SODIUM BICARBONATE INGESTION AUGMENTS THE INCREASE IN PGC-1α mRNA EXPRESSION DURING RECOVERY FROM INTENSE INTERVAL EXERCISE IN HUMAN SKELETAL MUSCLE

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TITLE: Sodium bicarbonate ingestion augments the increase in PGC-1α mRNA expression during recovery from intense interval exercise in human skeletal muscle

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ABSTRACT

We investigated the hypothesis that ingestion of sodium bicarbonate prior to an acute session of high-intensity intense interval training (HIIT) would augment signalling cascades and gene expression linked to mitochondrial biogenesis in human skeletal muscle. On two occasions separated by ~1 wk, nine active men (22 ± 2 y; 78 ± 13 kg, VO_{2peak} = 48 ± 8 mL/kg/min; mean ± SD) performed a HIIT protocol that involved 10 x 60 s cycling efforts at an intensity eliciting ~90% of maximal heart rate (263 ± 40 W), interspersed by 60 s of recovery. In a double-blind, crossover manner, participants ingested either 0.2 g kg^{-1} b.w. sodium bicarbonate (BICARB) or an equimolar dose of a placebo, sodium chloride (PLAC), 90 and 60 min prior to exercise. Blood samples confirmed that bicarbonate concentration, as well as pH and lactate, were all elevated during and following exercise after BICARB *vs.* PLAC ($p < 0.05$). Needle biopsies from the *vastus lateralis* obtained immediately post HIIT revealed that glycogen utilization was greater after BICARB (27%) *vs*. PLAC (11%). Phosphorylation of acetyl-CoA carboxylase, a downstream marker of AMPK activity, and p38 MAPK were increased immediately after exercise compared to rest with no differences between treatments ($p > 0.05$). However, compared to rest, PGC-1α mRNA expression was increased after 3 h of recovery to a greater extent in BICARB vs. PLAC (~7- vs. 5-fold, $p < 0.05$). We conclude that $NaHCO_3$ ingestion altered skeletal muscle metabolism during HIIT and augmented the expression of PGC-1α mRNA during recovery. These results provide novel insights into potential mechanisms responsible for enhanced adaptations after chronic supplementation.

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LIST OF ABBREVIATIONS

ACC	–	acetyl-CoA carboxylase
ADP	–	adenosine diphosphate
AMP	–	adenosine monophosphate
AMPK	–	5'AMP-activated protein kinase
ANOVA	–	analysis of variance
ATF2	–	activating transcription factor-2
ATP	–	adenosine triphosphate
β-HAD	–	3-hydroxyacyl-CoA-dehydrogenase
BICARB	–	sodium bicarbonate trial
BMI	–	Body Mass Index
Ca^{2+}	–	calcium
CAMKK	–	Ca^{2+}/CaM-dependent protein kinase kinase
CHO	–	carbohydrate
COX	–	cytochrome c oxidase
Cr	–	creatine
CS	–	citrate synthase
CYT	–	cytosolic fractions
eNOS	–	endothelial nitric oxide synthase
$FABP_{pm}$	–	fatty acid binding protein
FAT/CD36	–	fatty acid translocase
GAPDH	–	glyceraldehyde-3-phosphate dehydrogenase
GLUT 4	–	glucose transporter 4
H^+	–	hydrogen ion
HCO_3^-	–	bicarbonate

HDAC	–	histone deacetylase
HIIT	–	high-intensity interval training
HR_{max}	–	maximum heart rate
LDH	–	lactate dehydrogenase
LT	–	lactic threshold
MCT	–	mono-carboxylate transporter
MEF2	–	myocyte enhancer factor-2
MHC	–	myosin heavy chain
MICT	–	moderate intensity continuous training
mtDNA	–	mitochondrial DNA
NRF	–	nuclear respiratory factor
NaCl	–	sodium chloride
$NaHCO_3$	–	sodium bicarbonate
NBC	–	sodium bicarbonate co-transporter
NUC	–	nuclear fractions
O_2	–	oxygen
p38 MAPK	–	p38 mitogen-activated protein kinase
PCr	–	phosphocreatine
PDH	–	pyruvate dehydrogenase
PDK	–	pyruvate dehydrogenase kinase
PFK	–	Phosphofructokinase
PGC-1α	–	peroxisome proliferator-activated receptor γ co-activator -1α
PHOS	–	glycogen phosphorylase
PHR	–	peak heart rate

Pi	–	inorganic phosphate
PLAC	–	sodium chloride trial
PPAR-δ	–	peroxisome proliferator-activated receptor-delta
ROS	–	reactive oxygen species
S.D.	–	Standard Deviation
SIT	–	sprint interval training
SR	–	sarcoplasmic reticulum
TBS-T	–	tris buffer saline with tween
TFAM	–	mitochondrial transcription factor A
TTE	–	time to exhaustion
VO_{2max}	–	Maximal oxygen uptake
VO_{2peak}	–	peak volume of oxygen uptake
W_{max}	–	maximum power output

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Chapter I – Review of the Literature

I. INTRODUCTION

Interval training as an exercise modality has received renewed interest in scientific, athletic and public health circles, owing in part to its potential to induce numerous adaptations that are usually associated with continuous, moderate-intensity exercise training (42, 44). Interval exercise fundamentally involves repeated bouts of relatively intense exercise, interspersed by periods of rest or lower intensity exercise for recovery. A number of acronyms have been used to describe the vast array of interval protocols that have been developed, leading to a general lack of standardization in the literature. Recently, Weston et al. proposed a relatively simple terminology scheme to categorize interval training, and the nomenclature proposed will be adopted for the purposes of this review. The authors recommended that "sprint-interval training" (SIT) be used to describe "all-out" efforts or any workload that exceed that which elicited 100% maximal oxygen uptake (VO_{2max}). In contrast, "high-intensity interval training" (HIIT) was proposed to describe work intensities that elicited 80-100% of peak heart rate (PHR). The authors also recommended "moderate-intensity continuous training" (MICT) to describe traditional endurance-type exercise that evokes 60-75% PHR (134). The first section of this review will focus on some of the major physiological adaptations to interval training, with a particular emphasis on structural and metabolic changes in skeletal muscle and the functional consequences in healthy individuals.

II. SKELETAL MUSCLE ADAPTATIONS TO INTERVAL TRAINING

a. Structural adaptations

One of the most well characterized adaptations to interval training is a robust increase in skeletal muscle mitochondrial content. As little as 6 sessions of HIIT or SIT over a 2 wk training

period has been demonstrated to increase the maximal activity and/or protein content of representative mitochondrial markers including citrate synthase (CS) and cytochrome oxidase (COX) (19, 20, 43, 59, 74). For example, a HIIT protocol that entailed 6 sessions of training over 2 wk, each involving 8-12 x 60 s intervals at ~100% peak power interspersed with 75 s of recovery, led to increases in the protein content and activity of COX and CS by ~30% and ~20%, respectively (74). Further research investigating a direct functional role of these adaptations have shown increases in respiration from isolated mitochondria (59) and elevated muscle oxidative capacity through phosphorus magnetic resonance spectroscopy (68).

There is also evidence that interval training may result in a phenotypic transition of the skeletal muscle fiber type. For example, Joanisse et al. demonstrated that 6 weeks of HIIT consisting of 10 x 60 s cycling bouts interspersed by 60 s of active recovery resulted in an increase in hybrid fibres expressing both myosin heavy chain (MHC) I and II isoforms. This was associated with a reduction in type II fibres suggesting there was a fast-to-slow transition, representative of a greater oxidative phenotype (63). These results are supported by Simoneau et al., who showed increases in type I fibers along with a decrease in type IIB after 15 wks of SIT (118). Conversely, Jansson et al. reported a decline in type I fibers and an increase in type IIA fibers after 6 wks of Wingate based SIT training (61). It is unclear what may be underlying these seemingly paradoxical results, however, it has been suggested that the methodology to classify these fiber type transitions (i.e. MHC or myofibrillar actomyosin ATPase staining) may be inherently flawed and therefore the topic is still under much scrutiny (17).

A more consistent finding in interval training studies has been a noted change in proteins involved in carbohydrate (CHO) and fat metabolism. Specifically, proteins involved in glycolysis and CHO oxidation including; phosphofructokinase (PFK), pyruvate dehydrogenase

kinase (PDK), lactate dehydrogenase (LDH) and pyruvate dehydrogenase (PDH), have been elevated after training (20, 94). Research also supports increases in the whole muscle expression of transport proteins associated with the import of CHO as well as export of by-products such as glucose transporter (GLUT 4) and mono-carboxylate transporter (MCT 1 and 4) (131). With respect to fat oxidation, interval training has been shown to elevate the protein content of various proteins associated with the multiple levels of regulation. For example, plasma membrane fatty acid binding protein ($FABP_{pm}$) and fatty acid translocase (FAT/CD36) are believed to be important regulators of transport across the sarcoplasmic reticulum (SR) and mitochondria, respectively (reviewed in 123). Both have been shown to be higher in response to HIIT (95, 128, 129). It appears that intramuscular triglyceride handling is also altered as the perilipins PLIN 1 and PLIN 5, hypothesized to regulate IMTG lipolysis, are expressed more after SIT (117). In addition, the maximal activity of 3-hydroxyacyl-CoA-dehydrogenase (β-HAD) a key regulatory enzyme in the breakdown of long-chain fatty acids through β-oxidation is also elevated after SIT and HIIT interventions (20, 94–96, 128).

Emerging evidence suggests interval training can also lead to remodelling of skeletal muscle microvasculature. Cocks et al. comprehensively compared the microvascular response to MICT and SIT training protocols by investigating the angiogenesis of new capillaries, as well as changes in endothelial nitric oxide synthase (eNOS). Their results demonstrated that SIT was as effective as MICT at stimulating the growth of new capillary beds as evidenced by an increase in the capillary to fiber ratio and capillary density. Furthermore, the observed elevation of eNOS protein content after training and reduced eNOS phosphorylation in response to a single exercise bout suggests an increase in endothelial function at the microvasculature level. The results are in

support of previous work in animal models (reviewed in 69) but represent the first demonstration in humans (31).

b. Functional adaptations

Structural adaptions can converge to translate into functional adaptations. Perhaps there is no greater example of this than the improvements in VO_{2peak} and performance that has been seen after interval training. For example, a mean increase in VO_{2max} of ~8% was reported in a comprehensive meta-analysis that summarized the findings from 16 studies that implemented SIT protocols over a 2-10 wk intervention period (47). A different meta-analysis that incorporated both HIIT and SIT investigations determined a mean of 13 sessions resulted in VO_{2max} improvements of 10 and 7% in untrained men and women, respectively (135). VO_{2max} tests are an integrative measure of the cardiovascular systems ability to take up, transport and deliver oxygen to the muscle and for the muscles to extract and use the oxygen for ATP production. Due in part to its integrative nature, enhancement of any part of the pathway is believed to provide the potential to increase VO_{2max}, yet, the cardiovascular system is arguably considered to be limiting (8). It has been suggested that the increase in VO_{2max} associated with interval training is believed to at least initially be mediated by peripheral cardiovascular adaptations rather than central changes (75, 119). This is because a number of studies have demonstrated improved muscle O_2 utilization kinetics (4, 59, 75, 82) while the majority have reported no change in cardiac output, at least over the short-term (59, 75). In contrast, Esfandiari et al. recently reported cardiac outputs determined by Doppler imaging were increased in response exercise after 2 wks of HIIT, highlighting the need for future research in this area (39).

As discussed by Gibala and Jones, it is intriguing to put the adaptions noted in response to interval training into the context of what is agreed to be the determinants of endurance performance (42). The VO_{2max} sets the upper limit of an individual's maximal ability to utilize O_2 and therefore to increase it can theoretically raise the upper limit of performance. However, performance VO_2 is the O_2 consumption that is able to be sustained during more prolonged exertions and is a better determinant of performance. In contrast to the cardiovascular limitations to VO_{2max}, performance VO_2 is partially dictated by substrate utilization and muscle metabolism during exercise that helps explain performance enhancement in the absence of increases in VO_{2max} (65).

Therefore, due to the gross structural adaptations to skeletal muscle after interval training it is not surprising that athletic performance is enhanced with and without noted changes in VO_{2max}. For example, Burgomaster et al. showed a 100% increase in cycling time to exhaustion without an increase in VO_{2max} after 2 weeks of SIT in recreationally active individuals (21). The results mirror those in athletic populations that have shown replacing a portion of a training block with relatively higher intensity exercise enhances performance measures independent of VO_{2max}, although the specific mechanisms involved are likely different (reviewed in 42).

Enhanced performance after interval training is likely related to altered metabolism. For example, as reflected by the structural adaptations in skeletal muscle, there is a global increase in enzymes regulating ATP production. Those involved with glycogenolysis and glycolysis are likely increased to maximize the potential for glycolytic flux; however, increases in PDH and its regulatory kinase PDK allow for a greater match between glycolysis and oxidative phosphorylation. As a result, there is a shift away from non-oxidative metabolism towards oxidative phosphorylation in response to interval training. This is evidenced after a training

intervention by a reduction in the PCr degradation, glycogenolysis and glycolysis during performance tests (19, 20, 94, 95, 128). In addition, there is a shift towards a higher fat oxidation at submaximal workloads (95). All of these functional adaptations converge to limit glycogen depletion and are believed to be a consequence of tighter matching between ATP supply and demand. A reduction in lactate accumulation at the same exercise intensity observed with HIIT and SIT provides support for this (19, 40, 95). Furthermore, not only is there an alteration in fuel utilization to spare glycogen during exercise, there is also a marked elevation in the resting content of glycogen (19–21, 43, 74, 94, 95) and IMTGs (117). Both of which have implications in exercise performance (reviewed in 64).

III. METABOLIC BASIS FOR MUSCLE REMODELLING IN RESPONSE TO INTERVAL TRAINING

A number of signalling cascades have been identified linking the cellular perturbations associated with interval training to the plasticity of skeletal muscle in response to repeated training sessions. It is now generally accepted that the training induced adaptations, for example, increases in mitochondrial protein content, are due to the transient and repeated up-regulation of gene expression and protein translation in response to successive exercise bouts (reviewed in 32, 38). Perry et al. provided empirical support for this thesis when they investigated the temporal changes associated with single and repeated bouts of HIIT. They found that in general, increases in mRNA expression preceded changes in protein content and enzymatic activity (96). This part of the review will focus on the acute changes in skeletal muscle during interval training and the mechanisms of signal transduction that are hypothesized to underpin the adaptions discussed in

the previous section. While the focus will be on the control of mitochondrial biogenesis, many of the same signals and pathways are believed to be involved with other adaptations.

a. **Metabolic signals generated during interval training**

The metabolic stress associated with any form of exercise is dependent on the intensity and duration of the stimulus. This homeostatic disturbance associated with a single maximal effort or repeated intervals, as is the case with SIT and HIIT, imposes a huge stress to skeletal muscle because of its high relative work intensity. In order for skeletal muscle to generate the high force, it relies on the recruitment of a large proportion of muscle fibres and has an extreme demand for ATP. While muscle generally maintains ATP pools under stress, a single 30 s all-out exercise bout has been shown to result in a decline ATP concentration (13, 14, 27, 93, 142). Coinciding with the hydrolysis of ATP is the accumulation of lower energy nucleotides. Specifically, ADP and AMP concentrations can be raised 6- and 10-times the resting levels, respectively in response to a single Wingate (27, 93). In an attempt to buffer the initial demand for ATP, the near equilibrium enzyme creatine phosphokinase catalyzes the resynthesis of ATP from phosphocreatine (PCr), however, the cytosolic PCr pools are depleted 45%, 55% and 68% within the first 6, 10 and 15s of all-out cycling (14, 93). A single Wingate can reduce PCr stores by up to 90% (93). A number of metabolic by-products including, ADP, AMP, Cr inorganic phosphate (Pi) are involved with the activation of glycolysis in order to provide an additional supply of ATP. Glycogen is rapidly broken down and fluxed through glycolytic enzymes to produce pyruvate that then is converted to lactate (to restore redox balance) or enters the mitochondria (to be metabolised through oxidative phosphorylation). However, upon the first 15 s of intense exercise, PDH, an enzyme regulating the entry of pyruvate into mitochondria is not

fully activated and therefore the majority of pyruvate is converted to lactate. Furthermore, even once PDH is fully activated an imbalance between pyruvate production and oxidation persists due to the higher relative glycogenolytic flux (93). This is evident as a 30 s Wingate results in glycogen depletion of 20-35% and a subsequent 15-20 fold increase in muscle lactate (13, 27, 93). It is important to note that while substrate level phosphorylation provides a large fraction of the ATP supply during the onset of exercise, even the last half of 30 s sprint is mainly supported by oxidative phosphorylation as PDH becomes almost fully activated and pyruvate oxidation increases significantly (93). It is hypothesized that this occurs in part because of an inhibition of glycogenolysis in response to decreases in muscle pH associated with higher intensities of exercise (57, 93, 122). At the onset of subsequent intervals during a HIIT session, the cytosolic concentration of PCr remains depressed as it has yet to be fully re-synthesised (13, 93). As a result, it can't support the initial ATP demand to the same extent as the first bout and this difference is made up by a higher relative rate of oxidative phosphorylation (93). In fact, oxidative phosphorylation contributes the vast majority of ATP during repeated sprints (93).

