EFFECTS OF COMBINED SAGO–SOY SUPPLEMENTATION ON CYCLING TIME TRIAL PERFORMANCE IN THE HEAT

by

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KESAN GABUNGAN PENGAMBILAN SAGO–SOYA TERHADAP PRESTASI PERCUBAAN MASA BERBASIKAL DALAM PERSEKITARAN YANG PANAS

oleh

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Tesis yang diserahkan untuk

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	Degree Celsius
m	Meter
$km.h^{-1}$	Kilometer per hour
g, kg	Gram, kilogram
min	Minute
$kg.m^{-2}$	Body mass divided by the square of the body height for Body mass index
$mL.kg^{-1}.min^{-1}$	Millilitres per kilograms per minutes for Maximal oxygen consumption
$beats.min^{-1}$	Beats per minute for Heart rate
$pg.mL^{-1}$	Picograms per millilitres for F_2-Isoprostanes analysis
$U.L^{-1}$	Units per litre for Creatine kinase analysis
$mmol.L^{-1}$	Millimoles per litre for Lactate, free fatty acids, and glucose analysis
$\mu U.mL^{-1}$	Microunits per millilitre for Insulin analysis
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion

ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
CHO	Carbohydrate
PRO	Protein
CHO–PRO	Carbohydrate and protein combination
VO_2max	Maximal oxygen consumption
ROS	Reactive oxygen species
CK	Creatine kinase
GI	Glycaemic index
NADH	Nicotinamide adenine dinucleotide
FADH	Flavin adenine dinucleotide
SD	Standard deviation
p	The *p*–value
FFA	Free Fatty Acids

KESAN GABUNGAN PENGAMBILAN SAGO–SOYA TERHADAP PRESTASI PERCUBAAN MASA BERBASIKAL DALAM PERSEKITARAN YANG PANAS

ABSTRAK

Kesan pengambilan karbohidrat serta kombinasi karbohidrat dan protein terhadap prestasi daya tahan telah dikaji oleh ramai penyelidik tetapi keputusan kajian adalah tidak sekata. Dalam kajian semasa produk tempatan, iaitu kanji sago (88 % karbohidrat) dan protein soy (mengandungi asid amino) telah digunakan. Tujuan kajian ini adalah untuk menyiasat kesan pengambilan iso–kalori sago, soya, dan sago + soya semasa berbasikal 90 minit berbanding dengan plasebo terhadap ujian masa berbasikal sejauh 20–km di persekitaran yang panas (~ 31 °C; 70 % kelembapan relatif). Dua belas pelumba basikal lelaki terlatih (umur: 19.0 ± 5.6 tahun, berat badan: 60.1 ± 11.2 kg, tinggi: 170.8 ± 7.6 cm, dan VO_2max: 56.5 ± 6.5 $mL.kg^{-1}.min^{-1}$) yang mewakili pasukan lumba basikal Kelantan, telah dipilih untuk mengambil bahagian dalam kajian ini. Kajian ini dijalankan secara rawak dan berbentuk 'single–blind' dan terkawal dengan plasebo. Berikutan ujian–ujian awal untuk menyiasat kecergasan fizikal dan untuk menentukan beban kerja berbasikal dalam keadaan mantap, semua peserta melakukan empat ujian eksperimen. Ujian–ujian eksperimen terdiri daripada kayuhan selama 90 minit pada intensiti 60 % daripada VO_2max, diikuti oleh ujian masa berbasikal sejauh 20–km. Dari awal ujian eksperimen dan pada setiap 20 minit semasa berbasikal keadaan mantap (0, 20, 40, 60, dan 80 minit), peserta diberi minuman sebanyak 200 mL sama ada: 7.5 % sago, 7.5 % soya, 6.0 % sago + 1.5 % soya, ataupun plasebo. Tindak balas kardiorespiratori termasuk kadar denyutan jantung, pengambilan oksigen, nisbah pertukaran pernafasan, dan penilaian tahap keletihan telah dipantau. Keputusan yang diperolehi menunjukkan bahawa tiada perbezaan yang signifikan ditemui dalam masa untuk mengkayuh 20–km tanpa mengira jenis minuman yang diberikan (p > 0.05). Tindakbalas metabolik termasuk kepekatan plasma glukosa dan insulin meningkat semasa berbasikal

dalam ujian sago dan sago + soya berbanding soya dan plasebo. Pada akhir ujian masa berbasikal sejauh 20–km, kepekatan plasma laktat telah meningkat dengan ketara dalam semua ujian (p <0.001), dan plasma asid lemak bebas adalah jauh lebih tinggi dalam ujian plasebo dan soya berbanding dengan sago dan sago + soya (p <0.001). Di samping itu, perbezaan dalam kepekatan plasma kreatin kinase dan F_2–Isoprostanes adalah tidak signifikan di antara ujian–ujian selepas eksperimen (p > 0.05). Data ini menunjukkan bahawa pengambilan sago dan soya secara iso–kalori tidak memberi kesan untuk meningkatkan prestasi percubaan masa berbasikal berbanding dengan minuman plasebo.

EFFECTS OF COMBINED SAGO–SOY SUPPLEMENTATION ON CYCLING TIME TRIAL PERFORMANCE IN THE HEAT

ABSTRACT

The effects of carbohydrate intake and a combination of carbohydrate and protein intake on endurance performance have been studied by many researchers with equivocal findings. In this study, local food products such as sago starch (containing 88% carbohydrate) and a soy–protein isolate (containing amino acids) has been used. The purpose of the current study was to examine the effects of sago, soy, and iso–caloric combination of sago+soy supplementations during 90 minutes steady–state cycling at 60 % VO_2max, on a subsequent 20–km cycling time trial performance in the heat (~31 °C; 70 % relative humidity). Twelve well–trained male cyclists (age: 19.0 ± 5.6 years, body weight: 60.1 ± 11.2 kg, height: 170.8 ± 7.6 cm, and VO_2max: 56.5 ± 6.5 $mL.kg^{-1}.min^{-1}$) representing Kelantan (state level) cycling team were recruited for this randomised single–blind placebo–controlled crossover study. Following preliminary trials, to investigate the VO_2max and to determine workload of the steady–state cycling, participants performed four experimental trials. The experimental trials consisted of a 90–minute steady–state pedalling at 60 % of VO_2max, followed by a 20–km cycling time trial. From the beginning of the experimental trials and at 20–minute intervals during the steady–state cycling (0, 20, 40, 60, and 80 minutes), participants consumed 200 mL of either: 7.5 % Sago, 7.5 % Soy, 6.0 % Sago + 1.5 % Soy, or placebo.

