The Physiological and Performance Effects of Honey Consumption in Sport and Exercise: A Systematic Review

by

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A Thesis

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Declaration

The candidate confirms that the work submitted is his/her/their own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Hills, S.P., Mitchell. P., Wells, C. and Russell, M. 2019. Honey supplementation and exercise: a systematic review. *Nutrients*, **11**(7), E1586, pp.1-22.

> Hills, S.P., lead author and researcher on the publication. Wells. C. and Russell. M., were lead supervisors and also responsible for authoring and editing the manuscript.
>
> Personally responsible for data collection and analysis conducted to validate lead authors contributions. Additionally, responsibilities included checking for errors and amending changes to manuscript prior to publication.

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Abstract

Introduction: Honey is a naturally occurring foodstuff composed from several mono- and di-saccharides, but predominantly, fructose (~35-40 %) and glucose (~30-35 %) together with other constituents such as water, polyphenols, vitamins and minerals. Research has identified ergogenic effects on exercise performance when such constituents have been consumed independently. Therefore, the rationale exists for a functional food containing a myriad of potentially ergogenic components to improve exercise performance. Accordingly, this review aimed to assimilate the current evidence pertaining to the performance and/or physiological responses to honey supplementation in sports and exercise settings.

Methods: In accordance with PRISMA guidelines, database searches (MEDLINE, PubMed, SPORTDiscus) were conducted to locate relevant research publications. Articles were initially scanned (titles, abstract), and then 24 full texts screened in agreement with pre-defined inclusion criteria. Having excluded three records following a quality assessment, 11 studies were selected for the final review.

Results: Five of the 11 studies assessed physical performance and/or physiological outcomes concomitantly across a single exercise session, while six investigated physiological responses of chronic honey supplementation over several weeks. Honey was as equally effective as high glycaemic index carbohydrates regarding the time to complete a simulated stage of the Tour de France (128.8 ± 3.5 vs. 128.3 ± 3.8 min respectively). Similar patterns have been observed in soccer, whilst running distances improved versus water alone (3420 ± 350 vs. 3120 ± 340 m). Honey ingested chronically from 20 $g.d^{-1}$, or 70 g ingested ~ 90 min before exercise, attenuated oxidative biomarkers (i.e., lipid peroxidation; 2.9 ± 0.9 vs. 4.7 ± 0.8 $nmol \cdot mL^{-1}$), while pro-inflammatory interleukin cytokines (Tumour Necrosis Factor -TNF-α, IL-1β) and reduced DNA damage have been identified at several time points versus no supplementation. Increases in T-lymphocyte cells (+16.2 % vs. pre-test), antioxidant enzymes (catalase, superoxide dismutase) and total antioxidant capacity were also evident.

Discussion and Conclusion: The evidence suggests favourable improvements to immunological biomarkers and indices of exercise induced oxidative stress following chronic honey ingestion. Similarly, acute honey supplementation demonstrated efficacy in maintaining physical performance comparable to high glycaemic index carbohydrates. Research opportunities exist to further investigate the physical performance and physiological effects of honey ingestion in the sports and exercise sciences. Researchers may wish to consider homogeneity in terms of the carbohydrate and exercise stimuli examined.

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Abbreviations

Abbreviations	Meaning
CD4	Immune White Blood Cells
CD8	Lymphocyte
DNA	Deoxyribonucleic acid
g	Grams
$g \cdot h^{-1}$	Grams per Hour
$g \cdot kg \cdot h^{-1}$	Grams per Kilogram per Hour
$g \cdot min^{-1}$	Grams per Minute
GI	Glycaemic Index
GLUT5	Glucose Transporter Five
HR	Heart Rate
HSP-70	Heat Shock Protein 70
IL-1	Pro-inflammatory Interleukin Cytokine 1
IL-1 β	Pro-Inflammatory Interleukin Cytokine 1 β
IL-1ra	Anti-Inflammatory IL-1 Receptor Antagonist
IL-6	Pro-Inflammatory Interleukin Cytokine 6
IL-10	Anti-Inflammatory Cytokine
$km \cdot wk^{-1}$	Kilometres per Week
m	Metres
MDA	Malondialdehyde
MeSH	Medical Subject Heading
$mmol \cdot L^{-1}$	Millimoles per Litre
$nmol \cdot mL^{-1}$	Nanomoles per Millilitre
ml	Millilitres
$ml \cdot d^{-1}$	Millilitres per Day
$mOsm \cdot L^{-1}$	Measure of Osmolarity
MTC	Multiple Transportable Carbohydrate
NCAA	National Collegiate Athletic Association
PEDro	Physiotherapy Evidence Database Scale
$pg \cdot mL^{-1}$	Picograms per Millilitre
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Abbreviations cont.

Abbreviations	Meaning
ROS	Reactive Oxygen Species
RPE	Rating of Perceived Exertion
SGLT1	Sodium Dependent Glucose Transporter
SOD	Superoxide Dismutase
TAC	Total Antioxidant Capacity
TNF-α	Tumour Necrosis Factor
TT	Time-Trial
$\dot{V}O_{2max}$	Maximal Oxygen Uptake

1. INTRODUCTION

According to The Council of the European Union, honey is a naturally occurring sweet substance produced by the Apis mellifera honeybee (Council Directive 2014/63/EU). The bees collect nectar from plants (e.g., blossom honey), or living plant secretions, or excretions of insects feeding on living plants (e.g., honeydew honey), which is then combined with specific substances and stored in honeycombs to dehydrate and mature (Council Directive 2001/110/EC). As a foodstuff, honey consists of carbohydrate (~80 %), composed predominantly of the monosaccharides fructose (~39 %) and glucose (~31 %), oligosaccharides and disaccharides (10 %), and 14-18 % water (EFSA, 2010). Additionally, honey is known to contain up to 200 different components including amino acids and enzymes (0.5 %), vitamins, minerals and antioxidants (e.g., phenolic and flavonoid compounds), which may independently elicit positive effects on metabolic, oxidative and immunological physiology (Patzhold and Bruckner, 2006; Dimitrova et al., 2007; Alvarez-Suarez et al., 2010; Eteraf-Oskouei and Najafi, 2013; Cianciosi et al., 2018). However, considerable between-batch variation exists in the carbohydrate and nutritional content of honey that is attributable to factors associated with the plant ecology (e.g., geographical origin) from which the nectar is extracted, and thus must be clearly indicated according to EU legislation (Council Directive 2001/110/EC).

Carbohydrates are now synonymous with fuelling exercise performance (Burke et al., 2011; Jeukendrup, 2014) and have been since seminal research published in 1920 postulated the biochemical importance of exogenous carbohydrate ingestion (Krogh and Lindhard, 1920 in Hawley et al., 2015). Whilst it had

previously been theorised, Krogh and Lindhard (1920) noted a dietary effect on muscle substrate utilisation. Specifically, the oxygen cost of exercise was 11 % lower when carbohydrate diets were consumed pre-exercise versus fat. Additionally, in the mid-late 20th century and early 21st century, several lines of inquiry concluded that reductions in muscle glycogen concentrations, recorded at the end of a soccer match, were a likely determinant of impaired high-intensity physical performance (Agnevik, 1970; Saltin, 1973; Krustrup et al., 2006). Today, extensive evidence supports high carbohydrate provision to maintain euglycaemia and preserve muscle glycogen concentrations while underpinning fuelling recommendations (Jeukendrup, 2014; Thomas et al., 2016). Specifically, single carbohydrate solutions (i.e., glucose), ingested at a rate of 30-60 g·h^{-1} (~0.7 g·kg·h^{-1}) for exercise 60-150 min in duration are advocated (Thomas et al., 2016); although it is accepted that carbohydrate oxidation can become saturated at 60 g·h^{-1} (Burke et al., 2011).