Although a causal link has yet to be found, the high reliance on oxidative phosphorylation may also contribute to an elevation in reactive oxygen species (ROS) that can act as a signalling molecule. For example, complex I and III of the mitochondrial electron transport chain are believed to be major sites of ROS production during exercise (reviewed in 99). In addition, to signals generated from the production of ATP, calcium (Ca^{2+}) may also be an important factor to consider for signalling. As has been well documented, depolarization of the SR results in the release, and eventual re-uptake, of Ca^{2+} that regulates excitation-contraction coupling (reviewed in 83). However, a single bout of high-intensity exercise transiently reduces the ability of the SR to release and uptake Ca^{2+} (56, 76, 77). Based on these

observations one hypothesis is that interval training may result in a greater accumulation of cytosolic Ca^{2+} in comparison to continuous moderate exercise per se, that has implications for cellular signalling (6).

b. Metabolic and contractile sensors signalling cellular energy demands

As noted earlier, shifts in substrate utilization (i.e., glycogen breakdown and lactate accumulation), ATP turnover (i.e., ADP & AMP), mechanical stress, redox status and calcium flux are all potent cellular signals induced by contraction. Protein sensors are regulated by these cellular stresses and initiate several mechanisms of signal transduction ultimately leading to the transcription of genes encoding proteins regulating cellular bioenergetics and muscle phenotype. This intricate network is deemed excitation-transcription coupling and has been thoroughly reviewed elsewhere (38, 48), however, two putative sensors with particular relevance are discussed below.

The 5'AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase that is a critical cellular energy sensor that can regulate metabolism (51, 90). In addition, it has been well established that AMPK plays a critical role in the adaptations to exercise in skeletal muscle (62) including mitochondrial biogenesis (90). In terms of acute modulation of metabolism, the role of AMPK is ultimately ATP conservation. Activation (ie. phosphorylation) of AMPK leads to the down-regulation of energy consuming pathways and conversely the up-regulation of energy producing pathways. For example, skeletal muscle protein (15, 49) and glycogen (22) synthesis are negatively modulated with AMPK activation. In contrast, pathways implemented in the rapid production of ATP such as contraction induced glucose uptake (91) have at least in part been associated with increased AMPK activity.

In order to maintain energy homeostasis against future insults, the activation of AMPK also acts as an upstream signal for muscle adaptation. AMPK can phosphorylate and thus inactivate histone deacetylase 5 (HDAC5) (79), a protein that has been proposed to be critical in regulating the metabolic adaptive response by modulating gene expression (81). In addition, AMPK can enhance the activity of peroxisome proliferator-activated receptor γ co-activator -1α (PGC-1α) (60) and effect its subcellular localization (120) both of which have implications for mitochondrial biogenesis (discussed further below). Overall, AMPK can activate a number of different pathways ultimately converging on transcriptional regulation, however, a full description is outside of the scope of this review and has been extensively reviewed elsewhere (80).

Due to its implications to muscle adaptation, health and disease, there is particular interest in the control of AMPK activity. AMPK is a heterotrimer composed of an α (1 or 2), β (1 or 2) and γ (1,2 or 3) subunit, the majority of which are α2, β2 and γ1 in human skeletal muscle (137). The major level of regulation is through phosphorylation at threonine 172 on the catalytic α subunit by several upstream kinases including Ca^{2+}/CaM-dependent protein kinase kinase (CaMKK) in response to intracellular Ca^{2+} (reviewed in 92). In addition, elevated ADP and AMP concentrations allosterically regulate AMPK by binding to the γ subunit. This binding has also been proposed to protect activated AMPK complexes from dephosphorylation, making ADP and AMP potent regulators (112). A relatively less understood mechanism modulating AMPK activity is the sequestering of the β subunit by oligiosacharides such as glycogen (78). When sequestered, the β subunit may become unavailable to act as a scaffold for AMPK complex formation resulting in decreased activity (78, 113). Interestingly, mice lacking muscle β2 are

deficient in the exercise-induced activation of PGC-1α transcription and target genes co-activated by PGC-1α signalling, highlighting its importance in muscle regulation (33).
The high cellular stress associated with intense aerobic exercise, including; high Ca^{2+} flux, ATP turnover and glycogen depletion, makes it no surprise that a number of interval training protocols robustly activate AMPK (6, 45, 116, 139, 141). In fact, AMPK is activated with exercise in an intensity dependent manner suggesting that SIT arguably may be the most potent activator (28, 37, 136). Therefore, AMPK and its activation of downstream signalling may be one method which interval training stimulates an increase in mitochondrial biogenesis after training.

The p38 mitogen-activated protein kinase (p38 MAPK) is another protein kinase implicated in the signalling of skeletal muscle adaption. However, its activation is in response to stimuli associated with stress rather than metabolic perturbation. For example, a wide range of cellular stresses relevant to exercise such as cytokine release, changes in osmolality and ROS production have been linked to MAPK signalling (26). As with many other signalling molecules, p38 MAPK, exerts its effects on mitochondrial adaptations in part through the regulation of PGC-1α (1). Specifically, p38 MAPK activation can phosphorylate PGC-1α directly (102) or signal its up-regulation through the phosphorylation of various transcription factors such ATF2 and MEF2 (1). Interestingly, the isoform highly expressed in skeletal muscle (i.e., p38γ) is indispensable for the training induced increases in mitochondrial biogenesis (98). However, it has been suggested that p38 activation may play a permissive role in PGC-1α regulation (and thus mitochondrial biogenesis) more so than the direct signal itself. This is because p38 MAPK phosphorylation is observed to the same extent at both low and high-intensity endurance exercise, in contrast to other kinases that are only activated at high-intensity which ultimately resulted in an augmented expression of PGC-1α (37). In either case, interval

training has been shown to act as a potent activator of p38 MAPK implicating it as another possible mechanism underlying mitochondrial biogenesis after training with HIIT or SIT (6, 30, 45, 73).

c. Mechanism of signal transduction with interval training through PGC-1α

PGC-1α does not contain intrinsic enzymatic activity or DNA binding capability; however, through the interaction with transcription factors it enhances gene expression by co-activating their promoters (103). The term "master regulator" of mitochondrial biogenesis befits PGC-1α because a number of these transcription factors interact with promoters encoding structural and enzymatic proteins essential for mitochondrial function. For example, it has been shown to interact with the family of nuclear respiratory factors (NRF-1 and 2), which regulate genes encoding subunits of each protein in the electron transport chain as well as mitochondrial transcription factor A (TFAM) (reviewed in 114, 115). The influence on TFAM expression is of particular importance because TFAM is necessary for the activation of mitochondrial gene transcription. This suggests a possible mechanism for co-ordinating transcription of both nuclear and mitochondrial DNA (mtDNA) which is required for mitochondrial biogenesis (115). More recently it has been demonstrated that PGC-1α itself is localized to the mitochondrial matrix to interact with TFAM directly at the D-loop of mtDNA (3). These findings are intriguing because it suggests that PGC-1α plays a direct role in the crosstalk between, and up-regulation of, nuclear and mtDNA rather than just indirectly through the NRF's and other transcription factors. It also suggests a possible mechanism underlying the regulation of PGC-1α.

Exercise is a potent stimulus for the redistribution of PGC-1α within the cell. Wright et. al showed that an acute bout of endurance exercise could induce changes consistent with a

cytosolic to nuclear translocation in rat muscles (138). Subsequently, work by Little et al. provided evidence of this mechanism in humans after performing HIIT showing that whole muscle PGC-1α content was unaltered, however, the nuclear abundance was significantly increased. Correspondingly, the authors noted significant increases in the mRNA of both nuclear and mtDNA encoded genes during recovery that later translated to functional increases in protein content and activity (73). Safdar et al. further clarified the role of PGC-1α in coordinating nuclear-mitochondrial cross talk when they illustrated that PGC-1α is also translocated to the mitochondrial matrix in response to an acute bout of endurance exercise (109). To date, there is no evidence that interval training induces mitochondrial translocation, however, it is hypothesized that this is likely the case.

As alluded to previously, many upstream signalling cascades converge on PGC-1α in order to regulate its function. As discussed above, one way to accomplish this is through regulating the subcellular localization. For example, AMPK is required for translocation to the subsarcolemmal mitochondria. Smith et al. elegantly demonstrated a lack of mitochondrial redistribution in response to exercise in mice lacking AMPK activity. In addition, they demonstrated enhanced translocation in rat and human muscle after pharmacological activation of AMPK and exercise interventions, respectively, highlighting the conservation of regulatory mechanisms across species (120). Little is known regarding the regulation of PGC-1α translocation to the nucleus, however, the underlying mechanisms of NT- PGC-1α (a PGC-1α splice variant) transport have begun to unfold and may provide some insight. Chang et al. placed the regulation of NT- PGC-1α on nuclear export, in contrast to import. In other words, certain stimuli, potentially exercise for example, inactivate the export of NT- PGC-1α from the nucleus subsequently leading to a gradual accumulation of nuclear protein content and enhanced gene

expression (24). It is important to note that the nuclear control has only been investigated in vitro and therefore further investigation is warranted, especially in the context of exercise as a stimulus.

An abundance of data supports post-translational modifications as an additional mechanism in which upstream signalling molecules converge to regulate PGC-1α. For example, the half-life of PGC-1α is relatively short under basal conditions, but upon the stimulation of p38 MAPK its stability is greatly increased (102). Therefore, it is possible that protection from proteasomal degradation through p38 MAPK phosphorylation is what gives p38 its permissive effects and allows other regulatory proteins to regulate PGC-1α further (37). AMPK phosphorylation is one such example, as it directly phosphorylates PGC-1α at thr-177 and ser-538 and enhances transactivation activity (60). Conversely, PGC-1α is also subject to a number of negative regulators through post-translational modifications. It has been demonstrated that phosphorylation (70) and acetylation (41) at specific amino acid residues can inhibit downstream signalling by PGC-1α.

It should be noted that a recent report has provided evidence that the exercise induced activation of mitochondrial biogenesis is not dependent on PGC-1α (108). However, this is not necessarily surprising since co-activators may facilitate the regulation of transcription but not necessarily act as a gatekeeper since they cannot interact with the DNA directly. In either case, it is obvious that exercise induced adaptions in skeletal muscle represents an elaborate network of signalling cascades subject to many levels of regulation. It would be naïve to believe that the complexity of transcriptional, translational and post-translational modifications that regulate the process would occur through a single pathway. Nonetheless, it appears that PGC-1α, and thus the

control of its activity, plays an intricate role in cellular bioenergetics and the adaptions of skeletal muscle to endurance and interval exercise.

IV. NUTRITIONAL AND SUPPLEMENT MANIPULATION OF SKELETAL MUSCLE ADAPTATION TO INTERVAL TRAINING

a. Introduction

Nutritional interventions and supplementation can modulate the adaptive response to training (52). Broadly speaking, the influence on training adaptations could be manifested in one of two ways: 1) the intervention could facilitate a greater total work and thus greater training stimulus; and/or 2) the intervention could enhance signalling through energy sensors, or increase impulses of gene expression after a single session, leading to greater adaptations over time (46). Many different potential candidates have been identified and are discussed in detail elsewhere (11, 87, 124).

One nutritional intervention that has garnered substantial interest and has the potential to influence training adaptation is manipulation of glycogen through dietary CHO intake (or lack thereof), and specifically performing some sessions of training with reduced CHO availability (53, 54). The original concept, first reported by Hansen et al. proposed that repeated bouts of exercise commenced in the low glycogen state would have enhanced adaptations due to the greater relative stress to the skeletal muscle. The authors had subjects perform single leg knee extensor exercise on one leg twice daily and every other day with the contralateral leg. They demonstrated that performing half of the sessions with reduced glycogen (i.e., twice daily) resulted in greater adaptations than if the same work was performed over two days. Specifically, they noted a greater resting glycogen, β-HAD activity and time to exhaustion during a fatigue

test (50). Similar protocols of training twice every other day vs. once every day to induce a "reduced" glycogen state have also demonstrated augmented fat oxidation during steady state exercise (58, 140) and elevated markers of mitochondrial adaptations (58, 86, 140). However, criticism of low glycogen interventions is that training intensity is commonly reduced, perceived effort is higher and there is a lack of evidence to support an enhancement in performance (reviewed in 54).

Despite these caveats, CHO restriction provides researchers mechanistic insights into how mitochondrial content can be enhanced in response to exercise, even in well-trained athletes. For example, HIIT and MICT commenced with CHO restriction has been shown to augment the acute activation of AMPK (139), p38 MAPK (30) and the mRNA expression of PGC-1α (101), all of which have been extensively discussed above. Furthermore, other pathways with implications for mitochondrial biogenesis have been noted to be elevated in response to exercise in a low glycogen state. For example, Bartlett et al. recently showed that p53 activity is elevated (7). Furthermore, glycogen content in conjunction with contractile activity has recently been demonstrated to regulate the transcription factor peroxisome proliferator-activated receptor-delta (PPAR-δ) which has implications for PGC-1α protein content and the expression of fat oxidation enzymes (97). All of these mechanisms are implicated in mediating skeletal muscle adaptations in response to exercise and therefore an acute augmentation after a single bout may undermine the observed enhancements.

The restriction of CHO provides a strong example of a nutritional intervention that can be used to optimize the adaptations to exercise, including HIIT and SIT. Other possible interventions with particular interest to interval training are supplements that manipulate buffering capacity due to the extreme challenge to the bodies buffering systems. The effects of

pH on skeletal muscle and how nutritional buffers may play a role in enhancing performance and/or adaptations are discussed in the subsequent section.

b. pH and the effects on skeletal muscle function

i. Factors contributing to skeletal muscle pH during interval training

Regulation of hydrogen ions (H^+) or pH within homeostatic concentrations is critical for proper physiological function. As noted previously, interval training is a potent stimulus to induce many physiological disturbances, including changes in intramuscular pH. For example, repeated sprints have the potential to reduce intramuscular pH, usually 7.2, to as low as 6.5-6.8 (13, 84, 93, 122). The factors contributing to the change in muscle pH seen during intense exercise are numerous and the role of each factor remains hotly debated (16, 67, 71, 100, 106, 107). However, classically it is believed that a large contributor of H^+ is through the accumulation of lactate produced from glycolysis (16). Due to the low relative pKa, lactate is fully dissociated at physiological pH and therefore contributes to the elevation in H^+. However, it should be noted that others have suggested disturbances in muscle pH are caused mainly by the hydrolysis of ATP (105) or changes in strong ion difference (72).

