Title of Article: Carbohydrate oxidation and glucose utilisation under hyperglycaemia in aged and young males during exercise at the same relative exercise intensity

Preferred Running Head: Carbohydrate metabolism and ageing

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Abstract

Purpose: The purpose of the present study was to investigate the age related carbohydrate oxidation and glucose utilisation rate response during exercise at the same relative intensity under hyperglycaemia in aged and young males

Methods: 16 endurance trained aged (n = 8; 69.1 ± 5.2 yr) and young (n = 8; 22.4 ± 2.9 yr) males were studied during 40 minutes of cycling exercise (60% $\dot{V}O_{2max}$) under both hyperglycaemic and euglycaemic (control) conditions. Venous blood samples were collected at baseline, post infusion, mid and post exercise. Carbohydrate and fat oxidation rates were determined at both 15 and 35 mins during exercise and glucose utilisation rates were calculated.

Results: The aged group displayed significantly lower rates of carbohydrate oxidation during exercise during maintained hyperglycemia (15 mins = 2.3 ± 0.4 vs. 1.6 ± 0.5 $g.min^{-1}$; 35 mins = 2.3 ± 0.5 vs. 1.5 ± 0.5 $g.min^{-1}$) and control (15 mins = 2.2 ± 0.4 vs. 1.6 ± 0.7 $g.min^{-1}$; 35 mins = 1.9 ± 0.7 vs. 1.3 ± 0.7 $g.min^{-1}$) conditions ($P = 0.01$). The rate of glucose utilisation during exercise was also significantly reduced (85.76 ± 23.95 vs 56.67 ± 15.09 $uM.kg^{-1}.min^{-1}$). There were no differences between age groups for anthropometric measures, fat oxidation, insulin, glucose, NEFA, glycerol and lactate ($P > 0.05$), although hyperglycemia resulted in elevated glucose and insulin, and attenuated fat metabolite levels.

Conclusion: Our findings highlight that ageing results in a reduction in carbohydrate oxidation and utilisation rates during exercise at the same relative exercise intensity.

Keywords: Carbohydrate Oxidation, Fat Oxidation, Glucose Clamp, Insulin, Ageing, Endurance

Abbreviations

AMPK = adenosine 5'-monophosphate-activated protein kinase
ANOVA = analysis of variance
DXA = dual-energy X-ray absorptiometry
ELISA = enzyme-linked immunosorbent assay
FPG = fasting plasma glucose
GLUT-4 = insulin-sensitive glucose transporter
HOMA2 = homeostasis model assessment
HOMA2-%B = β-cell function
HOMA2-%S = insulin sensitivity
HOMA2-IR = insulin resistance
HR_{max} = Maximal heart rate
INF = glucose infusion rate
NEFA = non-esterified fatty acids
$\dot{V}CO2$ = volume of carbon dioxide
$\dot{V}O2$ = volume of oxygen
$\dot{V}O_{2max}$ = maximal oxygen uptake
RER = respiratory exchange ratio
SC = space correction
UC = correction for urinary loss of glucose

INTRODUCTION

Ageing is associated with a decline in physical activity which may lead to alterations in carbohydrate metabolism, particularly in terms of glucose utilisation (DiPietro et al. 2006) and oxidation rates (Sial et al. 1996). Skeletal muscle is the major site relating to insulin-mediated glucose disposal, with the level of oxidative capacity suggested to relate to the effectiveness of insulin action (Short et al. 2003). The rate of glucose uptake by muscle depends on both exercise and circulating levels of insulin concentration (Kern et al. 1990). Previously untrained aged adults display a reduced carbohydrate oxidative capacity compared to young untrained adults at the same relative intensity (Meredith et al. 1989; Coggan et al. 1992b). This is particularly problematic as a lack of exercise training in aged adults may lead to reduced insulin sensitivity and ultimately insulin resistance (Lee et al. 2015; Colberg et al. 2016).

