference(31, 32, 66, 67, 161). No obvious differences in subject number, exercise protocol, or fasting durations explain these disparate results; it is thus difficult to endorse any concrete conclusions about the effect of sex on RER. In contrast, studying the effect of menstrual cycle phase on RER has produced more consistent results, as the majority of studies (27, 156, 162, 163) have found no effect on RER. Still, more rigorous investigations, well-controlled for potential confounding variables, are needed to better understand the influence of sex and sex hormones on RER and how this may vary during different exercise contexts.

Despite evidence that females may have a lower RER and oxidize more fat, there are no significant effects of sex on palmitate R_a during exercise (Table 6)(67, 161). There is also no evidence menstrual cycle phases significantly affect palmitate R_a during exercise(163). However, the markedly higher fatty acid flux responses to exercise in post-menopausal females(88) suggests there could be interesting hormonal or age-related influences on exercise-induced fatty acid flux that are not captured in experiments using younger subjects.

Studies examining the effect of sex on glucose flux in exercising human subjects have found inconsistent results (Table 2). Some groups have found no significant effect of sex on glucose R_a (31, 32, 155). Others have found glucose R_a is higher in males than females when expressed per unit of body weight, but this effect is abrogated when corrected for differences in fat free mass (FFM)(26). In contrast, several groups have found a persistently higher glucose R_a in males during exercise even after accounting for FFM(66, 67, 156). The latter studies used long-duration (90 min.), mid-intensity (~60% VO_{2max}) cycling protocols after an overnight fast, while the studies failing to find sex differences used either shorter protocols or shorter fasting periods (3 hours), suggesting that sex differences may only arise during more nutrient depleted states. The effect of sex (outside of differences in FFM) could be partially attributable to estrogen, as short-term 17β-estradiol administration to

men decreases their glucose flux during exercise(155). Given the potential impact of sex hormones, the effects of menstrual cycle phases on glucose flux have also been tested, to again conflicting results. Some groups have found no effect of menstrual cycle phase on glucose R_a(27, 163). Others have found glucose R_a was higher in the follicular phase compared to the luteal phase, but this difference was only apparent at certain exercise intensities and durations(30, 64, 156). While both sex and menstrual cycle phases may have some effect on glucose kinetics, both seem subtle compared to the larger effect of exercise and nutrition.

As previously mentioned, there is evidence that females may have a lower RER during exercise and rely more heavily on fat oxidation. Tracer studies, however, have generally failed to find sex differences in the relative contributions of carbohydrates and plasma FFA to TEE during steady-state exercise(26, 66, 67) (Table 10). There are differences, however, in how each sex responds to *changes* in exercise intensity. Both males and females increase absolute rates of carbohydrate oxidation with higher exercise intensity. Females, though, also increase the absolute rate of fat oxidation while male subjects do not, and thus male subjects increase the relative contribution of carbohydrates to TEE at higher exercise intensities(26). There are conflicting findings about the effect of sex on use of carbohydrate sources. Two groups found no sex differences in the relative contributions of plasma glucose and glycogen to total carbohydrate oxidation(66, 67), while one study using a shorter exercise protocol (30 min. compared to 90 min.) found the relative contribution of muscle glycogen was lower in females(26). Another observed that muscle glycogen contribution was higher in females compared to males, but only when females were in the mid-follicular phase of the menstrual cycle(156). These conflicting results suggest the effect of sex on tracer-based measurements of substrate oxidation could be context-specific and influenced by a myriad of other variables that warrant further study.

Influence of Carbohydrate Ingestion on Fuel Use During Exercise

Nutrient supplementation substantially impacts substrate oxidation, in an exercise intensity and time-dependent manner. Carbohydrate ingestion is the most well-studied method of nutrient supplementation (Table 11). During prolonged, low intensity exercise (25% VO_{2peak}), glucose ingestion suppresses mildly lipolysis and increases the relative contribution of carbohydrates to TEE by the end of exercise(164). Some studies using glucose ingestion during mid-intensity exercise have observed glucose ingestion mildly suppresses lipolysis(164) and decreases fat oxidation(35, 63, 165). The decrease in fat oxidation corresponds to an increase in glucose R_d(164). Interestingly, this decrease in fat oxidation is specific to long-chain fatty acids(165), which supports the idea that fatty acid oxidation is at least in part regulated by transport into the mitochondrial matrix. In other studies at similar exercise intensities, however, glucose ingestion does not suppress fat oxidation(65, 164). Similarly, glucose ingestion increased the relative contribution of plasma glucose to total carbohydrate oxidation in most(35, 63, 64, 166) but not all(164) studies. Differences in reporting methods make it difficult to determine whether the total CHO consumed by subjects differs between trials, but this important variable could explain the inconsistencies. Higher doses of glucose ingestion can help prevent or attenuate the prolonged exercise-induced fall in the relative contribution of carbohydrates to TEE

observed in fasting conditions(63, 65). Interestingly, however, there is no effect of glucose ingestion on skeletal muscle glycogen oxidation(35, 63, 65, 166).

Influence of Age on Fuel Use During Exercise

Ample evidence (both empirical and anecdotal) has shown that exercise capacity declines with age(167), but much less is known about how age changes fuel use, and only a handful of studies have compared substrate flux and oxidation across age groups. At the same absolute exercise intensity, older adults (~73 years) oxidized less fat and more carbohydrates compared to younger adults (~26 years)(168). This difference did not persist at the same relative exercise intensity, however, and thus likely reflects a difference in fitness level rather than fuel oxidation. During high-intensity exercise, middle-aged adults (~61 years) with metabolic syndrome had blunted exercise-induced lipolysis and plasma glucose clearance compared to young, healthy controls(169). It was unclear, however, whether age or metabolic syndrome drove these differences. There was no difference between groups at lower exercise intensities. Together, the scarce available evidence suggests that aerobic fitness, rather than age itself, may dictate substrate use, but more comprehensive studies are sorely needed.

Pathway Flux is an Important Determinant of Substrate Oxidation

There are two important factors that determine nutrient oxidation rates: the relative contribution of a nutrient to its oxidative pathway (in the great majority of cases the TCA cycle) and the rate at which the pathway is operating (i.e. TCA cycle flux). These are distinct determinants, akin to which fuel an engine is receiving versus how fast the engine is burning the fuel. While many studies have examined the contribution of various nutrients to oxidation, there is less evidence for how exercise affects TCA cycle flux- or how fast the TCA cycle reactions are occurring. Studies using A-V sampling techniques use oxygen consumption rates (calculated from the O_2 gradient) as a proxy for TCA cycle flux. Skeletal muscle has immense capacity for increasing TCA flux. During short, exhaustive exercise in fasted men, TCA cycle intermediate pool size increases 5-fold and TCA cycle flux increases 100-fold in leg muscle by maximum effort(13). However, using A-V O_2 gradients only provides information about the organ bed a given vein drains, and cannot differentially measure TCA flux in specific tissues (e.g. different muscle fiber types) or cell types. Further, not all organs or tissues of interest have readily available veins from which to sample.

Future Directions

The critical importance of exercise to metabolic health has generated considerable interest over the years in studying exercise metabolism. The current literature clearly shows exercise produces a wide range of stimuli that activates downstream signaling pathways and marked physiological responses across many organs. These physiological responses are accompanied by changes in how organs take up, produce, and use nutrients. Most of the research so far has focused on skeletal muscle, which primarily relies on glycogen stores, circulating glucose produced from the liver, lactate both released from and taken up by skeletal muscle (i.e. compartmentalization), and fatty acids liberated from adipose tissue. The relative proportions of dependence on these fuel sources heavily depend on exercise

intensity and duration, and to a lesser (yet still important) extent on nutritional status, sex, and age. Liver-derived ketones and proteolysis-derived amino acids serve only as minor fuel sources during most aerobic exercise. It is evident that other organs also experience notable metabolic perturbations, but a lack of rigorous studies means our understanding of fuel use in these organs during exercise is more limited. Recent advances in stable isotope tracing, chromatography, and mass spectrometry techniques allow for more comprehensive, sensitive, and specific quantification of *in vivo* cell metabolism. Several lacunae and thus opportunities for future studies exist, e.g.:

- Studies that focus on broader populations, e.g. females (pre and post menopause), people with metabolic disorders, people with metabolically healthy vs. unhealthy obesity, older adults, etc.
- Evaluation of specific metabolic pathways in tissue samples, leveraging isotope tracers and bulk mass spectrometry, or the rapidly improving 2D imaging mass spectrometry
- Integration of metabolic data with other ‘omics data (e.g. combining isotopic tracing with metabolomics or gene expression data)
- Broadening the range of cell types/tissues/organs examined, beyond skeletal muscle, to better investigate the holistic effects of exercise

Exercise remains our best therapy for numerous diseases. Understanding the metabolic underpinnings of exercise could thus be foundational for development of new therapies targeting the prevention, management, and treatment of many chronic metabolic diseases.