No significant differences were found in time–to–complete the 20–km time trial regardless of the drink ingested ($p > 0.05$). Metabolic responses including plasma concentrations of glucose and insulin were increased during the steady–state cycling in the sago and sago+soy trials compared with soy and placebo trials. At the end of the 20–km cycling time trial, the plasma lactate concentration was increased significantly in all trials ($p < 0.001$), and the plasma free fatty acids concentration was significantly higher in the

placebo and soy trials than the sago and sago+soy trials (p < 0.001). There were no significant differences between the trials in plasma concentrations of creatine kinase and F_2–Isoprostanes after the experimental trials (p > 0.05). The present study demonstrated that ingestion of sago, soy, and iso–caloric sago+soy combined supplementation did not improve the 20–km cycling time trial performance in the heat despite the availability of a higher level of plasma glucose and insulin. Hence, carbohydrate alone or a carbohydrate+protein combined supplementation may not be beneficial in exercise performance, specifically in the heat.

CHAPTER ONE

INTRODUCTION

1.1 Background

Endurance athletes consume supplements to have higher performance during sports events (Close *et al.*, 2016; Knapik *et al.*, 2015). They often try a different supplement to maximise their endurance capacity in order to compete to their fullest capability (Greenwood *et al.*, 2015). Such athletes intend to achieve their optimal performance during competitions by combining nutritional supplements with specific training. They require special dietary interventions and additional nutrients, as it is not easy to have the sufficient amount of macronutrients via daily food only. In addition to natural talent and appropriate training, proper diet has also been identified as the next most significant aspect. A diet plan undertaken by endurance athletes before or during a competition impacts their performance (Bompa and Buzzichelli, 2015). Therefore, prolonged exercise and supplementation are highly interrelated for endurance athletes (Maughan and Shirreffs, 2013). The effort to obtain a full physical performance or desired aesthetics has been present since ancient times. In the history of sports, nutrition has been thought of as an important factor in ancient games and former varieties of public spectacle (Katch *et al.*, 2012). In this regard, countless quests have continued to find more ways to train and eat accordingly.

The primary concern of nutrition is meeting up with energy demand in the body to maintain lean body mass, cardiovascular, and immune functions, and the most efficient muscular work. Every day between 60 to 75 % of the energy expended is allocated to basal metabolism (Poehlman, 1989). About 10 % of energy expenditure accounts for the digestive system that is known as the thermic effect of food (Bijlani and Manjunatha, 2010), but 15 to 30 % constitutes for physical activity (Poehlman, 1989). Energy expenditure depends on the level of physical activity as the activity is lesser in inactive people and higher in athletes who are extremely active (Torun, 2005). Furthermore, different kinds of food that an athlete consumes ultimately provide the energy necessary for them (Sherman and Lamb, 1988). At the beginning of a physical activity, muscle cells need greater energy due to the elevated contraction of muscles under conscious control. The substrates stored in active cells deliver this energy. However, if the exercise duration is prolonged, the energy required to maintain the exercise comes from other stored energy compounds (Katch *et al.*, 2012). Adenosine triphosphate (ATP) holds chemical energy from the breakdown of food contents in the body and supplies it to active cells to fuel some processes, such as to drive metabolic reactions in order to produce energy for physical activity.

The predominant substrates to synthesise ATP during physical activity are carbohydrate (CHO) and fat. CHO is stored as glycogen both within the muscle and the liver, and its role as an energy source in physical activity is well–known (Ivy, 2013). However, the CHO stores compared to that of the fat or protein (PRO) are limited in an athlete's body (Horton and Terjung, 1988). During exercise performance, when intensity increases, the reliance on muscle glycogen is enhanced in order to produce sufficient calories for active muscles. As the intensity of training is increased from 60 % to 80 % of VO_2max, all main substrates contribute toward ATP synthesis during high–performance exercise (Winter *et al.*, 2016). In this regard, the PRO oxidation does not contribute considerably to energy production (Coggan and Mendenhall, 2001). However, when glycogen levels during endurance exercise are low, PRO contributes as a source of fuel (Ivy, 2008). In addition, fatigue perception in endurance performance is related to the decline in the concentration of muscle glycogen (Manabe *et al.*, 2013). Coyle et al. (1986) studied the effect of CHO supplementation on endurance performance. They observed improvement during endurance cycling while the muscle glycogen was not reduced. The supplementation of CHO enhanced performance by maintaining blood glucose concentration and CHO oxidation, rather than sparing muscle glycogen.

1.2 Improvement in Endurance Performance

The ability to perform endurance activity depends on the maintenance of homoeostasis condition in the performance. Endurance activity causes a quick alteration from resting condition to constant activity, a decreased reliance on restricted muscle and liver glycogen supplies, and several thermoregulatory and cardiovascular changes to support and maintain the body's homoeostasis. There are few adaptations that have reported improvement in endurance performance. Some of these adaptations include increased proportion of slow twitch fibres, increased the number of mitochondria in muscle cells, greater ability for active muscles to use fat as a metabolite, and increased antioxidant capacity in the muscles (Hawley, 2009). Also, several reports have documented that nutritional supplements may enhance endurance performance in sports event and training bouts. Endurance exercise can benefit from a proper diet plan by preventing or reducing fluid loss, supplying extra fuel for muscular contraction, enhancing recovery (Ivy, 2008), reducing muscle damage (Clarkson and Hubal, 2002), and suppressing the immune system (Nieman *et al.*, 1990). However, ingestion and suitability of the supplied nutrient are as important as the ingestion timing. Thus, supplementing proper nutrients at a suitable period of physical activity can affect endurance performance and exercise adaptation.

The benefits of CHO supplementation during intermittent and continuous exercise has been reported previously (Ivy, 1999). The rate of decrease in muscle glycogen was reduced because of CHO ingestion during endurance cycling, and the performance was improved by 45 % as compared to placebo drinks (Hargreaves *et al.*, 1984). In another study, athletes were given CHO ingestion during one trial at the beginning and 20 minutes after the onset of exercise; and were given a placebo in another trial. The point of fatigue was defined as the time at which the exercise intensity the athletes could maintain declined below their work rate by 10 % of VO_2max. Fatigue was delayed by 17 % when athletes had CHO ingestion (Coyle *et al.*, 1983). The CHO consumption was shown to supply the blood glucose concentration to prevent hypoglycaemia and compensate for muscle glycogen depletion in continuous exercise.