Despite the reported reductions in carbohydrate oxidation due to ageing at the same relative intensity, evidence suggests that long-term endurance training can attenuate the effects of ageing (Coggan et al. 1992a; Dube et al. 2008). Comparisons between trained and sedentary aged adults have revealed an improvement in insulin sensitivity and glucose disposal within trained aged adults (Pratley et al. 1995; Lanza et al. 2008). Training intervention studies have also found improvements in insulin-mediated glucose disposal and physical fitness parameters in previously sedentary aged adults (Cox et al. 1999; Evans et al. 2005; DiPietro et al. 2006; Bloem and Chang 2008). Thus it may be possible to offset some of the adverse physiological effects of ageing through regular endurance exercise. Bassami et al. (2007) previously found that a relative exercise intensity of 60% $\dot{V}O_{2max}$ produced peak fat oxidation rates in aged adults. Subsequently, this would allow for the measurement of fat oxidation and the relative contribution of carbohydrate oxidation during exercise.

Previous work investigating carbohydrate metabolism in trained aged adults compared to young have utilised the hyperinsulinemic clamp technique at rest (Dubé et al. 2016). However, no study to date has investigated the response under conditions of hyperglycaemia to quantify insulin resistance/sensitivity alongside oxidation rates during exercise. In this study we investigated the age-related carbohydrate oxidation and glucose utilisation rate response during a steady state exercise protocol at the same relative exercise intensity under maintained hyperglycaemia. We hypothesized that trained young males would demonstrate higher rates of

carbohydrate oxidation and glucose utilisation rates in comparison to trained aged males at the same relative exercise intensity.

METHODS

Participants

Sixteen trained males who regularly participated in endurance exercise (running = 6 and cycling = 10, >3 times per week) were recruited for the study and separated into the aged (n = 8) and young (n = 8) group categories (Table 1). Participants were recruited from local running and cycling clubs through online advertisements and follow up via email/phone. The participants were medically screened by a qualified physician prior to study commencement. Following the initial screening and testing process, four aged participants declined to take any further part in the study (i.e. 12 recruited, with 8 aged participants completing the study). All participants were non-smokers, free from any metabolic conditions (e.g. diabetes, cardiovascular disease and musculoskeletal issues) and not on any medications known to influence glucose/fat metabolism. Informed consent was obtained from all individual participants and ethics approval was granted by the Liverpool John Moores University ethics committee prior to beginning the study.

*****Insert Table 1 Around Here*****

Experimental Design

This study was a single-blind, repeated measures design that took place over a 3-week period. The study consisted of an initial anthropometric and physiological assessment with protocol familiarisation, followed by two steady state exercise protocols during which glucose or saline infusion took place in a sequential order. The glucose infusion trial took place first because the rate of saline infusion needed to be equivalent for that of glucose infusion (MacLaren et al. 1999). However, the participant was not informed of which infusion condition they were undertaking. Each visit to the laboratory was separated by a 7-day period to ensure sufficient recovery between the trials. Figure 1 outlines the protocols and sampling time points of the experimental protocol. Participants were required to refrain from drinking alcohol and

strenuous exercise 24 hours before trials. Furthermore, participants were required to fast for 12 hours prior to commencement, with all trials carried out in the morning (08:00).

*****Insert Figure 1 Around Here*****

Protocols

Anthropometric and Physiological Assessment

On arrival at the laboratory, participants were assessed for height and weight using a calibrated stadiometer and digital scale, respectively (Seca, Gmbh & Co., Germany). Following this initial assessment, fat and lean body mass were determined using dual-energy X-ray absorptiometry (DXA) (Hologic QDR Series Discovery A, Bedford, MA). Prior to scanning, the DXA unit was calibrated using criterion phantom devices provided by the manufacturer to minimise scanning errors. Participants followed standard protocols of food and fluid intake prior to each scan. The participants were placed with their hands in a pronated flat position, with the legs secured with straps to avoid overlap within the lower limbs in minimal clothing (i.e. t-shirt and shorts). The same technician analysed all scans using manual analysis to determine total body fat and lean body mass.