A less contentious issue relates to the physiological mechanisms which act to buffer the decrease in pH. In terms of direct intracellular regulation; inorganic phosphates, proteins containing histidine residues, carnosine, bicarbonate (HCO_3^-) and regulators of ion concentrations are crucial intramuscular buffers. In addition, during intense exercise the shuttling of H^+ and lactate across the sarcolemma is also believed to play an important role in the maintenance of pH (66). This is due to the extracellular buffering capacity HCO_3^- which is believed to promote the efflux of H^+ from active muscles (9, 55).

ii. Role of pH in muscle function and exercise performance

Fatigue is generally defined as the reversible decrement of performance during activity, and the underlying mechanisms are multifactorial in nature (2). Muscle acidosis has been associated, at least in part, with fatigue development with a concomitant detriment to performance. Specifically, it has been suggested that reductions in pH alter the capacity of the sarcoplasmic reticulum to handle calcium (88), negatively effects cross-bridge cycling (25, 29), disturbs ion regulation (126) and reduces glycolytic flux thus limiting fuel supply (93, 122). Although, due to the complexity of the fatigue process there is no consensus as to the extent to which lower pH contributes (5, 84, 89, 132).

The majority of research suggests that the regulation of pH is an important determinant for intense excise performance. For example, Messonnier et al. highlighted this by finding that the ability to perform supramaximal work is negatively correlated with a decrease in muscle pH and the accumulation of lactate. The authors also found positive correlations of work capacity with a number of proteins involved with pH control (ex MCTs) (85). Furthermore, performance during repeated sprint tests (10) and short duration endurance cycling (133) is associated with greater muscle buffering capacity. This is in line with findings comparing trained and untrained individuals, which noted greater capacity in those that are trained (36, 110). In light of these results, nutritional or supplemental interventions to supplement the body's buffering capacity in attempts to augment performance have been an area of great interest.

iii. Ergogenic effects of sodium bicarbonate

One method to maximize the extracellular buffering capacity that has garnered significant attention is the consumption of sodium bicarbonate ($NaHCO_3$). A recent meta-analysis by Carr et al., based on 59 studies, provides evidence of the ergogenic effects of $NaHCO_3$ (23). Specifically,

a mean performance enhancement of 1.7% was observed during a single 60 s sprint in male athletes who consumed 0.3 g/kg body mass. Additional enhancements in performance were noted with higher doses (0.5% increase at 0.4 g/kg) and repeated sprints (0.6% increase with 5 additional bouts) (23).

Mechanistically, it is hypothesized that the ergogenic effects of $NaHCO_3$ are due to an elevation in pH that creates a higher concentration gradient across the sarcolemma that then facilitates lactate and H^+ efflux from the active muscle (9, 55). Evidence suggests that this may indeed occur as a greater elevation in blood lactate concentrations when performing exercise after ingesting $NaHCO_3$ is a common finding (9, 18, 55, 127). Hollidge-Horvat et al. provided further support when they used arterial and venous samples to calculate lactate efflux, which they found to be greater with $NaHCO_3$ (55). It has also been suggested the ergogenic effects of alkalosis may be associated with better maintenance of muscle membrane excitability through improved strong ion regulation (104, 121, 126).

Regardless of how sodium bicarbonate imposes its ergogenic effects, the ability to allow athletes to work harder may enhance the exercise stimulus. In addition, there is evidence that $NaHCO_3$ supplementation can improve adaptations independent of greater work output (12, 35, 130). This suggests that it may be an effective nutritional intervention to augment training effects as put forth by Gibala and is the focus of the subsequent section (46).

c. Manipulating buffering capacity through $NaHCO_3$ ingestion to augment training adaptations

A thought-provoking study by Edge et al. examined the effects of chronic $NaHCO_3$ ingestion during 8 wks (3 sessions wk^{-1}) of interval training in active women. The authors

recruited team sport athletes and divided them into two groups matched for fitness based on lactic threshold (LT). All training sessions were preceded by the consumption of either $NaHCO_3$, to induce alkalosis, or NaCl as a placebo. Each pair of matched athletes completed all training sessions simultaneously and was required to complete the same amount of work. Therefore, training intensity and volume were matched between the alkalosis and placebo groups. The main focus of their study was to investigate effects on muscle buffering capacity, which they did not detect any differences. Surprisingly, however, the authors noted greater training induced adaptations in the females ingesting $NaHCO_3$. Short-term endurance capacity as assessed by TTE at 100% VO_{2max} was elevated ~40% more and was associated with an augmented lactic threshold. It should be noted that a tapering period prior to post testing was included to wash out any lingering effects of treatment. The authors proposed that smaller deviations from homeostatic pH during exercise after $NaHCO_3$ ingestion may have reduced the impact on mitochondria and could promote improved adaptations (35).

Recently, Driller et al. looked to further these findings in a more applied manner with internationally competitive rowers. The authors recruited 12 elite rowers, matched for peak power output during a graded exercise test, and had them complete 4 wks (2 sessions wk^{-1}) of HIIT on a rowing ergometer. Half the group ingested $NaHCO_3$ and the other an equimolar dose of NaCl prior to each work matched training session. While both groups had notable improvements in 2000-m rowing performance, there were no effects of treatment in any of the measured variables. What is interesting is that while not significant, the authors did observe nearly a 1 s greater improvement in the $NaHCO_3$ supplemented athletes (34). To put this improvement into perspective, the 2012 Olympic Men's 2000-m single skulls saw 1st through 4th decided by less than 7 s. Although outside of the researchers control, it should be

highlighted that the study was limited in their power (n=6 per group) and consisted of only 8 training sessions over 4 wks. Therefore, it is possible that more subjects, a longer intervention or perhaps unmatched worked throughout training may have led to greater adaptations with $NaHCO_3$ supplementation, although this is only speculative.

In order to gain further insight the potential mechanistic basis for the performance improvement observed by Edge et al., two follow up papers by Jacques Mercier's group investigated combined HIIT and $NaHCO_3$ supplementation in rats (12, 130). Thomas and colleagues had rats run 7-12 x 2 min intervals, 5 x wk^{-1} for 5 wks while being supplemented with either $NaHCO_3$ or a placebo. Each group was matched for performance and each training session consisted of fitness matched rats training simultaneously. The focus of the study was to examine buffering capacity and the expression of proteins involved with regulating ion concentrations including; MCT-1, -4 and sodium bicarbonate co-transporter (NBC). They found that HIIT was a potent stimulus for all measures of interest and that $NaHCO_3$ enhanced the increase in MCT-4 expression. Furthermore, a significant 7% greater elevation in maximal CS activity was seen. These results suggest that chronic $NaHCO_3$ supplementation during training may potentiate greater adaptations to handle lactate shuttling and mitochondrial content (130).

Bishop et al. provided further support for the potential benefit of training using $NaHCO_3$ when they completed a study identical to that of Thomas and colleagues, in which performance and mitochondrial adaptations were the focus (12, 130). Both exercising groups increased their endurance capacity as evident by significant increases in treadmill running time to exhaustion in comparison to unexercised control rats. Coinciding with the human findings by Edge et al., there was a significantly greater improvement in the rats that consumed $NaHCO_3$. In addition, the rats

that were supplemented with $NaHCO_3$ had elevated markers of training-induced mitochondrial oxidative capacity (12).

Overall, there is good evidence to support the notion that $NaHCO_3$ is an effective supplementation to augment the mitochondrial and performance enhancements associated with HIIT. Despite the numerous implications of these findings, few studies have investigated the acute mechanisms which may be mediating these adaptations.

d. Potential mechanisms underpinning augmented adaptations

Several studies investigating the potential ergogenic basis for $NaHCO_3$ ingestion have examined its effect on muscle metabolism, which may in turn provide insight into signalling adaptations. Table 1 provides a summary of the studies which have looked at both muscle and blood metabolites in exercising humans. Hollidge-Horvat and colleagues produced the most comprehensive of the analysis by looking at a wide range of enzymes and metabolites within the intracellular milieu. A significant finding by the authors was that $NaHCO_3$ supplementation resulted in a greater glycolytic flux due to a lack of inhibition of key regulatory enzymes. Of particular interest, they noted significantly higher glycogen depletion and a concomitant increase in muscle/blood lactate concentrations at higher cycling intensities (55). This is in-line with a number of other studies which have reported similar glycogen and lactate findings (9, 125, 127). Furthermore, Hollidge-Horvat noted elevated concentrations of [ADP], [AMP] and [Pi] while [PCr] was reduced in with $NaHCO_3$ supplementation by comparison with a placebo (55).

As discussed earlier, glycogen and adenosine phosphates have been implicated in the acute signalling of mitochondrial biogenesis, particularly due to their role in activating AMPK (111). Therefore, at least theoretically, the augmented adaptations seen after training with

$NaHCO_3$ supplementation may stem from enhanced signally through protein kinases (such as AMPK) that may mediate greater gene expression. Over repeated sessions, these transient increases in transcription may lay the foundation for greater protein expression and eventual performance enhancement.

V. SUMMARY AND RATIONALE FOR RESEARCH

Acute and chronic high-intensity interval exercise is a potent stimulus to influence a number of physiological adaptations with implications for health and athletic performance (42, 44). The acute mechanisms regulating these adaptations in skeletal muscle appear to be mediated by transient increases in gene expression as a result of cellular perturbations from homeostasis that activate various signalling cascades (38). Evidence indicates that nutritional interventions or supplementation that can improve any aspect of the adaptive process may potentiate greater adaptations than interval training alone (52, 53). Due to the intense nature of this training modality and associated disturbance to muscle pH, which has been implicated in fatigue, it has been hypothesized that augmenting the body's natural buffering capacity through nutritional means may be a strategy to augment training adaptations (46). One way of doing this is through the ingestion of $NaHCO_3$ prior to exercise, which has shown to have ergogenic effects allowing athletes to perform more work with each training session (23). In addition, greater mitochondrial and performance adaptations are seen when HIIT is preceded by $NaHCO_3$ ingestion even when work is matched (12, 35, 130). Research suggests that altered metabolism within the muscle during exercise may underpin the adaptations (55). However, the underlying mechanisms have yet to be thoroughly examined. Therefore, the purpose for this study was to

investigate the acute perturbations in skeletal muscle metabolism in response to an acute bout of HIIT when $NaHCO_3$ is ingested.

VI. STATEMENT OF RESEARCH QUESTION AND HYPOTHESIS

Our a priori research question was: can $NaHCO_3$ ingestion enhance the transcription of genes associated with mitochondrial biogenesis after an acute bout of HIIT? Secondary measures assessed whether muscle metabolism is altered, and if signalling pathways implicated in gene expression are activated to a greater extent after $NaHCO_3$. These data will shed light on the potential underlying mechanistic basis for augmented training adaptations observed after several weeks of HIIT when combined with chronic $NaHCO_3$ supplementation.

It was hypothesized that acute metabolic alkalosis, induced through the ingestion of $NaHCO_3$ will augment the transcription of a number of key proteins associated with mitochondrial biogenesis including PGC-1α, citrate synthase and cytochrome c oxidase. Furthermore, it was hypothesized that an elevation in mRNA expression will be the result of greater signalling through AMPK, as evidenced by protein markers of enzyme activation.

Table 1 – Summary of papers investigating both blood and muscle metabolites after acute exercise supplemented with sodium bicarbonate

REF	Subjects		Protocol		Adaptations					
					Blood		Muscle			
	Type	VO_{2max}	Dosage	Exercise	pH	[Lac]	pH	[Gly]	[Lac]	Nucleosides
Hollidge-Horvat et al. (2000)	8 ♂	42.5	ALK 0.3 g/kg $NaHCO_3$ CON 0.3 g/kg $CaCO_3$	Continuous, constant-rate cycling 15 min @ 30%, 60% & 75% VO_{2max} Work matched	ALK 7.43 to 7.24 CON 7.36 to 7.19	ALK ~14.5 x ↑ CON ~14 x ↑	ALK 6.3% ↓ CON 5.6% ↓	ALK 58% ↓ CON 47% ↓	ALK ~7 x ↑ CON ~6.5 x ↑	[ATP] ALK ↔ CON ↔ [ADP] ALK ~11 x↑ CON ~8 x ↑ [AMP] ALK ~11 x ↑ CON ~5 x ↑
Sutton et al. (1981)	5 ♂	48.8	ALK 0.3 g/kg $NaHCO_3$ CON 0.3 g/kg $CaCO_3$ ACID 0.3 g/kg NH_4Cl	Continuous, constant-rate cycling 20 min at 33%, 66% & 95% VO_{2max} Not work matched (to exhaustion)	ALK 7.43 to 7.33 CON 7.38 to 7.26 ACID 7.21 to 7.13	ALK ~8 x ↑ CON ~6 x ↑ ACID~4 x ↑	N.R.	ALK 88% ↓ CON 91% ↓ ACID 71% ↓	ALK ~9 x ↑ CON ~7.5 x ↑ ACID ~4 x ↑	N.R.
Bishop et al. (2004)	10 ♀	41.0	ALK 0.3 g/kg $NaHCO_3$ CON 0.207 g/kg NaCl	5x6-s all out cycle sprints every 30s (~300% VO_{2max}) Not matched work	ALK 7.5 to 7.3 CON 7.4 to 7.2	ALK ~13 x ↑ CON ~9 x ↑	ALK 3.5% ↓ CON 3.5% ↓	N.R.	ALK ~11 x ↑ CON ~7 x ↑	N.R.
Stephens et al. (2002)	7 ♂	63.4	ALK 0.3 g/kg $NaHCO_3$ CON 0.3 g/kg $CaCO_3$	30 min at 77% VO_{2max} + as much work as possible in 30 min (~30 min at ~80%) Each session matched work but told to complete as fast as possible	ALK 7.5 to 7.4 CON 7.4 to 7.3	ALK ~10 x ↑ CON ~8 x ↑	ALK 2% ↓ CON 2% ↓	ALK 64% ↓ CON 55% ↓	ALK ~9 x ↑ CON ~8 x ↑	[ATP] ALK 19% ↓ CON 14% ↓
Bouissou et al. (1988)	6 ♂	58.6	ALK 0.3 g/kg $NaHCO_3$ CON 0.3 g/kg $CaCO_3$	Single cycle sprint at ~125% VO_{2max} until 90 RPM couldn't be maintained Not work matched	ALK 7.35 to 7.15 CON 7.25 to 7.05	ALK ~7 x ↑ CON ~4 x ↑	N.R.	N.R.	ALK ~7 x ↑ CON ~5 x ↑ estimated	N.R.

VO_{2max} is in mL min^{-1} kg^{-1}; ALK , alkalosis (bicarbonate); CON, Control; N.R., not reported; [Lac], lactate concentration; [Gly] glycogen concentration.

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CHAPTER II

Sodium bicarbonate ingestion augments the increase in PGC-1α mRNA expression during recovery from intense interval exercise in human skeletal muscle

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II. INTRODUCTION

Interval training has garnered renewed interest in the general population and athletic circles due to its time efficient nature and potential to induce adaptations traditionally associated with prolonged moderate intensity exercise. Owing to a general lack of standardization in the literature, Weston et al. recently proposed a relatively simple classification scheme to categorize interval exercise training, which will be employed here. "Sprint interval training" (SIT) is used to describe "all-out" efforts or those performed at intensities that exceed 100% of maximal oxygen uptake ($VO2_{max}$), whereas "high-intensity interval training" (HIIT) refers to work intensities that elicit 80-100% of peak heart rate (51).

Nutritional state and supplementation can influence both the acute and chronic responses to exercise in humans, including metabolic adaptation in skeletal muscle (21). While most studies have focused on traditional aerobic/endurance or strength/resistance exercise, nutrition and supplements can also alter the response to interval training, which generally refers to repeated bouts of brief, intense exercise interspersed by periods of rest or lower intensity exercise for recovery. For example, manipulation of carbohydrate (CHO) availability during short-term HIIT can alter skeletal muscle metabolic adaptations (20, 25, 35, 55). Morton and colleagues (36) studied 3 groups of subjects who completed 4 sessions of HIIT wk^{-1} for 6 wks; with one group completing training every other day (with "normal" glycogen), while the other two groups trained twice d^{-1}, 2 d wk^{-1} in order that half of the training sessions were initiated in a reduced glycogen state. To tease out the effects of exogenous versus endogenous CHO availability, one of the low glycogen groups consumed CHO between and during the second training session (low glycogen + CHO) while the other consumed a placebo (low glycogen + PLAC). HIIT increased $VO2_{max}$, performance and mitochondrial content, as reflected by succinate dehydrogenase (SDH)

maximal activity. The increase is SDH was greatest in the low glycogen + PLAC group, but there were no differences between treatments in time to exhaustion (35). Other studies utilizing once daily/every day or twice daily/every other day protocols, have also reported greater increases in mitochondrial content after training with reduced glycogen (25, 55). The altered muscle adaptive response may be due in part to greater protein signalling and transcriptional activation after each training session. Signalling through AMPK (54), p38 MAPK (12) and p53 (2) is activated to a greater extent when HIIT is commenced with CHO restriction. It was also recently shown that the mRNA of PGC-1α, considered to be a "master regulator" of mitochondrial biogenesis, is further elevated in response to an acute session of interval exercise commenced in a low glycogen state (41).