1.3 Improvement in Endurance with Carbohydrate Supplementation

Endurance athletes should train based on the purposes of personal and the point in time within the training plans (preseason, in–season, and postseason) supplementation is considered. Their nutritional requirements should emphasise on a daily intake which focuses on a mixture of all nutritive substances. CHO is one of the major sources to supply additional fuel for energy production during extended physical activity. It is important that adequate amount of CHO is available for such activities due to enhanced rate of conversion of adenosine diphosphate (ADP) to ATP, which is an essential step for providing energy to active muscle cells. The relationship between endogenous CHO stores and endurance exercise is well–documented (Close *et al.*, 2016; Coyle, 1995; Temesi *et al.*, 2011; Tsintzas and Williams, 1998; Yen *et al.*, 2013). Some studies have shown the beneficial effects of CHO supplements, but others showed deterioration in endurance exercise with different protocols of training (Coletta *et al.*, 2013; Cramer *et al.*, 2015; Macdermid *et al.*, 2012; Stannard *et al.*, 2009). In a study by Macdermid et al. (2012) a 6 % CHO ingestion did not improve endurance performance in elite endurance cyclists. In addition, Piacentini et al. (2012) studied the effect of a 4–week low CHO ingestion on endurance exercise in elite athletes. This ingestion was based on the Zone diet (Tobias *et al.*, 2015), which altered the macronutrient ingredients (40 % CHO, 30 % PRO, and 30% fat) and controlled the PRO to CHO ratio in the range of 0.60 to 0.7. They concluded that their supplementation had no effect on endurance exercise.

A major amount of glycogen is being stored in the liver and skeletal muscles. The depletion of glycogen stores leads to fatigue during prolonged exercise, and influences training capacity. Intrinsically, it is beneficial to maintain or enhance CHO availability such as CHO consumption before, during, and after physical activity (Maughan and Shirreffs, 2016). Glycogen stores in the muscle cells are altered by diet and physical activity. The rate of glycogen breakdown and subsequent synthesis in the muscles is clearly related to its primary concentration in the skeletal muscle (Bergstrom and Hultman, 1966; Frentzel and Reach, 1901; Galbo *et al.*, 1979). It has been shown that muscle glycogen is an important factor for optimum endurance performance (Berning and Steen, 1998; Ehrman *et al.*, 2013). In contrast to the glycogen in the muscles, in the liver, glycogen reconverts into glucose, and is then released into the blood circulation system to deliver energy to tissues throughout the human body. Additionally, in the bloodstream and extracellular space, CHO is kept in a small amount, but the major source of CHO in the human body is the muscle glycogen storage (Close *et al.*, 2016; Ivy, 1999).

Fatigue is a consequence of alterations at the level of the active muscles, and athletes often experience an aversion to the limited form of human deficiency. This phenomenon is observed as a decrease in neural drive to the muscle, resulting in a declined in body's ability to provide enough energy during repeated muscle contractions (Amann and Calbet, 2008; Powers and Howley, 2012). Dehydration, hyperthermia, and CHO depletion induce fatigue by processes that can compromise neuromuscular, metabolic, cardiovascular, and thermoregulatory functions (Coggan and Coyle, 1987; Jeukendrup *et al.*, 1997). In fact, some of the training variables such as intensity and duration of exercise provoke fatigue. The sensations of fatigue and exhaustion in workout show that one has to decline the training workload or even stop the physical activity. Consuming energy during any physical activity decreases or depletes the energy sources in an athlete's body. Without replenishing the sources, the body will face deleterious effects. Thus, some nutrients, such CHO and PRO, could be supplied to active muscle cells to delay the early stages of fatigue, and to prevent the training cessations in endurance exercise. Replenishing glycogen perhaps increases the importance of its stores if endurance training is coupled with a diet plan involving inadequate dietary CHO to supply sufficient calories for the physical activity (Hawley, 2013; Ivy, 1991). In three successive days of endurance exercise with a poor diet plan of CHO, which contained 40 % of its calories of CHO, progressive reduction in resting muscle glycogen prior to exercise was observed (Costill *et al.*, 1971). The muscle glycogen content in endurance athletes significantly reduced by 30 to 36 % during one week of prolonged exercise. In contrast, CHO ingestion of 10 $g.kg^{-1}$ of body weight maintained muscle glycogen concentrations (Sherman *et al.*, 1993). Other studies have also reported similar effects of the improvement of endurance exercise by CHO supplementation (Jeukendrup, 2007; McCleave *et al.*, 2011; Meeusen, 2014; Pascoe *et al.*, 1990; Pottier *et al.*, 2010). CHO supplementations are able to decrease fatigue appearance in endurance exercise and improve the duration of training, so athletes can increase their endurance performance by glycogen loading and delay the onset of fatigue (Jeukendrup, 2007; Siegler *et al.*, 2013).

1.4 Improvement in Endurance with Carbohydrate and Protein Combination

Recent trends in CHO supplementation during exercise have led to a proliferation of studies that the addition of PRO to a CHO supplement has been reported to improve endurance performance (Coletta *et al.*, 2013; Espino-Gonzalez *et al.*, 2015; Ghosh *et al.*, 2010; McCleave *et al.*, 2011; Saunders *et al.*, 2004). It causes considerable adjustments to the flowing metabolites and the hormonal milieu like insulin hormone along with glycogen balance and response of muscle PRO during prolonged exercise (Volek, 2004). In a study, the effects of a CHO and a CHO–PRO ingestions on aerobic endurance performance were compared. Athletes pedalled at intensities that varied between 45 % and 75 % VO_2max for 180 minutes, and then at 85 % VO_2max up to fatigue. All supplements were given at every 20 minutes and consisted of a 7.75% CHO ingestion, a 7.75 % CHO + 1.94 % PRO ingestion, and a placebo. It was found that the addition of PRO to a CHO ingestion improved endurance performance above that which occurred with CHO ingestion (Ivy *et al.*, 2003).