In order to determine maximal oxygen uptake ($\dot{V}O_{2max}$), each participant completed an incremental maximal cycling test on a Monark cycle ergometer (874E, Vansbro, Sweden). The test began at 90 W and was increased by 30 W every 3 minutes until volitional exhaustion. Expired gas was collected for the final 60 seconds of each 3-minute stage using the Douglas bag method (Douglas 1911). A mouthpiece connected to a two-way valve was used for collection of gas samples (Cranlea & Company, Birmingham, England), which were subsequently analysed for oxygen and carbon dioxide concentrations (Servomex 5200S, Crowborough, UK). $\dot{V}O_{2max}$ was confirmed using established physiological criteria from the British Association of Sport and Exercise Science (BASES), and included oxygen uptake reaching a plateau with increasing work rate, a heart rate close to age predicated maximal values, and a rating of perceived exertion (RPE) of 20. Following determination of individual $\dot{V}O_{2max}$ values, work rates equivalent to 60% $\dot{V}O_{2max}$ were calculated by interpolating the relationship of $\dot{V}O_{2max}$ and work rate (W).

Steady State Exercise Infusion Protocol

On arrival to the laboratory, participants were instructed to void urine before lying on a medical bed in a supine position for insertion of a 16-gauge IV cannula into the antecubital vein of the right hand under local anaesthetic for infusion of glucose (Infusion Pump, Colleague, Baxter Healthcare, IL, USA). The left hand was placed in a hotbox in order to arterialise the blood and a 20-gauge cannula was inserted retrogradely into the dorsal vein of the left hand for sampling arterialised venous blood. The use of a heated superficial hand vein as a replacement for an artery has been validated for measurement of glucose kinetics in humans (Abumrad et al. 1981). A baseline blood sample (20ml) was taken after 20 minutes rest, after which a priming infusion of 20% dextrose was initiated into the right vein for 30 minutes to increase the blood glucose concentration to 10 mM in accordance to the method by Defronzo et al. (1979). During this period, blood glucose concentration was measured (Hemocue AB, Angelholm, Sweden) and the infusion rate adjusted every 5 minutes based on the negative feedback principle. At the end of the 30 minute prime infusion of glucose a further blood sample (20ml) was taken.

Thereafter, participants cycled at 60% $\dot{V}O_{2max}$ for 40 minutes on a Monark cycle ergometer (874E, Vansbro, Sweden) following a 5 minute warm up period at 90 W. Dextrose was continually infused to maintain blood glucose concentration at 10mM, with glucose concentration measured and infusion rate adjusted every 5 minutes accordingly. Participants were required to maintain a constant cycle cadence of between 60 – 70 rpm on the cycle ergometer during the exercise period. Two more venous blood samples (20 ml) were taken during exercise (20 min) and immediately after exercise (40 min). Indirect calorimetry was performed during exercise for the measurement of oxygen volume ($\dot{V}O_2$), carbon dioxide volume ($\dot{V}CO_2$) and respiratory exchange ratio (RER). Samples were collected at 15 and 35 minutes using the Douglas bag method previously detailed and whole-body substrate oxidation rates (carbohydrate and fat) were determined using the stoichiometric equations of Frayn (1983).

On completion of the exercise protocol, participants lay down on a medical bed whilst the glucose infusion rate was gradually slowed. Participants were provided with exogenous carbohydrate sources (e.g. sandwiches, chocolate and sports drinks) to prevent rebound hypoglycaemia. During this recovery period, blood glucose was continually measured every 5

minutes until glucose levels were stable. A urine sample was collected immediately following the recovery period to measure any spill over of glucose.

In the saline control trial, participants were infused with a 0.9% saline solution instead of receiving the dextrose solution. The exercise protocol, indirect calorimetry and blood sample collection time points were identical to the previous dextrose infusion trial.

Blood Sample Analysis

Venous blood samples were withdrawn using a sterile plastic syringe and gauge needle, which were treated using different anticoagulants specific to each variable. Blood samples were centrifuged (Sigma 3-18K, Osterode am Harz, Germany) for 15 minutes (speed = 3950 rpm, RCF = 3000, temperature = 4^{o}C). Plasma was removed and stored in an ultra-low temperature freezer (Thermoforma, Ohio, USA) at -80^{o}C until further analysis. Plasma samples were defrosted and analysed for non-esterified fatty acids (NEFA), glycerol, glucose and lactate concentrations using a fully automated bench top clinical chemistry analyser (Daytona RX, Randox Laboratories Ltd, Crumlin, UK). Plasma samples were also analysed for insulin concentration using a solid phase enzyme-linked immunosorbent assay (ELISA) kit (Demeditec Diagnostics GmbH, Germany) based on the sandwich principle. For the lactate analysis, only four of the eight participants in the elderly group were successfully analysed for lactate concentrations.