A potential limitation of reduced CHO training protocols is the fact that they can also result in reductions in power output during training, higher perceptions of effort and the lack of a clear performance enhancement (22). Ingestion of sodium bicarbonate ($NaHCO_3$) may be an alternative intervention to enhance the adaptations to HIIT, without the limitations associated with manipulating CHO availability. A meta-analysis showed that performance during a single 60 s sprint is enhanced 1.7% with a further 0.6% modifying effect when sprints are repeated following the consumption of ~300 mg kg^{-1} $NaHCO_3$ (9). One potential explanation for a beneficial effect of $NaHCO_3$ is thus the capacity to produce more total work, which in turn leads to a greater cumulative training stimulus. Alternatively, there is evidence that $NaHCO_3$ supplementation per se favourably alters the metabolic response to HIIT such that adaptations are enhanced even if total work is maintained constant. Edge et al. showed this by having team sport athletes perform HIIT 3 x wk^{-1} for 8 wks while consuming $NaHCO_3$ or a placebo prior to each session (16). Despite matching training volume and intensity, the authors found the

$NaHCO_3$ supplemented group had greater improvements in endurance capacity and lactic threshold (16). Follow up work by some of the same authors using a rat model provided insight into potential underlying mechanism. Specifically, Bishop et al. reported that mitochondrial respiratory capacity was increased to a greater extent following HIIT supplemented with $NaHCO_3$ as compared to a placebo in rats. This was associated with a 52% greater increase in running time to exhaustion in the supplementation group (6).

Few data are available regarding the potential mechanistic basis for superior performance adaptations observed after $NaHCO_3$-supplemented HIIT in humans; however, some studies have provided insightful clues in this regard. For example, a rather common finding is that $NaHCO_3$ supplementation results in higher rates of glycogen utilization and elevated lactate concentrations in muscle (5, 8, 23, 48, 49). These data suggest an enhanced contribution from non-oxidative energy metabolism, which in turn could alter metabolically-sensitive signalling mechanisms during recovery that are linked to cellular remodelling. In the present study, we investigated the hypothesis that ingestion of sodium bicarbonate prior to an acute session of HIIT would augment signalling cascades and gene expression linked to mitochondrial biogenesis in human skeletal muscle.

III. METHODS

Participants and Ethics

Ten healthy active men were recruited through poster advertisement. The purpose of the study and potential risks of participation was explained to all subjects, and written informed consent was obtained prior to their participation. Subjects also completed a Physical Activity Readiness

Questionnaire, which was designed to identify potential symptoms that might preclude their participation. All experimental procedures were approved by the Hamilton Integrated Research Ethics Boards. One subject was removed as a precaution, owing to an adverse reaction to a muscle biopsy procedure. Descriptive characteristics for the nine men who completed the study are presented in Table 1.

Pre-experimental Procedures

Subjects completed baseline testing and familiarization procedures a minimum of 1 wk prior to the main experimental trials. Baseline testing included the determination of fat and fat-free mass through air displacement plethysmography (BodPod®, COSMED Inc., Concord, CA, USA). A ramp test to volitional fatigue was also performed on a cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) to determine peak oxygen uptake (VO_{2peak}) using continuous online gas collection (Moxus modular oxygen uptake system, AEI technologies, Pittsburgh, PA, USA), as well as maximum power output (W_{max}) and maximal heart rate (HR_{max}). The protocol involved a 2 min warm-up at 50 W and thereafter resistance was increased by 1 W 2 s^{-1} until volitional exhaustion or the point at which pedal cadence fell below 40 rpm as previously described (12). Heart rate (HR) was assessed continually using telemetry (Polar A3, Lake Success, NY, USA). After a brief recovery, subjects remained in the lab in order to be fitted with an Actiheart device (CamNtech, Boerne, Tx, USA) per the manufacturer's instructions as previously described (46). The Actiheart allows for the non-invasive estimation of activity energy expenditure based on activity counts, determined by an accelerometer, and HR (14). During a subsequent visit, subjects completed a familiarization trial which consisted of 4 x 60 s cycling efforts at an intensity designed to elicit ~90% of HR_{max}, interspersed with 60 s of active

recovery at 50 W, on a cycle ergometer set in constant watt mode (LifeCycle R1, Life Fitness, Schiller Park, IL, USA).

Experimental Protocol

An overview of the experimental protocol is outlined in Figure 1. Subjects completed two trials in random order, separated by at least 1 wk. Each trial involved an acute session of HIIT, with repeated blood and muscle biopsy sampling to assess the metabolic response to exercise. The only difference between trials was the nature of a supplement that was ingested in two doses, at 90 min and 60 prior to exercise. On one occasion, subjects ingested a dose of $NaHCO_3$ equivalent to 0.2 g kg b.w^{-1} at each time point (BICARB), whereas the other trials involved an equimolar dose of NaCl, which served as the placebo condition (PLAC). This dosing protocol was based on previous studies and in order to minimize the potential for gastrointestinal discomfort (16).

Subjects reported to the laboratory 24 h prior to each experimental trial to have the Actiheart attached. They were also provided with an activity log and asked to abstain from physical activity other than activities of daily living, prior to the trial. 24 h energy expenditure was not different between trials (BICARB vs. PLAC; 57.3 ± 19.5 vs. 56.7 ± 12.3 kJ 24 h^{-1} kg^{-1}, p = 0.95). The self-reported activity logs reflected this lack of difference. In addition, diet logs were provided along with instructions to record all food and drink consumption, in order that the same nutritional pattern could be replicated prior to the second trial. Subjects were asked to refrain from alcohol or caffeine prior to the trial. All diet logs were subsequently analysed using an on-line diet analysis tool (myfitnesspal, San Francisco, CAL, USA) in order to estimate total energy

intake and macronutrient composition. Neither the estimated total energy intake nor diet composition was different between trials (Table 2).

On the morning of each trial, subjects reported to the laboratory after a 10 h overnight fast and a catheter was inserted for an antecubital vein. After an initial fasting blood sample was obtained, subjects ingested a standardized breakfast, which consisted of ~ 600 Kcal, derived from ~59% CHO, ~25% Fat and ~16% protein. Immediately following the breakfast, subjects ingested the first dose of either BICARB or PLAC followed 30 min later by a second dose. Ninety minutes after the initial dose, a second blood sample was obtained and a resting muscle biopsy was obtained from the vastus lateralis under local anaesthesia (1% Lidocaine) using a Bergrstom needle adapted for manual suction as previously described (27). Muscle tissue was quickly removed from the needle, cleaned of excess blood, sectioned and then frozen in liquid nitrogen and stored at -80°C for subsequent analyses.

Subjects then completed the HIIT intervention, which involved a 5 min warm up at 50 W, followed by 10 x 60 s cycling intervals at an individualized absolute workload that elicited ~90% HR_{max} (263 ± 40 W), interspersed with 60 s of active recovery at 50 W. Total work during each trial was identical for a given subject. HR was continuously measured throughout each trial with a heart rate monitor (Polar A3, Lake Success, NY, USA). In addition, subjects were asked for a rating of perceived exertion on a scale of 6-20 after the completion of each interval (7).

Immediately upon completion of exercise, a blood sample was collected, and a second muscle biopsy was obtained. Additional blood samples were collected after 15, 30, 60, 120 and 180 min of recovery, followed by a third and final muscle biopsy 3 h after exercise. Subjects also completed a 15-item questionnaire that was designed to assess potential gastrointestinal or other

discomforts on a 1-10 Likert scale, with total discomfort scores ranging between 15-150 as previously described (10). Subjects were permitted to consume water ad libitum throughout the first trial day, with the approximate timing and volume recorded in order to match the pattern of intake during the second trial.

Blood Analyses

After an initial saline flush of the catheter to remove any residual clots, ~1 mL of venous blood was collected in a lithium heparin syringe and was immediately analysed by the Hamilton Regional Medicine Program Core lab for acid-base variables using a blood-gas analyzer (Radiometer ABL 800, Radiometer Medical, Bronshoj, Denmark). While acid-base variables are generally measured from arterial samples, recent reports suggest that venous samples can be an acceptable surrogate for the measures of interest in this study (3, 34). Plasma, serum and whole blood samples were collected with the appropriate collection tubes, processed as per the manufacturer's recommendations (BD Vacutainer®: Becton, Dickinson and Company, Franklin Lakes, NJ, USA), aliquoted and stored at -20°C for future analysis. Sodium fluoride and potassium oxalate treated samples were analysed for glucose and lactate concentrations using a YSI 2300 STAT Plus Glucose and Lactate Analyzer (Xylem, White Plains, NY, USA).

Muscle Analyses

Glycogen. A portion of the muscle samples ~20 mg in size was freeze-dried, powdered, and dissected free of blood and connective tissue. Glycogen was determined as has been previously described (12). Briefly, ~2 mg of powdered muscle was incubated for 2 h at 98˚C in 2 M HCl in order to hydrolyze glycogen to glucose. Samples were cooled to room temperature and an equal volume of 2 M sodium hydroxide was subsequently added to neutralize samples. Glucose

concentration was determined using a standard hexokinase enzymatic assay adapted for a plate reader according to methods outlined in the commercially available kit (Pointe Scientific, Canton, MI, USA).

Signalling proteins. Nuclear and cytosolic fractions were prepared from ~50 mg of wet muscle using a commercially available kit (NE-PER, Pierce, Rockford, IL, USA) as has been previously described with further modifications (29). All incubations and centrifugations were done on ice or at 4˚C, respectively. Homogenization was performed in CER-I buffer supplemented with protease (cOmplete mini, Roche Applied Science, Laval, PQ, Canada) and phosphatase (PhosStop, Roche Applied Science, Laval, PQ, Canada) inhibitors using an electronic homogenizer. Homogenates were vortexed for 15 s at max speed to ensure full suspension and then incubated for 15 min. CER-II was then added to the homogenate. Samples were vortexed 5 s, incubated for 5 min and vortexed an additional 5 s. A crude cytosolic fraction was obtained by removing the supernatant after samples were centrifuged at 20 000 g for 10 min. The pellets containing nuclei were cleaned of contamination by resuspending in PBS supplemented with the previously mentioned inhibitors and centrifuging at 20 000 g for 10 min. Additional purifications were performed by gently washing the tube and pellet with excess PBS three times. Washed pellets were then resuspended in NER buffer by vigorous pipetting followed by repeated 15 s vortexes over a 40 min incubation. Samples were centrifuged at 20 000 g for 10 min and then disrupted by gentle agitation with a micro pestle and repeated 1 s pulses of sonication. A final 10 min centrifugation at 20 000 g removed insoluble membranes and the supernatant was taken as the nuclear fraction. Crude cytosolic fractions were purified by repeatedly centrifuging at 20 000 g for 5 min and transferring the supernatant to clean tubes. Aliquots were taken prior to storage at -80 ˚C in order to quantify protein concentration which was determined using a commercially

available bicinchoninic acid assay kit (Pierce, Rockford, IL, USA). The absence of LDH and α-tubulin and enrichment of histone H3 determined by western blotting was used as confirmation of the purity of the nuclear fractions (Figure 2).

Samples were prepared for western blot analysis by diluting in 4xLamelli buffer (240 mM Tris-HCl, pH 6.8, 40% glycerol, 8% SDS, 0.04% bromophenol blue, 5% β-mercaptoethanol) and boiling for 5 min at 100˚C. Equal amounts of protein were loaded onto either 10 or 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and were separated by electrophoresis for 1.5 h at 120 V. Proteins were then wet transferred to nitrocellulose membranes for 1-2 h at 120V. In order to ensure proper transfer and equal loading in all lanes, nitrocellulose blots were stained and imaged using Ponceau S. Upon removal of the stain by gentle agitation in tris-buffered saline with tween (TBS-T), membranes were blocked at room temperature by incubating in 3% fat-free milk dissolved in TBS-T. Blots were then sectioned and incubated with primary antibodies against; PGC-1α, phospho-p38 mitogen-activated protein kinase (MAPK) (Thr 180/Tyr 182), total-p38 MAPK, phospho-AMPK (Thr 172), phospho-acetyl-CoA carboxylase (ACC) (Ser 79), lactate dehydrogenase (LDH), histone H3 and α-tubulin, which were purchased from Cell Signalling Technology (Beverly, MA, USA). In addition, a primary antibody specific for total-AMPKα2 was purchased from Upstate (Millipore, Billerica, MA, USA). Primary antibodies were incubated at a dilution of 1:1000 overnight at 4 °C in 3% fat-free milk or BSA in TBS-T. Blots were washed 5 x 3 min with TBS-T to remove excess antibody. After incubation in the appropriate species-specific secondary antibody conjugated to horseradish peroxidase for 1 h at room temperature diluted 1:5000 in TBS-T, blots were again washed 5 x 3 min with TBS-T and proteins were detected by chemiluminescence

(Supersignal® West Dura, Pierce). Band intensity was quantified by spot densitometry using NIH ImageJ software.

Gene expression. RNA was isolated from ~30 mg of frozen muscle using a combination of TRIzol (Life Technologies, Carlsbad, CA, USA) and RNeasy (Qiagen Inc., Valencia, CA, USA) as per the manufacturer's protocol. Briefly, the FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals, Solon, OH, USA) in conjunction with Lysing Matrix D tubes (MP Biomedicals, Solon, OH, USA) were used to homogenize samples by rapidly agitating at a speed of 6 m/s for 40 s. Samples were visibly inspected to ensure thorough homogenization and then incubated at room temperature for 5 min followed by the addition of chloroform (Sigma-Aldrich, Oakville, ON, CAN). After vigorous mixing for 15 s by hand, an additional 5 min incubation at room temperature followed by centrifugation at 12 000 g for 10 min at 4˚C forced the partitioning of the aqueous and organic phase. RNA was purified from the aqueous phase using a commercially available kit (RNeasy Mini Kit, Qiagen Inc., Valencia, CA, USA). RNA purity and quantity were determined using the Nano-Drop 1000 Spectrophotometer (Thermo Fisher Scientific, Rockville, MD, USA). Furthermore, RNA integrity was quantified using the Agilent 2010 Bioanalyzer (Agilent Technologies, Toronto, ON, CAN). The RNA integrity number (RIN) was 7.4 ± 0.6. cDNA was synthesized using a commercially available High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) in an Eppendorf Mastercycler epgradient thermal cycler (Eppendorf, Mississauga, ON, Canada) and stored at -20˚C. Gene expression was quantified as has been previously described with slight modification (1, 12). Briefly, quantitative real-time PCR was performed on the prepared cDNA samples using SYBR Green Fluorescence (Quanta Biosciences, Gaithersburg, MD) and the corresponding oligonucleotide primers for the genes of interest. The following primers were used for gene

quantification; PGC-1α, Fwd 5'- CAC TTA CAA GCC AAA CCA AC AAC T -3', Rev 5'- CAATAGTCTTGTTCTCAAATGGGGA-3' (37): CS Fwd 5'- CCT GCC TAA TGA CCC CAT GTT – 3', Rev 5'- CAT AAT ACT GGA GCA GCA CCC C -3' (37): COX IV Fwd 5'- CGA GCA ATT TCC ACC TCT GT -3', Rev 5'- GGT CAC GCC GAT CCA TAT A -3' (12) and GAPDH Fwd 5'- CCT CCT GCA CCA CCA ACT GCT T -3', Rev 5'- GAG GGG CCA TCC ACA GTC TTC T -3' (47). All reaction mixtures were prepared using the epMotion 5075 Eppendorf automated pipetting system (Eppendorf, Mississauga, ON, Canada) and ran on an Eppendorf realplex2 Master Cycler epgradient S (Eppendorf, Mississauga, ON, Canada). Changes in gene expression were analyzed using the $2^{-\Delta\Delta CT}$ method as has been previously described (44), where GAPDH was used as a housekeeping gene as it was unchanged across time and treatments (data not shown). Within-subject samples were normalized to their respective pre-exercise placebo values.