In another study, cyclists ingested supplementation of 60 g of CHO, 52.4 g CHO + 15 g PRO, or placebo at 20–min intervals of steady–state during 60 min ride at 60 %

VO_2max followed by exhaustion pedalling at 90 % VO_2max (Ghosh *et al.*, 2010). Almost 84 % CHO–PRO trial was longer than placebo trial because of the added PRO to the consumption. Although, some evidence have supported this kind of supplement leading to performance improvement (Cermak and van Loon, 2013; Espino-Gonzalez *et al.*, 2015; Jeukendrup and McLaughlin, 2011). However, the improvements in endurance capacity by the CHO–PRO ingestion is not a well–established fact. It has been speculated that improved endurance performance with CHO–PRO feeding may be because of enhanced PRO oxidation when muscle glycogen is depleted (Koopman *et al.*, 2005), or due to decreases in central fatigue (Blomstrand *et al.*, 1991). The findings are controversial and none of these reasons appears plausible. Hence, the evidence is lacking (Betts *et al.*, 2009; Breen *et al.*, 2010; Green *et al.*, 2008), and more study is necessary to investigate the effects of CHO–PRO ingestion on exercise.

A number of studies have shown no difference in performance when a CHO–PRO supplementation or a CHO alone was consumed (Jeukendrup *et al.*, 2008; Madsen *et al.*, 1996; van Essen and Gibala, 2006). In some studies, the ingestions prepared in their protocols were not iso–caloric, because the CHO–PRO ingestion provides additional energy than CHO ingestion alone (Espino-Gonzalez *et al.*, 2015). However, other studies have shown a greater insulin response with a CHO–PRO drink as compared with CHO drink (Ghosh *et al.*, 2010; Niles *et al.*, 2001; Volek *et al.*, 2016; Williams *et al.*, 2003). Therefore, some investigators have provided an iso–caloric supplement such that the calorie content of these types of drinks was matched. These studies reported that an iso–caloric CHO–PRO supplement did not improve endurance exercise (McLellan *et al.*, 2014; Richardson *et al.*, 2012; Romano-Ely *et al.*, 2006). A few of these studies suggested that CHO–PRO supplement can delay gastric emptying (Murray *et al.*, 1999), or higher insulinaemic responses that inhibit lipolysis leading to a greater reliance on muscle glycogen as well as performance deterioration exercise.

1.5 Endurance Exercise in Hot and Humid Conditions

Environmental situations, especially in extended duration and in spite of endurance competition are able to alter physiological mechanisms of heat loss in thermal stress and help to cause heat stress symptoms such as electrolyte imbalance, exertional heat–related illnesses, and dehydration (McDonagh and Zideman, 2015). Exercising in a hot and humid environment can affect thermoregulatory, circulatory, cardiovascular, and endocrine systems (Hargreaves, 2008). To keep up with the most desirable rhythm of physical activity, many interrelated physiological processes work side by side, such as regulating fluid volume, cooling the body, and sustaining blood pressure (Casa, 1999). However, the primary consequence of dehydration and minor exertional heat illness is fatigue, which leads to performance impairment (Nybo *et al.*, 2014). Furthermore, it appears that heat–induced fatigue is not caused by a single reason, but possibly due to a combination of events in the metabolic processes. Some of the important reasons for muscle fatigue under heated condition are fluid balance impairment, muscle metabolism alteration, and a decrease in cardiovascular function (King *et al.*, 2016; Powers and Howley, 2014).

Dehydration (Powers and Howley, 2014), hyperthermia (Maughan and Meyer, 2013), and reduced mental function (Lee *et al.*, 2014c) are the consequences of continuous exercise in the heat. Dehydration and depletion of muscle glycogen sources are two main factors which may influence the capacity of endurance activities (Bergstrom *et al.*, 1967; Von Duvillard *et al.*, 2004). Athletes can improve their performance by adding CHO to the drinks during exercise (Carter *et al.*, 2003; Cherif *et al.*, 2016). On the other hand, there is a successful key for endurance athletes to train in a hot and humid environment when trying to maintain the hydration status (Rothenberg and Panagos, 2008). CHO feeding during a 40–km run in the heat (32 °C) elevated blood glucose concentration compared to placebo (Millard-Stafford *et al.*, 1992). There was a significant improvement in endurance exercise once athletes consumed 4 to 6 % CHO supplements in cool condition, while, in warm condition (30 °C) only 6 % CHO supplement improved the performance (Watson *et al.*, 2012). In addition, a 6 % CHO supplement is a proper drink selection for anyone who is participating in endurance exercise in the heat (30 °C), in terms of combining perceptual and exercise benefits (Corbett *et al.*, 2015). As such, it is assumed that CHO needs are enhanced when exercising in the heat due to the change in substrate utilisation to CHO oxidation (Jentjens *et al.*, 2003). It indicates that the CHO requirements for endurance athletes increase in the heat. This proposal of increased CHO oxidation is principally due to the breaking down of glycogen in active muscles. Furthermore, blood flow redistribution to the skin and muscles, with a decrease in blood flow to the intestines could be another reason for that reduction. This blood redistribution would likely adversely change CHO absorption. However, the combination of multiple CHOs is able to overcome this problem, and a high rate of exogenous CHO oxidation can be achieved even in the heat (Jentjens *et al.*, 2002).

1.6 Exercise–Induced Oxidative Stress and Muscle Fatigue and Damage

Numerous benefits of a moderate level of physical activity are well known. But, the recent growth in evidence on the relationship between injuries and exercise has revealed that exercise is likely to produce an adverse effect on the body. There is also an additional amount of free radicals and reactive oxygen species (ROS) production in the athletes' body (Jeukendrup and Gleeson, 2009). It has been shown that ROS are associated with muscle damage during exercise; also the process of many chronic diseases, including some cancers, is initiated by free radicals (Davies *et al.*, 1982; Fang *et al.*, 2002). Free radicals exist as reactive molecules that hold one or more unpaired electrons (Chen, 2015). These electrons are not stable, and can oxidise certain cell components constituting nucleic acids, PROs, and lipids. Excessive production of ROS causes oxidative stress (Quindry *et al.*, 2013; Ulrich *et al.*, 2012), which has been involved in oxidative damage of macromolecules (Veskoukis *et al.*, 2008), or muscle damage (Nikolaidis *et al.*, 2007). Still, such conditions could remain for few days after the activities (Maruhashi *et al.*, 2007; Nikolaidis *et al.*, 2008).