Studies over the past two decades have investigated the ingestion of multiple transportable carbohydrates (MTC) during exercise for the purposes of increased carbohydrate oxidation (e.g., beyond 60 g·h^{-1}). Specifically, Jentjens et al. (2004) investigated the effects of combined fructose and glucose beverages (0.6 g·min^{-1}; 1.2 g·min^{-1}, respectively) when compared with two glucose beverages void of fructose when consumed at rates equivalent to 1.2 g·min^{-1} and 1.8 g·min^{-1}, during 2 h of cycling. Interestingly, the authors documented peak carbohydrate oxidation rates of ~1.26 g·min^{-1} when glucose was co-ingested with fructose versus approximately ~0.80 g·min^{-1} when single-source carbohydrates were consumed irrespective of ingestion rate. When glucose is consumed as a single carbohydrate, its uptake is mediated by sodium dependent glucose transporters

(SGLT1), which become burdened at 60 $g \cdot h^{-1}$ (Juekendrup, 2010; Burke et al., 2011). However, the addition of fructose promotes additional absorption through a separate diffusive transport pathway (e.g., glucose transporter five – GLUT5), increasing total carbohydrate oxidation, in this case by ~55 % (Jentjens et al., 2004; Burke et al., 2011; Röder et al., 2014). Similarly, increases in oxidation (~36 %) have been reported elsewhere (Jentjens et al., 2006), while a physical time-trial performance benefit (~8 %) exists during an ultra-endurance event lasting >2.5 h (Currell and Jeukendrup, 2008; Rowlands and Houltham, 2017). Considering that honey naturally contains fructose and glucose, its application as a potential MTC solution may appeal to athletes seeking food first fuelling strategies.

Fructose has a glycaemic index (GI) of just 19, which is much lower than that associated with glucose (100), so its inclusion in MTC solutions has implications for glycaemic control (Bobiş et al., 2018; Sadeghi et al, 2019). The GI is a ranking index of glucose responses to independent carbohydrate ingestion (Jenkins et al., 1981; Aston et al., 2010), so high GI carbohydrates (≥ 70) typically instigate a rapid postprandial release of glucose into the blood (Williams and Rollo, 2015). Conversely, low GI values (≤ 55) limit the release of blood glucose and the corresponding insulin response due to delayed gastric emptying and absorption (Moore et al., 2010; Bobiş et al., 2018). While exact mechanisms are uncertain, some researchers have posited that fructose and glucose combinations may aid metabolism of each other (e.g., glucokinase from fructose stimulates hepatic glucose uptake (Bobiş et al., 2018)). Thus, given that exercise may negatively affect intestinal transit time (Fujisawa et al., 1993), and that honey is known to span low, medium, and high GI categories (see review by Bogdanov et al., 2008),

the varying GI of honey and high carbohydrate availability make it a prime candidate for applied ergogenic research.

High GI carbohydrates ingested immediately before, and continuously during, prolonged and/or high-intensity intermittent exercise can help to maintain physical performance (Burke et al., 2011; Galloway et al., 2014; Thomas et al., 2016). However, continuous high GI feeding (i.e., every 15 min) during soccer-specific exercise has been shown to influence hypoglycaemia (i.e., blood glucose concentrations <4.0 $mmol \cdot L^{-1}$) in the second-half of simulated soccer-specific exercise (Kingsley et al., 2014). Interestingly however, when investigating high and low GI sports drinks (8 %) consumed prior to, and at half-time (45 min), during 120 min of soccer-specific exercise, Stevenson et al. (2017) noted that isomaltulose (GI: 32) better maintained glucose concentrations in the second-half (i.e., 75-90 min) versus maltodextrin (GI: 90-100). While continuous feeding is not appropriate in practical soccer settings, exogenous carbohydrates must be ingested to avoid depletion of endogenous glycogen (i.e., muscle and liver) stores (Jensen et al., 2011; Ørtenblad et al., 2013). Thus, the prolonged metabolism associated with low GI carbohydrate ingestion may be of benefit to athletes seeking ergogenic strategies for prolonged exercise demands and restricted feeding. Given that pre-exercise low GI carbohydrates have previously been linked to improved physical performance (Moore et al., 2010), honey may be of interest to practitioners and researchers looking for practical exogenous carbohydrate solutions when feeding restrictions exist.

It is well established that intense physical training and competition typically seen among athletes, is a risk factor for a number of health-related outcomes. Notably,

exercise volume and immunological biomarkers are inversely related (Rahim et al., 2017). Similarly, inflammation induced by training adaptions may accelerate fatigue (e.g., muscle and signalling cells/proteins), while it has also been posited that frequent exercise at high-intensity and/or prolonged bouts of aerobic activity generate excessive reactive oxygen species (ROS) and contribute to subsequent tissue damage (Abbey and Rankin, 2009; He et al., 2016). Nonetheless, it has been speculated that honey has the potential to attenuate immunological perturbations (e.g., Interleukin cytokines- IL) due to the prevalence of anti-inflammatory and immunomodulatory compounds (Rahim et al., 2017; Pasupaleti et al., 2017). Similarly, bioavailable phenolic (e.g., allagic, ferulic and syringic acid), and flavonoid (e.g., hesperetin, pinobanksin, luteolin) compounds of honey enrich the antioxidant effect and play an important role in inhibiting serum lipoprotein oxidation (Alvarez-Suarez et al., 2010; Pasupaleti et al., 2017; Ahmed et al., 2018). Indeed, this was evidenced when researchers noted that chronic honey ingestion (1.2-1.5 $g \cdot kg^{-1} \cdot d^{-1}$) positively influenced serum antioxidant status (Schramm et al., 2003 in Bogdanov et al., 2008). Thus, considering these components have potential health benefits, a theoretical basis for honey consumption to augment an athlete's physiological profile exists.

Because honey is a naturally occurring complex carbohydrate comprised of both fructose and glucose (~80 %), it has high carbohydrate availability, with a low GI, and is a prime candidate for use as a MTC solution. Since exogenous low GI carbohydrates help to control glucose metabolism and preserve endogenous glycaemia (e.g., blood glucose, muscle glycogen), and while MTC solutions may facilitate carbohydrate oxidation beyond the 60 $g \cdot h^{-1}$ upper limit, it is likely that honey possesses ergogenic potential. In a similar fashion, honey contains over

200 components that are purported to have positive health effects, such as antioxidant and immunomodulatory properties. Accordingly, it is likely that honey has the potential to attenuate biomarkers of oxidative stress and immune function exacerbated by prolonged and/or intensive exercise. With these proposed benefits in mind, the aim of this research was to systematically review published research articles pertaining to the physiological responses and/or physical performance effects of combined exercise and honey supplementation. The null hypothesis (H_0) associated with this review was that honey would have no effect on physiological and/or physical performance responses.

2. METHODS

The research review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocols (Liberati et al., 2009). Briefly, the PRISMA statement is a checklist of minimum standards for the collection, analyses and synthesis of published research studies (Moher et al., 2009). These standards have been validated, are widely accepted and utilised in journals publishing human nutrition research. In agreement with institutional guidelines, full ethical review was not necessary for this research.

2.1. Search and study selection

Between May 2018 and July 2019, electronic literature searches were conducted systematically within databases that likely contained published research pertinent to human/sports nutrition. Specifically, the electronic literature searches aimed to obtain peer-reviewed research publications that experimentally investigated the physiological responses and physical performance effects of honey ingestion in sports and exercise settings. PubMed, MEDLINE, and SPORTDiscus databases were chosen based on the aforementioned criteria and an early scoping review of potentially feasible research data. Some databases (e.g., PsycINFO) were excluded due to the central subject focus being outside of the scope of the research question. In addition to the electronic database search, it was agreed that any papers previously identified by the research team, would be integrated into the review if not retrieved in the search. Furthermore, no pre-defined published date restrictions were set as it was unknown when the earliest studies

of honey supplementation for sports performance were dated, thus this action was deemed restrictive.