Statistical Analyses

All data are reported as mean ± standard deviation (SD). HR, RPE, activity energy expenditure and dietary analyses data were compared using a student's paired t-test. All other data were analysed using a two-factor (time x condition) repeated-measures analysis of variance (ANOVA). A Mauchly's sphericity test was used to validate data sets prior to interpreting ANOVA results. A Greenhouse-Geisser correction was applied to data sets that violated the assumption of sphericity. Statistical significance was set at $p \leq 0.05$. Significant effects based on ANOVA were further analyzed using a Tukey's Honestly Significant Difference post hoc test.

IV. RESULTS

Physiological responses to treatments and HIIT

Interval workload, HR and RPE are summarized in Table 3. Interval workload was 263 ± 40 W, which elicited 89 ± 5 and 88 ± 5 % HR_{max} for the BICARB and PLAC trials, respectively ($p = 0.37$). RPE was not different between treatments (BICARB vs PLAC; 15.2 ± 1.6 vs 14.9 ± 1.8; $p = 0.69$). Ratings of gastrointestinal and other discomforts were generally low, with little change from baseline with the exception of immediately after the first dose (BICARB vs PLAC: 22 ± 3 vs 24 ± 8), although the overall scores were similar between trials (BICARB vs PLAC: 18 ± 2 vs 18 ± 3; $p = 0.53$).

Blood metabolites

Blood data are summarized in Figure 3. Blood bicarbonate concentration was higher compared to PLAC at all-time points after ingestion ($p < 0.05$; Figure 3 A). This was associated with a marked increase in blood pH, which remained elevated above PLAC until 3 h of recovery ($p < 0.05$; Figure 3 B).

Blood glucose decreased to the same extent in both trials post-ingestion (main effect for time, $p < 0.05$; Figure 3 D). However, post hoc analysis revealed no differences and values returned to pre-ingestion levels after HIIT. In contrast, HIIT resulted in a significant increase in blood lactate post-exercise which persisted 15 min into recovery (main effect for time, $p < 0.05$; Figure 3 C). Ingestion of BICARB exacerbated the increase post-exercise (BICARB vs PLAC; 12.84 ± 2.55 vs 10.45 ± 2.79 mmol L^{-1}; $p < 0.05$) and 15 min into recovery (BICARB vs PLAC; 8.24 ± 3.58 vs 5.65 ± 2.71 mmol L^{-1}; $p < 0.05$).

Muscle glycogen

Muscle glycogen content was reduced after HIIT and remained lower after 3 h of recovery compared to rest (main effect time, $p < 0.05$). The reduction in glycogen was greater in BICARB (27% reduction, Pre: 451 vs Post: 325 mmol/kg dry mass) compared to PLAC (11% reduction, Pre: 449 vs Post: 396 mmol/kg dry mass) (interaction, $p < 0.05$; Figure 4).

Muscle signalling proteins

The ratio of phosphorylated (phos-) to total AMPK ($p = 0.07$) and p38 MAPK ($p < 0.05$) was increased immediately following HIIT but returned to resting levels after 3 h of recovery, with no differences between treatments (Figures 5A and B). A downstream marker of AMPK activity, phos-ACC, followed a similar pattern as it was elevated immediately following HIIT but returned to baseline after 3 h of recovery (Figure 5C). The nuclear abundance of PGC-1α was not different at any point (data not shown).

Gene expression

PGC-1α mRNA expression was higher after 3 h of recovery compared to rest, but the increase was greater in BICARB compared to PLAC (Figure 6 A). There was no effect of exercise or treatment on the expression of CS or COX IV mRNA (Figure 6 B and C, respectively).

V. DISCUSSION

The major novel finding from this study was that $NaHCO_3$ supplementation prior to an acute bout of interval exercise increased PGC-1α mRNA expression during recovery in human skeletal muscle. Our data provide novel insights into possible acute mechanisms underlying enhanced

muscle and performance adaptations seen with chronic $NaHCO_3$ ingestion during training (6, 16, 50). Contrary to our hypothesis, we did not find greater activation of AMPK or p38 MAPK, two signalling proteins linked to PGC-1α signalling and mitochondrial biogenesis.

mRNA Expression. While the mechanisms that regulate adaptations to exercise are obviously complex (reviewed in 18), transient yet repeated increases in gene expression could lay the foundation for increases in mitochondrial protein content. Some empirical evidence for this notion stems from work by Perry et al. (37) who investigated the temporal changes associated with single and repeated bouts of HIIT. They found that in general, transient increases in mRNA expression, and clearly protein translation, preceded changes in protein content and enzymatic activity (37). Therefore, greater PGC-1α mRNA expression observed with $NaHCO_3$ ingestion may lead to elevated PGC-1α protein content that can then act to stimulate mitochondrial biogenesis during subsequent training sessions. In the current study, we did not observe elevation of other markers of mitochondrial gene expression (i.e. CS or COX IV mRNA). While some studies have reported increases in these markers 3 h after an acute bout of interval exercise (28), others have found that the timing of expression occurs later into recovery (37). Therefore, while the timing of the biopsy was selected to facilitate potential detection of a divergent PGC-1α response, it may not have been optimal to detect potential temporal differences in the response of other proteins.

Blood and Muscle Metabolites. The supplementation protocol resulted in elevated blood pH and bicarbonate concentrations, similarly to those reported in previous studies (9, 16). The HIIT-induced decline in blood pH was also attenuated in the BICARB trial. Consistent with previous data on sprint (5, 8) and continuous (23, 48) exercise with $NaHCO_3$ supplementation, we also observed a higher plasma lactate concentration. The elevated lactate may be due to a greater

concentration gradient across the sarcolemma that facilitates lactate and H^+ transport out of the active muscle (5, 23). Despite the potentially greater efflux of lactate, $NaHCO_3$ supplementation commonly results in elevated intramuscular lactate content as well (5, 8, 23, 48). While muscle lactate was not determined in this study, Hollidge-Horvat et al. previously reported that $NaHCO_3$ supplementation resulted in greater lactate accumulation during cycling at 75% VO_{2max} without a change in pyruvate oxidation. This was indicative of a greater allosteric upregulation of glycogen phosphorylase (PHOS) and phosphofructokinase (PFK) potentiating glycolytic flux while pyruvate dehydrogenase (PDH) was already fully activated and unable to facilitate greater pyruvate oxidation (23). Consistent with this previous study (23), we observed 16% greater glycogen depletion with BICARB. Hollidge-Horvat also noted altered cellular energetics as [ADP] and [AMP] were elevated (23), but these measurements were not included in the present study.

Muscle Signalling. It is well documented that [ADP] and [AMP] allosterically regulate AMPK by binding to the γ subunit and have been proposed to protect activated complexes from dephosphorylation, making them potent regulators (42). AMPK has also been proposed to be regulated by the sequestering of the β subunit by oligiosacharides such as glycogen (31). For example, high glycogen content may make the β subunit unavailable to act as a scaffold for AMPK complex formation resulting in decreased activity (31, 43). Conversely, under conditions of glycogen depletion (i.e. CHO restriction or BICARB in this study), the β subunit may be liberated to facilitate AMPK activation and exacerbate its downstream steam signalling. Despite the proposed elevation of [ADP] and [AMP] as well as observed glycogen reductions with BICARB, we did not observe a greater activation of AMPK. This may be due to the use of threonine 172 phosphorylation assessed through western blotting as a surrogate measure of

AMPK activity. However, no differences in ACC phosphorylation were observed, providing further support that AMPK activation was not altered between treatments. AMPK has been shown to be activated in an intensity dependent manner (11, 17, 52), therefore, the HIIT protocol may have been a sufficient stimulus to fully activate AMPK and not allow an enhancement with BICARB.

We sought to investigate if p38 MAPK phosphorylation was influenced by BICARB, owing to its acute activation with a number of HIIT protocols (1, 12, 19, 28) and putative role in training induced mitochondrial biogenesis (39). As with AMPK, we did not observe any differences between trials in the acute activation. It has been suggested that p38 activation may play a permissive role more so than the direct signal itself. This is because p38 MAPK phosphorylation is seen to the same extent at both low and high-intensity endurance exercise, in contrast to other kinases that are only activated at high-intensity that ultimately results in an augmented expression of PGC-1α (17).

Despite the lack of evidence for a link between reduced glycogen and elevated PGC-1α mRNA expression in this study, speculation can be made regarding possible alternative mechanisms that were not examined. For example, Philp et al. recently demonstrated in rats that glycogen content in conjunction with contractile activity regulates the activity of the transcription factor peroxisome proliferator-activated receptor-delta (PPAR-δ) (38). It has been shown that PPAR-δ can regulate PGC-1α mRNA expression through a PPAR response element in the PGC-1α promoter (24). Furthermore, mice myocytes in which PPAR-β (analogous to PPAR-δ) is ablated have a reduction in PGC-1α content (45). Therefore, the increased PGC-1α gene expression observed with the BICARB trial may be mediated by the altered activity of transcription factors including PPAR-δ.

In addition to the findings regarding the regulation of PPAR-δ, Philp and colleagues also observed that PGC-1α nuclear translocation only occurred in conditions of low glycogen (38). The regulatory mechanisms dictating PGC-1α nuclear localization in response to exercise are not well understood, however, both acute endurance (29) and SIT (28) can initiate the process in human muscle. These observations are important because it is believed that mitochondrial biogenesis in response to exercise is likely mediated in part by PGC-1α subcellular redistribution (53). However, changes in PGC-1α localization in response to treatment or exercise were not observed in the current study and the reasoning is unclear.

Study Limitations. Our research provides novel insight into acute changes within the muscle that may underpin the aforementioned adaptations with chronic $NaHCO_3$ supplementation. However, we were unable conclusively to elucidate a complete mechanism underpinning greater PCG-1α expression. A more comprehensive analysis of the intramuscular metabolic environment likely would have given greater direction to investigate potential signalling cascades. Adenosine nucleotides, redox status, pH and ion concentrations (i.e. K^+, Ca^{2+}) have all been implicated in adaptations to exercise (reviewed in 18) and also been suggested to be affected by $NaHCO_3$ supplementation but were not investigated here.

The pathways known to induce mitochondrial biogenesis that we investigated, the AMPK and p38 MAPK, were only assessed by looking at the acute phosphorylation status. Both kinases can impose their effects on muscle oxidative capacity in a number of different ways. AMPK, for example, regulates the activity of histone deacetylase 5 (HDAC5) (32), a protein proposed to control metabolic adaptive response by modulating gene expression (33). Therefore, AMPK and p38 MAPK may still be involved with enhancing training adaptations but through a mechanism not examined here.

As was discussed previously we only detected greater mRNA expression of PGC-1α and therefore the degree of conclusions that can be drawn is limited. Full transcriptomic analysis, such as using microarrays, may have provided greater insight. Furthermore, a single biopsy provides a "snapshot", though it would be of great interest to investigate a time-course based expression of other genes and subsequent protein translation, in response to BICARB.

Finally, due to the relatively high dose of $NaHCO_3$ commonly consumed to elicit ergogenic and metabolic effects, great consideration has to be made regarding placebos. NaCl is a common choice in order to match the Na^+ content (4, 5, 15, 16). It is well established that dietary sodium can effect plasma volume and water distribution in the body (30). However, it has been suggested that NaCl is not necessarily "chemically inert" due to its effects ion redistribution and hyperchloremic acidosis (13, 26). Despite this argument, we chose NaCl in order to mimic the protocols showing enhanced adaptations with training (16).

Future Directions. Our investigation stems from a thought-provoking study by Edge et al. (16), which examined the effects of chronic $NaHCO_3$ ingestion during 24 sessions of HIIT spread over 3 wk. The authors recruited female team sport athletes and divided them into two groups matched for fitness based on lactic threshold (LT). Pairs of matched athletes simultaneously consumed $NaHCO_3$ or a placebo followed by the completion of identical intervals in order to match training stimuli between groups. Interestingly, greater training induced adaptations were observed in the athletes ingesting $NaHCO_3$. Specifically, short-term endurance capacity assessed by TTE at 100% VO2max was elevated ~40% more in the bicarbonate supplemented group and was associated with a ~10% greater increase in LT (16). In light of these findings, we recruited recreationally active men to assess the acute changes that may underpin these enhanced training adaptations in an untrained population. However, future research should focus on the acute

mechanisms that may occur in elite athletes with $NaHCO_3$ ingestion and if it would translate to enhanced adaptations as well. Evidence suggests that at the same relative workload gene expression is not different between trained and untrained individuals (36, 40). Therefore, we may hypothesize that we would see similar results, at least acutely. Driller et al. questioned if the results of Edge and colleagues would have been observed to the same extent in elite athletes due to their already well adapted lactic threshold and mitochondrial oxidative capacity (15). To investigate this thesis, 12 elite rowers matched for peak power output during a graded exercise test were recruited. Half the group ingested $NaHCO_3$ and the other an equimolar dose of NaCl prior to HIIT on a rowing ergometer 2 x wk^{-1} for 4 wks. Both groups noted improvements in 2000-m rowing performance without a difference between groups. Based on these findings, the authors concluded that there was no further advantage to chronically supplementing with $NaHCO_3$ in elite athletes, at least when training work output was matched (15). However, it is interesting to note that while statistically non-significant, a 0.8 s greater enhancement was observed in $NaHCO_3$ supplemented athletes. Considering the study was limited in their power (n=6 per group) and consisted of only 8 training sessions over 4 wks; more subjects, a longer intervention or perhaps unmatched worked throughout training may have led to greater adaptations, although this is only speculative. Therefore, clear evidence for $NaHCO_3$ supplementation to be an effective nutritional intervention exists; however, the implications for the athletic community remain to be determined.

Conclusion. We found that ingestion of a total dose of 0.4 g kg^{-1} b.w. of $NaHCO_3$ prior to an acute session of interval training altered muscle metabolism during exercise and enhanced the expression of PGC-1α mRNA during recovery. Thereby we provide evidence of potential mechanisms mediating enhanced adaptations seen with chronic $NaHCO_3$ ingestion during

training. Furthermore, the increased gene expression does not appear to be associated with greater activation of AMPK or p38 MAPK.

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VII. TABLES

Table 1 Physical characteristics and performance test data

Age (years)	22 ± 2
Height (cm)	180 ± 6
Body mass (kg)	78 ± 13
Body fat (%)	15 ± 5
Fat free body mass (%)	85 ± 5
VO_2 peak (mL min^{-1} kg^{-1})	48.1 ± 8.0
W_{max} (W)	345 ± 53
HR_{max} (bbm)	193 ± 7

Data are mean ± SD. W max, maximum workload; HR max, maximum heart rate

Table 2 Diet analysis of 24 h preceding HIIT with BICARB or PLAC

		BICARB	PLAC
Energy (kcal)		2220 ± 501	2249 ± 520
CHO	g	300 ± 119	309 ± 107
	% total cal	52 ± 10	51 ± 8
FAT	g	65 ± 23	84 ± 35
	% total cal	26 ± 8	31 ± 11
PRO	g	120 ± 50	104 ± 31
	% total cal	22 ± 9	18 ± 6

Data are mean ± SD. BICARB, bicarbonate; PLAC, placebo; CHO, carbohydrates; PRO, protein; g, grams.

Table 3 Interval workload, heart rate and RPE during HIIT with BICARB or PLAC

	BICARB	PLAC
Interval Workload (W)	263 ± 40	
Average % of HR_{max}	89 ± 5	88 ± 5
Average RPE	15.2 ± 1.6	14.9 ± 1.8

Data are mean ± SD. BICARB, bicarbonate; PLAC, placebo; HR_{max}, maximum heart rate; RPE, rating of perceived exertion.

