J Physiol 596.8 (2018) pp 1319–1330

The Journal of Physiology

SYMPOSIUM REVIEW

Sedentary behaviour is a key determinant of metabolic inflexibility

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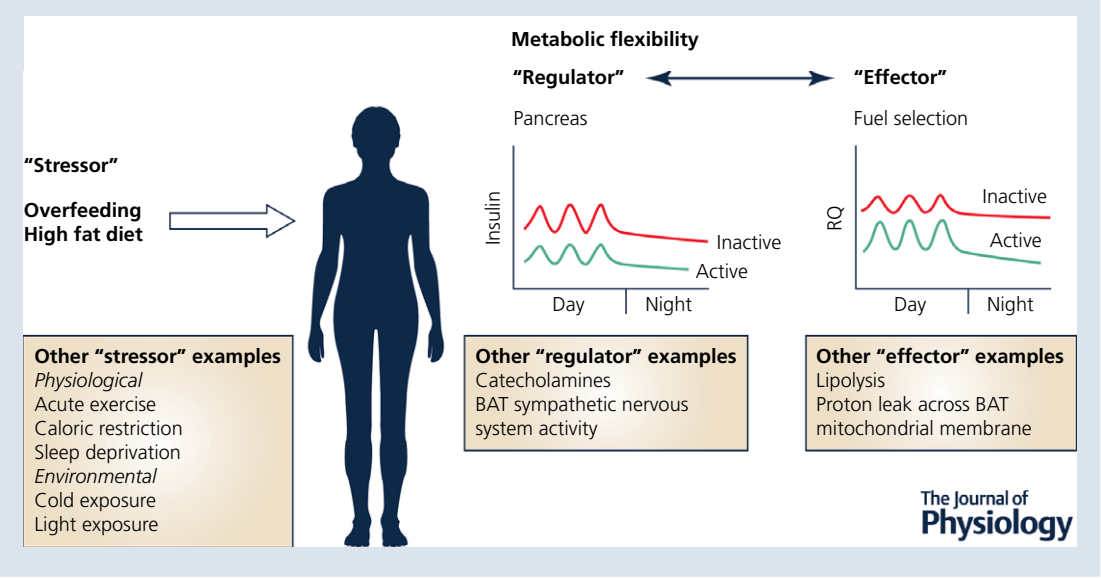

The authors are a group of researchers from the University of Colorado in the USA and the French National Centre for Scientific Research (CNRS, Strasbourg) who are experts in metabolism, energetics, exercise and physical inactivity physiology. **Corey Rynders** is a senior postdoctoral fellow working with Daniel Bessesen on studies of circadian rhythms, exercise, and metabolism. **Stéphane Blanc** is studying the role of environment on the etiology of obesity using unique human and animal models. **Nathan DeJong** is a graduate student working under the mentorship of Audrey Bergouignan. **Daniel Bessesen**, MD, is an endocrinologist who studies the regulation of body weight at the University of Colorado. The group is building an international lab including members from the two institutions to address the role of sedentary behaviour in the onset and progression of metabolic diseases. **Audrey Bergouignan**, PhD, is a researcher who completed her graduate studies and postdoctoral training under the mentorship of Stéphane Blanc and Daniel Bessesen, respectively.

This review was presented at the symposium 'Physiological approaches to study the science of human sedentariness', which took place at Physiology 2016, Dublin, Ireland, 29–31 July 2016.

DOI: 10.1113/JP273282

Abstract Metabolic flexibility is defined as the ability to adapt substrate oxidation rates in response to changes in fuel availability. The inability to switch between the oxidation of lipid and carbohydrate appears to be an important feature of chronic disorders such as obesity and type 2 diabetes. Laboratory assessment of metabolic flexibility has traditionally involved measurement of the respiratory quotient (RQ) by indirect calorimetry during the fasted to fed transition (e.g. mixed meal challenge) or during a hyperinsulinaemic–euglycaemic clamp. Under these controlled experimental conditions, 'metabolic inflexibility' is characterized by lower fasting fat oxidation (higher fasting RQ) and/or an impaired ability to oxidize carbohydrate during feeding or insulin-stimulated conditions (lower postprandial or clamp RQ). This experimental paradigm has provided fundamental information regarding the role of substrate oxidation in the development of obesity and insulin resistance. However, the key determinants of metabolic flexibility among relevant clinical populations remain unclear. Herein, we propose that habitual physical activity levels are a primary determinant of metabolic flexibility. We present evidence demonstrating that high levels of physical activity predict metabolic flexibility, while physical inactivity and sedentary behaviours trigger a state of metabolic 'inflexibility', even among individuals who meet physical activity recommendations. Furthermore, we describe alternative experimental approaches to studying the concept of metabolic flexibility across a range of activity and inactivity. Finally, we address the promising use of strategies that aim to reduce sedentary behaviours as therapy to improve metabolic flexibility and reduce weight gain risk.

(Resubmitted 28 February 2017; accepted after revision 22 May 2017; first published online 24 May 2017)
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Abstract figure legend Sedentary behaviour is a key determinant of metabolic inflexibility. In our view, metabolic flexibility can be assessed by examining the allostatic relationship between a *regulator* (e.g. insulin, catecholamines, brown adipose tissue sympathetic nervous system) and an *effector* (respiratory quotient, lipolysis, or proton leak brown adipose tissue mitochondrial membrane) in response to a stressor (e.g. meal consumption, exercise, or cold exposure). Stressors can be of physiological or environmental origin. In general, a physically inactive individual responds to a stressor with either an increase in the amplitude/mean concentration of the regulator or a decrease in the amplitude/mean concentration of the effector.

Abbreviations OGTT, oral glucose tolerance test; RQ, respiratory quotient.