Acknowledgements

JA is supported by F31NR019925-01 and ZA is supported by the NIH (DK135958, HL152446).

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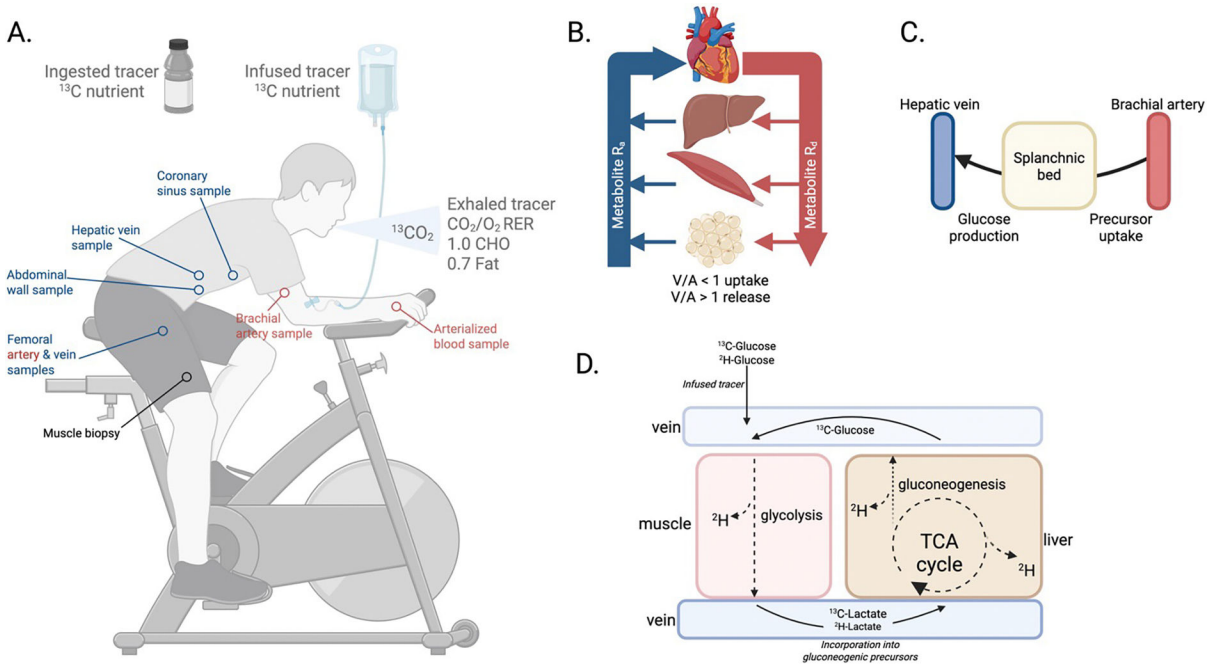

Figure 1.

Methods to quantify metabolic responses to exercise in human subjects. (A) Tracers can be introduced via an intravenous infusion or oral ingestion and detected in biological samples collected from blood, tissue, or breath. The ratio of inhaled O_2 to exhaled CO_2 can also be used to calculate the Respiratory Exchange Ratio (RER) and quantify systemic fuel oxidation. (B) Comparing metabolite levels in arterial (A) vs. venous (V) samples can be used to measure fuel uptake and release across the organ bed that a given vein drains. A warmed venous hand sample is often used as a less-invasive proxy for arterial blood, as labeled in (A)(170). Metabolite R_a is the rate at which a metabolite is collectively released from tissues into the bloodstream. Metabolite Rd is the rate at which a metabolite is collectively consumed by tissues from the bloodstream. (C) Hepatic gluconeogenesis has been estimated by catheterizing the brachial artery and hepatic vein to measure gluconeogenic precursor uptake and glucose release, respectively. (D) Gluconeogenesis has also been estimated by measuring the difference in R_a between ^{13}C labeled glucose, in which the ^{13}C gets recycled in the Cori cycle, and ^{2}H glucose, which does not get recycled.

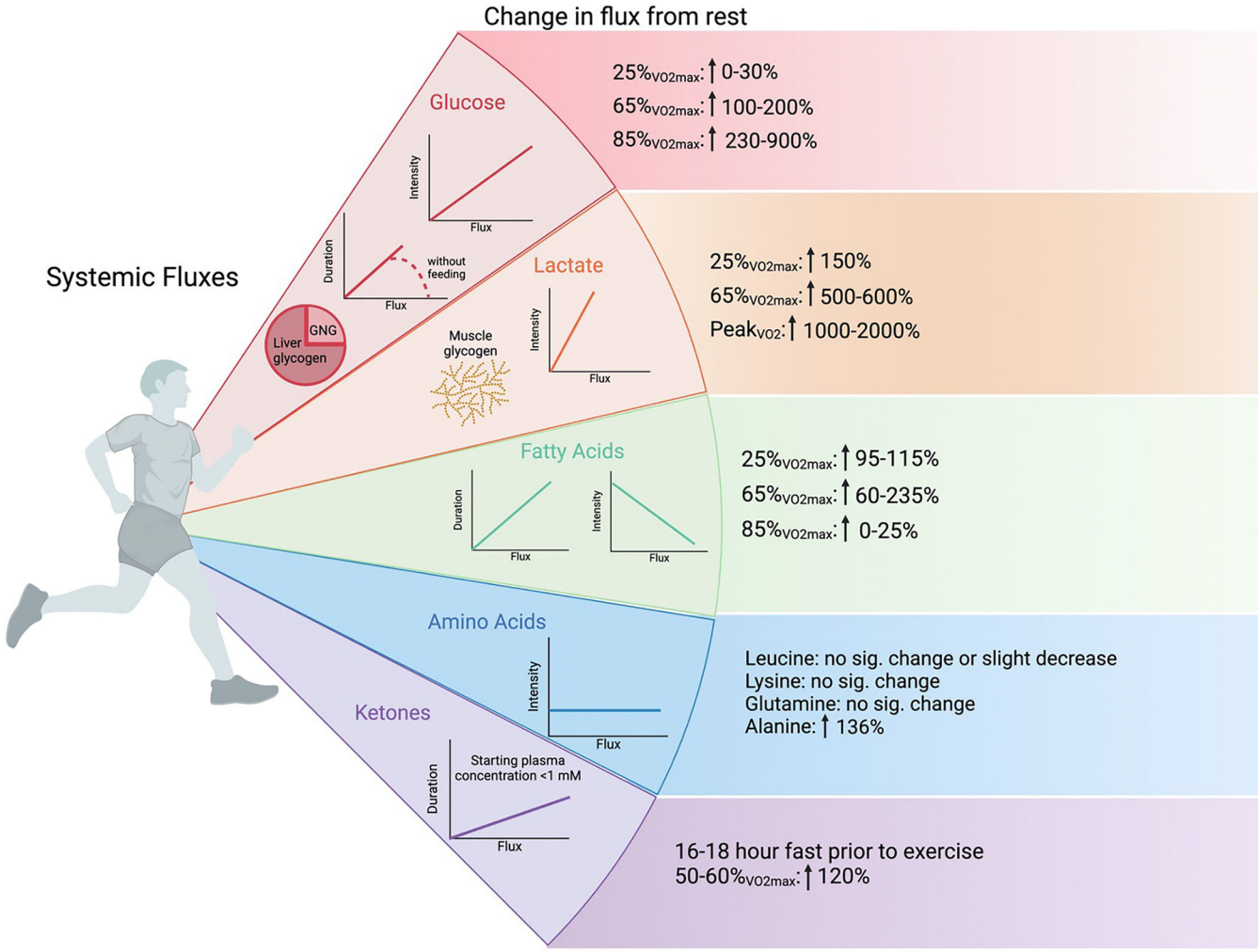

Figure 2.

Model of findings summarized from studies measuring systemic nutrient fluxes during acute aerobic exercise in humans. GNG: gluconeogenesis, VO_{2max}: maximum volume of oxygen consumed (ml/min or ml/kg/min).