The search strategy consisted of terms directly related to sports-specific physical performance and physiological outputs, and honey as a foodstuff. The search strategy was primarily developed for the MEDLINE database and adapted to other databases based on unique database keyword categories. Where relevant, the EBSCO research platform was used to conduct database searches. Boolean, wildcard and proximity key-word search terms were used to optimise search results. Medical Subject Headings (MeSH) were also used where necessary. A tabulated version of the MEDLINE search list has been included in the appendix.

Following implementation of the database search, full scans of titles and abstracts were conducted. In line with our aims, journal articles were deemed not relevant to the research question if no exercise stimulus was present and/or if the ingestion of honey was absent. The screened articles were then exported from EBSCO and PubMed to EndNote (EndNote X9; Clarivate Analytics, USA) where duplicates were removed and full text versions obtained.

2.2. Inclusion and exclusion criteria

Full text articles were eligible for inclusion in the review if they: 1) were conducted on human participants, 2) involved an exercise stimulus, 3) required honey ingestion by participants, either chronically or acutely, 4) were published in the English language, 5) included comparator treatments and/or placebo conditions, and 6) reported the results of at least one physiological or physical performance

outcome. An example of the search and selection tool has been included in the appendices for reference (Appendix 1).

2.3. Quality assessment

Screened full text journal articles were assessed for quality using the Physiotherapy Evidence Database Scale (PEDro). This scale has been used and validated in published systematic reviews of randomised control trials, thus was deemed to represent a model of efficacy in appraising selected studies for a potentially publishable review of our own (de Morton, 2009). The scale assesses intra-study quality based on a subjective rating (maximum of 10) relative to experimental research standards; this strategy was employed to minimise quality bias and ensure treatment effectiveness/integrity of the systematic review (Maher et al., 2003). Specifically, a subjective rating of 6/10 was required as a minimum score to underpin the integrity of inferable research quality and has consistently been applied by authors in our research group (Russell and Kingsley, 2014; Hills et al., 2019).

2.4. Risk of bias

In support of implementing the PEDro scale, a risk of bias assessment was also completed. This was completed in line with guidelines and criteria set within the Cochrane Handbook for Systematic Reviews of Interventions (Higgins et al., 2019). Specifically, judgements were made regarding sequence generation and allocation sequence concealment (i.e. selection bias), blinding of participants and personnel (i.e., performance bias), blinding of outcome assessment (i.e.,

detection bias), short- and long-term incomplete outcome data (i.e., attrition bias). Selective outcome reporting (i.e., reporting bias), and other threats to study validity were also considered.

2.5. Blinding

The results of the searches were blinded between the lead author and another member of the research team who analysed findings upon data extraction to reduce researcher bias and validate the review. Eliminated studies were agreed between authors based on PEDro ratings. Included studies were discussed between reviewers and if disagreements existed, discrepant publications were presented to a third member of the research team who had not previously been involved in the search process.

3. RESULTS

A full PRISMA summary is presented in Figure 1. Searches retrieved 1598 results from three electronic databases. Specifically, MEDLINE (1070), SPORTDiscuss (264), and PubMed (264). Having applied an initial title and abstract scan, and eliminated studies that were unrelated to the subject-specific keyword parameters, and duplicate removal (12), 39 abstracts were further screened and studies eliminated based on the exclusion criteria identified above. Of these, five were unavailable for full text, two were not written in the English language, four were review/summary papers, one did not administer honey, and three were animal studies. Thus, 24 studies were selected for full text retrieval. Having applied exclusion criteria to the full texts, a further two studies were eliminated since they did not include an exercise stimulus, one study administered only a variant of honey (i.e., royal jelly, propolis), five additional studies did not involve human participants, while two studies were identified as a review paper or secondary publication. Application of the quality assessment tool to the remaining 14 papers resulted in 11 studies being eligible for review.

The three eliminated studies that failed to meet the pre-defined PEDro standards were classified as providing insufficient evidence of participant eligibility criteria, not disclosing random allocation and concealment, omission of baseline data presentation, and/or not using participant/researcher blinding.

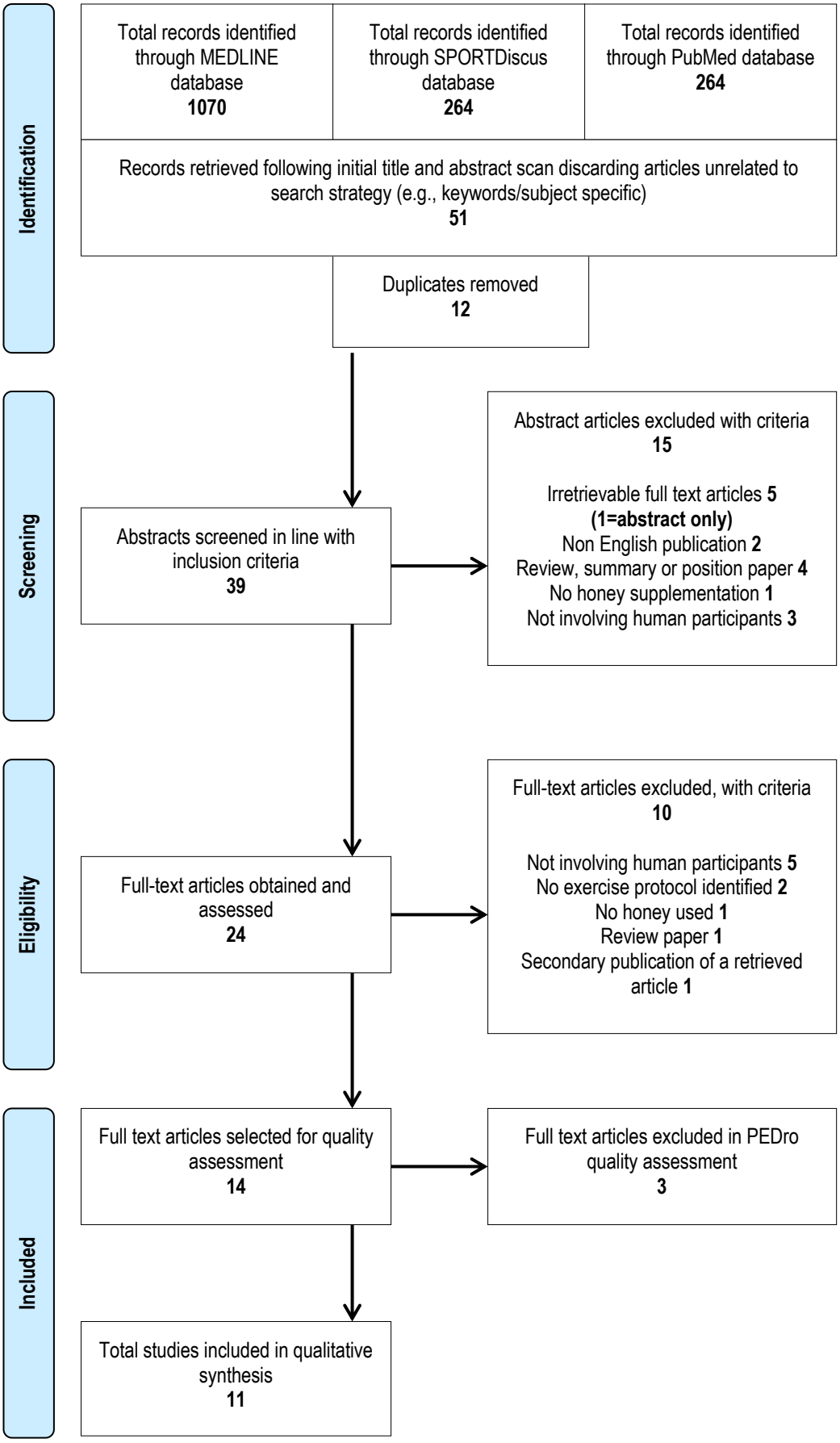

Figure 1. PRISMA flow diagram

3.1. Study details

Of the studies selected for analysis in the review, five investigated physical performance and/or physiological responses to acute honey ingestion (i.e., supplementation and/or outcome measures over a single exercise session). Conversely, six studies investigated physical and/or physiological responses to chronic honey supplementation (i.e., ingestion and exercise stimuli over a period of several weeks). Randomised control trials were pre-defined as the preferred study design for research analysed in this review. Most of the studies selected upheld this status.