Introduction

Metabolic flexibility refers to the capacity to modulate the level of daily fuel oxidation to changes in fuel availability (Kelley & Mandarino, 2000). Impairments in metabolic flexibility have been observed in chronic disorders such as obesity (Kelley *et al.* 1999; Astrup, 2011), insulin resistance (Corpeleijn *et al.* 2008; Faerch & Vaag, 2011), and type 2 diabetes (Kelley & Mandarino, 1990; Kelley *et al.* 1992; Kelley & Simoneau, 1994). The metabolically 'inflexible' state in these disease conditions is typically characterized by decreased fat oxidation during fasting and a reduced ability to upregulate carbohydrate oxidation during feeding (Kelley & Mandarino, 2000). However, the intrinsic and extrinsic factors that regulate the inability of fuel oxidation (carbohydrate and fat oxidation) to respond to changes in fuel availability (either by diet or infusion protocols) are not well understood. The present review focuses on the hypothesis that physical activity status is a main predictor of metabolic flexibility. We present evidence to support the notion that a shift towards more sedentary time in modern society is a cause of multiple metabolic diseases because of this inflexibility. Furthermore, we demonstrate the power of the metabolic flexibility concept as a detailed, integrative biology approach to understanding the mechanisms that link sedentary behaviours to states of dysmetabolism. Finally, we argue that increasing daily physical activity and/or reducing sedentary time is an inexpensive and practical approach for enhancing metabolic flexibility and preventing adverse weight and diabetes-related outcomes.

In 2008, 9.4% of all 57 million deaths in the world could be attributed to physical inactivity (Lee *et al.* 2012). It was also concluded that physical inactivity has a deleterious effect on health that is comparable in magnitude to smoking and obesity (Lee *et al.* 2012). Physical activity has increasingly been engineered out of contemporary society with the emergence of passive leisure activities (e.g. TV viewing, video games) and work-related activities (e.g. extended commute times, computer work). Traditional approaches to decreasing the prevalence of physical inactivity have focused on exercise training and increasing time spent in activities of moderate to vigorous intensity. However, daily sedentary time has

emerged as a distinct form of physical inactivity that appears to exert deleterious effects on metabolism even in individuals who meet current intensity-based physical activity guidelines (Hamilton *et al.* 2014; Bouchard *et al.* 2015). A growing number of epidemiological studies have observed positive relationships among sedentary behaviours, such as time spent sitting, and the risk of developing obesity, type 2 diabetes, and cardiovascular diseases independent of age, sex, ethnicity and adiposity (Levine *et al.* 2006; Oldridge, 2008; Dunstan *et al.* 2012*a*)

Physical activity–inactivity status exists along a broad continuum that likely covers a range of different levels of fuel integration. In the present review, we highlight emerging data to suggest that physical activity levels predict metabolic flexibility. While exercise training and high levels of daily physical activity improve metabolic flexibility, physical inactivity and sedentary behaviours trigger varying states of metabolic inflexibility (Bergouignan *et al.* 2013*a*). For the purposes of this review we compare and contrast measures of metabolic flexibility in endurance trained adults and those meeting current physical activity guidelines (i.e. 150 min of weekly moderate-intensity activity) with untrained adults who meet criteria for high levels of sedentary time (i.e. inactive adults). Here, sedentary activities refer to any waking behaviour characterized by an energy expenditure less than 1.5 metabolic equivalents while in a sitting or reclining posture (1 metabolic equivalent is defined as the energy expended while sitting at rest). We will consider the effects of sedentary behaviours on metabolic flexibility in adults who accumulate more than 6–8 h of sedentary time per day. We also consider studies that have included periods of bed rest in healthy adults as a way to measure physiological responses to physical inactivity under controlled laboratory conditions.

In this review, we discuss (i) the definition of and common methods for measuring metabolic flexibility, (ii) the effects of different levels of physical activity and inactivity on metabolic flexibility, and (iii) recent data from protocols testing the metabolic effects of frequent 'breaks' in sedentary time as a novel approach to prevent the negative health effects induced by physical inactivity.

Original definition and assessment of metabolic flexibility

Kelley and Mandarino originally defined the term metabolic flexibility as 'the capacity for an organism to adapt fuel oxidation to fuel availability' (Kelley & Mandarino, 2000). The most common experimental approach used to assess metabolic flexibility involves measuring the change in the respiratory quotient (ΔRQ; indirect calorimetry) during a euglycaemic–hyperinsulinaemic clamp. An increase in RQ under insulin-stimulated conditions reflects the suppression of lipid oxidation and greater utilization of glucose for oxidation and storage. Using the clamp method, metabolic flexibility to glucose has been shown to be reduced in a number of clinical conditions and physiological states, including type 2 diabetes, obesity and ageing (Kelley & Mandarino, 2000). The physiological mechanisms that explain impairments in metabolic flexibility are multifactorial, but appear to be due to reduced skeletal muscle glucose disposal rate (Kelley & Mandarino, 1990; Thyfault *et al.* 2006; Galgani & Ravussin, 2008; Galgani *et al.* 2008*a*); impaired suppression of adipose tissue lipolysis (Thyfault *et al.* 2006; Gaster, 2007; Sparks *et al.* 2009*a*,*b*); reduced suppression of hepatic glucose output (Thyfault *et al.* 2006); and/or skeletal muscle mitochondrial dysfunction (Ukropcova *et al.* 2007; Boyle *et al.* 2012). Collectively, impaired glucose transport, lower fatty acid turnover, and reduced oxidative capacity favour the accumulation of ectopic lipid and multi-organ metabolic dysfunction.