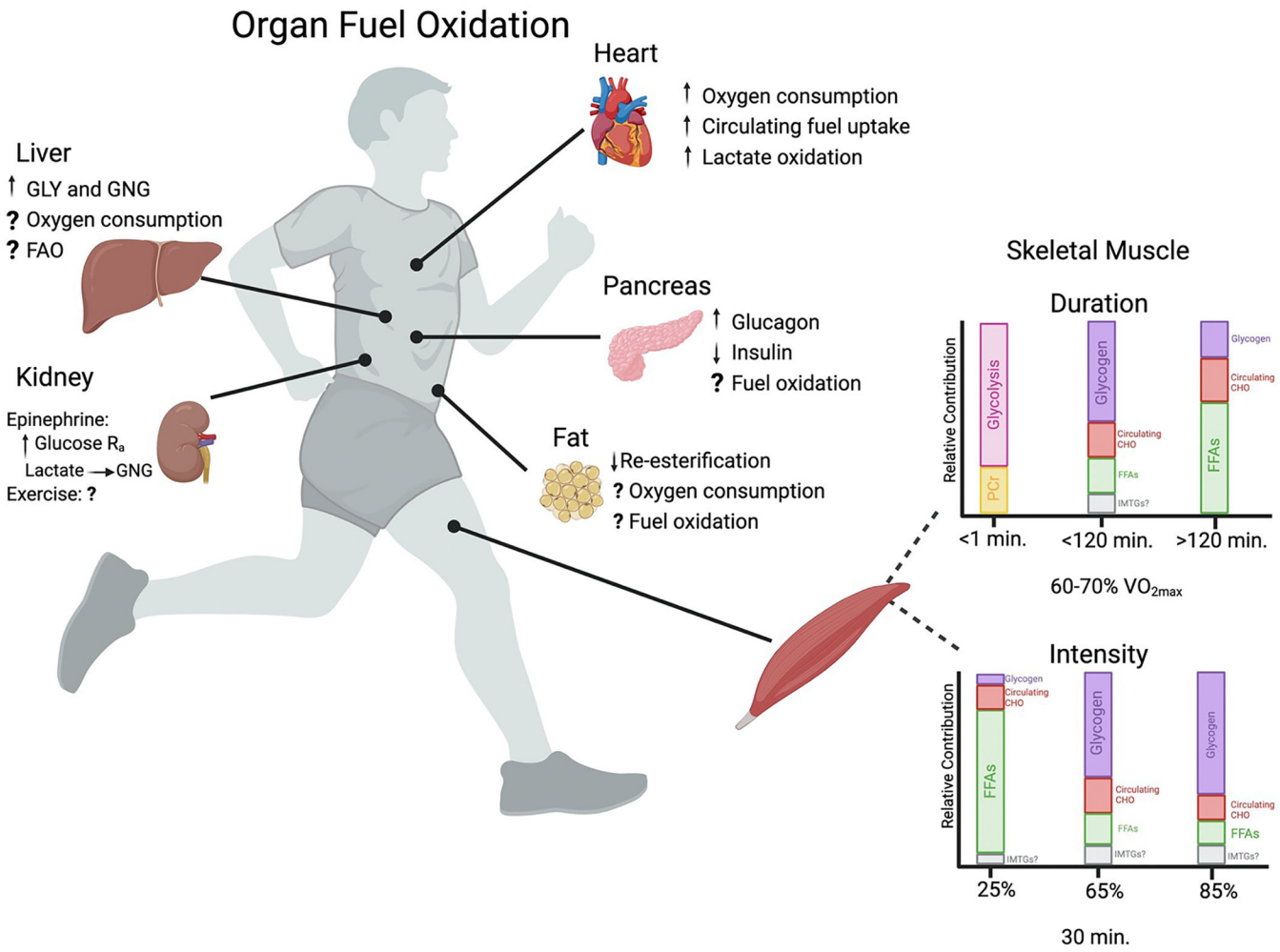

Figure 3.

Model of findings summarized from studies examining physiologic roles and/or fuel oxidation of specific organs during acute aerobic exercise in humans. GLY: glycogenolysis, GNG: gluconeogenesis, CHO: carbohydrate, FFA: free fatty acids, IMTGs: intramuscular triglycerides, VO_{2max}: maximum volume of oxygen consumed (ml/min or ml/kg/min).

Table 1. Glucose flux during acute exercise in fasted male subjects.

Glucose Ra is expressed as percent increase from rest unless stated otherwise. OF: overnight fast. Wmax: maximum power. T: trained. UNT: untrained. M: male. A ~ symbol indicates estimated from figure.

Cite	Study Design	Glucose R_a		RER
(25)	5 T M 10–12 hr. fast Cycling at: Trial 1: 120 min. at 25% VO_{2max} Trial 2: 120 min. at 65% VO_{2max} Trial 3: 30 min. at 85% VO_{2max}	25% VO_{2max}	At 30 min.: ↑ ~30% At 60 min.: ↑ ~20% At 120 min.: ↑ ~18%	0.73
		65% VO_{2max}	At 30 min.: ↑ ~114% At 60 min.: ↑ ~125% At 120 min.: ↑ ~210%	0.83
		85% VO_{2max}	At 30 min.: ↑ ~420%	0.91
(36)	6 UNT M OF 120 min. cycling at 60% VO_{2peak}	↑ ~200% Peaked at 120 min.		0.91
(33)	6 T M 5–6 hr. fast 30 min. cycling at 80–85% VO_{2max}	↑ ~228% from 20–30 min.		Not provided
(171)	11 T M OF Cycling to fatigue at: Trial 1: 80% VO_{2max} Trial 2: 100% VO_{2max}	No difference between intensities		Not provided
(21)	9 UNT M 4.5–5 hr. fast 60 min. of cycling at: Trial 1: 45% VO_{2peak} Trial 2: 65% VO_{2peak}	Trial 1: ↑ ~60% Trial 2: ↑ ~130%		Trial 1: 0.93 Trial 2: 0.96
(24)	8 T M OF 90 min. of cycling, 30 min. each: Stage 1: 40% W_{max} Stage 2: 55% W_{max} Stage 3: 75% W_{max}	Stage 1: ↑ ~49% Stage 2: ↑ ~130% Stage 3: ↑ ~324%		Not provided
(172)	6 T M OF Roller skiing at~75% VO_{2max}: 40 min. continuous arm and leg (CAL), 10 min. arm double poling (A), 10 min. arm and leg (AL)	CAL: ↑ 91% increase from rest Plateaued 24–40 min. No significant change with A and AL		CAL: 0.93 A: 1.00 AL: 0.93
(50)	8 T M OF 120 min. cycling at 60% VO_{2max}	↑ 89% Peaked at 120 min.		15 min.: 0.91 120 min.: 0.85
(86)	10 UNT M OF 120 min. cycling at 50% W_{max}	↑ 101% Peaked at 120 min.		Not provided

Table 2. Glucose flux during acute exercise in female subjects.

Glucose R_a is expressed as percent increase from rest unless stated otherwise. OF: overnight fast. MC: menstrual cycle phase. M: male. F: female. T: trained. UNT: untrained. A ~ symbol indicates estimated from figure.

Cite	Study Design	Glucose R_a		RER
(31)	16 M, 12 F OF MC: Follicular Cycling 14 min. 88% VO_{2max}	↑ 900% No sig. sex diff. Peaked at 14 min.		M: 0.99 at exhaust F: 1.00 at exhaust No sig. sex diff.
(32)	8 T F 10–12 hr. fast Cycling Trial 1: 60 min. 25% VO_{2max} Trial 2: 30 min. at 85% VO_{2max} Trial 3: 60 min. at 65% VO_{2max}	25% VO_{2max}: No change 65% VO_{2max}: ↑ ~120% Plateaued 5–60 min. 85% VO_{2max}: ↑ ~400% Peaked at 15 min. No sig. sex diff.		25% VO_{2max}: 0.756 65% VO_{2max}: 0.816 85% VO_{2max}: 0.906 No sig. sex diff.
(155)	8 M, 8 F UNT, MC: mid-follicular 3 hr. fast Cycling 90 min. 60% VO_{2peak}	Males: ↑ ~148% Females: ↑ ~115% No sig. sex diff.		Males: ~0.89 Females: ~0.84 Females sig. lower RER
(64)	8 T F MC: luteal & follicular OF Control or 6% Glucose Solution ingestion during exercise Cycling 120 min. 70% VO_{2peak}	Control	At 60 min.: ↑ ~70% At 120 min.: ↑ ~60% luteal phase ↑ ~120% follicular phase Peaked at 120 min.	Not provided
		6% GS	At 60 min.: ↑ ~111% At 120 min.: ↑ ~150% No effect of MC Peaked at 120 min.	
(30)	6 F UNT MC: luteal & follicular 10–12 hour fast Cycling 50 min. 25 min. at 41% VO_{2peak} 25 min. at 52% VO_{2peak}	41% VO_{2peak}:	↑ 43%, no effect of MC	41% VO_{2peak}: 0.83, no effect of MC 52% VO_{2peak}: 0.86 Follicular and 0.84 Luteal
		52% VO_{2peak}:	Follicular: ↑ 143% Luteal: ↑ 107%	
(163)	13 F MC: early follicular, mid-follicular, midluteal 12–13 hour fast Cycling 90 min. 50% VO_{2max}	At 20 min.: ↑ ~70% At 90 min.: ↑ ~100% No effect of MC		Rest: ~0.72 Exercise: ~0.85 No effect of MC
(67)	7 M, 7 F T MC: mid-follicular OF Cycling 90 min. 58% VO_{2peak}	Males: ↑ 105% Females: ↑ 78%		Rest: 0.79 Exercise: ~0.89 No sig. sex diff.
(26)	6 M, 6 F UNT MC: luteal phase 10 hr. fast Cycling 30 min. Trial 1: 39% VO_{2peak} Trial 2: 50% VO_{2peak}	Males	39% VO_{2peak}: ↑ 65% 50% VO_{2peak}: ↑ 191%	Males 39% VO_{2peak}: 0.83 50% VO_{2peak}: 0.84
		Females	39% VO_{2peak}: ↑ 51% 50% VO_{2peak}: ↑ 131%	Females 39% VO_{2peak}: 0.82 50% VO_{2peak}: 0.85
(27)	8 F UNT MC: luteal and follicular 3 hr. fast Cycling 60 min. Trial 1: 45% VO_{2peak} Trial 2: 65% VO_{2peak}	45% VO_{2peak}: ↑ ~120% 65% VO_{2peak}: ↑ ~200% No effect of MC		45% VO_2 peak: 0.9 65% VO_2 peak: 0.93 No effect of MC
(156)	11 M 13 F UNT MC: luteal & follicular 10–12 hour fast Cycling 90 min. 65% VO_{2peak}	Males	↑ ~170% Peaked at 90 min.	Males 0.93
		Females	Luteal phase: ↑ 120% Follicular phase: ↑ 95% Peaked at 90 min.	Females Luteal: 0.9 Follicular: 0.91 No effect of MC
(66)	12 M 10 F UNT MC: mid-luteal OF	Males: ↑ ~165% Females: ↑ ~80% Peaked at 90 min.		Males: 0.88 Females: 0.865 No sig. sex diff.