HOMA Scores

Insulin resistance (HOMA2-IR), insulin sensitivity (HOMA2-%S) and β-cell function (HOMA2-%B) in fasting state were determined using a homeostasis model assessment (HOMA2). These values were calculated using a computer programme based on the equations of Levy et al. (1998). The programme is based on a corrected non-linear model that has been calibrated in line with current insulin assays. The calculations are made from fasting plasma insulin (FPI, measured in μIU/mL) and fasting plasma glucose (FPG, measured in mmol/l) amounts.

Rate of Glucose Utilisation

The rate of glucose utilisation was calculated using a series of formula described by DeFronzo et al. (1979b). The utilisation rate is based on the computation of an M value, which is a measure of glucose tolerance. The M value is calculated for 5 minute intervals throughout the hyperglycaemic clamp according to the equation:

$$\mathbf{M = INF - UC - SC}$$

Where INF is the glucose infusion rate, UC is the correction for urinary loss of glucose and SC is the space correction, with all values computed in dimensions of uM.kg-1.min-1. The M value is calculated from the mean of the 5 minute intervals from 0 to 20 minutes and 20 to 40 minutes of the glucose infusion trial.

Statistical Analysis

A three-way mixed design ANOVA for trial (glucose and saline), age (young and aged) and time (15 and 35 minutes), were employed to examine the differences in the mean values of fat and carbohydrate oxidation measured during exercise. In addition, blood parameters (glucose, NEFA, glycerol, lactate and insulin) measured at -30, 0, 20 1and 40 minutes (time) across two trials (glucose and saline) and two age groups (young and aged) were analysed using a three-way mixed design ANOVA. Glucose utilisation rates were analysed across two time points (0 – 20 and 20 – 40 minutes) and age group (young and aged) using a two-way mixed design ANOVA. Where the ANOVA revealed a significant effect, post hoc tests were completed using the Bonferroni correction method. Anthropometric and training data, HOMA2-IR, HOMA2-%B and HOMA2-%S were compared between age groups (young and aged) using an independent t-test or non-parametric equivalent (Mann-Whitney test) after checking for normality. HR_{max} was the only parameter that was found to violate the assumption of normality and therefore analysed using the non-parametric equivalent test. Alpha significance level was set at 0.05 for all analyses. All data was analysed using Statistical Package for Social Sciences (version 24.0, SPSS Inc., Chicago, IL). Assumptions for all statistical analyses were explored according to the methods of Field (2009). Values are reported as mean ± SD.

RESULTS

Anthropometric and physiological assessment

297

298 The aged group displayed lower $\dot{V}O_{2max}$, HR_{max} and higher HOMA2-%B scores in comparison
299 to the young group ($P < 0.05$) (Table 1). No differences were observed for body mass, body
300 mass index, body fat %, lean body mass, HOMA2-%S and HOMA2-IR ($P > 0.05$).

301

302 **Oxidation rates**

303

304 *Carbohydrate oxidation*

305

306 The young group displayed significantly higher overall rates of carbohydrate oxidation
307 compared to the aged group during both maintained hyperglycaemia (15 mins = 2.3 ± 0.4 vs.
308 1.6 ± 0.5 $g.min^{-1}$; 35 mins = 2.3 ± 0.5 vs. 1.5 ± 0.5 $g.min^{-1}$) and control (15 mins = 2.2 ± 0.4
309 vs. 1.6 ± 0.7 $g.min^{-1}$; 35 mins = 1.9 ± 0.7 vs. 1.3 ± 0.7 $g.min^{-1}$) conditions ($P = 0.01$; Figure
310 2a). Both groups displayed significantly higher carbohydrate oxidation rates at 15 minutes
311 compared to 35 minutes during exercise ($P = 0.04$). There was a significant reduction in
312 oxidation rates during the saline control trial at 35 mins in both groups compared to the glucose
313 infusion trial ($P = 0.01$).