Our view of the metabolic flexibility concept

The underlying mechanisms and factors triggering the onset of metabolic inflexibility are not fully elucidated, potentially because the definition of metabolic flexibility is still under debate in the scientific community. For many years, researchers studied the pathophysiology of obesity and related metabolic diseases by focusing on the main metabolic pathways involved in the regulation of metabolism at different levels of integration. These studies have included measures of glucose uptake by peripheral organs, glycolysis, glycogen synthesis in skeletal muscle and liver, mitochondrial fat uptake and oxidation, and whole-body and skeletal muscle fat oxidation in different metabolic situations (e.g. fasting, postprandial conditions, exercise, beta-adrenergic stimulation). To understand the independent role of each pathway in the aetiology and development of metabolic diseases, scientists have used different paradigms to create highly controlled metabolic conditions to isolate one pathway or another. While informative, these studies provide a limited view of the holistic regulation of metabolism. The regulation of energy and fuel homeostasis are intrinsically intertwined and require the integration of a multitude of extrinsic and intrinsic metabolic regulators and effectors. In this line, the concept of metabolic flexibility is powerful as it represents a framework within which changes in fuel oxidation can be examined along with changes in fuel availability that result from modifications in dietary intake, energy demand, or peripheral metabolic processes. It thus represents an integrated assessment of the interaction between environmental factors (diet, physical activity, others) and regulation of the metabolism (organism). Understanding the interaction between environmental and biological factors in the development of metabolic flexibility is

important to improve our understanding of the aetiology and progression of metabolic diseases.

In their comprehensive review of metabolic flexibility and insulin resistance, Galgani *et al.* (2008*b*) suggested that a number of additional metabolic challenges (besides the clamp) should also be considered in the assessment of metabolic flexibility. For example, the switch from glucose to lipid oxidation during an overnight fast (i.e. metabolic flexibility to fasting) or fuel shifts in response to dietary challenges varying in macronutrient composition (e.g. high-fat and high carbohydrate diets) could be considered in the context of metabolic flexibility. Our group ascribes to the view that any response of fuel metabolism to a stress or challenge, be it environmental or physiological, might provide information about metabolic flexibility. In our view, any paradigm which aims to assess metabolic flexibility must have three components: a *stressor*, a *regulator* and an *effector*. It is the allostatic relationship between the *regulator* and *effector* in response to *stressor* that is measured experimentally and informs on metabolic flexibility. For example, under the original definition of Kelley & Mandarino (2000), the conditions of the clamp serve as the metabolic stressor, and the allosteric relationship between the insulin infusion rate (*regulator*) and ΔRQ (*effector*) provides information on metabolic flexibility (Fig. 1). Within this construct, the artificial hyperinsulinaemic condition of the clamp is just one challenge to the system, and an important one, but other stressors and challenges, like exercise, fasting, meals, catecholamines infusion, etc., can also be used to examine the response of an effector to a regulator providing new insights into metabolic dysregulation. For example, one could study the effects of exercise (*stressor*) on the relationship between sympathetic nervous system activity (*regulator*) and lipolysis (*effector*). Another hypothetical example would be a study of the relationship between the proton leak through brown adipose tissue mitochondrial membrane (*effector*) and sympathetic nerve activity (*regulator*) in response to cold exposure (*stressor*). Although these hypothetical scenarios might be considered an over-simplification of the *in vivo* metabolic situation we believe that the stressor–regulator–effector model of metabolic flexibility will ultimately lead to new insights into integrative pathophysiology of obesity and diabetes.

In summary, we believe that it is important for the scientific community to consider a broader definition of metabolic flexibility that includes a variety of potential metabolic and physiological challenges/stressors that could result in perturbations in the fine tuning between metabolic regulator and effector, which ultimately impact fuel homeostasis. It is within this framework that we review evidence supporting the role of physical activity and inactivity on fuel selection and metabolic flexibility.

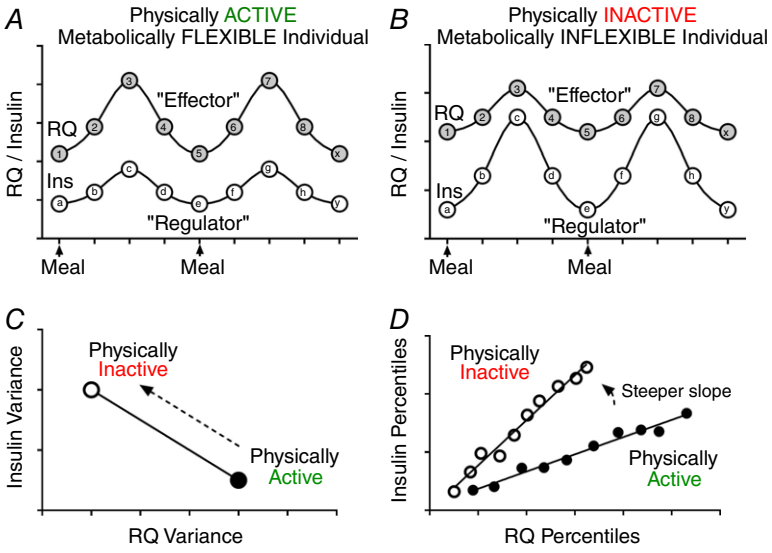

Figure 1. The variance-based concept of metabolic flexibility
Physical activity and inactivity levels modulate the response to challenges (stressors) to the system. The variation in 24 h insulin and respiratory quotient (RQ) represent the regulator and effector components of the system, respectively. It is the allostatic relationship between the regulator and effector responses that are measured experimentally and inform on the degree of metabolic flexibility. A physically active metabolically flexible individual is characterized by a high variance in RQ and low variance in plasma insulin concentration (*A*). To the contrary, a physically inactive, metabolically inflexible individual has a low amplitude RQ response to marked changes in insulin levels (*B*), resulting in a low variance in RQ for a high variance in insulin. By plotting the variances of 24 h RQ and insulin, we obtain a clear graphic representation of the effects of physical activity status on metabolic flexibility status (*C*). Another way of graphically representing the data is a log transformation plot of the RQ and insulin percentiles (*D*; see text for details).