Cite	Study Design	Glucose R_a	RER
	Cycling 90 min. 51% VO_{2max}		
(88)	10 F UNT, post-menopausal 1.5 hr fast Cycling 60 min. 65% VO_{2peak}	↑ 41%	Not provided

Table 3. Glucose flux during acute exercise in male subjects with carbohydrate supplementation.

Glucose R_a is expressed as percent increase from rest unless stated otherwise. OF: overnight fast. W_{max}: peak power. T: trained. UNT: untrained. M: male. A ~ indicates estimated from figure.

Cite	Study Design	Glucose R_a		RER	
(166)	14 M T 3 hr. fast Placebo or 10% CHO drink at 500 mL/hour Cycling 180 min. 70% VO_{2max}	Placebo: ↑ 60% Peaked at 180 min. CHO: ↑ 100% Peaked at 180 min. ~75% of Glucose R_a came from exogenous glucose in CHO group		5 min: 180 min:	Placebo: 0.91 CHO: 0.94 Placebo: 0.88 CHO: 0.88
(173)	6 M T OF Placebo or 10% CHO drink (200g glucose total) Cycling 120 min. 69% VO_{2peak}	Placebo: ↑ ~315% Peaked at 120 min. CHO: ↑ ~608% Peaked at 120 min. 51% reduction in hepatic glucose production with CHO		Placebo: 0.92 CHO: 0.93	
(164)	6 M T OF Control or CHO ingestion during exercise * Cycling 120 min. Trial 1: 25% VO_{2peak} Trial 2: 65% VO_{2peak}	25% VO_{2peak}:	Control: no change CHO feeding: ↑ ~116% Peaked at 120 min.	Not provided	
		65% VO_{2peak}	↑ ~246% No effect of CHO feeding Peaked at 120 min.	Not provided	
(63)	6 M T OF Control, 4% glucose, or 22% glucose ingestion during exercise Cycling 120 min. 50% VO_{2max}	4% Glucose: ↑ 45% compared to control-state exercise ~71% of Glucose R_a came from exogenous glucose 22% Glucose: ↑ 138% compared to control-state exercise 100% of Glucose R_a came from exogenous glucose Glucose R_a plateaued 75–120 min.		Water: 0.807 4% glucose: 0.831 22% glucose: 0.864	
(65)	7 M T OF Ingestion of a beverage 30 min. before & during exercise **: Trial 1: Placebo before & during (PP) Trial 2: Placebo before & CHO during (PC) Trial 3: CHO before & placebo during (CP) Trial 4: CHO before & during (CC) Cycling 120 min. steady state followed by a time trial 63% of W_{peak}	PP: ↑ 67% compared to rest Plateaued 60–120 min. PC: ↑ 122% compared to rest Peaked at 120 min. CP: ↑ 161% after feeding during rest and decreased 38% during exercise Plateaued 60–120 min. CC: ↑ 121% after feeding during rest and no change by end of exercise Peaked at 120 min.		PP: 0.91 PC: 0.91 CP: 0.92 CC: 0.94	

* (0.8 g/kg at 30 min., 0.4g/kg at 60 min., and 0.4 g/kg at 90 min.)

** 6% glucose solution or aspartame placebo

Table 4. Estimates of gluconeogenesis during acute exercise.

R_a expressed as percent increase from rest unless otherwise stated. OF: overnight fast. Ex.: exercise. TR: trial. HGP: hepatic glucose production. T: trained. UNT: untrained. M: male. TR: trial. A ~ indicates estimated from figure.

Cite	Study Design	R_a	Gluconeogenesis (GNG)		RER
Percent of glucose production represented by GNG					
(34)	19 M OF Cycling 40 min. TR1: 400 kg-m/min. TR2: 800 kg-m/min. TR3: 1200 kg-m/min.	Not measured	HGP derivable from precursor uptake (%):		TR1 10 min: 0.79 40 min: 0.80
			Onset	TR1: 24% TR2: 29% TR3: 20%	TR2 10 min: 0.85 40 min: 0.82
			40 min	TR1: 16% TR2: 11% TR3: 6%	TR3 10 min: 0.87 40 min: 0.87
(39)	10 M T, 11–15 hr. fast Cycling 30– 50 min. 43% VO_{2max}	Glucose R_a ↑ ~39%	Lactate → Glucose GNG 23% of glucose R_a during exercise		Not provided
(36)	6 M UNT OF Cycling 120 min. 60% VO_{2peak}	Glucose R_a. ↑ ~200% Peaked 120 min.	HCO_3 → Glucose GNG 23% of glucose R_a by 120 min. exercise		0.91
(35)	6 M T OF TR1: Water TR2: 4% glucose TR3: 22% glucose Cycling 120 min., 50% VO_{2max}	Glucose R_a TR2: ↑ 39% vs. water TR3: ↑ 257% vs. water Plateaued from 90–120 min.	Glucose recycling method GNG 4–10% of glucose R_a across all trials		TR1: 0.83 TR2: 0.86 TR3: 0.89
(22)	9 M UNT 4.5–5 hr. fast Cycling 60 min. TR1: 45% VO_{2peak} TR2: 65% VO_{2peak}	Glucose R_a TR1: ↑ 59% TR2: ↑ 129%	Lactate → Glucose GNG % glucose R_a: Rest ~4% TR1 ~8.5% TR2 ~4.5%		Not provided
(28)	8 M T 12 hr. fast Cycling 90 min. TR1: 45% VO_{2peak} TR2: 65% VO_{2peak}	Glucose R_a TR1: ↑ ~100%, peak 75 min. TR2: ↑ ~200%, peak 90 min.	Glucose recycling method GNG of glucose Ra: Rest: 24% TR1: 16% TR2: 12% Glycerol 13.6% of gluc. R_a		TR1: 0.87 TR2: 0.9
(38)	6 M T 12 hr. fast Cycling 90 min. TR1: 55% VO_{2peak} TR2: 65% VO_{2peak}	Lactate R_a TR1: ↑ ~275% TR2: ↑ ~430% Glucose R_a TR1: ↑ ~300%	Lactate → Glucose GNG % of glucose R_a: Rest ~35% TR1: ~25%		TR1: 0.89 TR2: 0.91
(29)	8 M T 12 hr. fast Cycling 90 min. TR 1: 45% VO_{2peak} TR 2: 65% VO_{2peak}	Glucose R_a TR1 30 min.: ↑ ~50% 60 –90 min.: ↑ ~100% TR2 30 min.: ↑ ~160% 60 min.: ↑ ~210% 90 min.: ↑ ~200%	MIDA method GNG % of glucose R_a Rest: ~25% TR1: ~24% TR2: ~22%		Not provided
(37)	6 M T OF Cycling 60 min. ~69% VO_{2max}	Glucose R_a ↑ ~230%	Lactate → Glucose GNG 40% of glucose R_a at rest and during exercise		0.93
Percent change in estimated GNG rate					

Cite	Study Design	R_a	Gluconeogenesis (GNG)	RER
(23)	19 M UNT 12 hr. fast Cycling 60 min. TR 1: 45% VO_{2peak} TR 2: 65% VO_{2peak}	Glucose R_a: TR1: ↑ ~73% TR2: ↑ ~110%	Glucose recycling method Estimated GNG rates TR1: ↑ ~63% TR2: ↑ ~186%	TR1: 0.91 TR2: 0.94
(42)	7 M UNT OF Cycling 60 min., 50% VO_{2peak}	Lactate R_a 10 min.: ↑ ~539% 60 min.: ↑ ~204%	Lactate → Glucose conversion ↑ 326%	Not provided

Table 5. Lactate flux during acute exercise.