314

315 *Fat oxidation*

316

317 Fat oxidation rates were significant higher at 35 minutes compared to 15 minutes during control
318 conditions for the young group (0.6 ± 0.3 vs. 0.4 ± 0.2 g.min-1, $P = 0.04$) and aged group (0.4
319 ± 0.4 vs. 0.3 ± 0.2 g.min-1, $P = 0.04$) (Figure 2b). However, there were no time differences
320 found for both groups during maintained hyperglycaemia ($P < 0.05$). In addition, there was no
321 significant difference found in fat oxidation rates between young and aged groups ($P < 0.05$).

322

323 *****Insert Figure 2 Around Here*****

324

325 **Blood analysis**

326

327 *Glucose*

328

329 There were no significant differences found between age groups across both trials ($P < 0.05$)
330 (Figure 3a). Glucose infusion resulted in a significantly elevated glucose levels compared to

the control trial in both young (9.3 – 10.1 vs. 4.6 – 4.7 mmol/l, respectively) and aged (10.4 – 10.7 vs. 4.7 – 5.3 mmol/l, respectively) groups (P = 0.01).

Insulin

Despite displaying higher overall values in the young compared to aged group during maintained hyperglycaemia (13.6 ± 11.0 and 8.9 ± 8.9 µIU/mL), this did not reach statistical significance (P = 0.08) (Figure 3b). There were significantly higher values found during maintained hyperglycaemia compared to control in both groups when comparing baseline (-30 mins) and post exercise (40 mins) values (P = 0.01). No difference was found both within and between groups during exercise (i.e. 0 – 40 mins) for either trial (P < 0.05).

NEFA

There was a significant increase observed during control compared to maintained hyperglycaemia in both age groups (P = 0.01) (Figure 3c). Specifically, differences were found during exercise (20 mins; P = 0.04) and post-exercise (40 mins; P = 0.01) compared with baseline (-30 mins). The aged group displayed higher NEFA concentrations during exercise within the control trial (0.29 – 0.50 vs. 0.57 – 0.78 mmol/l). However, this differences were determined as non-significant (P = 0.07). No differences were observed between age groups during maintained hyperglycaemia (P < 0.05).

Glycerol

The aged group displayed higher concentrations of glycerol during both trials, however there was no statistical significance found between age groups (P = 0.67) (Figure 3d). The control trial resulted in higher concentrations for both groups, with differences found at -30 vs. 40 mins (P = 0.01), 0 vs. 40 mins (P = 0.01) and 20 vs. 40 mins (P = 0.01).

Lactate

Blood lactate concentrations were similar between both age groups across both trials (P = 0.94) (Figure 3e). Values were found to be significantly elevated immediately post-exercise compared to both baseline (-30 mins; P = 0.01) and post-infusion (0 mins; P = 0.01) across

both trials. There was also a significantly higher lactate production during maintained hyperglycaemia compared to control conditions (P = 0.02).

*****Insert Figure 3 Around Here*****

Glucose utilisation rates

Significantly higher overall utilisation rates were observed in the young compared to aged group at both 0 – 20 mins (85.76 ± 23.95 and 56.67 ± 15.09 uM.kg-1.min-1, respectively) and at 20 – 40 minutes (104.87 ± 17.79 and 57.60 ± 29.30 uM.kg-1.min-1, respectively) (P = 0.01) (Figure 4a). However, there were no significant differences found within groups at 0 - 20 mins and 20 - 40 mins following glucose infusion (P < 0.05). When glucose utilisation rates were expressed relative to total carbohydrate oxidation, the aged group displayed lower percentages at both 0 – 20 minutes (47.8 vs. 52.1%) and 20 – 40 minutes (54.4 vs. 63.7%) compared to the young group, respectively (Figure 4b).