Visualizing the metabolic flexibility concept in relation to physical activity status

We previously proposed that physical activity is a relevant systemic challenge that potently modulates metabolic flexibility (Bergouignan *et al.* 2011, 2013*a*). In an analysis of one of our previous interventional studies, we considered the effects of daily physical activity (*stressor*) on the dynamic nature of postprandial metabolic responses to standardized meals (*regulator* and *effector* systems). We established an index that accounts for the intra-individual variances in daily insulin (*regulator*) and RQ (*effector*) responses to standard meals (as measured by repeated indirect calorimetry measures or during whole room calorimetry) and stratified these responses based on physical activity levels (Bergouignan *et al.* 2013*a*). Using this analysis approach, exercise training was found to correlate with an increase in the daily variance in RQ and a decrease in the daily variance of insulin (i.e. a large shift in the fuel mix being oxidized at a low insulin signal; Fig. 1*A*). In contrast, a sedentary metabolically inflexible subject is characterized as having a large daily variance in insulin for a small variance in RQ (i.e. a small shift in the fuel mix being oxidized at a high insulin signal; Fig. 1*B*).

The variances in insulin and fuel oxidation can be related mathematically to compare subjects contrasting in physical activity status (or other relevant stressors). One approach is to calculate the product of the 24 h RQ and insulin variance (Fig. 1*C*). Another objective approach to examine changes in metabolic flexibility is to test for differences in the slope of the relationship between the *effector* and the *regulator* obtained after linearization of the data (Fig. 1*D*). Following a log transformation, percentiles ranging from the 1st to 99th can be calculated for each individual. The average of every 5th percentiles can then be plotted to create a percentiles–percentiles linear relationship. A metabolically flexible state characterized by a large shift in the fuel mix being oxidized in response to small changes in insulin is thus associated with large differences between the 1st and 99th percentiles of the postprandial RQ values and small differences between the two extreme percentiles for postprandial insulin. A metabolically inflexible state is associated with a decrease in the range between the 1st and 99th percentile in RQ concomitant with an increase in the range between the 1st and 99th percentile insulin values, thus resulting in an increase in the slope of the percentiles–percentiles linear relationship. The whole goal of these mathematical calculations is to provide an easy and objective way to detect changes in metabolic flexibility by integrating the changes in both the *effector* and the *regulator* in response to a *stressor*. This definition of metabolic flexibility is consistent with the original definition of Kelley & Mandarino (2000), but broadens the concept and assessment techniques to include other relevant determinants of metabolic flexibility.

Effects of exercise training on metabolic flexibility

Although the term 'metabolic flexibility' is not always used, a number of studies provide strong evidence to indicate that exercise training improves components of metabolic flexibility. It is well known that exercise increases dietary fat trafficking towards oxidation in muscle and away from storage in adipose tissue (Calles-Escandon *et al.* 1996; Friedlander *et al.* 1998), and towards ectopic storage in other peripheral tissues, and improves insulin sensitivity (Bruce *et al.* 2006). For example, metabolic flexibility defined as the insulin-stimulated (clamp) RQ minus fasting RQ improved after 12 weeks of aerobic exercise training (60 min day^{-1} for 5 days week^{-1} at ∼85% heart rate maximum) in 24 older adults with pre-diabetes and obesity (Malin *et al.* 2013*a*). Also, young, obese individuals lack metabolic flexibility in terms of increasing fatty acid oxidation in skeletal muscle in response to a high fat diet; however, just 10 consecutive days of aerobic exercise training (1 h day^{-1}, 70% $\dot{V}_{O_2peak}$) increased fat oxidation in skeletal muscle during a high fat meal challenge in obese individuals in a manner that was similar to lean subjects (Battaglia *et al.* 2012). Finally, we previously showed that 1 month of exercise training performed at currently recommended levels improved metabolic flexibility in lean sedentary subjects as indicated by shifts in the insulin/RQ variance indices described above (Bergouignan *et al.* 2013*a*,*b*). Together, these findings support the beneficial role of exercise and physical activity on metabolic flexibility, which may partially explain the well-known preventive effect of exercise-training programmes on unhealthy weight, the onset of obesity, diabetes, metabolic syndrome, and mortality risk. If exercise improves metabolic flexibility, the direct corollary is that physical inactivity and sedentary behaviours impair metabolic flexibility.

Physical inactivity, sedentary behaviours and metabolic flexibility

The scientific community has only begun to appreciate the fundamental role of sedentary behaviours in the development of metabolic diseases. However, the hazards of physical inactivity have been known for a long time (Martin, 1966). The cascade of events triggered by physical inactivity and leading to the development of metabolic inflexibility has been summarized in a previous review by our group (Bergouignan *et al.* 2011). Briefly, physical inactivity *per se*, independent of detectable changes in energy balance, triggers insulin resistance (Blanc *et al.* 1998, 2000*a*,*b*; Bergouignan *et al.* 2009, 2011); hyper-lipidaemia; decreased clearance of dietary lipids; and

reduced fasting and post-prandial lipid oxidation (Blanc *et al.* 2000*b*; Bergouignan *et al.* 2006, 2009) in favour of greater use of carbohydrate as fuel and ectopic fat storage (Dirks *et al.* 2016). Collectively, these abnormalities define the main tenants of the metabolic inflexibility concept.

We have previously used the bed rest model to explore the effects of activity and inactivity on metabolic flexibility. Using an oral glucose tolerance test (OGTT), we showed that following 7 days of bed rest both men and women transitioned from a metabolically flexible state characterized by high variance in RQ in response to low variance of insulin to a metabolically inflexible state characterized by low variance in RQ for high variance in insulin (Fig. 2*A*) (Blanc *et al.* 2000*b*). The decrease in metabolic flexibility was also confirmed by an increased slope of the daily insulin–RQ percentile relationship (Fig. 2*B* and *C*) resulting from a simultaneous reduction in the RQ amplitude for a greater change in insulin following meal consumption, which indicates an overall negative effect of physical inactivity on metabolic flexibility.