VIII. FIGURE LEGENDS

FIGURE 1 – Overview of experimental trial, which subjects completed twice in a counterbalanced manner with at least 7 d in between. 24 h prior to each session Actihearts (Acti) and diet logs (DL) were given. The following morning a fasting blood (Bld) sample was acquired followed by the ingestion of a standardized breakfast (FD) and the first dose (DS 1) of either $NaHCO_3$ or an equimolar amount of NaCl. A second dose was provided 0.5 h later. Bld and a muscle biopsy (Bx) were obtained prior to an acute bout of HIIT with subsequent specimen collection upon completion of exercise and throughout recovery. A gastrointestinal distress and related complaints questionnaire (GI) was completed throughout trials to assess discomfort.

FIGURE 2 – Representative western blot following muscle fractionation to demonstrate nuclear and cytosolic enrichment and purity. Primary antibodies targeting LDHA and α-tubulin were used as representative cytosolic proteins which were lacking in nuclear fractions (NUC). In contrast, histone H3 was highly expressed in NUC but absent in the cytosolic fractions (CYT). A whole muscle homogenate was used as a positive control.

FIGURE 3 – Response of various blood measures to HIIT with ingestion of sodium bicarbonate (BICARB) or a placebo (PLAC). Panels depict (A) Blood pH and (B) bicarbonate concentrations pre ingestion (PRE ING), post ingestion (POST ING), post HIIT (POST EX) and 2h- (2Hr REC) 3h-recovery (3 Hr REC). Additional measure at 15-, 30-, and 1 hr – recovery were made for (C) blood lactate and (D) glucose concentrations. * Main effect for time compared to PRE ING ($p < 0.05$). † Significant difference between BICARB and PLAC at the time point designated ($p< 0.05$). Main effect for treatments were observed in blood pH, bicarbonate concentration and lactate ($p < 0.05$)

FIGURE 4 – Muscle glycogen content prior to (PRE), immediately post (POST) and 3 h into recovery (3 Hr REC) after the ingestion of sodium bicarbonate (BICARB) or a placebo (PLAC). * Main effect for time compared to PRE ($P<0.05$). † Significant difference between BICARB and PLAC at the time point designated ($P<0.05$).

FIGURE 5 – Acute protein phosphorylation in response to HIIT in conjunction with sodium bicarbonate (BICARB, open bars) or a placebo (PLAC, closed bars) supplementation. Western blots were used to analyse muscle biopsied acquired prior to commencing HIIT (PRE), immediately upon completion (POST) and 3 h after (3 Hr REC). (A) Ratio of phosphorylated p38 mitogen activated protein kinase (p38 $MAPK^{Thr180/Tyr182}$) to total p38 MAPK. (B) Phosphorylated AMP-activated protein kinase ($AMPK^{Thr172}$) relative to total AMPK. (C) Phosphorylation of acetyl-CoA carboxylase (phos-ACC ^{Ser79}). * Main effect for time compared to PRE ($P<0.05$). $ Trend towards main effect for time compared to PRE ($p = 0.071$).

FIGURE 6 – mRNA expression after an acute bout of HIIT supplemented with sodium bicarbonate (BICARB, open bars) or a placebo (PLAC, closed bars). (A) peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1α), (B) citrate synthase and (C) cytochrome c oxidase subunit IV (COX IV) mRNA expression before (PRE) and 3 h after (3 Hr Rec) an acute bout of HIIT. Main effect for treatment on PGC-1α mRNA was observed ($p < 0.05$). * Main effect for time compared to PRE ($P<0.05$). † Significant difference between BICARB and PLAC at time point designated ($P<0.05$).

IX. FIGURES

FIGURE 1

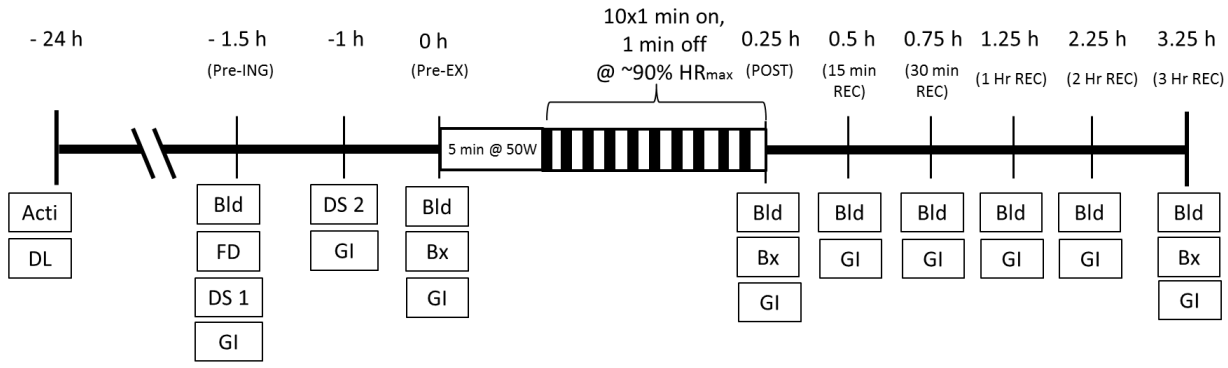


FIGURE 2

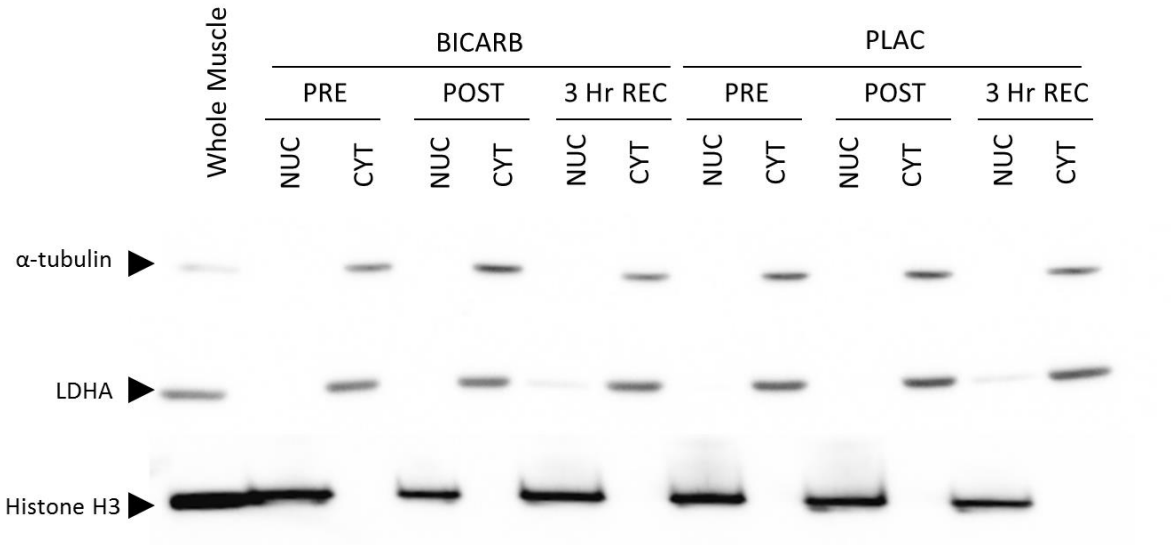

Whole Muscle
BICARB
PLAC
PRE
POST
3 Hr REC
PRE
POST
3 Hr REC
NUC
CYT
NUC
CYT
NUC
CYT
NUC
CYT
NUC
CYT
NUC
CYT
α-tubulin
LDHA
Histone H3

FIGURE 3

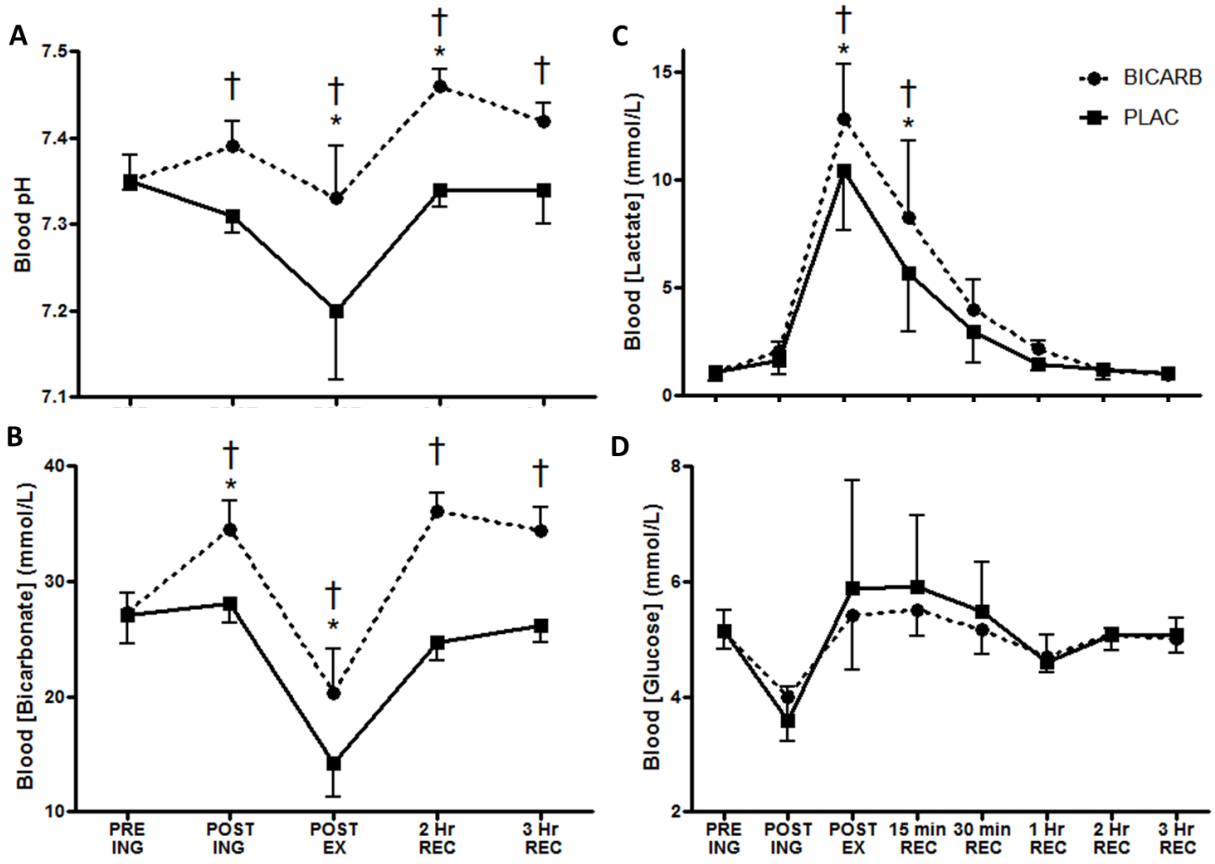

A
Blood pH
7.5
7.4
7.3
7.2
7.1
C
Blood [Lactate] (mmol/L)
15
10
5
0
BICARB
PLAC
B
Blood [Bicarbonate] (mmol/L)
40
30
20
10
D
Blood [Glucose] (mmol/L)
8
6
4
2
PRE ING
POST ING
POST EX
2 Hr REC
3 Hr REC
15 min REC
30 min REC
1 Hr REC

FIGURE 4

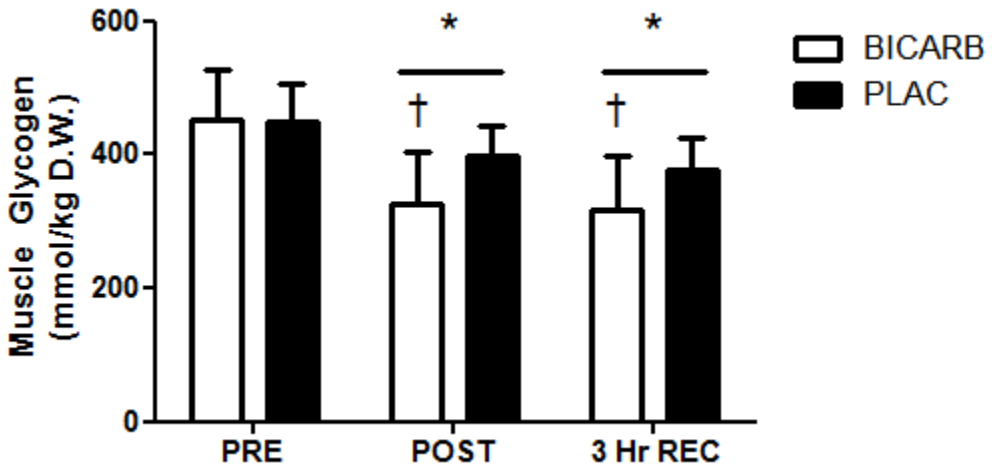

600
400
200
0
Muscle Glycogen
(mmol/kg D.W.)
*
*
†
†
PRE
POST
3 Hr REC
BICARB
PLAC

FIGURE 5

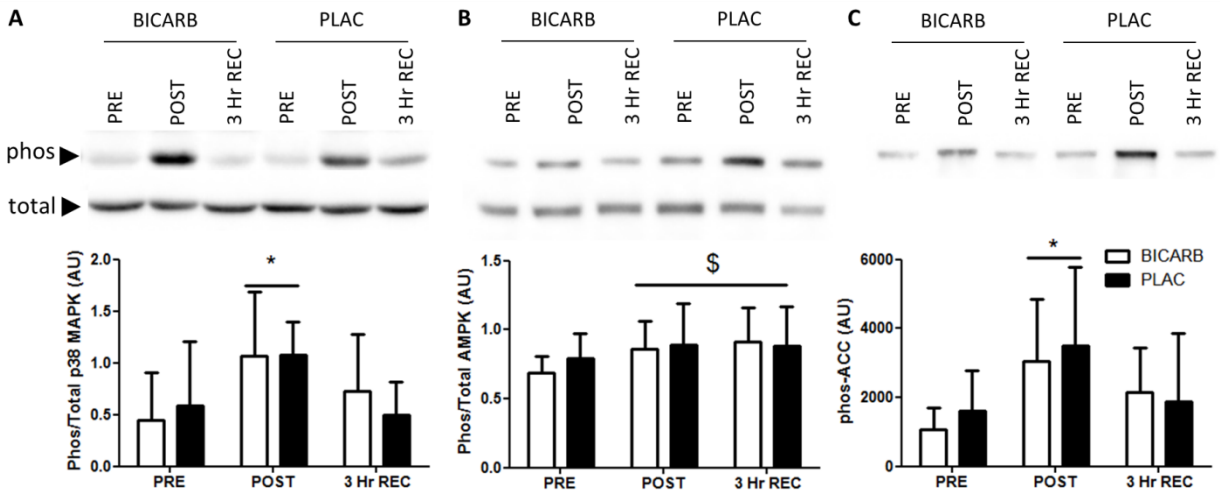
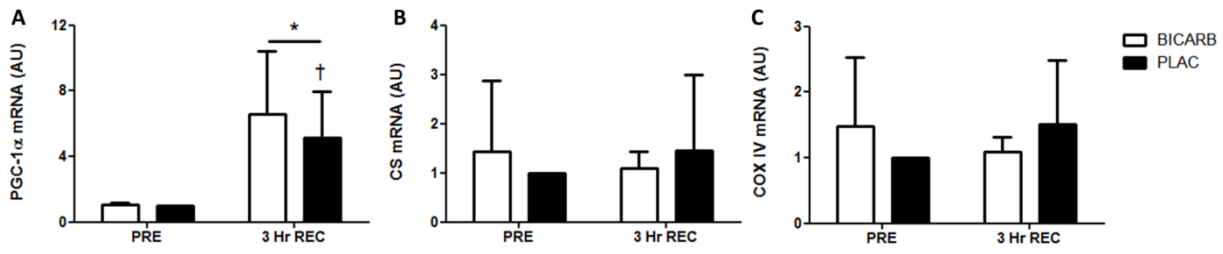

A
BICARB
PLAC
PRE
POST
3 Hr REC
phos
total
B
C
Phos/Total p38 MAPK (AU)
Phos/Total AMPK (AU)
phos-ACC (AU)
*
$
BICARB
PLAC