3.2. Participant characteristics

In its entirety, this review includes summary data from 254 participants (n=135 male; n=119 female) across all comparator intervention groups. In acute honey research studies, ~74 % were male participants (n=59/80). In chronic investigations, ~56 % were female participants (n=98/174). In totality, participants were well-trained rowers (n=11), experienced soccer players (n=10), resistance trained (n=40), recreational runners (n=26), endurance cyclists (n=72), sedentary individuals (n=77), or recreational athletes (n=18). Further information is presented in Table 1.

Table 1. Baseline Characteristics

Reference	Athlete status	Participant Characteristics	
Abbey and Rankin (2009)	n=10: Male soccer players (NCAA div 1)	Age - 22.5 (± 3.3 SD) yrs Mass - 74.0 (± 6.3 SD) kg Height – 177.0 (± 5.4 SD) m BMI – 23.6 (± 1.3 SD) kg·m^{-2} $\dot{V}O_{2max}$ – 50.1 (± 4.4 SD) ml·kg^{-1}·min^{-1}	
Ahmad et al. (2015)	n= 10: Male recreational runners (no standards defined)	Age – 21.8 (± 1.4 SD) yrs Mass – 59.9 (± 7.8 SD) kg $\dot{V}O_{2max}$ - 51.7 (± 4.1 SD) ml·kg^{-1}·min^{-1}	
Earnest et al. (2004)	n=9: Male endurance amateur cyclists (category II, III) and triathletes	Age – 30 .0 (± 1 SD) yrs Mass – 77.0 (± 2.6 SD) kg Height – 169.9 (± 4.6 SD) cm Power – 329 (± 20 SD) W	
Kreider et al. (2007)	n=40: Male (n=19) and female (n=21) resistance trained athletes with at least 1 years experience	Sucrose supplement	Age – 24.0 (± 1.0 SD) yrs, Mass – 71.2 (±4.0 SD) kg Height – 171.3 (± 5.0 SD) cm
		Honey supplement	Age – 23.3 (±1.1 SD) yrs Mass – 70.7 (± 4.8 SD) kg Height – 171.2 (± 3.7 SD) cm
		Maltodextrin supplement	Age – 24.7 (± 1.6 SD) yrs Mass – 84.5 (± 7.1 SD) kg Height – 175.8 (± 3.6 SD) cm
		Control condition	Age – 20.9 (± 0.7 SD) yrs Mass – 72.4 (± 5.9 SD) kg Height – 171.2 (± 3.4 SD) cm
Łagowska et al. (2017)	n=11: Well trained male rowers with at least 3 years of training experience	Age = 20.2 (± 2.0 SD) yrs Mass = 86.6 (± 10.1 SD) kg Fat free mass = 74.0 (± 6.6 SD) kg Height = 189.1 (± 3.3 SD) cm Max power output = 368 (±36 SD) W	
Deneghian et al. (2019)	n=18: Female participants	Honey (n=8)	Age - 23.25 (± 5.14 SD) yrs Mass - 58.58 (± 8.6 SD) kg Height – 1.65 (± 0.5 SD) m BMI - 21.39 (± 2.09 SD) kg·m^{-2} Body fat – 25.78 (± 1.87 SD) % $\dot{V}O_{2max}$ – 35.11 (± 6.02 SD) ml·kg^{-1}·min^{-1}
		PLA (n=10)	Age - 22.70 ± (± 4.37 SD) yrs Mass- 62.59 (± 6.07 SD) kg Height -1.65 (± 0.3) m BMI - 22.73 (± 1.61 SD) kg·m^{-2} Total Body fat - 26.52 (± 1.37) % $\dot{V}O_{2max}$ – 38.02 (± 6.45 SD) ml·kg^{-1}·min^{-1}
Gmünder et al. (1990)	n=16: Male (n=13) and female (n=3) long distance runners	Honey (n=8)	Age - 24 yrs Mass – 65.5 kg Height – 175 cm
		PLA (n=8)	Age - 27.5 yrs Mass – 62.5 kg Height – 169 cm
Hajizadeh-Maleki et al. (2016)	n=29: Male non professional cyclists	Honey with exercise (n=15)	Age – 23.3 (± 5.7 SD) yrs Mass – 71.1 (± 9.7 SD) kg Height – 178 (± 8.1 SD) cm BMI – 21.5 (± 1.9 SD) kg·m^{-2} Fat – 7.1 (± 3.3) % $\dot{V}O_{2max}$ – 63.6 (± 5.5 SD) ml·kg^{-1}·min^{-1}

Table 1. Cont.

Reference	Athlete status	Participant Characteristics	
		Exercise only (n=14)	Age – 23.1 (± 6.2 SD) yrs Mass – 70.7 (± 9.1 SD) kg Height – 180 (± 7.4 SD) m BMI – 21.8 (± 1.7 SD) $kg \cdot m^{-2}$ Fat – 7.2 (± 3.1) % $\dot{V}O_{2max}$ – 63.8 (± 5.2 SD) $ml \cdot kg^{-1} \cdot min^{-1}$
Ooi et al. (2011)	n=37: Females	Control (n=10)	Age – 21.6 (±2.7 SD) yrs Mass – 52.3 (± 8.2 SD) kg Height – 156.9 (± 6.7 SD) cm
		Honey (n=10)	Age – 20.7 (± 2.3 SD) yrs Mass– 53.5 (± 9.9 SD) kg Height – 155.5 (± 6.7 SD) cm
		Exercise only (n=9)	Age – 22.2 (± 2.0 SD) yrs Mass – 53.9 (± 11.2 SD) kg Height – 156.7 (± 6.7 SD) cm
		Honey and exercise (n=8)	Age – 23.5 (± 1.7 SD) yrs Mass – 50.5 (± 8.2 SD) kg Height – 157.9 (± 2.6 SD) cm
Rahim et al. (2017)	n=40: Healthy sedentary females		Age 29.7 (± 5.3 SD) yrs
		Control (n=11)	Height – 154.2 (± 5.6 SD) cm Mass– 56.0 (± 9.9 SD) kg Body fat – 32.5 (± 9.8 SD) %
		Honey (n=9)	Height – 153.8 (± 4.8 SD) cm Mass – 54.5 (± 7.8 SD) kg Body fat – 33.0 (± 7.2 SD) %
		Exercise only (n=11)	Height – 154.6 (± 6.1 SD) cm Mass – 55.3 (± 5 SD) kg Body fat – 32.7 (± 5 SD) %
		Honey and exercise (n=9)	Height – 156.4 (± 6.0 SD) cm Weight – 53.4 (± 7.7 SD) kg Body fat – 30.0 (± 7.4 SD) %
Tartiban and Hajizadeh-Maleki (2012)	n=39: Male non-professional long distance road cyclists with minimum 1 year experience	Honey (n=20)	Age – 23.9 (± 5.3 SD) yrs Mass – 71.1 (± 4.9 SD) kg Height – 1.8 (± 5.7 SD) m BMI – 21.9 (± 1.1 SD) $kg \cdot m^{-2}$ Fat – 8.1 (± 2.2) % $\dot{V}O_{2max}$ – 63.8 (± 2.5 SD) $ml \cdot kg^{-1} \cdot min^{-1}$
		Exercise only (n=19)	Age – 23.6 (± 5.7 SD) yrs Mass – 72.3 (± 5.3 SD) kg Height – 1.8 (± 7.1 SD) m BMI – 22.1 (± 1.4 SD) $kg \cdot m^{-2}$ Fat – 8.0 (± 2.0) % $\dot{V}O_{2max}$ – 64.2 (± 3.1 SD) $ml \cdot kg^{-1} \cdot min^{-1}$