While our bed rest studies have focused on the effects of physical inactivity on postprandial variability in insulin and RQ, it is important to consider other metabolic markers that might inform on metabolic flexibility. For example, one of the earliest detectable changes in the transition from normal glucose tolerance to diabetes is the decline in pancreatic β-cell function. β-Cell function is characterized by a two-pool model consisting of an immediate first phase insulin secretion response upon initial glucose stimulation and a second phase insulin response resulting from the synthesis of 'new' insulin during the remainder of the postprandial period. First phase insulin release is one of the earliest detectable defects in β-cell function in individuals at risk of developing type 2 diabetes (Gerich, 2002) and can be measured during the early postprandial period as well as from C-peptide responses. According to our expanded definition, the loss of the first phase insulin response (*regulator*) to a meal challenge (*stressor*) could be considered an index of metabolic inflexibility. A number of studies have demonstrated that exercise (both acute and chronic) is sufficient to augment the first phase insulin response in adults with impaired β-cell function (Slentz *et al.* 2009; Malin *et al.* 2013*b*; Rynders *et al.* 2014). To our knowledge, the independent effects of sedentary behaviours on first phase insulin responses and β-cell function have not been investigated. However, 5 days of detraining increased glucose-stimulated insulin, proinsulin and C-peptide levels in trained young men (Mikines *et al.* 1989). Also, 6 days of prolonged sedentary time increased postprandial C-peptide levels, reflecting increased endogenous insulin, in healthy young men (Altenburg *et al.* 2016).

In summary, the direct effects of physical inactivity and sedentary behaviours on metabolic flexibility using the standardized method of the euglycaemic–hyperinsulinaemic clamp or alternative methods (e.g. meal tolerance tests) are only starting to be investigated in controlled studies. In addition, the respective effects of physical activity level, time spent sedentary, body composition, sex and age on metabolic flexibility remain to be elucidated.

From oxidative balance to metabolic flexibility

Consideration of outcomes from training, detraining, exercise and bed rest studies has the potential to reveal

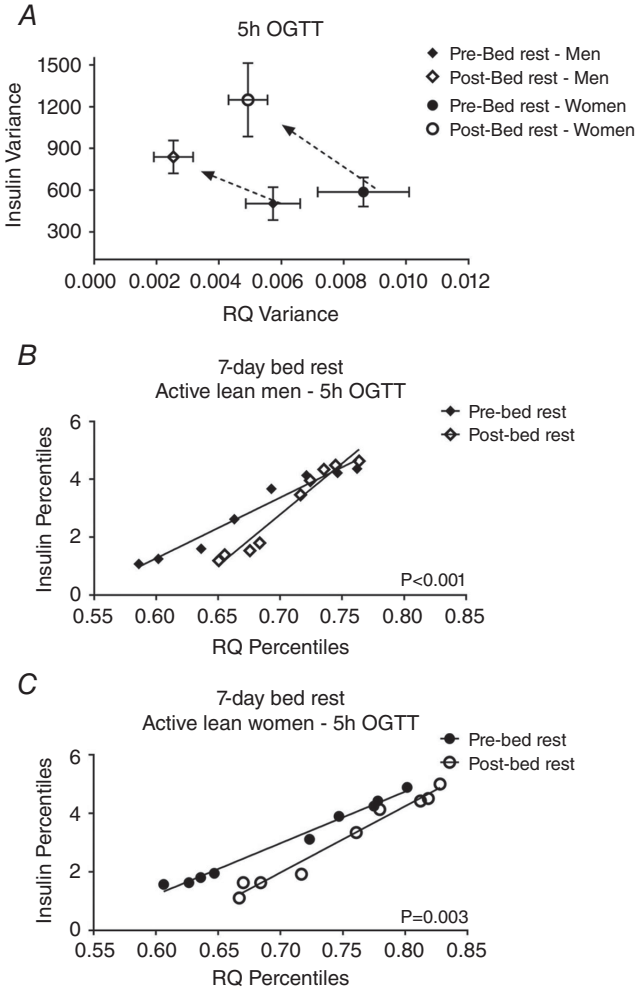

Figure 2. Metabolic flexibility represented by variances and percentile–percentile plots of RQ and insulin
Data shown were collected on 8 women and 8 men who performed a 5 h oral glucose tolerance test before and after a 7 day bed rest study (Blanc *et al.* 2000*b*). In *A*, the decrease in metabolic flexibility after bed rest is indicated by a shift in the relationship between the insulin and RQ variance. Values plotted in *B* and *C* represent the 1st, 5th, 10th, the lower quartile, the median, the upper quartile, 90th, 95th and 99th percentiles of RQ and insulin. The significantly steeper slope in the percentile–percentile plots indicate a decrease in metabolic flexibility following bed rest.