*****Insert Figure 4 Around Here*****

DISCUSSION

The present study examined the age-related carbohydrate and fat oxidation response and glucose utilisation rates during a steady state relative exercise protocol under maintained hyperglycaemia and control conditions. We hypothesized that trained young males would demonstrate higher rates of carbohydrate oxidation and glucose utilisation in comparison to trained aged males. Our findings were in agreement with this hypothesis, with the trained aged group displaying lower rates of carbohydrate oxidation despite similar rates of fat oxidation. In addition, glucose utilisation rates were significantly reduced in trained aged adults throughout the exercise protocol. The trained aged adults displayed a reduced insulin response to hyperglycaemia, although this was not deemed statistically different to trained young adults. There were no age-related differences in NEFA, glycerol and lactate concentrations between both groups.

During moderate exercise aged adults displayed a reduction in carbohydrate oxidation despite similar fat oxidation rates to younger adults. This is in agreement with previous studies

399 which observed reduced carbohydrate oxidation using indirect calorimetry in aged athletes
400 (Sial et al. 1996; Dubé et al. 2016). Dubé et al. (2016) found aged athletes are able to maintain
401 similar levels of fat oxidation at moderate intensity exercise in comparison to their young
402 counterparts. However, when the intensity of exercise increased, the level of carbohydrate
403 oxidation was blunted in aged adults. This may be explained by the possible reduced glycogen
404 storage capacity of trained aged adults (Dubé et al. 2016). Sial et al. (1996) reported reduced
405 fat oxidation rates despite similar carbohydrate oxidation rates as observed in the present study.
406 One possible explanation for such discrepancies may be due to the lower training status of the
407 aged adults used in the study ($\dot{V}O_{2max}$ = 41.1 vs. 31.4 $ml \cdot kg^{-1} \cdot min^{-1}$). Training intervention
408 studies have also reported an improvement in fat oxidation rates in previously sedentary aged
409 adults (Sial et al. 1998). Therefore, it would appear that despite regular endurance training
410 protecting the ability to oxidise fat, there is an aged-related decline in carbohydrate oxidation.
411
412 Having stated the above, it must be borne in mind that the aged athletes exercised at a
413 lower absolute exercise intensity than the young athletes (i.e. 85W vs 127W; a 33% lower
414 exercise intensity). The difference in carbohydrate oxidation between the groups therefore
415 could be associated with the fact that the 33% lower intensity undertaken by the aged group is
416 reflected by a 30% reduction in carbohydrate oxidation. So, most of the reduced carbohydrate
417 oxidation may be associated with lower absolute exercise intensity. However, other factors
418 need to be considered since not all the reduced carbohydrate oxidation is due to exercise
419 intensity. Sial et al. (1996) previously compared both relative and absolute exercise intensities
420 in aged and young groups 60 minutes of cycle ergometer exercise under euglycaemic
421 conditions. The authors found the aged group displayed 35% higher carbohydrate oxidation
422 rates at the same absolute intensity but 40% lower when compared at the same relative intensity
423 as the young group. This would agree with our present findings and thus the absolute intensity
424 at which aged adults exercises may play a key role in how they handle and oxidise carbohydrate
425 under both hyperglycaemic and euglycaemic conditions.
426
427 Ageing has previous been associated with a decrease in glucose tolerance (Scheen
428 1997; Meneilly and Tessier 2001) and increased peripheral resistance to the action of insulin
429 (Broughton and Taylor 1991; Ryan 2000). The HOMA2 data revealed that trained aged adults
430 had significantly higher HOMA2-%B values compared to the trained young group. Despite a
431 trend towards lower HOMA2-%S and higher HOMA2-IR values in the trained aged group, this
432 was deemed to not be statistically different to the young group. Similar findings were evident

for the insulin response during exercise, with a trend towards lower values in the aged group but was not deemed statistically significant. As the glucose utilisation rates were significantly lower in the aged group, this would suggest that there may be evidence of reduced insulin sensitivity within the aged group. One possible cause may be due to lower levels of GLUT-4 in skeletal muscle, which has previously been associated with adults demonstrating reduced insulin sensitivity (Dela et al. 1994). However, training intervention studies have found that aged adults maintain the ability to increase concentrations of GLUT-4 in skeletal muscle (Cox et al. 1999; Kim et al. 2004; Biensø et al. 2015). Although speculative as muscle biopsies were not taken in the present study, it may be pertinent that aged adults can increase their GLUT-4 concentrations but are still lower than their younger counterparts which limits the oxidation and disposal of exogenous carbohydrate sources during exercise.