3.3. Study designs

Acute supplementation studies have applied crossover (80 %), or counterbalanced (20 %) experimental research designs, with outcomes measured over two or three sessions/visits (see Table 2). However, researchers in an additional acute investigation did not adopt a crossover design and instead

participants were aligned to experimental groups representative of control, sucrose, honey or maltodextrin trials.

Where longer methodologies were applied, the intervention/observational periods ranged from 4-16 weeks with honey ingested chronically, and exercise sessions implemented over several days as highlighted in Table 2. Participants in these investigations were assigned to experimental groups only.

3.4. Risk of bias

Results of the risk of bias assessment for each study have been summarised in Appendix 4 and Figure 2. Regarding random sequence generation, 27 % of studies were identified as low risk as they stated procedures of randomisation, while 64 % were judged to be unclear as they suggested random sequences existed but did not clarify any details. Similarly, 36 % of studies were judged to have a low risk of bias and 55 % were judged have unclear risk of bias for the same reasons regarding adequate sequence concealment protocols (e.g., randomisation performed by a third party). One study (9 %) was regarded as high risk as it made no reference to random sequence generation or how allocation was concealed. Although the study was not excluded as an earlier PEDro score deemed it worthy of inclusion.

In addition, the risk of performance bias was assessed through blinding procedures. Specifically, 54 % of studies were classified low risk as blinding procedures regarding participants and personnel were documented, while 18 % were regarded as an unclear risk of bias, or classified as medium risk due to some mention of blinding, or blinding had been identified in earlier publications.

Thus, some ambiguity remains as to whether blinding had been disclosed to Cochrane standards. However, 27% of studies did not mention any suggestion of blinding. While these were not discounted from inclusion based on meeting PEDro standards, it is recognised that the frequency of reporting of blinding processes in some published studies is low (Montori et al., 2002). As such, these could also be classified as medium risk. Similar figures exist for blinding of subjective participant reported outcomes (i.e., detection bias).

Of the studies reviewed, most (80-100 %) were deemed to have a low short term (i.e., 2-6 weeks) and long term (i.e., 6-12 weeks) attrition bias, based on high percentages of participants completing honey supplementation periods. These studies provided reasons for loss of participants ranging from pregnancy to injury and/or illness. Those classified as high risk of attrition bias were categorised as long term and outcomes were incomplete in more than 20 % of participants outcomes due to loss of follow up, as per PEDro guidelines. In a similar fashion, 90 % of studies were judged to have a low reporting bias or have a low risk of validity to other sources. This is due to mainly to methods and results being well linked and well reported and baseline prognostic indicators being similar.

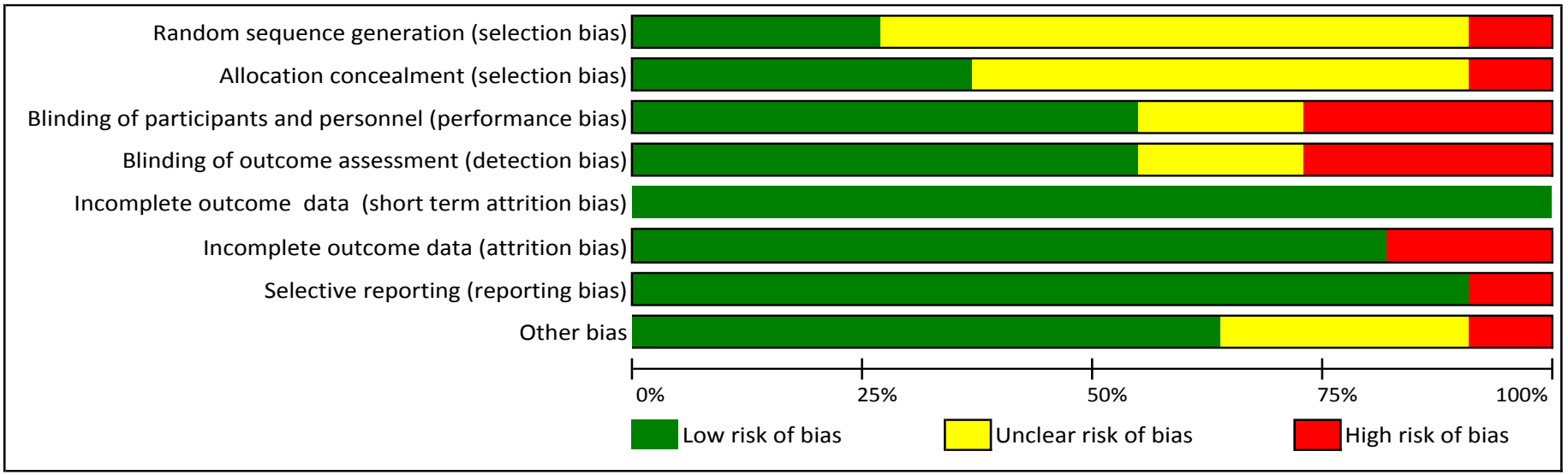

Figure 2. Cochrane risk of bias graph.

3.5. Exercise modalities investigated within a single session

Research studies have investigated the physical performance effects and/or physiological responses of honey supplementation across a range of exercise modalities. Specifically, the physical performance effects of honey consumption have been reported within a single exercise session, including a 64 km cycling time-trial, designed to replicate a pre-completed Tour de France stage. Moreover, a glycogen depleting run ~1 h at 65 % $\dot{V}O_{2max}$ preceding a running time-trial, 80 min of rowing, and resistance exercise including 10 repetitions of each of the following: chest presses, seated row, shoulder press, latissimus dorsi pulls, leg extensions, leg curls, bicep curls, and tricep extensions have all been documented. When a soccer match simulation was utilised as an exercise stimulus, participants performed 5 x 15 min blocks, an exhaustive run, and a warm-up and recovery phase.

3.6. Exercise modalities investigated over several weeks

In contrast to single exercise sessions, researchers have investigated the physiological responses of honey supplementation in exercise over several weeks. Notably, over a training period between 8 and 16 weeks which included cycling at low, moderate, and high-intensity from ~371 $km \cdot wk^{-1}$ to ~660 $km \cdot wk^{-1}$. When running was adopted as the exercise stimulus, participants completed a 21 km run following a 31 day supplementation period, or participants completed three active sessions per week (~38 min) at speeds corresponding to 60-65 % $\dot{V}O_{2max}$, including a warm-up (~10 min) and passive recovery phase (~ 8 min). In addition, two studies utilised aerobic dance as the mode of exercise, from which

participants completed one hour (i.e., 15 min warm up, 35 minutes dance, and cool down), three times a week over an eight-week supplementation period.