how metabolic flexibility integrates relationships among substrate availability and oxidation as well as among carbohydrate, fat and energy balance (Fig. 3). Maintaining a stable body weight not only requires that energy intake matches energy expenditure, but also that macronutrient intake must balance macronutrient oxidation. This has been well described and modelled by J. P. Flatt (2004) as follows. Regulation of macronutrient oxidative balance relies upon the storage and oxidative capacity of the macronutrients (Fig. 3). While proteins and carbohydrates have limited storage capacities in muscle and glycogen respectively, fat has an almost unlimited storage capacity in adipose tissue. Protein contributes a minor and fairly constant proportion of dietary energy, and biological evolution has led to regulatory features that spontaneously maintain protein balance (Flatt, 1995, 2004). Thus, it is the interaction between carbohydrate and fat intake and oxidation that primarily determines body weight maintenance. For example, changes in carbohydrate intake induce wide variations in the glycogen storage pool that rapidly adjust carbohydrate oxidation. To the contrary, changes in fat intake cause small changes in the lipid pool size and are therefore not compensated for by equivalent adjustments in lipid oxidation (Flatt, 2004). Different parameters regulate the flow in and out of the glycogen and lipid reservoirs. While inflow depends on the quantity and quality of meals, outflow can only be modulated by physical activity (Fig. 3). To illustrate this point, Schrauwen *et al.* (1997) studied the effect of low glycogen stores on fat oxidation after a switch from a reduced-fat diet to a high-fat diet. On a high fat diet, fat oxidation remained below fat intake, indicating the slow matching of oxidation to intake when switching to a high fat diet. However, after a bout of exercise that lowered glycogen levels, fat oxidation matched fat intake. This result demonstrates that it is possible to rapidly adjust fat oxidation to fat intake only when glycogen stores are reduced.

The role of physical activity in regulating the adjustment to lipid flux is a central aspect of the regulation of fat balance and hence, body mass. As shown by Smith *et al.* (2000) physical activity favours the adjustment of fat oxidation to fat intake when dietary fat increases (Fig. 4*A*). Similarly, using cross-over study design they showed that 7 days of sedentary behaviour or exercise modulated the ability to adjust dietary fat oxidation in response to a eucaloric high fat diet. In this study, healthy male subjects were asked either to remain sedentary in a whole-room calorimeter (physical activity level = 1.4) or to perform treadmill walking at moderate intensity to reach a physical activity level of 1.8, a value representative of physically active individuals in the general population. In sedentary conditions, several days were required to increase fat oxidation to match fat intake when dietary fat intake increases. Furthermore, there was a high inter-individual variation in the ability to adjust fat oxidation to fat availability. Increasing total energy expenditure from 1.4 to 1.8 times resting metabolic rate accelerated the adaptation

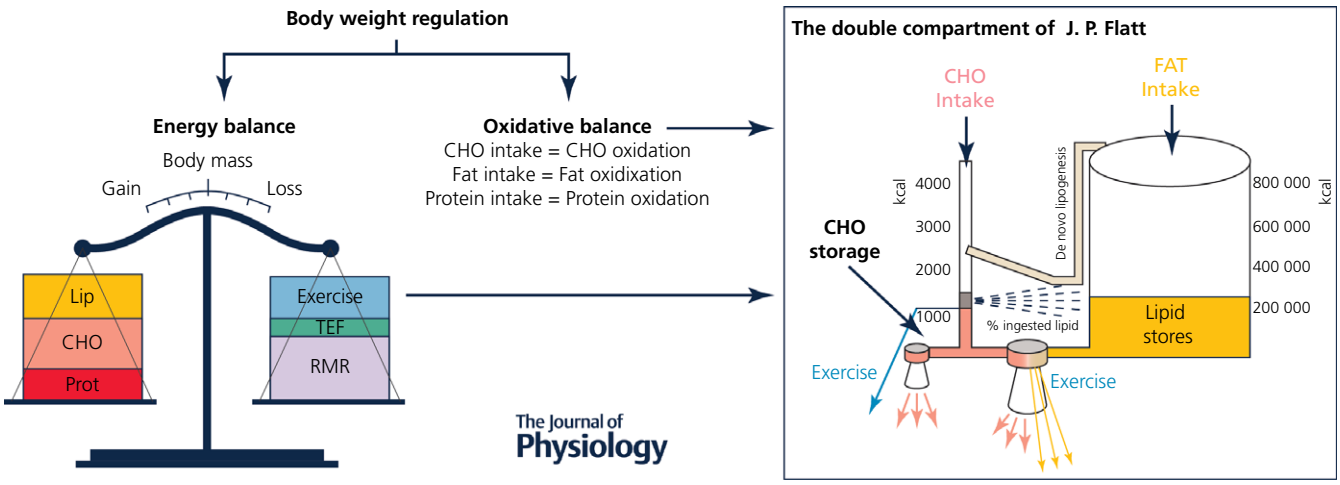

Figure 3. Physical activity and exercise exert influences on metabolic flexibility through regulation of energy balance and fuel homeostasis
Based on J. P. Flatt's theory (Flatt, 1995, 2004), the main regulator of lipid oxidation, and hence balance, is carbohydrate availability and oxidation. While an increase in carbohydrate intake leads to an increase in carbohydrate oxidation, an increase in fat intake does not trigger fat oxidation and results in fat storage. By utilizing glycogen stores, exercise increases fat oxidation. By being one of the main components of total energy expenditure, physical acivity and exercise further contribute to the regulation of energy balance. Energy balance is the result of energy intake composed of lipids, carbohydrates and proteins on one side and energy expenditure composed of physical activity energy expenditure, thermic effect of food (TEF) and resting metabolic rate (RMR). Both energy and oxidative balance are key factors involved in the regulation of body weight.

to high fat diet and reduced inter-individual variability. Along the same lines but in the opposite direction, Olsen *et al.* (2008) have reported that a decrease from 6200 to 1400 in daily steps impairs insulin sensitivity and increases plasma triglycerides, suggesting a reduced fat oxidation, in healthy lean men after 3 weeks of intervention. To date, the long term effects of such changes in physical activity behaviour on body weight regulation have not been tested.