Lactate R_a is expressed as percent increase from rest unless stated otherwise. OF: overnight fast. W: Watts. T: trained. UNT: untrained. M: male. A ~ indicates estimated from figure.

Citation	Study Design	Lactate R_a	RER
(46)	6 M T 12 hr. fast Cycling 24 min.- 6 min. at each workload: 300, 600, 900, and 1200 kg-m/min	300 kg-m/min: ↑ 128% 600 kg-m/min: ↑ 310% 900 kg-m/min: ↑ 910% 1200 kg-m/min: ↑ 2049%	Not provided
(133)	10 M T 11–15 hr. fast Cycling 30–50 min. 43% VO_{2max}	↑ 181% from 25– 50 min.	Not provided
(174)	8 M UNT 12–14 hr. fast Cycling to exhaustion 30–36 min. total 60 W + 40 W every 6 minutes	50% VO_{2max}: ↑ ~415% 60% VO_{2max}: ↑ ~565% Peak VO_2: ↑ ~975%	1.0 at exhaust
(171)	11 M T OF Cycling to fatigue TR 1: 80% VO_{2max} Mean time: 28.4 min TR 2: 100% VO_{2max} Mean time: 6.5 min.	TR1: ↑ 100% compared to TR2 No effect of exercise duration	Not provided
(44)	9 M UNT 4.5– 5 hr. fast Cycling 60 min. TR 1: 25% VO_{2peak} TR 2: 65% VO_{2peak}	TR1: ↑ 150% TR2: ↑ 500%	Not provided
(172)	6 M T OF Roller skiing 60 min. 40 min. continuous arm and leg (CAL) 10 min. arm double poling (A) 10 min. arm and leg (AL) ~76% VO_{2max}	CAL: ↑ 1000% from rest Plateaued 12–40 min. A: further. ↑ 70% from CAL AL: ↓ slightly from A (~6%)	CAL: 0.93 A: 1.00 AL: 0.93
(45)	6 M UNT 12 hr. fast Cycling 60 min. ~69% VO_{2max}	Lactate R_a ↑ 600%	Not provided
(175)	5M 4F UNT No fasting Cycling 30 min. 70% W_{max}	Lactate R_a ↑ 922% Sex differences not reported	Not provided

Table 6. Fatty acid flux during acute exercise.

Palmitate R_a is expressed as percent increase from rest unless stated otherwise. OF: overnight fast. M: male. F: female. T: trained. UNT: untrained. MC: menstrual cycle phase. TR: trial. A ~ indicates estimated from figure.

Cite	Study Design	Palmitate Ra	RER
(87)	5 M T 10 hr. fast Walking 4 hours at 40% VO_{2max}	↑ ~500% at 4 hours Peaked at 4 hours	0.83
(25)	5 M T 10–12 hr. fast Cycling 30– 120 min. TR1: 120 min. at 25% VO_{2peak} TR 2: 120 min. at 65% VO_{2peak} TR 3: 30 min. at 85% VO_{2peak}	TR1: ↑ ~115% Plateaued 10–120 min. TR2: ↑ ~147% Peaked at 120 min. TR3: No change from rest	TR1: 0.73 TR2: 0.83 TR3: 0.91
(33)	6 M T, 5–6 hr. fast, cycling 30 min. at 80–85% VO_{2max}	No change	Not provided
(165)	6 M T OF, cycling 40 min. at 50% VO_{2max} TR1: Control TR2: 1.4 g/kg glucose 60 and 20 min. prior to exercise (~200g glucose total)	TR2: ↓ 32– 36% during 20–40 min. ex. vs. TR1	TR1: 0.86 TR2: 0.91
(47)	8 F UNT MC: midfollicular, 2.5–6 hr. fast Cycling 60 min. TR1: 45% VO_{2peak} TR2: 65% VO_{2peak}	TR1: ↑ 100% TR2: ↑ 75%	TR1: 0.88 TR2: 0.91
(48)	10 M UNT 4.5–6 hr. fast, cycling 60 min. TR1: 45% VO_{2peak} TR2: 65% VO_{2peak}	TR1: ↑ 89% TR2: ↑ 56%	TR1: 0.89 TR2: 0.94
(161)	6 M 6 F T MC: follicular OF Cycling 90 min. 45% VO_{2peak}	F: ↑ 86%, last 30 min. M: ↑ 167%, last 30 min. No sig. sex diff.	F: 0.85 M: 0.89 No sig. sex diff.
(32)	8 F T, 10–12 hr. fast, cycling 30–60 min. TR 1: 60 min. 25% VO_{2max}, 60 min. bed rest, 30 min. 85% VO_{2max} TR 2: 60 min. 65% VO_{2max}	25% VO_{2max}: ↑ 95% and peak at 5 min. 65% VO_{2max}: ↑ 98% and peak at 60 min. 85% VO_{2max}: ↑ 25% and peak at 30 min.	25% VO_{2max}: 0.756 65% VO_{2max}: 0.816 85% VO_{2max}: 0.906
(24)	8 M T OF Cycling 90 min., three 30 min. stages Stage 1: 40% W_{max} Stage 2: 55% W_{max} Stage 3: 75% W_{max}	40% W_{max} : ↑ ~65% 55% W_{max} : ↑ ~60% 75% W_{max} : ↑ ~35%	Not provided
(67)	7 M 7 F T OF MC: midfollicular Cycling 90 min. 58% VO_{2peak}	Males: ↑ 70% Females: ↑ 126% No sig. sex diff.	Rest: 0.79 Exercise: ~0.89 No sig. sex diff.
(84)	6 M T, 10 hr. fast Cycling 60 min. 60% W_{max}	↑ ~160% Peaked at 60 min.	0.85
(50)	8 M T, OF Cycling 120 min. 60% VO_{2max} Peaked at 120 min.	↑ ~133%	0.90 15 min. 0.85 120 min.
(163)	13 F T MC: early follicular, midfollicular, midluteal 12–13 hr. fast Cycling 90 min. 50% VO_{2max}	↑ 89% Peaked at 90 min. No effect of MC	Not provided
(86)	10 M T, OF Cycling 120 min. 50% W_{max}	↑ ~126% Peaked at 120 min.	Not provided
(49)	8 M UNT 4.5–6 hr. fast Cycling 60 min. TR 1: 45% VO_{2peak} TR 2: 65% VO_{2peak}	TR1: ↑ 78% TR2: ↑ 59%	TR1: 0.93 TR2: 0.95

Cite	Study Design	Palmitate Ra	RER
(88)	10 F, UNT, 1.5 hr. fast Cycling 60 min. 65% VO_{2peak}	↑ 235%	Not provided

Table 7. Ketone and amino acid fluxes during acute exercise.

R_a is expressed as percent increase from rest unless stated otherwise. OF: overnight fast. M: male. F: female. T: trained. UNT: untrained. TR: trial. A ~ indicates estimated from figure.