3.7. Acute honey ingestion

Five studies investigated honey supplementation prior to and continuously throughout exercise (2), prior to and at half-time (1), or post-exercise (2) administered acutely in beverages and/or gels. When ingested prior to and continuously throughout exercise, 6.7 % (i.e., 6 × 150 ml) and 60 g (i.e., 4 × 15 g) of honey ingestion has improved time-trial performance (~2.5 min) when compared to water, which was similar to a dextrose comparator. No significant time effects were observed in high-intensity running performance compared to a sports drink or an energy free placebo when honey was consumed. However, plasma IL-1ra concentrations were reduced (~28 %) compared with a sports drink (~65 %) and a placebo (~64 %), when a 6 % honey solution was ingested prior to (0.5 $g \cdot kg^{-1}$), and at half-time (0.5 $g \cdot kg^{-1}$). Following exercise, honey has been ingested *ad libitum* immediately or in several boluses over 2 hours between ~480 ml (120 g) to ~1400 ml, which increased blood glucose concentrations up to ~2 $mmol \cdot L^{-1}$ versus energy free placebos or carbohydrates (i.e., sucrose or maltodextrin). This equated to ~10 % improvement in running distance when compared to water.

3.8. Chronic honey ingestion

In the eligible research, honey was administered chronically alongside other carbohydrates (i.e., honey, plasmolysed herbal yeast, malt, and orange juice).

When administered as an ingredient alongside other carbohydrates, daily doses (i.e., 3 x 10 $ml \cdot d^{-1}$) from 4 weeks to 31 days had no effect on immunological status or had variable effects on attenuating oxidative stress (i.e., lipid peroxidation, enzyme activity). However, honey ingested independently on a daily basis (i.e., 20 g) or 90 min before exercise (i.e., 70 g) from 6 – 16 weeks attenuated exercise induced oxidative stress, DNA damage, lipid peroxidation (i.e., 2.9 vs. 4.7 $nmol \cdot mL^{-1}$ without supplementation) and pro-inflammatory cytokines. Whilst, increases were reported in antioxidant status, immunological lymphocyte and leukocyte (e.g., CD) cell counts, and/or bone formation serum alkaline phosphatase.

3.9. Assessments of physical performance outcomes

Five research studies reported outcome measures specific to physical performance indicators; these include comparative measurements of the time taken to perform tasks (2), total distance travelled (2), or the time spent performing in a particular speed velocity (1). Additionally, one study reported outcome measures on the quality of skilled/technical actions performed by participants with no significant effects. Several studies have also reported outcomes on subjective perceptions of effort post-ingestion although no detrimental effects were noted.

3.10. Assessments of physiological outcomes

All research studies included in this review presented data inclusive of at least one physiological marker. Outcomes were derived from laboratory-based

analyses in all instances. In most studies (90 %), blood samples were chosen for analyses and were obtained using intravenous (i.e., venepuncture) or capillary sampling techniques. A research team member then collected serum fluid for biochemical analysis and data were presented on a plethora of physiological biomarkers, including hormones (i.e., testosterone), blood serum physiology (i.e., metabolites and immunology), markers of oxidative stress (i.e., ROS), and antioxidant physiology (i.e., antioxidant enzyme glutathione) as per Table 2. One research study exclusively analysed semen samples and provided information on seminal cytokines and biomarkers of oxidative stress.

Table 2. Physical performance and physiological responses of acute and chronic honey ingestion in sports and exercise settings.

Ref and Aims	Study Design and Duration	Exercise Protocol Details	Dietary Intervention	Results Summary
Acute honey supplementation				
Abbey and Rankin (2009). Investigation into the performance and physiological effects of honey consumption on physical and technical performance in soccer-specific exercise.	Double blind, randomised, crossover controlled study. Three experimental trial days separated by 1 week (representing H, SP, PLA).	5 x 15 min blocks of 20 m shuttle runs with a 10 min half-time. Blocks included repeated running cycles of 55 % $\dot{V}O_{2max}$, running at 120 % $\dot{V}O_{2max}$, walking and a maximal sprint. Soccer-specific protocol validated by Kingsley et al. (2005), with modifications. A PSR to fatigue followed.	H & lemon flavoured drink (6 %) vs. Gatorade sports (SP) drink (6 %) vs. PLA (lemon flavoured - energy free). H & S assumed to deliver 0.5 g·kg^{-1} CHO before exercise and at half-time (1 g·kg^{-1} total). 8.8 ml·kg^{-1} ingested 30 min prior to exercise and during 10 min half- time.	Effects of time but not treatments for blood indices ($ORAC_{total}$; $ORAC_{pca}$; IL-6; IL-10; IL-1ra) but not Cortisol. Glucose → 15.6 % post-test vs. pre-test in all treatments. 1 hr post-test values ← below pre-test values. Significant time → in HIR performance in all timed periods (2-5) vs. initial period (1). ← time to exhaustion in all treatment. No influencing treatment effects. No time or trial interactions reported for agility and shooting. Progressive time → in RPE.
Ahmad et al. (2015). Investigation of the efficacy of honey supplementation to regulate glucose following exercise in warm conditions.	Randomised, placebo controlled, cross-over study design. Single blinded (researcher). Two trial days representing H and PLA.	Glycogen depleting run 65 % $\dot{V}O_{2max}$ for 60 min (run 1). A 2 hr rehydration phase with no activity. Following this, participants completed further run classified as a time-trial (run 2).	Acacia H (1395 ml) or Water PLA (1350 ml) solutions were ingested and compared in this intervention. Honey drink formulation was 6.8 % CHO equivalent to 150 % of body weight (lost), consumed during a 2 h rehydration phase (following a run and prior to a TT) at 0 min (60 %), 30 min (50 %) and 60 min (40 %).	Plasma glucose, higher in H vs. PLA throughout rehydration and TT run. Serum insulin significantly higher throughout rehydration phase. Lower in glycogen depleting run. Serum osmolarity consistently higher in H than PLA throughout rehydration and TT. Gut fullness similar. Post rehydration phase running distance → when H consumed vs. PLA. This equates to statistical significance as subjects ran 3420 m when consuming H vs. 3120 m when consuming PLA.
Earnest et al. (2004). An investigation into the performance responses of consuming different CHO supplements.	Randomised, placebo controlled, crossover, counterbalanced study design. Double blind. Three trial days separated by 1 week, representing supplemented PLA, H and D solutions.	A 64 km time-trial cycle simulation under lab conditions. Participants were instructed co complete as fast as possible.	H gel (GI=35) was compared against D (GI=100) and PLA. Gel supplements consumed every 16 km (15 g) with 250 ml water. Additional water consumption every 3.2 km. H constituted 38.5 % fructose, 31 % glucose, 17.1 % water, 7.2 % maltose, 4.3 % trisaccharides, 1.5 % sucrose.	No effects observed between treatments for HR/RPE. Significant→HR/RPE over time. No treatment effects noted between groups for glucose. Significant ← in glucose for D at 48 km vs. 16 km. No significant changes in insulin. Total time taken to complete 64 km TT was not significant between CHO treatments. However, trends noted to be longer 131.3 min PLA vs. D 128.3 min or H 128.8 min. Significant effects noted vs. PLA. W lower when placebo consumed and less pronounced overall. Last two segments tended to be slower in PLA corresponding to 48 and 64 km.

Table 2. Cont.