FIGURE 6


A
PGC-1α mRNA (AU)
12
8
4
0
*
†
PRE
3 Hr REC
B
CS mRNA (AU)
4
3
2
1
0
PRE
3 Hr REC
C
COX IV mRNA (AU)
3
2
1
0
PRE
3 Hr REC
BICARB
PLAC

APPENDIX A – SUBJECT CHARACTERISTCS

Subject ID	Age	Anthropometrics				
		Height (cm)	Mass (kg)	BMI (kg/m^2)	%FAT	%FFM
1	25	177	73.0	22.9	12.8	87.2
2	23	180	78.2	24.1	15.7	84.3
3	21	175	64.0	20.9	14.0	86.0
5	20	178	93.0	29.3	11.6	88.4
7	21	170	69.7	24.1	15.2	84.8
8	23	188	97.9	27.7	22.7	77.3
9	24	189	92.2	25.8	19.7	80.3
10	21	182	73.0	22.0	21.0	79.0
11	20	181	62.7	19.1	6.7	93.3
Mean	22.0	180.0	78.2	24.0	15.5	84.5
SD	1.8	6.0	13.1	3.2	5.0	5.0

Subject ID	Performance Data					
	VO2 peak (ml/min)	VO2 peak (mL/min/kg)	W max (W)	HR max (bpm)	90% HR max (bpm)	Interval Workload (W)
1	2763	38.6	240	192	173	180
2	3788	48.5	335	185	167	250
3	3656	57.1	350	193	174	270
5	4099	44.1	377	183	165	300
7	3240	46.5	300	190	171	240
8	4505	46.0	394	189	170	295
9	4225	45.8	416	200	180	310
10	3052	41.8	320	206	185	240
11	4047	64.5	369	195	176	280
Mean	3708.3	48.1	344.6	193	173	262.8
SD	583.3	8.0	53.4	7	6	40.4

APPENDIX B - DIETARY & ACTIVITY CONTROL

SELF REPORTED DIET ANALYSIS

Subject	CAL (kcal)		CHO (g)		FAT (g)		PRO (g)	
ID	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC
1	1,547	1,462	246	261	46	164	63	48
2	2,304	2,304	320	320	82	82	80	80
3	2,525	2,073	345	275	63	57	146	116
5	2,219	2,219	245	245	92	92	104	104
7	3,264	3,264	587	587	76	76	147	147
8	2,017	2,184	189	289	41	68	209	116
9	2,309	2,614	214	264	93	81	162	141
10	1,658	1,688	241	235	29	36	102	103
11	2,137	2,431	317	308	65	99	65	80
Mean	2220	2249	300	309	65	84	120	104
SD	502	521	119	108	23	35	49	31
TTEST	**0.71**		**0.58**		**0.20**		**0.17**	

Subject	CHO (%CAL)		FAT (%CAL)		PRO (%CAL)	
ID	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC
1	59.6	38.5	25.1	54.4	15.3	7.1
2	54.7	54.7	31.6	31.6	13.7	13.7
3	54.5	53.0	22.4	24.7	23.1	22.3
5	44.1	44.1	37.2	37.2	18.7	18.7
7	64.9	64.9	18.9	18.9	16.2	16.2
8	38.6	51.8	18.8	27.4	42.6	20.8
9	36.6	45.0	35.8	31.0	27.7	24.0
10	59.0	56.1	16.0	19.3	25.0	24.6
11	60.0	50.4	27.7	36.5	12.3	13.1
Mean	52.4	50.9	25.9	31.2	21.6	17.8
SD	10.2	7.7	7.7	10.9	9.5	5.8
TTEST	**0.66**		**0.15**		**0.16**	

24 h ENERGY EXPENDITURE PREDICTED BY ACTIHEART (KJ 24 h^{-1} kg^{-1})

Subject ID	BICARB	PLAC
1	-	-
2	50.26	70.13
3	29.52	47.23
5	-	-
7	55.87	48.67
8	80.21	75.31
9	79.78	41.62
10	66.24	56.59
11	38.88	57.60
Mean	57.25	56.74
SD	19.46	12.31
TTEST	**0.952073**	

APPENDIX C - EXERCISE VARIABLES: RAW DATA and STATISTICS

% HRmax ACHIEVED AFTER EACH INTERVAL AVERAGED OVER ALL INTEVALS

Subject ID	BICARB	PLAC
1	90	87
2	93	93
3	83	79
5	93	89
7	93	91
8	82	81
9	86	84
10	85	88
11	90	95
Mean	89	88
SD	5	5
TTEST	**0.37026**	

RPE AFTER EACH INTERVAL AVERAGED OVER ALL INTERVALS

Subject ID	BICARB	PLAC
1	16.30	12.60
2	17	17.8
3	12.20	12.20
5	17.3	14.10
7	14.6	15.30
8	15.4	15.3
9	14.90	16.00
10	13.7	14.6
11	15	16.2
Mean	15.2	14.9
SD	1.6	1.8
TTEST	**0.692408**	

TRIAL DAY GASTROINTESTINAL DISTRESS AND RELATED COMPLAINTS

GI Distress and Related Complaints questionnaire

Subject ID	PRE		POST DS 1		POST ING		POST EX		15 min REC		30 min REC		1 Hr REC		2 Hr REC		3 Hr REC	
	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC
1	15	18	18	33	18	27	18	22	15	19	15	17	15	17	17	16	16	15
2	16	15	25	37	25	17	27	20	26	18	23	22	30	15	28	15	15	15
3	15	15	24	17	21	15	17	15	15	15	15	15	15	15	15	15	15	15
5	15	15	19	30	15	21	20	15	15	15	15	15	17	15	17	15	17	15
7	15	15	20	20	16	22	20	15	19	15	15	15	15	15	15	15	15	15
8	19	15	22	23	17	21	15	22	17	20	15	17	15	16	15	17	18	15
9	19	18	24	16	35	29	21	21	20	21	24	19	24	18	18	19	17	15
10	15	15	27	26	26	15	15	15	16	15	15	15	15	15	15	15	15	15
11	15	15	23	18	19	17	15	18	15	16	15	15	15	15	15	15	15	15
Mean	16.0	15.7	22.4	24.4	21.3	20.4	18.7	18.1	17.6	17.1	16.9	16.7	17.9	15.7	17.2	15.8	15.9	15.0
SD	1.7	1.3	3.0	7.5	6.4	5.0	3.9	3.2	3.7	2.4	3.8	2.4	5.4	1.1	4.2	1.4	1.2	0.0

ANOVA Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	990.1	2.438	406.0	14.71	**0.000$**
Error (Time)	538.5	19.507	27.6		
Treatment	12.5	1	12.5	0.437	0.527
Error (Treatment)	228.8	8	28.60		
Time x Treatment	47.22	8	5.9	0.583	0.788
Error (Time x Treatment)	648	64	10.13		

$ Greenhouse-Geisser correction applied due to violation of sphericity

Pairwise comparisons with Tukey's HSD test : TIME

HSD = q(k,df) √(MSE/n)		p < 0.05
	k =	8
	dfE =	19.507
	q =	4.794
	MSE =	27.604
	n =	18
	HSD =	**5.9367**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE	vs	POST Dose 1	7.61	**Y**
PRE	vs	POST ING	5.06	N
PRE	vs	POST EX	2.56	N
PRE	vs	15 min REC	1.50	N
PRE	vs	30 min REC	0.94	N
PRE	vs	1 Hr REC	0.94	N
PRE	vs	2 Hr REC	0.67	N
PRE	vs	3 Hr REC	-0.39	N

APPENDIX D - BLOOD & MUSCLE ANALYSIS: RAW DATA and STATISTICS

Blood pH

Subject	PRE		POST ING		POST EX		2 Hr REC		3 Hr REC	
ID	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC
1	7.32	7.32	7.35	7.34	7.33	7.23	7.52	7.34	7.43	7.33
2	7.33	7.35	7.35	7.32	7.28	7.2	7.48	7.36	7.4	7.32
3	7.34	7.36	7.38	7.31	7.45	7.31	7.46	7.34	7.4	7.33
5	7.32	7.34	7.4	7.33	7.33	7.23	7.47	7.33	7.44	7.34
7	7.35	7.35	7.38	7.29	7.29	7.18	7.48	7.34	7.41	7.35
8	7.37	7.34	7.39	7.3	7.32	7.2	7.33	7.37	7.4	7.44
9	7.4	7.36	7.44	7.29	7.38	7.23	7.5	7.36	7.44	7.31
10	7.34	7.37	7.4	7.29	7.37	7.21	7.46	7.34	7.43	7.34
11	7.34	7.35	7.38	7.33	7.24	7.01	7.42	7.3	7.39	7.29
Mean	7.35	7.35	7.39	7.31	7.33	7.20	7.46	7.34	7.42	7.34
SD	0.03	0.01	0.03	0.02	0.06	0.08	0.06	0.02	0.02	0.04

ANOVA Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	.185	4	.046	20.676	**.000**
Error (Time)	.071	32	.002		
Treatment	.141	1	.141	90.252	**.000**
Error (Treatment)	.012	8	.002		
Time x Treatment	.049	4	.012	13.429	**.000**
Error (Time x Treatment)	.029	32	.001		

Pairwise comparisons with Tukey's HSD test : TIME

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	4
dfE =	32
q =	3.832
MSE =	0.002
n =	18
HSD =	**0.04269**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE	vs	Post ING	-0.001	N
PRE	vs	POST EX	0.081	**Y**
PRE	vs	2 Hr REC	-0.053	**Y**
PRE	vs	3 Hr REC	0.030	N

Pairwise comparisons with Tukey's HSD test : TIME x TREATMENT

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	5
dfE =	32
q =	4.086
MSE =	0.001
n =	9
HSD =	**0.0413**

Pairwise comparison			**Mean Difference**	**Significance MD>HSD**
BICARB PRE	vs	PLAC PRE	-0.003	N
BICARB POST ING	vs	PLAC POST ING	0.074	**Y**
BICARB POST EX	vs	PLAC POST EX	0.132	**Y**
BICARB 2 Hr REC	vs	PLAC 2 Hr REC	0.116	**Y**
BICARB 3 Hr REC	vs	PLAC 3 Hr REC	0.077	**Y**

Blood Bicarbonate Concentration mmol/L

Subject	PRE		POST ING		POST EX		2 Hr REC		3 Hr REC	
ID	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC
1	28	28	33	28	19	13	37	25	35	26
2	28	28	34	29	19	15	35	24	35	26
3	29	27	33	27	27	18	36	24	33	27
5	30	29	37	30	21	16	39	26	38	28
7	25	24	31	26	17	12	34	23	33	24
8	26	30	35	30	22	15	35	25	35	27
9	26	25	39	27	25	18	38	24	35	28
10	26	23	33	26	17	10	35	24	31	24
11	28	30	36	30	16	11	36	28	35	26
Mean	27.33	27.11	34.56	28.11	20.33	14.22	36.11	24.78	34.44	26.22
SD	1.66	2.57	2.46	1.69	3.77	2.91	1.62	1.48	1.94	1.48

ANOVA Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	2444.489	1.547	1579.754	120.931	**.000 $**
Error (Time)	161.711	12.379	13.063		
Treatment	940.900	1	940.900	384.041	**.000**
Error (Treatment)	19.600	8	2.450		
Time x Treatment	296.489	4	74.122	50.997	**.000**
Error (Time x Treatment)	46.511	32	1.453		

$ Greenhouse-Geisser correction applied due to violation of sphericity

Pairwise comparisons with Tukey's HSD test : TIME

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	4
dfE =	12.379
q =	4.199
MSE =	13.063
n =	18
HSD =	**3.57713**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE	vs	Post ING	4.11	**Y**
PRE	vs	POST EX	9.94	**Y**
PRE	vs	2 Hr REC	3.22	N
PRE	vs	3 Hr REC	3.11	N

Pairwise comparisons with Tukey's HSD test : TIME x TREATMENT

HSD = q(k,df) √(MSE/n) p < 0.05

k =	5
dfE =	32
q =	4.086
MSE =	1.453
n =	9
HSD =	**1.64203**

Pairwise comparison			Mean Difference	Significance MD>HSD
BICARB PRE	vs	PLAC PRE	0.22	N
BICARB POST ING	vs	PLAC POST ING	6.44	**Y**
BICARB POST EX	vs	PLAC POST EX	6.11	**Y**
BICARB 2 Hr REC	vs	PLAC 2 Hr REC	11.33	**Y**
BICARB 3 Hr REC	vs	PLAC 3 Hr REC	8.22	**Y**

Blood pCO2 mmHg

Subject	PRE		POST ING		POST EX		2 Hr REC		3 Hr REC	
ID	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC
1	54	55	59	52	36	30	45	45.5	53	49
2	53	51	61	56	40	38	47	42	57	51
3	54	48	56	54	39	36	50	44	54	51
5	59	53	59	56	40	37	54	49	56	52
7	46	44	53	54	36	31	45	43	52	44
8	45	55	57	60	42	38	51	43	57	52
9	42	44	57	57	42	42	49	43	52	55
10	48	40	53	54	30	26	49	44	47	45
11	51	54	60	56	38	45	56	56	57	54
Mean	50.22	49.33	57.22	55.44	38.11	35.89	49.56	45.50	53.89	50.33
SD	5.38	5.57	2.86	2.30	3.76	5.99	3.75	4.44	3.33	3.74

ANOVA Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	3765.900	2.238	1682.952	63.160	**0.000 $**
Error (Time)	477.000	17.901	26.646		
Treatment	140.625	1.000	140.625	16.520	**0.004**
Error (Treatment)	68.100	8.000	8.513		
Time x Treatment	30.278	4.000	7.569	1.050	0.397
Error (Time x Treatment)	230.622	32.000	7.207		

$ Greenhouse-Geisser correction applied due to violation of sphericity

Pairwise comparisons with Tukey's HSD test : TIME

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	4
dfE =	17.901
q =	3.997
MSE =	26.646
n =	18
HSD =	**4.8631**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE	vs	Post ING	6.56	**Y**
PRE	vs	POST EX	12.78	**Y**
PRE	vs	2 Hr REC	-2.25	N
PRE	vs	3 Hr REC	2.33	N

Blood pO2 mmHg

Subject	PRE		POST ING		POST EX		2 Hr REC		3 Hr REC	
ID	**BICARB**	**PLAC**	**BICARB**	**PLAC**	**BICARB**	**PLAC**	**BICARB**	**PLAC**	**BICARB**	**PLAC**
1	21	17	16	29	54	93	36	36	27	21
2	26	27	21	18	43	46	45	33	20	19
3	23	32	22	25	63	86	26	33	18	21
5	19	27	27	23	49	77	23	32	20	29
7	49	75	25	26	59	105	70	53	26	34
8	34	26	18	18	39	47	26	35	15	21
9	53	50	26	26	51	58	33	50	31	21
10	28	64	19	23	97	74	18	34	21	31
11	28	28	15	17	42	39	23	20	24	21
Mean	31.22	38.44	21.00	22.78	55.22	69.44	33.33	36.25	22.44	24.22
SD	12.08	19.84	4.36	4.24	17.56	23.14	15.98	9.87	4.98	5.52

ANOVA Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	18976.38	4	4744.097	26.292	**.000**
Error (Time)	5773.939	32	180.436		
Treatment	701.406	1	701.406	9.708	**.014**
Error (Treatment)	578.000	8	72.250		
Time x Treatment	510.264	4	127.566	1.339	.277
Error (Time x Treatment)	3047.861	32	95.246		

Pairwise comparisons with Tukey's HSD test : TIME

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	4
dfE =	32.000
q =	3.832
MSE =	180.436
n =	18
HSD =	**12.1325**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE	vs	Post ING	-12.94	**Y**
PRE	vs	POST EX	-27.50	**Y**
PRE	vs	2 Hr REC	-0.04	N
PRE	vs	3 Hr REC	-11.50	N