Oxidative stress is the result of the imbalanced production of free radicals and ROS, and antioxidant defense in the body (Hagen, 2003), so it is defined as a disorder ta the level of prooxidant/antioxidant systems in the human cells (Sies, 1991). Mechanical and metabolic stresses during physical activity increase oxygen consumption in active muscles up to two hundred times higher than at resting situations. The production of superoxide anion in the mitochondria of the muscle cells increases the respiration and energy production during exercise. This causes an increased rate of ROS generation and induced lipid peroxidation and oxidative adaptations of PRO and deoxyribonucleic acid (Radak, 2000). Muscle exposure of muscle to free radicals can lead to fatigue development and reduced muscle contractions. It has been shown that free radicals accelerate muscular fatigue during physical activity (Barclay and Hansel, 1991). Also, in a situation such as oxidative stress, increased ROS concentration is associated with fatigue in muscle contraction and in post–exercise muscle damage (Pasiakos *et al.*, 2014). During endurance exercise the production of ROS results in muscular fatigue, and it has been suggested that oxidative stress contributes to muscular fatigue (McKenna *et al.*, 2006).

A vast number of antioxidant supplements have been reported to possess powerful antioxidative capabilities and preserve the normal condition of cells from oxidation (Taylor, 2012). On the other hand, some findings have shown that ingesting high dosage of these supplements may hamper endurance exercise and its capacities, and the qualified nutritionisted must recommend the usage (Ristow *et al.*, 2009). In this respect, macronutrients have the same protective effect against oxidation during exercise if athletes ingest them as a supplement (Etheridge *et al.*, 2008). Some supplements similar to CHO and PRO ingestions are able to reduce the decrease in plasma glucose concentration, and release some hormones, such as cortisol and catecholamines, into the blood flow during endurance exercise (Niemen, 1998). This leads to greater production of contractile PROs (Phillips *et al.*, 1997). Other investigations have, however, reported different findings regarding such effects on stress. In comparison to a placebo, CHO supplementation provides no advantage for improving exercise–induced muscle damage following an endurance cycling performance (Valentine *et al.*, 2008), or a downhill treadmill running (Green *et al.*, 2008).

CHO–PRO supplementation has been observed to enhance PRO production and prevent an increased rate of PRO reduction following exercise (Bird *et al.*, 2006; Tang *et al.*, 2007). Thus, enhancing PRO turnover after an acute muscle damage using CHO–PRO supplementation shows a strong stimulatory effect to improve muscle repair (Phillips *et al.*, 1997), and leads to the reduction of exercise–induced muscle damage (Mastorakos and Pavlatou, 2005; Rowlands *et al.*, 2008), whereas other results showed no effect following CHO–PRO supplementations (Betts *et al.*, 2009).

1.7 Local Foods and Supplementation for Exercise

Some coaches have aimed to make a supplement that maximises athletic performance and seeks to increase knowledge and awareness through encouraging local ingestions along with training. Physical activity in addition to healthy eating habit are inseparable partners to prevent disease, decrease stress, and improve performance (Farooqui and Farooqui, 2015). This natural and healthy choice is usually less processed than imported diets. The use of local daily food as supplements over expensive drinks is cost effective. Studies have shown that locally–sourced and organic foods have more nutrients, including CHO (Ghosh *et al.*, 2010; Quindry *et al.*, 2008), PRO (Ghosh and Jusoh, 2010), and antioxidants (Batra *et al.*, 2014; Yavari *et al.*, 2015). Also, few studies have investigated the effect of different types of locally–sourced food on the body (Ahmad *et al.*, 2005; Dunne *et al.*, 2011; Ghosh and Jusoh, 2010; Ghosh *et al.*, 2010; Ghosh *et al.*, 2008). Based on the results of these studies, those who have allergies to food and its preservatives often find their symptoms declining or disappearing altogether when they consume locally–sourced and organic foodstuffs (Johnson and Endres, 2012). Previous researchers, who have combined CHO and PRO in a supplement have seen improvement in endurance performance (Ghosh *et al.*, 2010; Ivy *et al.*, 2003; Šatalić, 2016). Also, the effect of iso–caloric CHO–PRO supplementation on endurance performance has been investigated in several studies (Calders *et al.*, 1997; Ferguson-Stegall *et al.*, 2010; Ghosh *et al.*, 2010; Holliday and Blannin, 2014; Saunders *et al.*, 2004; Saunders *et al.*, 2007; Saunders *et al.*, 2009; Volek *et al.*, 2016). Commercially available supplementations are designed as extra energy to be used during exercise while others are consumed after the exercise to help recovery and improve subsequent training (Romano-Ely *et al.*, 2006).

1.8 Sago and Soy Supplementations for Exercise

Previously in our laboratory at Sports Science Unit, some investigators studied the effects of sago (Ahmad *et al.*, 2005; Ahmad *et al.*, 2006; Ahmad *et al.*, 2009; Ghosh *et al.*, 2008), or combined sago+soy supplementations (Faizal *et al.*, 2012; Ghosh *et al.*, 2010; Rahaman *et al.*, 2006) during exercise. The current study is a continuation of the earlier studies done in our laboratory concerning the effects of sago and soy supplementations on endurance exercise in the heat (~31 °C; 70 % relative humidity). Agro based locally available CHO and PROs are sago and soy PRO. One of the local sources of CHO in the tropical countries of Southeast Asia is sago starch. In Malaysia and India, sago is widely used to make local dishes and biscuit (Ahmad *et al.*, 2009; Ghosh *et al.*, 2010). But in other cultures, instead of potato or rice sago is used as a principal diet. The people in Malaysia consume sago together with potato, rice, and corn and in the manufacturing of pasta (Kueh *et al.*, 1991).

Soy is one of the commercially available local sources of PRO in a diet plan. Many athletes have added soy to their supplementations due to the evidence that soy may offer health and performance benefits (Ghosh and Jusoh, 2010). While sago holds 88 % of CHOs in the form of nutritional polysaccharides of amylose and amylopectin, and the soy PRO is a combination of many essential amino acids such as leucine, isoleucine, and valine. These specific sago and soy supplementations are able to be patterned in the laboratory with the expectation of indicating positive outcomes in endurance exercise in the heat. Additionally, the resources of the basic ingredient like sago and soy PRO are easily available at a low cost in a local market. For that reason, it can be potentially commercialised and very suitable to be developed as a new sports drink for endurance training in the heat. In all the studies, the CHO–PRO supplementation had higher calorie than the supplementation of CHO, resulting in an improvement in endurance performance. Moreover, literature on the studies on CHO–PRO combined supplementation in the heat on endurance performance are scanty. Additionally, it is postulated whether an iso–caloric CHO–PRO combined supplementation using a locally available source can improve endurance performance or not. Since, hot and humid environment deteriorate the endurance performance, a definitive investigation is required on supplementation of iso–caloric CHO–PRO combination during prolonged exercise in the heat.