Ref and Aims	Study Design and Duration	Exercise Protocol Details	Dietary Intervention	Results Summary
Kreider et al. (2007). Investigation into the effects of various carbohydrate solutions when consumed with protein on glucose and insulin to highlight opportunities for post-exercise recovery.	A randomised, double-blind, placebo-controlled study. 1 trial day for each participant. Participants only aligned to single intervention arm.	Three sets of 10 repetitions of nine exercises including chest press, seated row, shoulder press, lat pull, leg extension, leg curl, bicep curl, tricep extension and leg press.	Supplement ingested immediately post exercise. 40 g of whey protein consumed with 120 g of S, 120 g powdered H or 120 g M or a control group (PLA). 16 oz water added to supplements. H composition = 95 % mixed fructose (31.5 %), glucose (26 %), wheat starch (25.3 %), soluble fibre (12.5 %) and maltose (4.7 %).	H supplementation reported glucose concentrations higher +30 min post-exercise when compared with maltodextrin, sucrose and control. H values were always higher than baseline measures unlike comparator indices, maltodextrin and sucrose, which did in at least one time
Łagowska et al. (2017) An investigation into the hydration and physiological effects of a natural food supplement compared to a commercial sports drink.	A Two way, randomised, crossover study. Research across 2 experimental trial days.	A rowing test at 75 % OBLA load for 2 x 40 min periods interspersed with a 5 sec recovery period.	7.8 % commercial isotonic beverage (sucrose, glucose syrup, maltodextrin, dextrose, citric acid, sodium citrate) vs. 6.7 % natural drink (salt, banana, water, pineapple juice, lemon juice, honey). Unknown honey weight as a component.	H attenuated weight loss during exercise compared with SP. No differences between interventions for → lactate over time, lymphocytes, monocytes, granulocytes, creatine kinase and leukocytes. Haematocrit changes no different. Statistically significant post-exercise glucose response lower when H consumed vs. SP
Chronic honey supplementation				
Deneghian et al. (2019). An assessment of oxidative status, antioxidant activity and HSP-70 expression following consumption of a natural food supplement.	A randomised, double-blind, placebo-controlled trial. Study lasted for 4 weeks.	Exercise protocol consisted of warm up for 10 min to a targeted HR rate of 120 bpm, 2 x 10 minute running sessions interspersed by a 5 min recovery period for 4 weeks (12 sessions). Aim to achieve 60-65 % $\dot{V}O_{2max}$	H consisted of 2 g ginger root, 2 g cinnamon bark, 30 g raw almond fruit powder, 2 g rosemary leaf powder and honey (219 kcal), was compared with a PLA consisting of (no antioxidant) ingredients including roasted wheat flour, and sugar syrup (197 kcal).	HSP-70 increased when H ingested at all time points vs. PLA – significant P-value (p=0.001). Significant Time interactions for total antioxidant capacity and superoxide dismutase, but not trial. Time x Trial interactions not significant for MDA (p=0.19). No significant differences for GPx.
Gmünder et al. (1990). An investigation in the effect of food supplementation on immunological status of long distance runners.	A randomised, double-blind, placebo-controlled study. Study duration was over 31 consecutive days.	21 km run performed toward the end of supplement cycle (day 29).	Plasmolysed herbal yeast, malt, honey, and orange juice (H), was compared with a sucrose and caramel solution (PLA). Consumption of 3 x 10 ml per day with meal for 31 consecutive.	No differences reported between trials for c1-inactivator, complement c3c, c4, b, B2-microglobulin, white blood cells (leukocytes), B- and T-cells, lymphocytes, immunoglobulins, IL-2 receptors, and other cellular and humoral variables.

Table 2. Cont.

Ref and Aims	Study Design and Duration	Exercise Protocol Details	Dietary Intervention	Results Summary
Hajizadeh-Maleki et al. (2016). An investigation in the ability of honey to attenuate biochemical changes following road cycling.	A randomised controlled trial. 16 weeks of supplementation and exercise + 30 days recovery period.	Training intensities performed at low (<55.2 % $\dot{V}O_{2max}$), moderate (55.2 % - 82.9 % $\dot{V}O_{2max}$) and high-intensity (>82.9 % $\dot{V}O_{2max}$). This represents 22.4 %, 44.2 % and 33.4 % of total training intensity during the first 8w or 9.5 %, 51.5 %, 39 % during the second 8w. Distance covered in training was 371 ± 42.1 km wk $^{-1}$ over 12 hours for the first 8 wk and 660 ± 49.2 km wk^{-1} over 16 hours for the remaining 8 wk.	70 g of unprocessed H consumed with 250 ml distilled water 90 min before every training session vs. no supplement. H constituted 41 % fructose, 5.1 % sucrose, 24 % glucose and other vitamins/minerals/micronutrients.	Results report → values of lymphocytes, cytokines, DNA damage, and ← in antioxidant activity following exercise. Results of which, were attenuated when honey consumed. Performance improved significantly in both exercise and exercise + supplement group for (W), 5 km TT and 40 km TT when compared to baseline values. No statistical significance between controls.
Ooi et al. (2011). An investigation into the effects of aerobic exercise combined with honey supplementation on bone health.	A controlled trial. Anthropometrics defined group assignments. 6 weeks.	6 weeks of aerobic dance sessions (3 x 1 h·wk) in specific groups (i.e., aerobic dance only and aerobic dance with combined honey supplementation). Honey supplementation without exercise and a sedentary/no honey control group made up groups 3 and 4. Exercise was high and low impact.	Gelam H (20 g) was diluted in 300 ml of water and consumed for seven days a week for the duration of the trials – 6 wk vs. no supplement. In exercise treatment groups, participants were encouraged to consume fluids 30 min before activity.	Bone formation marker – ALP = results report significant → post-test when H consumed vs. pre-test in exercise and non-exercise groups. Bone resorption marker -1CTP = no significant changes/differences between groups at post-test or vs. pre-test.
Rahim et al. (2017). Investigation into the effects of physical activity and honey supplementation on immunological parameters.	A randomised controlled trial. Study lasted for 8 weeks and participants remained within their assignments.	Aerobic dance sessions conducted for 1 h, 3 days per week, over 8 weeks. 15 min warm up, 30-35 min activity and cool down period. Movements involved, side stepping, fast walking, forward and backwards, leg lifts, lunging, step board exercise, and high impact movement in line with musical speed prompts. Moderate intensity activity.	Malaysian Gelam H (20 g) consumed with 300 ml of plain water consumed every day over the 8 wk intervention period vs. no supplement. Drink to be consumed 30 min prior to commencing activity if in H with exercise group.	Post-test WBC concentration → in H & exercise vs. control. No change or neutrophil counts between groups post-exercise. H & exercise elicited → CD8 T cells vs. H without exercise or exercise without H; and lymphocyte, CD4 cells similarly. Average HR from 120 – 140 bpm (60-70 % HR_{max}).

Table 2. Cont.

Ref and Aims	Study Design and Duration	Exercise Protocol Details	Dietary Intervention	Results Summary
Tartiban and Hajizadeh-Maleki (2012). Investigation into the effects of supplemented honey on plasma cytokines, oxidative stress and antioxidant activity.	Randomised, double blind treatment and placebo control groups. 8 weeks supplement period (2 x 4 wk).	Cycling over an 8 wk period. Workload increased at 25 w·min^{-1}. Completion of test was achieved when an established threshold was completed or the participant voluntarily finished exercise.	Participant consumed a fluid solution of 70 g of H dissolved in 250 ml water, or 70 g energy free PLA (artificial sweetener) ingested 90 min before exercise activity.	Lower elevated levels of IL-1β, IL-6 at several time points in H & exercise group. Significantly lower TNF-α values in H and exercise. SOD and TAC values → significantly in several time-points vs. PLA. Catalase variable between time points and groups. MDA ←significantly in H & exercise while changes in ROS were slight. Differences reported between groups for cycle 1 and 2 in km·wk^{-1} (377 and 649 respectively) with an extra 4 hours per week in cycle 2 (12 vs.16 h·wk^{-1}). The time spent performing at Low-intensity was increased.