Another possible mechanism for the reduced uptake of glucose in aged adults may relate to skeletal muscle function involving adenosine 5'-monophosphate-activated protein kinase (AMPK). AMPK has previously been described as a key "master switch" in metabolism regarding the regulation of fuel transport for oxidation (Vigelsø et al. 2016). Whilst AMPK plays a major role in the regulation of intracellular fatty acid oxidation, it has previously been reported that it may also regulate insulin sensitivity via stimulating GLUT-4 expression (Jessen et al. 2003). Previous research has reported ageing-related insulin resistance associated with impaired AMPK-α activity (Qiang et al. 2007; Morris et al. 2010). It has been suggested that regular exercise may increase the recruitment of the AMPK signalling system and thus reduce the level of glucose intolerance in aged populations (Winder and Hardie 1999). In the present study, glucose utilisation rates expressed as a % of total carbohydrate oxidation were lower in the aged group across the exercise protocol. This would imply that ageing results in a reduction in the ability to utilise exogenous carbohydrate intake, possibly due to the limited activity of AMPK. However, further research is required investigating the AMPK response within endurance-trained aged adult humans.

Previous research has reported an increase in fat mass and decrease in lean body mass with advancing age in adults (Vermeulen et al. 1999). The present study revealed no difference between aged and young adults in terms of lean body mass, body fat % and body mass index when matched for exercise type and frequency. Longitudinal training data supports this notion, with significant reductions in fat mass reported in previously sedentary aged adults (Evans et al. 2005). Despite similar anthropometric characteristics between age groups, the aged adults

displayed significantly reduced fitness levels compared to young adults (e.g. $\dot{V}O_{2max}$ = 41.1 vs. 55.8 ml·kg^{-1}·min^{-1}, respectively). This is in agreement with previous research comparing trained adults across different age groups (Sial et al. 1996; Dubé et al. 2016). The decline in fitness levels is despite an apparent ability for aged adults to still evidence sufficient peripheral adaptation to exercise interventions (Coggan et al. 1992a; Sial et al. 1998). Dubé et al. (2016) suggested that this reduction in peak fitness is down to central factors rather than peripheral due to similar skeletal muscle capillarization and mitochondrial oxidative capacity between trained aged and young adults. Despite these differences between age groups, previous research would suggest that regular endurance exercise enhances overall fitness and metabolic capacity relative to sedentary adults in aged adults (Amati et al. 2011).

There were no significant differences observed between age groups for blood metabolic markers during exercise. Both lactate and glycerol values increased during exercise in both groups compared to baseline values. Hagberg et al. (1988) previously reported no difference in blood lactate values between trained older and younger runners during one hour of treadmill running at 70% $\dot{V}O_{2max}$. It has also been found that following a 16-week endurance training intervention on older adults, there were no significant differences in glycerol kinetics during exercise compared to younger adults (Sial et al. 1998). These findings would suggest that aged adults regularly engaged in endurance training maintain the ability to regulate glycerol metabolism during exercise. Conversely, Sial et al. (1996) reported that aged adults displayed reduced glycerol and free fatty acid kinetics in comparison to a young control group matched for anthropometric variables (e.g. lean body mass). However, the aged subjects used in this study were of a lower fitness level compared to the present study ($\dot{V}O_{2max}$ = 31.4 vs. 41.1 ml·kg^{-1}·min^{-1}). Therefore, this suggests that differences between age groups may relate to the level of fitness attained by aged adults later in life.