1.9 Objectives of the Study

Hence, the present study was undertaken to:

1. investigate the effects of sago (daily used local food containing mostly CHO), soy (daily used local food containing PRO), and iso–caloric sago+soy combined (CHO and PRO combined supplementation) supplementation during a 90–min endurance cycling at 60 % of VO_2max on cycling time trial, speed, and cadence in the heat, as compared to placebo.

2. observe the effects of sago (daily used local food containing mostly CHO), soy (daily used local food containing PRO), and iso–caloric sago+soy combined (CHO and PRO combined supplementation) supplementation during a 90–min endurance cycling at 60 % of VO_2max, on plasma concentrations of glucose, insulin, free fatty acids, and lactate as the metabolic variables during and after 20–km cycling time trial performance in the heat, as compared to placebo.

3. study the effects of sago, soy and iso–caloric sago+soy combined ingestion as local supplementations on plasma concentration of F_2-Isoprostanes as an

oxidative stress indicator following endurance cycling performance in the heat, as compared to placebo.

4. investigate the effects of sago, soy and iso–caloric sago+soy combined ingestion as local supplementations on plasma concentrations of creatine kinase as a muscle damage indicator following endurance cycling performance in the heat, as compared to placebo.

1.10 Hypotheses of the Study

With respect to the objectives of this study the following hypotheses to be evaluated are:

1. H_O: There is no significant difference in 20–km cycling time trial performance following sago, soy, iso–caloric sago+soy combined supplementations as compared to the placebo.

2. H_O: There is no significant difference in cycling speed following sago, soy, iso–caloric sago+soy combined supplementations as compared to the placebo.

3. H_O: There is no significant difference in cycling cadence following sago, soy, iso–caloric sago+soy combined supplementations as compared to the placebo.

4. H_O: There is no significant difference in plasma glucose concentration following sago, soy, iso–caloric sago+soy combined supplementations as compared to the placebo.

5. H_O: There is no significant difference in plasma insulin concentration following sago, soy, iso–caloric sago+soy combined supplementations as compared to the placebo.

6. H_O: There is no significant difference in plasma free fatty acids concentration following sago, soy, iso–caloric sago+soy combined supplementations as compared to the placebo.

7. H_O: There is no significant difference in plasma lactate concentration following sago, soy, iso–caloric sago+soy combined supplementations as compared to the placebo.

8. H_O: There is no significant difference in plasma creatine kinase concentration following sago, soy, iso–caloric sago+soy combined supplementations as compared to the placebo.

9. H_O: There is no significant difference in plasma F_2–Isoprostanes concentration following sago, soy, iso–caloric sago+soy combined supplementations as compared to the placebo.

1.11 Significance of the Study

The aim of this study was to determine the effects of sago, soy and iso–caloric sago+soy combined supplementations during 90 minutes steady–state cycling on 20–km time trial performance in the heat and to investigate the physiological responses to the exercise. This work follows the previous studies to examine the effects of iso–caloric sago and soy supplementations on exercise which is the first attempt in a hot and humid environment (~31 °C; 70 % relative humidity). This study will provide a new evidence if a local and daily use diet, sago and soy iso–caloric supplementations are able to improve endurance in cycling performance in the heat, especially by delaying the onset of fatigue, decreasing muscle damage or by reducing oxidative stress. Therefore, this iso–caloric ingestion will be beneficial in enhancing endurance performance and recovery of cyclists in the heat. Finally, the sago and soy iso–caloric supplements may be recommended to endurance cyclists who are competing in a hot and humid condition.

1.12 Operational Definitions

For the purpose of this study, the important terms that are involved in this study are:

1. Sago Supplementation – This test food is a supplement containing sago flour as the main ingredient. The chocolate flavour is added to improve palatability.

2. Soy Supplementation – This test food is a supplement containing soy flour as the main ingredient. The chocolate flavour is added to improve palatability.

3. Combined Sago+Soy Supplementation – This test food is a supplement containing sago and soy flours as the main ingredients. The chocolate flavour is added to improve palatability.

4. Placebo – This drink is an ingestion containing distilled water without any ingredient. The chocolate flavour is added to prevent participants' attention to the changes in the type of supplementation.

5. Iso–caloric Supplementations – These drinks have the same amount of calories. The energy content of the sago, soy, and sago+soy supplements are matched.

6. Cycling Time Trial – Pedalling at high intensity in the laboratory on a race bike which involves performing a set of 20–km distance in the shortest time possible.

7. Performance in the Heat – Cycling in a heat chamber, which is maintained in an ambient environment of 31 °C and 70 % relative humidity.

8. Metabolic Variables – The plasma variables relating to metabolism to synthesise energy by biochemical processes, such as glucose, insulin, lactate, and free fatty acids.

9. Oxidative Stress – A condition caused by an imbalanced production of reactive oxygen, an imbalance between pro–oxidants and antioxidant mechanisms.

10. Free Radical – Any species capable of individual existence containing one or more unpaired electrons.

11. Muscle Damage – A type of an injury in human muscles which frequently occurs after unfamiliar training, specifically if the exercise involves many eccentric

contractions. It leads to the release of muscle PROs into the blood, delayed onset muscle soreness, spasms, force loss, and inflammation.

12. Creatine Kinase – An energy dependent enzyme, which catalyses the conversion of creatine to phosphocreatine. The blood level of creatine kinase is widely used as a marker to reflect muscle breakdown.