In agreement with Flatt's two-compartment model (Flatt, 2004), we have shown a strong positive correlation between changes in activity energy expenditure and dietary fat oxidation (Fig. 4*B*), in association with greater metabolic flexibility (Bergouignan *et al.* 2013*b*). In this study, a decrease in total energy expenditure of −263 kcal day^{-1} in lean active men was associated with a reduction in total lipid oxidation of 47 kcal over 8 h following meal consumption. Because this study was conducted in stable energy balance, the decrease in fat oxidation was counterbalanced by a 52 kcal increase in carbohydrate oxidation over 8 h. We have also observed a negative correlation between carbohydrate and fat balance with both high and low amounts of physical activity, as would be expected under conditions of approximate energy balance (Fig. 4*C*). By increasing the use of dietary fat as fuel, physical activity and exercise are likely to provide a higher tolerance to high fat diet, which favours the control of fat balance and hence energy balance, as energy and fat balance are tightly intertwined (Schrauwen *et al.* 1997, 1998). In support of this, Stubbs *et al.* (2004) have shown that active adults were in a negative energy balance (−3.8 MJ day^{-1}) when facing 7 days of high fat intake, likely because of greater fat oxidation, while inactive individuals were in positive energy balance (+0.7 MJ day^{-1} or 167 kcal day^{-1}) and gain weight (+0.19 kg week^{-1}), likely because they are not able to increase fat oxidation to match fat intake (Fig. 4*D*).

In summary, these studies collectively suggest that metabolic inflexibility is one of the causes of weight gain and the related metabolic diseases. Furthermore, while physical activity is one of the key factor favouring metabolic flexibility and prevention of weight gain, low levels of physical activity trigger a transition from metabolic flexibility to metabolic inflexibility.

Effect of frequent interruptions of sedentary time with microbouts of activity on metabolic flexibility

While the relationship between physical activity and health has been considered to be linear for several decades, recent data suggest that sedentary behaviours play an

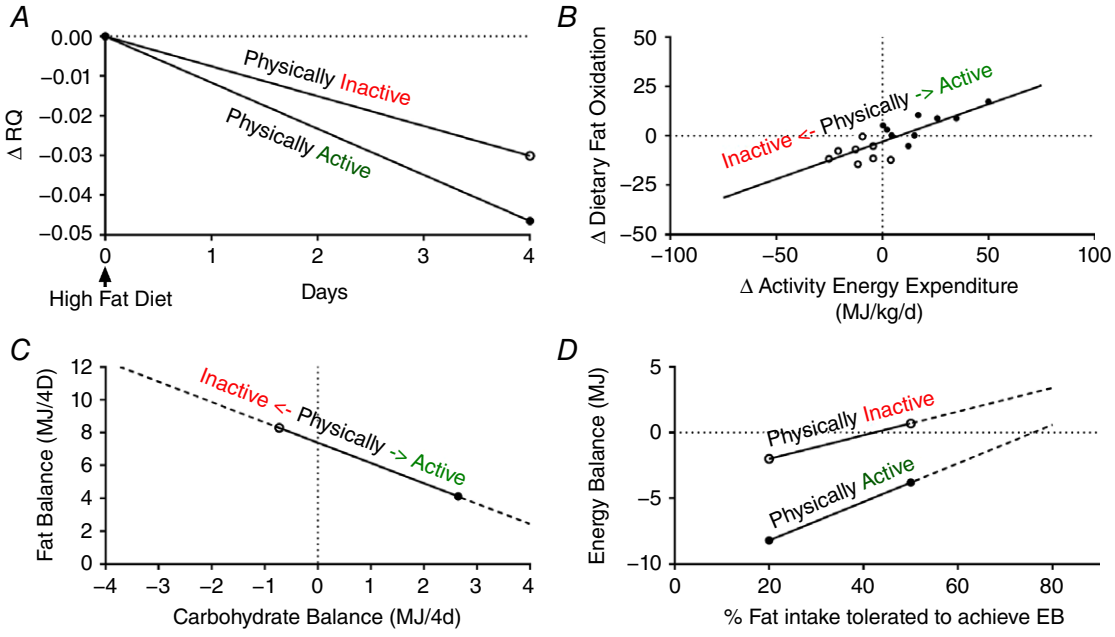

Figure 4. Contrasting levels of habitual physical activity predict adaptations to high fat feeding, dietary lipid oxidation and energy balance
In reponse to high fat intake, physical activity helps to adjust fat oxidation to fat availability (*A*; Smith *et al.* 2000). There is a positive association between changes in dietary fat oxidation and activity energy expenditure along this same physical activity continuum (*B*; based on Bergouignan *et al.* 2013*b*). Because of the effects on glycogen storage, there exists a tight negative relationship between fat and carbohydrate balance along the physical activity continuum (*C*; based on Smith *et al.* 2000). Together these relationships provide a higher fat tolerance to active individuals than inactive individuals, i.e. active individuals are able to achieve a stable energy balance at a higher fat intake compared to inactive individuals (*D*; based on Stubbs *et al.* 2004).

independent role in this relationship. In other words, too little exercise seems to have different health effects from too much sedentary activity (i.e. mainly sitting). Interestingly, recent population studies suggest that frequent interruptions to sedentary time with bouts of physical activity are associated with beneficial metabolic outcomes, even in individuals who regularly exercise (Healy *et al.* 2008, 2011). Breaking up sedentary time is a stimulus for improving metabolic health (flexibility) and has been suggested as a novel and promising strategy in the general population.