Ketone R_a			
Cite	**Study Details**	**Main Findings**	**RER**
(51)	7 subjects (both sexes) UNT 3 day fast Cycling 30 min. 60% VO_{2max}	0–7.5 min.: Decreased 22% 7.5– 30 min.: Returned to baseline values	Not provided
(52)	16 M 9 F UNT Group A: 16 hr. fast Group B: 3–5 day fast Treadmill exercise 120 min. 45–50% VO_{2max}	Group A (16h fast): ↑ ~125% Peaked at 120 min. Group B (3–5 day fast): Decreased ~100% at 10 min. but no sig. change from baseline 30–120 min.	Not provided
(53)	19 M 3 F UNT Group 1:18 hr. fast (n=10) Group 2:52 hr. fast (n=5) Group 3:112 hr. fast (n=6) Treadmill exercise 120 min. 49–57% VO_{2max}	Group 1: ↑ ~112%, peaked at 120 min. Group 2: ↑ ~27%, peaked at 105 min. Group 3: Decreased ~20% at 10 min. No change from baseline 20–120 min.	Not provided
Amino Acid R_a			
(176)	6 F, obese OF Cycling 120 min. 50 Watts, mild intensity	Decreased Leucine R_a 19%	0.82
(177)	4 M UNT Post-absorptive state (no fast duration provided) Cycling 105 min. 30% VO_{2max}	No sig. change in leucine R_a with exercise	Not provided
(178)	7 M UNT TR 1: 14 hr. fast TR 2: 3.5 day fast Cycling 45% VO_{2max} to exhaust Mean times: Trial 1: 132 min. Trial 2: 117 min.	Leucine R_a Trial 1 (14 hour fast): No sig. change in leucine R_a with exercise Trial 2 (3.5 day fast): decreased 17%	Not provided
(159)	6 M 6 F T 1 hr fast Treadmill running 90 min. 65% VO_{2max}	Leucine R_a Males: ↑ 18% Females: ↑ 14% Females lower leucine flux compared to males	Males: 30 min.: 0.87 60 min.: 0.86 90 min.: 0.83 Females: 30 min.: 0.83 60 min.: 0.83 90 min.: 0.8
(55)	6 M UNT OF Treadmill exercise 180 min. 45% VO_{2max}	Alanine R_a ↑ 136% No sig. change in glutamine R_a	Not provided
(179)	7 T and 6 UNT Both sexes OF 60 min. Cycling 50% VO_{max}	Trained: no sig. change in leucine R_a Untrained: decreased 7.7%	Trained: Rest: 0.74 15 min.: 0.79 60 min.: 0.81 UNT: Rest: 0.83 15 min.: 0.85 60 min.: 0.84
(180)	9 M 7 F 15 hr. fast Cycling 60 min. 50% VO_{max}	No sig. change in leucine R_a No sig. change in lysine R_a	0.84 at 60 min.

Ketone R_a			
Cite	**Study Details**	**Main Findings**	**RER**
(157)	7 M 7 F Training-level paired 15 hr. fast Cycling 60 min. 50% VO_{max}	No sig. change in leucine R_a No sig. effect of sex	F: ~0.81 M: ~0.86
(158)	6 M 6 F UNT 12 hr. fast Cycling 90 min. 60% VO_{2peak}	Overall trend towards decreased leucine flux during exercise F lower leucine flux vs. M	At 90 min. M: 0.91 F: 0.9

Table 8. Effect of acute exercise on substrate oxidation in fasted subjects.

OF: overnight fast. M: male. F: female. T: trained. UNT: untrained. TR: trial. A ~ indicates estimated from figure.

Cite	Study Details	Tracing Method	Main Findings		Relative Contributions of Substrates to TEE
Fatty acids					
(84)	6 M T 10 hr. fast Cycling 60 min. 60% W_{max}	[U-13C]palmitate [1,1,2,3,3-$^{2}H_{5}$]glycerol Breath & blood sampling Hepatic vein and abdominal wall catheterization	- FAO ↑ 550% - Extracellular re-esterification rate no change - Intracellular re-esterification rate ↑ 3-fold		Not provided
			% FA R_d oxidized:		
			Whole-body	Rest: 45% Exercise: 71%	
			Splanchnic bed	Rest: 12% Exercise: 15%	
			Adipose tissue	Not detected	
(87)	5 M T 10 hr. fast Walking 4 hours 40% VO_{2max}	[1-^{13}C]palmitate and D-5-glycerol Breath & blood sampling	FAO ↑ ~43% from 1–4 hrs.		Not provided
			% FA re-esterified Rest: 75% Exercise: 35%		
			% FA R_a Intracellular Recycling Rest: 20% Exercise: 12% Extracellular Recycling Rest: 50% Exercise: 20–25%		
(88)	10 F UNT Post-menopausal 1.5 hr. fast Cycling 60 min. 65% VO_{2peak}	[1-^{13}C]palmitate [1,1,2,2,3-$^{2}H_{5}$]glycerol Breath & blood sampling	Total FAO ↑ 514% from rest % of FFA R_d oxidized Rest: 7.6% Exercise: 60% % Re-esterification of fat flux Rest: ~85% Exercise: ~65%		% of total Fat Oxidation Rest: 18.9% plasma 81.1% other Exercise: 73.7% plasma 26.3% other
(86)	10 M T OF Cycling 120 min. 50% W_{max}	[U-^{13}C]palmitate [6,6-$^{2}H_{2}$]glucose Muscle biopsy Breath & blood sampling	% Palmitate R_d oxidized Rest: 34% Ex.: 92% Oxidation from 5–120 min. of exercise: Plasma FFA ↑ ~90% Non-plasma FA ↑ ~60% Plasma glucose ↑ ~50% Glycogen decreased ~25%		Rest: ~46% plasma FFA ~18% non-plasma FA ~36% CHO Ex. (2nd hour): ~35% plasma FFA ~10% other fat ~15% plasma glu. ~40% glycogen
Glucose and Lactate					
(39)	10 M T 11–15 hr. fast Cycling 30–50 min. 43% VO_{2max}	[6-^{14}C]glucose + [U-^{13}C]lactate or [U-^{13}C]glucose + [1-^{14}C]lactate Breath & blood sampling	-20% of glucose flux enters the blood lactate pool before undergoing complete oxidation - Lactate oxidation accounted for 22% of CHO oxidation (assuming 75% of lactate R_d oxidized)		Not provided
(37)	6 M UNT OF Cycling 60 min. ~69% VO_{2max}	[6,6-$^{2}H_{2}$]glucose and [3-^{13}C]lactate Breath & blood sampling	% Lactate R_d		Rest Lactate: 11% Glucose: 10% Glycogen: 9% Lipid: 69%
			Rest:	To glucose: 50% Total oxidation: 46% Direct oxidation: 13% Indirect oxidation: 33%	

Cite	Study Details	Tracing Method	Main Findings		Relative Contributions of Substrates to TEE
			Exercise:	To glucose: 25% Total oxidation: 89% Direct oxidation: 21% Indirect oxidation: 68%	Ex. Lactate: 26% Glucose: 17% Glycogen: 34% Lipid: 23%
			- Lactate oxidation increased ~1300%		
Glucose and Palmitate					
(33)	6 M T 5–6 hr. fast Cycling 30 min. 80–85% VO_{2max}	[6,6-$^{2}H_{2}$]glucose and [$^{2}H_{2}$]palmitate Breath & blood sampling	- Total fat oxidation ↑ ~377% from rest to exercise - Total CHO oxidation ↑ ~2500% from rest to exercise - Muscle glycogen accounted for 85% of total CHO oxidation during exercise - Plasma FFA concentrations decreased 45% during exercise - Restoring plasma FFA concentrations via a lipid infusion during exercise increased fat oxidation 27% and decreased glycogen oxidation 15%		Not provided
(50)	8 M T OF Cycling 120 min. 60% VO_{2max}	[U-^{13}C]palmitate and [6,6-$^{2}H_{2}$]glucose Muscle biopsy Breath & blood sampling	- % Palmitate R_a oxidized: Rest 35% Exercise 91% - Palmitate oxidation increased 506% with exercise - % change in oxidation rate from 30–120 min. of exercise: FFA: ~91% increase Other fat: ~50% decrease Plasma glucose: ~100% increase Glycogen: ~33% decrease		Rest FFA: 48% Other fat: 17% CHO: 34% Exercise FFA: 28% Other fat: 15% Plasma gluc.: 12% Glycogen: 45%

Table 9. Effect of exercise intensity and duration on substrate oxidation.

OF: overnight fast. M: male. F: female. MC: menstrual cycle phase. T: trained. UNT: untrained. TR: trial. A ~ indicates estimated from figure.