CHAPTER TWO

LITERATURE REVIEW

2.1 An Overview of Sports Nutrition

2.1.1 History

In recent years, most athletes from different sports have improved their skills and performance levels that have resulted from setting recent records during competitions (Knudson, 2013). Both coaches and athletes are seeking to achieve victory through several ways and methods, and one of these methods is by ingestion of ergogenic aids and consumption of adequate nutritious substances (Close *et al.*, 2016). The modern athletic period may be considered to have started in the 19th century. The physiology of the body was little understood, and the science of nutrition had not been born. The french cyclist François Faber, winner of the Tour de France winner in 1909, consumed six beefsteaks before the race (Mignot, 2016). However, at that time, the role of macronutrients as the primary fuel such as CHO for endurance exercise was unknown. Today, the ingestion of supplements in sports to benefit athletic performance is among the latest research achievements. The second half of the 20th century has brought a new scientific era titled 'Sports Nutrition' (Katch *et al.*, 1997). Many professional and amateur athletes have attempted to reap their benefits, so they have started to consume supplements during training sessions in their detailed dietary planning patterns, notably in sports competitions.

The importance of nutrition for athletes to optimise performance is well established (Close *et al.*, 2016; Williams and Rollo, 2015). Historically, the diet of earlier humans seemed to be rich in terms of quality that helped them to travel and migrate long distances through the mountains and deserts. Herodotus quoted in his inscription, referring to an epigraph of the Pyramids, which indicated the amount and type of foods needed for labours (Morton, 2006). However, Hippocrates's statement, "let food be thy the medicine and medicine be thy the food", has preoccupied scientists from Minoan, Assyria, Babylonia, Persia, Media and Mycenaean cultural backgrounds (Mayer and Bhikha, 2014). At that time, enough diet was recommended for maintaining the wellness of organs' function. Five centuries following Hippocrates, the ancient Greek physician named Claudius Galenus or Galen made specific recommendations in relation to health, nutrition and physical activity. His beliefs could be assigned to the fundamentals of applied exercise physiology, such as the seven "laws of health", which revealed the treatment of a healthy life by feeding on proper foods, consuming the right beverages, breathing fresh air, getting sufficient sleep, obtaining a daily bowel motion, self–control, and exercising (Katch *et al.*, 2010).

Athletes who were competing in the ancient Olympic Games used to plan their diet by benefiting from experiences of their coaches and previous successful athletes (Swaddling, 1999). For example, they ingested dried figs, moist cheese and wheat for sports events. Some athletes changed their diet pattern to rich–PRO diets, such as meat which was believed to improve strength and overall fitness (Grandjean, 1997; Grivetti and Applegate, 1997). In addition, they took these pieces of advice for the next 15 centuries. Nutrition and dieting were one of the most interesting topics in the 19th century with physical activity as a sub–branch of medical science (Katch *et al.*, 2010). It was then developed and the science of physiology was created by medical practitioners for the athletes, and exercise physiology has been established since then (Katch *et al.*, 2010).

In this regard, the science of nutrition was used as a concept in exercise physiology. One of the laboratories that pioneered nutrition experiments and energy metabolism was founded in 1904 at Carnegie Institute in Washington, DC, USA, in order to help exercise physiologists. A German physiologist, Nathan Zuntz who was the inventor of the early treadmill in 1889 used for studying physical activity, showed that fat and CHO could supply energy as metabolic fuels. He also created a portable metabolism device in cooperation with August Julius Geppert, which they named the Zuntz–Geppert respiratory apparatus. This device was used to provide the respiratory exchange ratio. He observed that if a fat–rich feeding was consumed before exercise, a low–intensity performance could be done by a small contribution of CHO metabolism. Also, the winner of the Danish Nobel prize in medicine, August Krogh, showed one of the most important physiological aspects in exercise. He proved that in resting conditions and physical activity, nutritional substrates of CHO and fat play an important part in energy production by increasing muscular work capacity (August and Johannes, 1920).

At Harvard Medical School, a study was conducted by a group of scientists to find out the CHO concentration after the 1924 Boston Marathon (Gordon *et al.*, 1925; Levine *et al.*, 1924). The participants of their study who crossed the finish line indicated a low glucose concentration of lower than 2.8 $mmol.L^{-1}$ in their blood (Levine *et al.*, 1924). In another study, participants ingested a rich–CHO content one day before the race, and sugar candy samples were taken by runners during the marathon race (Gordon *et al.*, 1925). In addition, tea, which included a large amount of CHO, was available at the stations along the race. It was reported that these patterns of ingestions did not cause hypoglycaemia or any other problems similar to the previous study results. CHO metabolism was reported to be associated with the physical activity workload (Marsh and Murlin, 1928), and it was proven that during the higher workloads, the CHO metabolism was greater than the lower workloads. Bøje revealed that ingesting CHO solutions improve exercise capacity in exhausted participants (Bøje, 1936). It obviously demonstrated the significance of CHO consumption in exercise performance (Christensen and Hansen, 1939a; Christensen and Hansen, 1939b).

Since the second half of the last century, these results have demonstrated that CHO is one of the important factors during physical activity. During the 1960s, by utilizing developed medical techniques, such as muscle biopsy or isotopic traces, a Swedish team tried these new processes of investigations to discover the medical changes across muscle tissues invasively. In 1966, Bergstrom and Hultman demonstrated that in an active leg, the glycogen content was less than a rested one (Bergstrom and Hultman, 1966). They also stated that fatigue happened in endurance exercise when muscle glycogen declined by pedalling time–to–exhaustion at 75 % VO_2max (Bergstrom *et al.*, 1967). Ahlborg (1967) indicated that starting the exercise with a high content of muscle glycogen leads to increased endurance capacity (Maughan, 1991b). One of the most important organs in the human body that play an active function in regulating glucose is the liver. Another study conducted in the late 1970s revealed a significant finding, that the liver secretes glycogen into the bloodstream (Coyle and Montain, 1992b; Montain and Coyle, 1993). This new outcome showed the consequences of heavy exercise on the liver and it was similar to glycogen depletion both in the muscle and liver. Numerous studies have confirmed the relationship between fatigue and glycogen depletion in prolonged, strenuous activity (Maughan, 1991b) as evidence for coaches and athletes. Consequently, the importance of CHOs as a supplement to support the physiological capacity during training was recognized.

Therefore, foods containing CHO like rice, grains, and potatoes were chosen as a major portion of a diet to provide beneficial strength during the exercise. This action led to modern considerations about uses of CHOs. It has been used as a dietary supplement for athletes, or development of beliefs such as CHO loading, which is a classic plan for athletes to consume CHOs during competitions. The University of Florida was one of the academic institutes that used CHO ingestion by manipulating the CHO content mixture in water for sports teams (Martin, 2007).