Blood Lactate Concentration mmol/L

Subject ID	PRE		POST ING		POST EX		15 min REC		30 min REC		1 Hr REC		2 Hr REC		3 Hr REC	
	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC
1	1.44	1.05	1.62	2.19	12.88	7.92	6.80	4.57	3.33	2.41	2.22	1.77	1.02	1.13	0.78	0.95
2	1.05	0.71	1.47	1.26	13.94	10.39	8.24	6.45	3.22	3.16	1.94	1.07	1.06	1.04	0.89	1.01
3	0.90	1.11	2.62	1.48	8.25	6.33	13.62	2.09	4.62	1.59	2.34	1.24	1.19	0.81	1.03	0.88
5	1.27	1.18	1.93	1.81	13.39	9.79	5.19	3.88	2.61	2.13	2.10	1.47	1.33	0.97	1.10	0.90
7	1.06	0.88	2.01	1.18	14.54	11.79	4.92	4.89	2.89	2.50	1.83	1.52	1.14	0.95	1.18	1.27
8	0.69	0.71	2.11	0.95	14.70	11.81	9.78	5.94	4.81	2.60	2.13	1.09	1.06	2.30	0.82	0.89
9	0.84	1.41	2.16	1.48	8.88	8.32	4.62	5.65	2.68	2.40	1.78	1.24	1.04	0.94	1.16	1.06
10	1.50	1.97	2.76	3.18	13.49	11.94	7.97	6.01	5.22	3.51	2.71	1.66	1.34	1.37	1.09	1.29
11	1.05	0.85	1.90	1.23	15.47	15.77	13.03	11.39	6.55	6.60	2.64	1.98	1.33	1.53	1.00	1.08
Mean	1.09	1.10	2.06	1.64	12.84	10.45	8.24	5.65	3.99	2.99	2.19	1.45	1.17	1.23	1.00	1.04
SD	0.27	0.40	0.42	0.68	2.55	2.79	3.35	2.53	1.37	1.46	0.33	0.31	0.14	0.46	0.14	0.16

ANOVA
Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	1815.363	1.891	959.793	89.997	**0.000$**
Error (Time)	161.371	15.131	10.665		
Treatment	27.959	1	27.959	16.007	**0.004**
Error (Treatment)	13.973	8	1.747		
Time x Treatment	35.666	7	5.095	4.992	**0.000**
Error (Time x Treatment)	57.159	56	1.021		

$ Greenhouse-Geisser correction applied due to violation of sphericity

Pairwise comparisons with Tukey's HSD test : TIME

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	7
dfE =	15.131
q =	4.782
MSE =	10.665
n =	18
HSD =	**3.6808**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE	vs	Post ING	0.76	N
PRE	vs	POST EX	10.55	**Y**
PRE	vs	15 min REC	5.85	**Y**
PRE	vs	30 min REC	2.40	N
PRE	vs	1 Hr REC	0.73	N
PRE	vs	2 Hr REC	0.11	N
PRE	vs	3 Hr REC	-0.07	N

Pairwise comparisons with Tukey's HSD test : TIME x TREATMENT

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	8
dfE =	56
q =	4.452
MSE =	1.021
n =	9
HSD =	**1.4993**

Pairwise comparison			Mean Difference	Significance MD>HSD
BICARB PRE	vs	PLAC PRE	0.01	N
BICARB POST ING	vs	PLAC POST ING	0.42	N
BICARB POST EX	vs	PLAC POST EX	2.39	**Y**
BICARB 15 min REC	vs	PLAC 15 min REC	2.59	**Y**
BICARB 30 min REC	vs	PLAC 30 min REC	1.00	N
BICARB 1 Hr REC	vs	PLA 1 Hr REC	0.74	N
BICARB 2 Hr REC	vs	PLA 2 Hr REC	0.06	N
BICARB 3 Hr REC	vs	PLA 3 Hr REC	0.03	N

Glucose Concentration mmol/L

Subject ID	PRE		POST ING		POST EX		15 min REC		30 min REC		1 Hr REC		2 Hr REC		3 Hr REC	
	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC
1	5.00	4.83	3.77	3.10	5.44	5.44	5.07	6.09	5.31	5.72	4.26	4.67	4.84	5.01	4.73	4.57
2	5.27	4.80	3.07	3.51	5.26	4.98	5.51	5.31	5.04	5.21	5.04	4.67	5.21	4.88	4.99	5.59
3	4.75	4.91	3.71	3.22	4.78	5.51	5.19	6.04	4.78	4.46	4.57	4.68	4.67	4.97	4.93	4.90
5	4.77	5.11	2.98	3.25	5.94	6.03	5.67	6.27	5.41	4.29	4.55	4.71	5.01	5.23	4.96	5.18
7	5.13	5.42	4.72	4.75	4.48	3.96	5.58	4.52	5.01	6.34	4.91	5.01	5.16	5.26	5.02	5.34
8	5.61	4.80	4.03	2.97	4.44	4.80	4.80	4.56	5.33	4.85	5.04	4.94	5.46	5.02	5.37	4.97
9	5.08	5.79	4.80	3.45	5.02	5.02	5.82	5.92	5.69	6.01	4.75	5.01	4.74	5.03	4.63	4.86
10	5.11	5.48	5.22	3.82	5.83	6.82	5.86	6.21	5.54	5.58	4.37	4.33	5.27	5.05	5.15	5.01
11	5.51	5.23	3.73	4.23	7.55	10.41	6.12	8.37	4.31	6.90	4.82	3.53	5.36	5.16	5.26	5.22
Mean	5.13	5.15	4.00	3.59	5.42	5.88	5.51	5.92	5.16	5.48	4.70	4.61	5.08	5.07	5.00	5.07
SD	0.29	0.36	0.77	0.58	0.96	1.87	0.42	1.14	0.43	0.87	0.28	0.46	0.28	0.12	0.23	0.30

ANOVA
Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	47.056	1.457	32.290	9.577	**.006$**
Error (Time)	39.308	11.658	3.372		
Treatment	.338	1	.338	.872	**.378**
Error (Treatment)	3.104	8	.388		
Time x Treatment	2.711	2.666	1.017	1.535	**.236**
Error (Time x Treatment)	14.129	21.330	.662		

$ Greenhouse-Geisser correction applied due to violation of sphericity

Pairwise comparisons with Tukey's HSD test : TIME

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	7
dfE =	11.658
q =	4.947
MSE =	3.372
n =	18
HSD =	**2.14106**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE	vs	Post ING	1.35	N
PRE	vs	POST EX	0.51	N
PRE	vs	15 min REC	0.57	N
PRE	vs	30 min REC	0.18	N
PRE	vs	1 Hr REC	0.49	N
PRE	vs	2 Hr REC	0.07	N
PRE	vs	3 Hr REC	0.11	N

Glycogen mmol/kg dw

Subject	PRE		POST		3 Hr REC	
ID	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC
1	445.96	404.87	324.22	385.09	207.04	299.87
2	484	488.57	330.3	476.39	281.61	374.43
3	484	528.13	379	444.44	453.57	424.65
5	497.7	445.96	340.95	360.74	324.22	377.48
7	389.65	401.83	252.69	360.74	278.56	353.13
8	312.04	383.56	176.6	342.48	228.34	327.75
9	382.9	534.07	309.47	423.21	328.19	464.96
10	559.99	440.49	352.66	364.18	400.17	394.42
11	506.72	411.69	459.2	407	364.18	367.06
Mean	451.44	448.80	325.01	396.03	318.43	375.97
SD	76.71	55.89	78.63	44.93	79.56	49.18

ANOVA Results

Source of Variation	SS	DF	MS	F	*p*
Time	112782.478	2	56391.239	31.115	**.000**
Error (Time)	28997.411	16	1812.338		
Treatment	23782.930	1	23782.930	4.038	.079
Error (Treatment)	47121.792	8	5890.224		
Time x Treatment	13845.202	2	6922.601	7.894	**.004**
Error (Time x Treatment)	14031.934	16	876.996		

Pairwise comparisons with Tukey's HSD test : TIME

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	3
dfE =	16
q =	3.649
MSE =	1812.34
n =	18
HSD =	**36.615**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE	vs	POST	-89.60	**Y**
PRE	vs	3 Hr REC	102.92	**Y**
POST	vs	3 Hr REC	13.32	N

Pairwise comparisons with Tukey's HSD test : TIME x TREATMENT

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	3
dfE =	16
q =	3.65
MSE =	877
n =	9
HSD =	**36.03**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE BICARB	vs	PRE PLAC	2.64	N
POST BICARB	vs	POST PLAC	71.02	**Y**
REC BICARB	vs	REC PLAC	57.54	**Y**

p38 MAPK (phos/total)

Subject ID	PRE		POST		3 Hr REC	
	BICARB	PLAC	BICARB	PLAC	BICARB	PLAC
1	0.53	1.83	1.98	1.57	1.72	0.49
2	0.36	0.03	1.59	0.76	0.59	0.10
3	0.10	0.05	0.79	1.11	0.20	0.10
5	1.63	0.45	1.92	1.33	1.35	0.88
7	0.31	0.62	0.71	1.33	0.43	0.81
8	0.20	0.13	0.68	0.99	0.67	0.67
9	0.39	1.13	1.02	0.83	1.08	0.71
10	0.34	0.10	0.17	1.25	0.44	0.61
11	0.15	1.01	0.72	0.56	0.04	0.08
Mean	0.45	0.59	1.07	1.08	0.73	0.50
SD	0.46	0.62	0.62	0.32	0.55	0.32

ANOVA Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	3.169	2	1.585	19.087	**.000**
Error (Time)	1.328	16	.083		
Treatment	.006	1	.006	.027	.874
Error (Treatment)	1.870	8	.234		
Time x Treatment	.333	2	.166	.962	.403
Error (Time x Treatment)	2.766	16	.173		

Pairwise comparisons with Tukey's HSD test : TIME

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	3
dfE =	16
q =	3.649
MSE =	0.083
n =	18
HSD =	**0.2478**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE	vs	POST	0.55	**Y**
PRE	vs	3 Hr REC	-0.09	N
POST	vs	3 Hr REC	0.46	**Y**

AMPK (phos/total)

	PRE		POST		3 Hr REC	
Subject	**BICARB**	**PLAC**	**BICARB**	**PLAC**	**BICARB**	**PLAC**
1	0.79	0.87	1.15	0.90	0.90	1.40
2	0.80	0.94	1.02	0.72	1.11	0.92
3	0.72	0.79	1.06	1.24	0.55	0.65
5	0.53	0.87	0.50	1.03	0.52	1.26
7	0.49	1.09	0.75	0.94	0.87	0.66
8	0.61	0.51	0.75	0.57	1.09	0.57
9	0.72	0.68	0.87	0.60	0.90	0.73
10	0.75	0.59	0.95	0.58	1.31	0.99
11	0.83	0.74	0.73	1.39	0.95	0.77
Mean	0.69	0.79	0.86	0.89	0.91	0.88
SD	0.12	0.18	0.20	0.30	0.25	0.29

ANOVA Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	.261	2	.130	3.136	**.071**
Error (Time)	.665	16	.042		
Treatment	.011	1	.011	.109	.750
Error (Treatment)	.827	8	.103		
Time x Treatment	.033	2	.017	.408	.672
Error (Time x Treatment)	.655	16	.041		

Pairwise comparisons with Tukey's HSD test : TIME (trending)

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	3
dfE =	16
q =	3.649
MSE =	0.042
n =	18
HSD =	**0.1753**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE	vs	POST	0.13	N
PRE	vs	3 Hr REC	-0.16	N
POST	vs	3 Hr REC	-0.02	N

pACC

	PRE		POST		3 Hr REC	
Subject	**BICARB**	**PLAC**	**BICARB**	**PLAC**	**BICARB**	**PLAC**
1	1240	1418	1207	6691	2313	1182
2	2497	3346	5099	2237	4091	7081
3	1358	2618	3069	4252	2588	1211
5	942	1239	2413	4890	1030	1272
7	1275	2331	2924	5875	4155	1440
8	450	316	5909	790	1421	722
9	775	2621	3990	4752	1958	1564
10	335	122	2745	583	1375	758
11	765	609	96	1344	376	1607
Mean	1070.70	1624.30	3050.14	3490.48	2145.05	1870.75
SD	643.41	1153.05	1798.95	2290.91	1302.67	1978.55

ANOVA Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	3.44E+07	2	1.72E+07	12.720	**.000**
Error (Time)	2.16E+07	16	1.35E+06		
Treatment	7.76E+05	1	7.76E+05	.447	.523
Error (Treatment)	1.39E+07	8	1.74E+06		
Time x Treatment	1.81E+06	1.255	1.45E+06	.347	.618$
Error (Time x Treatment)	4.18E+07	10.041	4.17E+06		

$ Greenhouse-Geisser correction applied due to violation of sphericity

Pairwise comparisons with Tukey's HSD test : TIME

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	3
dfE =	16
q =	3.649
MSE =	1.35E+06
n =	18
HSD =	**999.53**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE	vs	POST	1922.81	**Y**
PRE	vs	3 Hr REC	-660.40	N
POST	vs	3 Hr REC	1262.41	**Y**

PGC-1α mRNA expressed as fold change in Delta CT normalized to PRE PLA

	PRE		3 Hr REC	
Subject ID	**BICARB**	**PLAC**	**BICARB**	**PLAC**
1	1.01	1.00	12.38	8.22
2	1.23	1.00	3.43	3.68
3	1.12	1.00	4.17	3.12
5	1.01	1.00	3.14	3.07
7	0.89	1.00	7.94	8.06
8	0.97	1.00	8.51	5.24
9	1.17	1.00	2.50	1.00
10	1.16	1.00	12.21	9.25
11	0.84	1.00	4.86	4.63
Mean	1.04	1.00	6.57	5.14
SD	0.13	0.00	3.84	2.80

ANOVA Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	210.33	1	210.33	19.604	**.002**
Error (Time)	85.832	8	10.729		
Treatment	4.895	1	4.895	7.038	**.029**
Error (Treatment)	5.564	8	.696		
Time x Treatment	4.320	1	4.320	6.393	**.035**
Error (Time x Treatment)	5.405	8	.676		

Pairwise comparisons with Tukey's HSD test : TIME x TREATMENT

HSD = q(k,df) √(MSE/n) $p < 0.05$

k =	2
dfE =	8
q =	3.26
MSE =	.676
n =	9
HSD =	**0.893**

Pairwise comparison			Mean Difference	Significance MD>HSD
PRE BICARB	vs	PRE PLAC	0.04	N
REC BICARB	vs	REC PLAC	1.43	**Y**

CS mRNA expressed as fold change in Delta CT normalized to PRE PLA

	PRE		3 Hr REC	
Subject ID	**BICARB**	**PLAC**	**BICARB**	**PLAC**
1	1.0281	1	1	0.6878
2	0.895	1	0.9593	0.8645
3	1.0943	1	1.2924	1.2226
5	1.021	1	1.0718	1.1975
7	0.7792	1	0.669	1.0644
8	5.2416	1	1.879	5.5022
9	0.9794	1	1.057	0.8645
10	1.1892	1	1.0644	0.8467
11	0.7371	1	0.8586	0.8467
Mean	1.44	1.00	1.09	1.46
SD	1.43	0.00	0.34	1.53

ANOVA Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	.027	1	.027	.526	.489
Error (Time)	.409	8	.051		
Treatment	.014	1	.014	.412	.539
Error (Treatment)	.279	8	.035		
Time x Treatment	1.444	1	1.444	.820	.392
Error (Time x Treatment)	14.093	8	1.762		

COX IV mRNA expressed as fold change in Delta CT normalized to PRE PLA

	PRE		3 Hr REC	
Subject ID	**BICARB**	**PLAC**	**BICARB**	**PLAC**
1	1.04	1	1	1.13
2	0.95	1	0.91	0.96
3	1.39	1	1.26	2.79
5	0.78	1	0.99	0.97
7	0.81	1	0.73	0.81
8	4.14	1	1.53	3.53
9	1.13	1	1.06	0.96
10	1.8	1	1.18	0.96
11	1.28	1	1.12	1.51
Mean	1.48	1.00	1.09	1.51
SD	1.05	0.00	0.23	0.97

ANOVA Results

Source of Variation	**SS**	**DF**	**MS**	**F**	***p***
Time	.030	1	.030	.291	.604
Error (Time)	.824	8	.103		
Treatment	.007	1	.007	.059	.814
Error (Treatment)	.951	8	.119		
Time x Treatment	1.863	1	1.863	2.523	.151
Error (Time x Treatment)	5.907	8	.738		