Cite	Study Design	Tracing Method	Main Findings		Relative Contributions of Substrates to Energy Expenditure	
Fatty acids						
(48)	10 M UNT 4.5–6 hr. fast Cycling, 60 min. TR 1: 45% VO_{2peak} TR 2: 65% VO_{2peak}	[1,1,2,3,3-^{2}H]glycerol and [1-^{13}C]palmitate Breath & blood sampling	- Total fat oxidation ↑ ~300% compared to rest - Total fat oxidation did not change with exercise intensity - No effect of sex		% Energy from fat: Rest: 49.5% TR1: 36.4% TR2: 23.7%	
(49)	8 M UNT 4.5–6 hr. fast Cycling 60 min. TR1: 45% VO_{2peak} TR2: 65% VO_{2peak}	A-V leg catheterization [1-^{13}C] Palmitate Breath & blood sampling	Total fat oxidation (increase from rest):	TR1: 172% TR2: 202%	Rest	~8% leg lipid ~44% non-leg lipid ~48% CHO
			% FA R_d oxidized:	Rest: 19% TR1: 64% TR2: 96%	TR1	~18% leg lipid ~7% non-leg lipid ~75% CHO
			Leg contribution to total fat oxidation:	Rest: 14% TR1: 60% TR2: 28%	TR2	~5% leg lipid ~15% non-leg lipid ~80% CHO
			Contribution of plasma FFA to total fat oxidation:	Rest: 38% TR1: 77% TR2: 97%		
(47)	8 F UNT MC: midfollicular 2.5–6 hr. fast Cycling 60 min. TR1: 45% VO_{2peak} TR2: 65% VO_{2peak}	[1,1,2,3,3-^{2}H]glycerol [1-^{13}C]palmitate Breath & blood sampling	Total fat oxidation increase from rest:	TR1: 180% TR2: 272%	Rest	~5% plasma FFA ~55% other FFA ~40% CHO
			% of Palmitate R_d oxidized:	Rest: 11% TR1: 50% TR2: 51%	TR1	~18% plasma FFA ~22% other FFA ~60% CHO
			% Re-esterification of total fat flux	Rest: ~70% TR1: ~60% TR2: ~56%	TR2	~5% plasma FFA ~20% other FFA ~75% CHO
Fatty acids and Glucose						
(24)	8 M T OF Cycling 90 min. 30 min. stages S1: 40% max. power S2: 55% max. power S3: 75% max. power	[U-^{13}C]palmitate [6,6-$^{2}H_2$]glucose Muscle biopsy Breath & blood sampling	% Palmitate R_a oxidized: Rest: 35% S1: 77% S2: 85% S3: 78% - Fat oxidation peaked at 55%W_{max}		S1	FFA: 31% Other fat: 24% Plasma glucose: 10% Glycogen: 35%
					S2	FFA: 25% Other fat: 24% Plasma glucose: 13% Glycogen: 38%
					S3	FFA: 15% Other fat: 9% Plasma glucose: 18% Glycogen: 58%
(23)	19 M UNT 12 hr. fast Cycling 60 min. TR1: 45% VO_{2peak} TR2: 65% VO_{2peak}	[1-^{13}C]glucose and [6,6-^{2}H]glucose or [1-^{13}C]palmitate and [6,6-^{2}H]glucose, and [1,1,2,3,3-^{2}H]glycerol Breath & blood sampling	Glucose oxidation TR1: ↑ 338% TR2: ↑ 588% - Contribution of CHO TEE increased with exercise intensity		Rest	~10% glucose ~40% other CHO ~40% lipid
					TR1	~14% glucose ~56% other

Cite	Study Design	Tracing Method	Main Findings	Relative Contributions of Substrates to Energy Expenditure	
					CHO ~30% lipid
				TR2	1~5% glucose ~65% other CHO ~20% lipid
Fatty acids and Glucose, continued					
(25)	5 M T,10–12 hr. fast Cycling TR1: 120 min., 25% VO_{2max} TR2: 120 min., 65% VO_{2max} TR3: 30 min., 85% VO_{2max}	[6,6-$^2H_2$]glucose [$^2H_2$]palmitate [$^2H_5$]glycerol Breath & blood sampling	- Muscle TG oxidation peaked TR2 - Plasma substrates contribute more to EE as exercise duration increases - Muscle glycogen contributes more to EE as exercise intensity increases	TR1: 30 min Plasma FFA~80% Muscle TG: ~10% Plasma glu: ~10% 120 min Plasma FFA ~90% Plasma glu: ~10%	
				TR2: 30 min Plasma FFA: ~27% Muscle TGs: ~27% Plasma glu.: ~6% Glycogen: ~40% 120 min Plasma FFA: ~45% Muscle TGs: ~20% Plasma glu.: ~10% Glycogen: ~20%	
				TR3: 30 min. Plasma FFA: ~12% Muscle TGs: ~16% Plasma glu.: ~12% Glycogen: ~60%	
(32)	8 F T 10–12 hr. fast Cycling TR1: 60 min., 25% VO_{2max} 60 min. bed rest, 30 min. 85% VO_{2max} TR2: 60 min., 65% VO_{2max}	[6,6-$^2H_2$]glucose [$^2H_2$]palmitate Breath & blood sampling	- Fat oxidation peaked at 65% VO_{2max} - CHO oxidation peaked at 85% VO_{2max}	25% VO_{2max}:	Plasma FFA: ~80% Other fat: ~5% Plasma glucose: ~10% Glycogen: ~5%
				65% VO_{2max}:	Plasma FFA: ~35% Other fat: ~20% Plasma glucose: ~10% Glycogen: ~35%
				85% VO_{2max}:	Plasma FFA: ~10% Other fat: ~15% Plasma glucose: ~10% Glycogen: ~65%
Glucose and Lactate					
(21)	9 M UNT 4.5–5 hr. fast Cycling 60 min. T1: 45% VO_{2peak} T2: 65% VO_{2peak}	A-V leg catheterization [6,6-$^2H_2$]glucose Muscle biopsy Breath & blood sampling	- Whole-body CHO oxidation ↑ ~78% with intensity - Leg CHO oxidation ↑ ~92% with intensity		Not provided
			%Glucose R_d from leg net uptake:	Rest: ~ 25% T1: ~50% T2: ~60%	
			%CHO oxidation from leg:	T1: ~67% T2: ~70%	

Cite	Study Design	Tracing Method	Main Findings		Relative Contributions of Substrates to Energy Expenditure	
(171)	11 M T, OF Cycling to fatigue TR 1: 80% VO_{2max} Mean: 28.4 min. TR 2: 100% VO_{2max} Mean: 6.5 min.	[U-^{13}C]pyruvate, [1-^{13}C]pyruvate, or [1-^{13}C]lactate [6,6-$^{2}H_2$]glucose Muscle biopsy Breath & blood sampling	- 27% of Pyruvate R_a was oxidized - Pyruvate oxidation provided 78% of total energy demand from 0–3 min. at both intensities - Non-oxidative pyruvate metabolism increased with exercise intensity		Relative contribution to meet ATP demand	
					TR1	76.4% pyruvate oxidation 16.4% nonoxidized pyruvate 7.2% other (CP, ATP)
					TR2	76.0% pyruvate oxidation 19.2% nonoxidized pyruvate 4.8% other (CP, ATP)
Glucose and Lactate, continued						
(38)	6 M T 12 hr. fast Cycling 90 min. TR1: 55% VO_{2peak} TR2: 65% VO_{2peak}	[3-^{13}C]lactate or [6,6-$^{2}H_2$]glucose and $H^{13}CO_3^-$ Breath & blood sampling	- Lactate oxidation TR1: increased ~1200% TR2: increased ~1400%		% Contribution to total CHO ox.	
					Rest	~15% lactate ~27% blood glucose ~58% glycogen
					TR1	~30% lactate ~30% blood glucose ~40% glycogen
(73)	6 M T OF Cycling TR1: 140 min., 50% VO_{2max} TR2: 65 min., 75% VO_{2max}	[^{13}C]lactate Breath & blood sampling	- Lactate oxidation correlated with oxygen consumption (r= 0.81) - Percent of irreversible lactate disposal oxidized: Rest: 49% TR1: 82% TR2: 78%		% Contribution of lactate to total CHO oxidation: Rest: 48% TR1: 20% TR2: 11%	
(44)	9 M UNT 4.5–5 hr. fast Cycling 60 min. TR 1: 45% VO_{2peak} TR 2: 65% VO_{2peak}	A-V leg catheterization [6,6-^{2}H]glucose and [3-^{13}C]lactate Muscle biopsy Breath & blood sampling	- Whole-body lactate oxidation	TR1: ↑ ~1100% TR2: ↑ ~2900%	% of CHO oxidation from: - Leg lactate oxidation Rest: 57% TR1: 13% TR2: 18% - Whole-body lactate oxidation: Rest: 10% TR1: 15% TR2: 23%	
			-% whole-body lactate oxidation from leg	Rest: ~52% TR1: ~70% TR2: ~63%		
			- % Lactate R_d oxidized:	Rest: 21.5% TR1: ~61.6% TR2: ~69.5%		

Table 10. Effect of sex and/or menstrual cycle phase on glucose and palmitate oxidation.

OF: overnight fast. M: male. F: female. MC: menstrual cycle phase. T: trained. UNT: untrained. TR: trial. A ~ indicates estimated from figure.