2.1.2 Principles of Sports Nutrition

There are substances that supply essential nourishment for maintenance of life and growth in the body. These nutrients are divided into two groups, which are micronutrients and macronutrients (Powers and Howley, 2012). The micronutrients refer to nutritive substances required in tiny amounts to regulate metabolic processes effectively. They include vitamins and minerals which are mainly used for tissue synthesis and facilitation of energy transfer systems (Katch *et al.*, 2010). The human body needs only 350 g of micronutrients out of 862 kg of nutritional habits yearly (Hue, 2012). Since then, if athletes follow a proper diet of food sources, they are not required to take vitamins and/or mineral supplementations (Greenwood *et al.*, 2015). To maintain an organism's functional integrity and structure, a regimented eating plan gives macronutrients to the body, and they also contribute as biological fuels for both conditions of resting and physical activity.

There are six broad classifications of nutrients including CHOs, PROs, lipids, vitamins, minerals, and water (Campbell, 2013). Dietary macronutrients provide lipids or fats to the human body in order to be exploited in steroid hormone synthesis, cell membrane structure, fat–soluble vitamins absorption, vital organs protection, and insulation (Padilla *et al.*, 2000). In this regard, fats are a major storage energy substrate of the human body (Rauch *et al.*, 1999). It is considered that the acceptable fat intake ranges between 20 % to 35 % of total energy intake in adults, while it is recommended for moderate and highly active individuals to consume 30 % and 35 % respectively (Elmadfa and Kornsteiner, 2009). The body receives fat from foods in the forms of phospholipids, triglycerides, and cholesterol. Almost 98 % of dietary fats exist as triacylglycerol (Damodaran and Arora, 2013). Just about 90 % of the total fat is placed as a storage in the adipose tissue situated under the skin; based on the gender body–fat percentage is 15 to 25 % of body mass for young men and 25 to 28 % for young women (Jeukendrup and Gleeson, 2009). A perfect cellular fuels are fats which contain nine kcal of energy in one gram of unadulterated fat (Carter *et al.*, 2004). The amount of energy with more than twice the energy available in an equal amount of other macronutrients is in lipid molecules due to the higher quantity of hydrogen which is to be oxidised. For a young adult man with 80 kg in body weight, the potential energy stored in fats is about 110,700 kcal, and if he is a well–nourished person, lipolysis provides nearly 80 to 90 % of the energy requirement for bodily functions at rest conditions (Katch *et al.*, 2010).

In adipose tissues, triglycerides are broken down to shape fatty acids and glycerol, and fatty acids can be converted into triglycerides by reesterification functioning within a cell or they are even delivered into the blood stream in the form of free fatty acids (Hue, 2012). In fat cells, glycerol cannot be resynthesised as part of triglycerides by reincorporation due to the absence of some specific enzymes and existing glycerols in the blood circulation that indicate the rate of lipolysis (Fair, 2010). Free fatty acids are not soluble in water when entering the bloodstream. In fact, they are bound to a water–soluble PRO called albumin. Following that, free fatty acids are either reesterified mostly in the liver or oxidised (Katch *et al.*, 2010).

Similar to coupling glucose subunits to form glycogen, PROs are attained from amino acids' linkage as building blocks. Sequencing of amino acids in a PRO structure can alter the biochemical functions and properties of each PRO in cells that contain approximately 55000 varieties of PRO compounds. The body of a typical healthy adult constitutes almost 12 to 15 kg of PROs mainly located in the muscle mass. So, the normal amount of energy in the recommended food for this person should contain about 10 to 15 % of energy in the form of PRO. It means that adults should consume a daily of 0.8 g of their body weight (Kenney *et al.*, 2015).

Although energy is one of the most important elements in physical activity, there are some other issues due to the physiological adaptations to exercise when an athlete is training under a higher temperature (Coso *et al.*, 2008). So, water serves as an important role for rehydration when the body absorbs considerable heat via slight temperature changes in the environment. Consuming water on a regular basis is required to maintain a well–hydrated condition (Ivy, 2008). It is also required for the form and structure of the cells in the human body. The water of comprises a considerable great extent of total body mass, about 40 to 70 % (Katch *et al.*, 2010), and lean body tissues hold a constant fraction of water by mass, and constitute almost 65 to 75 % of muscle weight (Ehrman *et al.*, 2013). The adipose tissue contains a small amount of water, and it depends on gender, age, and body composition. The total body water in a healthy young male with 70 kg body weight is almost 42 litres (Maughan and Burke, 2008), and his body needs about 2.5 litres of water every day in thermoneutral conditions. Water is provided by three sources of foods, liquids, and metabolic processes in the body, and it is lost through the skin, urine, water vapour in expired air and excrement (Ivy, 2008).

As the metabolic rate increases during physical training, the body temperature rises, too. This common situation must be controlled by ingesting sufficient fluids (Maughan and Meyer, 2013). The amount of energy to apply exercise to is almost 25 % of energy utilisation, with the balance being scattered as heat, mostly through the skin (Plowman and Smith, 2013). For athletes who are training at a significant time, especially in warm weather conditions, body temperature is maintained through fluid loss by sweating which can restrain dehydration, depending on garments, exercise status, body size, and weather.

To date, several studies have investigated the relationship between physical activity and nutrition (Espino-Gonzalez *et al.*, 2015). Hence, it is clear that athletes need to consume appropriate nutrients before, during, and after endurance exercise (Birrer *et al.*, 2016). But, some of them do not follow nutritional principles due to insufficient support, and many of them cannot identify with the right diet (Torres-McGehee *et al.*, 2012). Thus, dietary habits and nutritional schemes that are planned in a correct approach are important, in which coaches and athletes have to be educated. These efforts are effective in providing the methods to recognize proper foods and applicable means to obtain the most efficient diets (Morente-Sánchez and Zabala, 2013).

2.2 Energy Sources for Skeletal Muscles During Prolonged Exercise

Many chemical reactions appear at any point of the human body during every single moment of life, and as a group, these responses are called metabolism (Gropper and Smith, 2012). Metabolism includes chemical pathways which cause synthesis of molecules, termed anabolic reactions, and likewise, the breakdown of molecules, termed catabolic reactions. All living organisms require energy to survive and the human body extracts energy from nutrients, whether from fresh greenery or steak. Nutrients are able to supply energy to the