In light of these epidemiological observations, a growing number of studies have examined the effects of frequent interruptions in prolonged sedentary activities with short bouts of activity varying in intensity, duration, mode and frequency on metabolism. For example, Dunstan *et al.* (2012*b*) compared in a cross-over study the effects of uninterrupted sitting with interrupted sitting (2 min bouts of activity every 20 min for 5 h) in overweight adults on plasma glucose and insulin incremental area under the curve. Sitting was either interrupted by light- (3.2 km h^{-1}) or moderate-intensity (5.8–6.4 km h^{-1}) treadmill walking. Compared to uninterrupted sitting, glucose and insulin area under the curve in response to standardized meal were both significantly reduced after the activity-break conditions. Notably, no statistical differences were observed between the light- and moderate-intensity bouts of activity. Taking all the recent studies in this area together, breaking up prolonged sedentary time with bouts of activity decreases both postprandial plasma glucose and insulin concentrations, regardless of the adiposity, sex and age of the subjects in these studies (Dunstan *et al.* 2012*b*; Peddie *et al.* 2013; van Dijk *et al.* 2013; Blankenship *et al.* 2014; Buckley *et al.* 2014; Holmstrup *et al.* 2014; Thorp *et al.* 2014; Bailey & Locke, 2015; Larsen *et al.* 2015; Lyden *et al.* 2015; Dempsey *et al.* 2016, 2017). Even standing instead of sitting has been reported to be enough to significantly attenuate blood glucose variations over a day as measured by continuous glucose monitoring (Buckley *et al.* 2014). However, reducing time spent sitting through frequent 2 min bouts of standing every 20 min did not reduce 5 h postprandial glycaemia while frequent 2 min bouts of light-intensity walking did (Bailey & Locke, 2015), suggesting that muscle contraction is a key factor in the use of glucose by the body. Mechanistic studies looking at insulin-dependent and muscle contraction-mediated glucose uptake pathways at the skeletal muscle levels support the role of contraction in glucose uptake by muscle (Bergouignan *et al.* 2016).

One could argue that a reduction in plasma insulin and glucose, which suggests greater use of carbohydrate as fuel, would have been expected as the energy expended during the bouts of activity was not replaced in the diet. However, when energy expended was replaced, a decrease in plasma insulin concentration was still detected. This suggests independent beneficial effects on insulin sensitivity from reduced sitting. Even greater health benefits by placing subjects in negative energy balance were observed (Stephens *et al.* 2011). A similar question is whether the observed metabolic benefits were due to the frequent interruptions to prolonged sitting or the addition of physical activity and/or energy expenditure. To address this question, Blankenship *et al.* (2014) examined in sedentary overweight adults the effects on postprandial glycaemia following a day with either frequent long breaks incorporating standing/stepping *vs.* taking fewer breaks by walking as per activity guidelines, with total energy expenditure matched between conditions. A third condition, frequent short breaks, with the same number of breaks as the frequent long breaks condition but with standing/stepping instead of walking was added to delineate the respective role of energy expenditure *vs.* breaking up sitting position. While no differences were observed between all conditions for both post-meal glucose and insulin responses, glycaemic variability measured by continuous glucose monitoring was reduced to a greater extent in the two frequent break conditions rather than in the walking condition, suggesting greater glucose control.

Other recent studies (Peddie *et al.* 2013) have compared the metabolic effects of microbouts of activity to one isocaloric continuous bout. Overall, these studies showed that while microbouts of activity reduce plasma glucose concentrations, one continuous bout of activity is associated with lower plasma lipid concentrations, mainly triglycerides and in some instances free fatty acids. These findings suggest the differential metabolic responses to one continuous bout or microbouts of activity are associated with a greater reliance upon lipid or carbohydrate, respectively, independent of differences in energy expenditure and/or energy balance. Only a few studies have examined the effects of frequent interruptions to prolonged sitting on both postprandial respiratory quotient and plasma insulin, the respective effector and regulator in response to meal consumption. Peddie *et al.* (2013) observed an increase in 9 h RQ with microbouts of activity but no change in RQ with one bout of activity. This however did not inform on changes in metabolic flexibility as only the average values were considered in this study.

In summary, a number of studies have linked time spent sitting to adverse health effects and increased risk of all-cause mortality, while others have found no association. Also, a growing number of studies have examined the effects of frequent interruptions to sedentary activities on postprandial glucose, lipid and insulin concentrations. It will be important to perform detailed physiological studies into the role of physical inactivity on fuel selection as new strategies to break up sedentary behaviours emerge.

Conclusions and future directions

It seems clear that physical activity level is a key determinant of metabolic flexibility and metabolic flexibility plays an important role in the regulation of fuel homeostasis and metabolic health. However, it is surprising to observe that few studies have directly examined the role of physical activity and sedentary behaviours on the regulation of metabolic flexibility. This may be due to the constraints of the initial definition and methods for assessing metabolic flexibility established by Mandarino and Kelley (i.e. changes in RQ in response to a hyperinsulinaemic euglycaemic clamp). We believe that by broadening this definition and offering alternative methods of assessing metabolic flexibility in a wide variety of physiological conditions will allow for a more comprehensive examination of this relationship in the future. Overall, the goal of this line of research is to generate sufficient data to help refine the guidelines on physical activity; to recommend the most appropriate approach to breaking up sedentary time either as a continuous bout or in total time; and to understand how manipulations in frequency, duration, intensity and volume of daily physical activity differentially impact various components of the metabolic flexibility concept. Future studies will need to confirm our hypothesis that reducing time spent sedentary and/or frequently breaking up time spent sedentary with multiple short bouts of activity has an equivalent or more favourable effect than an isoenergetic continuous bout of physical activity on the many facets of metabolic flexibility (e.g. improved 24 h glucose control, lower postprandial glycaemia, higher nocturnal fat oxidation).

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Additional information

Competing interests

The authors have no conflicts of interest to disclose.

Author contributions

C.A.R. and A.B. wrote the first draft of the review, and all authors contributed to the revising and editing of the paper. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

NIH/NIDDK Colorado Nutrition Obesity Research Center 5 P30 DK048520-21, K24 DK02935, R01 DK62874 (D.H.B.); NIH T32 HL116276 (C.A.R.); NIH/NIDDK K99DK100465 (A.B.).

Acknowledgements

We would like to thank Chantal Simon for her scientific input particularly on the mathematical approach using the percentiles/percentiles relationship to assess metabolic flexibility.