Cite	Study Design	Tracing Method	Main Findings	Relative Contributions to TEE	
(26)	6 M 6 F UNT MC: luteal 10 hr. fast Cycling 30 min. TR1: 39% VO_{2peak} TR2: 50% VO_{2peak}	[6,6-^{2}H]glucose Breath & blood sampling	- No sex diff. in the relative contributions of CHO and fat to TEE - M increased relative contribution of CHO to TEE with exercise intensity - Relative contribution of muscle glycogen to total CHO oxidation was lower in F	TR1	Males 59.5% fat 49.5% CHO (76.8% glycogen, 24.2% glucose)
					Females 56.6% fat 43.4% CHO (64.2% glycogen, 35.8% glucose)
				TR2	Males 48.4% fat 51.6% CHO (75.2% glycogen, 24.9% glucose)
					Females 52.1% fat 47.9% CHO (60.1% glycogen, 39.9% glucose)
(156)	11 M 13 F UNT MC: luteal and follicular 10–12 hour fast Cycling 90 min. 65% VO_{2peak}	[6,6-^{2}H]glucose Muscle biopsy Breath & blood sampling	- LP females degraded less glycogen compared to FP	% Contribution to Total CHO oxidation:	
				LP F	68% glycogen, 32% plasma glucose
				FP F	71% glycogen, 29% plasma glucose
				M	62% glycogen, 38% plasma glucose
(66)	12 M 10 F T OF MC: mid-luteal Cycling 90 min. 51% VO_{2max}	[6,6-^{2}H]glucose Breath & blood sampling	- No sex diff. in the relative contributions of CHO and fat to TEE - No effect of sex on the relative contributions of glycogen and plasma glucose to total CHO oxidation	Rest	Males 4.8% CHO 65.9% fat 29.3% protein
					Females 9.1% CHO 67.7% fat 23.3% protein
				Ex.	Males 61.9% CHO (73.6% glycogen) 35.2% fat 2.9% protein
					Females 57% CHO (75.7% glycogen) 40.6% fat 2.4% protein
(67)	7 M 7 F T OF MC: midfollicular phase Cycling 90 min. 58% VO_{2peak}	A-V leg catheterization [6,6-$^{2}H_{2}$]glucose NaH[^{13}C]O_{3} [U-^{13}C]palmitate	- Plasma FFA oxidation increased 395% during exercise - No sex differences in plasma FFA oxidation, muscle FA oxidation, or glycogen degradation	Male	~14% blood glucose ~43% glycogen ~10% plasma FFA ~5% IMTGs

Cite	Study Design	Tracing Method	Main Findings	Relative Contributions to TEE	
		Muscle biopsy Breath & blood sampling	- Relative contribution of intramuscular FAs was higher in females (25%) compared to males (5%)		~28% not accounted for
				Fem.	~14% blood glucose ~49% glycogen ~12% plasma FFA ~25% IMTGs ~1% not accounted for
(163)	13 F T MC: early follicular, midfollicular, midluteal 12–13 hr. fast Cycling 90 min. 50% VO_{2max}	[1,1,2,2,3-$^{2}H_5$]glycerol [1-^{13}C]palmitate Breath & blood sampling	- Plasma NEFAs 30–60% of total fat oxidation - TGs 45–65% of total fat oxidation - No effect of MC on the relative contributions of plasma NEFAs and TGs to total fat oxidation	Not provided	

Table 11. Effect of carbohydrate ingestion on glucose and palmitate oxidation.

OF: overnight fast. M: male. F: female. MC: menstrual cycle phase. T: trained. UNT: untrained. TR: trial. A ~ indicates estimated from figure.

Cite	Subjects	Method	Main Findings		Relative Contributions of Substrates to TEE
(64)	8 F T MC: luteal and follicular OF followed by Control or 6% Glucose Solution Cycling 120 min. 70% VO_{2peak}	[6,6-^{2}H]glucose 6% glucose solution Breath & blood sampling	Effect of MC	- Relative contribution of CHO to TEE higher in the FP compared to LP - Time trial performance better in FP	Control Trial FP: Plasma glucose: 11.9% Other CHO: 68.1% Fat: 19.9% LP: Plasma glucose: 9.8% Other CHO: 67.2% Fat: 23%
			Effect of glucose	- ↑ Relative contribution of CHO to TEE - ↑ Relative contribution of blood glucose to total CHO oxidation - Decreased the relative contributions of fat and other CHO to total CHO oxidation - Improved performance 19% in FP and 26% in LP compared to the control trial	Glucose Trial FP: Plasma glucose: 19.7% Other CHO: 64.9% Fat: 15.4% LP: Plasma glucose: 19.6% Other CHO: 65.3% Fat: 15.1%
(164)	6 M T OF followed by Control or CHO ingestion Cycling,120 min. TR1:25% VO_{2peak} TR2:65% VO_{2peak}	[6,6-$^{2}H_2$]glucose [$^{2}H_5$]glycerol Breath & blood sampling	TR1	- CHO dec. lipolysis 22% - CHO dec. fat oxidation from 80–120 min. - CHO ↑ relative contribution of plasma glucose to total CHO oxidation from 80–120 min.	Not provided
			TR2	- CHO dec. lipolysis ~20% - CHO no effect on fat or plasma glucose oxidation	
(35)	6 M T OF 3 trials: TR1: no glucose TR2: 4% glucose (70g) TR3: 22% glucose (350g) Cycling 120 min. 50% VO_{2max}	[6,6-$^{2}H_2$]glucose Glucose solution contained [U-^{13}C]glucose Breath & blood sampling	- Exogenous CHO oxidation limited to 1g/min - Glucose decreased total fat oxidation - Higher glucose dose ↑ plasma glucose oxidation compared to a lower glucose dose - No sig. differences in muscle glycogen oxidation		TR1: Fat: ~55% Hepatic glucose:~15% Glycogen: ~30%
					TR2: Fat: ~50% Exo. glucose.: ~10% Hepatic glucose: ~5% Glycogen: ~35%
					TR3 Fat: ~45% Exo. Gluc.: 25% Hepatic gluc.: 0% Glycogen: ~30%
(63)	6 M T OF 3 trials: TR1: no glu. TR2: 4% glu. (72g) TR3: 22% glu. (360g) Cycling 120 min. 50% VO_{2max}	[6,6-$^{2}H_2$]glucose [U-^{13}C]glucose Breath & blood sampling	- Glucose no effect on glycogen oxidation - Glucose ↑ plasma glucose to TEE - High glucose prevented the exercise-induced decrease in CHO oxidation - Glucose suppressed total fat oxidation		TR1: Fat: ~55% Plasma glu.: ~17% Glycogen:~28%
					TR2: Fat: ~40% Plasma glu.: ~30% Glycogen: ~30%
					TR3: Fat: ~27%

Cite	Subjects	Method	Main Findings	Relative Contributions of Substrates to TEE	
				Plasma glu.: ~50% Glycogen: ~23%	
(166)	14 M T, 3 hr. fast Placebo or 10% CHO drink Cycling 180 min. 70% VO_{2max}	[2-^{3}H]glucose [U-^{14}C]glucose Muscle biopsy Breath & blood sampling	- 180 min. blood glucose ox. 40.7% higher in CHO group - 180 min. blood glucose 65% of total CHO ox. in CHO group compared to 53% in placebo - No significant differences in total CHO oxidation, total fat oxidation, or glycogen degradation between groups	Not provided	
Effect of carbohydrate ingestion on glucose and palmitate oxidation, continued					
(65)	7 M T OF followed by ingestion of a beverage 30 min. before & during exercise: TR 1: PP TR 2: PC TR 3: CP TR 4: CC Cycling 120 min. steady state followed by a time trial 63% of peak power	[6,6-^{2}H]glucose Breath & blood sampling	- Glucose ingestion helped attenuate the exercise-induced decrease in CHO oxidation over time - Total CHO oxidation was increased in CC group compared to other trials only during 105–120 min. of exercise - No difference in muscle glycogen or fat oxidation between trials	PP	Fat: ~14% Glucose: ~8% Other CHO: ~78%
				PC	Fat: ~13% Glucose: ~9% Other CHO: ~78%
				CP	Fat: ~13% Glucose: ~10% Other CHO: ~77%
				CC	Fat: ~11% Glucose: ~11% Other CHO: ~78%
(173)	6 M T OF followed by Placebo or 10% CHO drink 200g glu. total Cycling 120 min. 69% VO_{2peak}	[6,6-^{2}H]glucose 1 Ci 6-[^{3}H]glucose/g glucose drink Breath & blood sampling	- No difference in total CHO oxidation between groups - Exogenous glucose supplied 18% of total CHO oxidation - Exogenous glucose supplied 14% of total substrate oxidation	Not provided	
(165)	6 M T OF TR 1: Control TR 2: ingesting 1.4 g/kg glucose 60 and 20 min. prior to exercise (~200g total) Cycling 40 min. 50% VO_{2max}	[1-^{13}C]palmitate or [1-^{13}C]octanoate Breath & blood sampling	- Glucose supplementation caused a 34% decrease in total fat oxidation during exercise - Glucose ingestion equally suppressed both plasma and non-plasma FA oxidation - Glucose supplementation decreased palmitate but not octanoate oxidation	Not provided	