There are several limitations that must be noted relating to the present study. Firstly, the type and intensity of the exercise protocol used in the present study (i.e. 60% of $\dot{V}O_{2max}$) was chosen as it has been previously shown to produce peak fat oxidation levels in aged adults (Bassami et al. 2007). This doesn't account for individual variation and the difference with the younger group. However, controlling the intensity in this way allows direct comparison of metabolic variables across age groups. The exercise protocol was only limited to 40 minutes to allow sufficient time for changes in metabolism during exercise. It must be noted that longer duration protocols have been used previously when investigating the metabolic response during

exercise (MacLaren et al. 1999). Therefore, the findings from this study are limited to type and duration of protocol utilised. In addition, we chose to compare the two age groups relative to their own individual $\dot{V}O_{2max}$ values (i.e. at 60% $\dot{V}O_{2max}$). Future work should investigate the same relative and absolute exercise intensities in order to tease out the ageing effects and the intensity of effort effects. The sample size of this study was relatively small per group and it must be acknowledged that further work with larger sample sizes would further enhance this research area in the future.

In conclusion, the results from the present study demonstrate that trained aged adults display lower rates of carbohydrate oxidation despite similar rates of fat oxidation when exercising at the same relative intensity. The effects of exercise intensity per se probably accounts for the major difference reported here. Likewise, glucose utilisation rates were significantly reduced in trained aged adults during steady state exercise. Trained aged adults displayed a reduced insulin response to hyperglycaemia during exercise, although this was not deemed statistically significant. There were no age-related differences in NEFA, glycerol and lactate concentrations between both aged groups. As trained aged adults appear to have a reduced ability to oxidise and utilise exogenous carbohydrate during exercise, it would be suggested that the use of carbohydrate supplements (e.g. energy gels and drinks) must be taken with caution. Over-use of such supplements may lead to an increase in fat mass and subsequently lead to aged-related health issues in the future.

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Table 1. Participant anthropometric and physical characteristics according to age group (mean ± SD)

Variable	Young	Aged
Age (years)	22.4 ± 2.9	69.1 ± 5.2*
Training Frequency (sessions per week)	4 ± 1	4 ± 1
Training Duration (total per week – mins)	258 ± 30	251 ± 31
Height (m)	1.8 ± 0.04	1.7 ± 0.1*
Body Mass (kg)	78.6 ± 4.3	76.3 ± 10
Body Mass Index (kg/m^2)	24.8 ± 1.7	25.9 ± 2.4
Body Fat (%)	18.9 ± 3.9	20.8 ± 4.3
Lean Body Mass (kg)	59.5 ± 4	57.7 ± 7
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	55.8 ± 5.1	41.1 ± 12.2*
HR_{max} (beats/min)	190.5 ± 8.7	160 ± 10.3*
HOMA2-%B	63.6 ± 22.4	125.3 ± 78.2*
HOMA2-%S	230.6 ± 102.0	146.0 ± 95.2
HOMA2-IR	0.5 ± 0.3	1.0 ± 0.5

$\dot{V}O_{2max}$ = maximal oxygen uptake; HR_{max} = maximal heart rate; HOMA = homeostatic model assessment. %B = β cell function; %S = insulin sensitivity; IR = insulin resistance. * denotes significant difference ($P < 0.05$) between age groups.

Figure Captions

Figure 1. Schematic representation of the experimental protocol. Abbreviations: BS = blood sample; RES = respiratory gas collection; U = urine sample.

Figure 2. Oxidation rates during glucose and saline infusion trials for young and aged groups. **a)** carbohydrate and **b)** fat. G = Glucose; S = Saline. ***** denotes significant difference for age; **#** denotes significant difference for time (15 vs. 35 mins); **$** denotes significant difference for trial (saline vs. glucose).

Figure 3. Blood analysis measures at baseline (-30 mins), post-infusion (0 mins), during exercise (20 mins) and post-exercise (40 mins) during glucose and saline infusion trials for young and aged groups. **a)** glucose; **b)** insulin; **c)** NEFA; **d)** glycerol; **e)** lactate concentrations. # denotes significant difference for time (compared to baseline); **$** denotes significant difference for trial (saline vs. glucose).

Figure 4. a) Glucose utilisation rates during exercise for young and aged during the glucose infusion trial. **b)** Glucose utilisation rates relative to overall carbohydrate oxidation rates during exercise for young and aged groups. * denotes significant difference between age groups ($P < 0.05$).