← = Lower than/decrease. → = Higher than/increase. 1CTP = Carboxyl-Terminal Telopeptide of Type 1 Collagen. Acute supplementation - responses recorded on day of ingestion. ALP = Serum Alkaline Phosphatase. b = Complement Factor b. c3c & c4 = Complement Factor c3c & c4. CD4 & CD8 = Cytotoxic T-cells. CHO = Carbohydrate. Chronic supplementation - responses recorded over a time period of habitual ingestion. D = Dextrose. DNA = Deoxyribonucleic Acid. DOMS = Delayed Onset Muscle Soreness. GI = Glycaemic Index. GPx = Glutathione Peroxidases. H = Honey. HIR = High-Intensity Running. HR = Heart Rate. HSP-70 = Heat shock Protein. HT = Half-Time. IL = Interleukin Leukocytes. kcal = Kilocalorie. M = Maltodextrin. MDA = Malondialdehyde. OBLA = Onset of Blood Lactate Accumulation. ORAC = Oxygen Radical Absorbance Capacity. PLA = Placebo. PPO = Peak Power Output. RCT = Randomised Controlled Trial. ROS = Reactive Oxygen Species. RPE = Rating of Perceived Exertion. SOD = Superoxide Dismutase. SP = Sports Drink. TAC = Total Antioxidant Capacity. TNF-α = Tumour Necrosis Factor. TT = Time-Trial. W = Watts. WBC = White blood Cells.

4. DISCUSSION

The purpose of the present study was to systematically review the current body of literature pertaining to the physical performance and/or physiological responses to honey supplementation when combined with exercise. Based on the primary findings, it is plausible that consumption of honey may yield an ergogenic benefit on markers of physical performance. Additionally, habitual ingestion of honey attenuated the negative effects of some immunological and oxidative biomarkers caused by exercise. However, there is a lack of homogeneity relative to study design, methodologies, nutritional interventions (e.g. acute/chronic), assessment indices and participant characteristics. Therefore, although research trends towards a beneficial effect of honey consumption on physical performance and physiological responses, more research is needed.

4.1. Effect of honey supplementation on biochemical markers

4.1.1. Acute honey consumption around a single exercise session

Blood glucose concentrations have been reported in research studies investigating physiological responses to honey supplementation within a single exercise session, although results have lacked significance. Nevertheless, statistically significant ($p=0.04$) differences were observed (i.e., post-exercise vs. pre-exercise) following 80 min of rowing (2 x 40 min) when a 6.7 % honey solution (~15 min) and 7.8 % sucrose derived sports drink was ingested (Łagowska et al., 2017). Blood glucose concentrations were higher post-test (vs. pre-test) when

sucrose was consumed compared to honey. The osmolarity of the honey solution (402 $mOsm \cdot L^{-1}$) was much higher than sucrose (258 $mOsm \cdot L^{-1}$) and was postulated to likely delay gastric emptying and inhibit carbohydrate absorption in this case. It is true that delayed gastric emptying and glucose metabolism may occur when fruit is consumed exclusively (Vermeulen et al., 2011). This may explain the lower glucose concentration given the ingredients appear to be disproportionately weighted towards fructose (i.e., salt, banana, water, pineapple juice, lemon juice and honey). However, since the study did not disclose quantities of ingredients in relation to total composition, the metabolic effects of ingested honey can only be inferred. A honey and lemon beverage (i.e., as used by Abbey and Rankin, 2009), matched for carbohydrate content and digestive feasibility, may however improve physiological outcomes (e.g., glucose concentration).

In many team sports like soccer, it is recommended that optimal exogenous carbohydrates be consumed immediately before and at half-time to prevent hypoglycaemia (Hills and Russell, 2018). Only one eligible study investigated honey supplemented within these feeding windows (i.e., 0.5 $g \cdot kg^{-1}$ prior to and at half-time), which caused an increase in glucose concentration at post-test versus pre-test, and between trials, when 6 % honey or sports drinks (5.7 vs. 4.8 $mmol \cdot L^{-1}$) were consumed (Abbey and Rankin, 2009). However, due to methodological issues when reporting measurements (i.e., pre-exercise and post-exercise only), and non-disclosure of nutritional contributions, the glycaemic response of low GI honey during exercise when restricted feeding exists remains hypothetical. Comparatively, when high GI carbohydrates (i.e., 9.6 % and 5.6 % or 9.6 $g \cdot L^{-1}$ and 5.6 $g \cdot L^{-1}$) have been ingested continuously (i.e., every ~15 min) during a

soccer match simulation, a transient lowering of blood glucose ~60 min, indicative of hypoglycaemia (<4.0 $mmol \cdot L^{-1}$), had not been prevented (Russell et al., 2012; Kingsley et al., 2014). Such physiological responses have been linked to depressed physical performance due to changes in mood and/or cognitive impairment (Kingsley et al., 2014). Accordingly, more research investigating low GI honey supplementation, with outcomes reported continuously during soccer-specific exercise, would help ascertain its efficacy in preventing hypoglycaemia. Researchers should also disclose the GI of honey used to help clarify carbohydrate provision in order to translate results.

Exposure to prolonged and intensive exercise is recognised to induce transient increases in cytokine inflammatory biomarkers (Nieman, 2012). When assessed acutely, Abbey and Rankin (2009) noted that honey (6 %) lowered interleukin cytokines (i.e., IL-1ra, IL-6) within 1 h of soccer-specific exercise versus 6 % sucrose (i.e., 1.40 vs. 1.67, 3.16 vs. 3.67 $pg \cdot ml^{-1}$). Additionally, while pro-inflammatory IL-6 concentrations were higher immediately after exercise when honey was consumed versus sucrose or a placebo (6.90; 5.92; 6.63 $pg \cdot ml^{-1}$ respectively), there was a significantly higher concentration of anti-inflammatory IL-1ra when sucrose consumed (1.66 $pg \cdot ml^{-1}$; $p \leq 0.05$) versus honey (1.26 $pg \cdot ml^{-1}$). Whilst IL-6 produced by muscle fibres acts as a stimulant for IL-1ra (Nieman, 2012), the provision of antioxidants and phenolic compounds derived from honey were cited to delay the onset of IL-1ra in this instance. However, given that IL-1ra may attenuate the pro-inflammatory effects of cytokine IL-1 (Fischer, 2006), which were not reported, researchers may wish to consider reporting more immunological variables to ascertain the broad physiological immunological potential of acute honey ingestion to contrast.

4.1.2. Honey supplementation over multiple weeks

When the physiological effects of chronic honey supplementation and exercise have been studied over several weeks, attenuations to some immunological biomarkers are apparent. Notably, 70 g honey ingested ~90 min prior to cycling tended to lower pro-inflammatory plasma concentrations (IL-1 β , IL-6, IL-8, IL-10 and TNF- α) across eight time points over 16 weeks versus no supplementation (Hajizadeh-Maleki et al., 2016). Similar physiological outcomes were reported over eight weeks from semen samples (Tartiban and Hajizadeh-Maleki, 2012). However, given that pre-exercise diets were not controlled, it is unknown whether attenuations were influenced by honey or by pre-exercise carbohydrate that has been shown to moderate cytokine responses (Bishop et al., 2001; Chen et al., 2009). Moreover, it is posited that increased IL-6 concentrations indicates a need for energy substrate, and given the absence of a comparator carbohydrate in these studies, its increased presence would be expected (Proschinger and Freese, 2019). As such, it is important to be mindful of the possible bias in these results, and it would be prudent for researchers to implement better-controlled studies with ecologically valid carbohydrate and honey supplementation so physiological outcomes can be adequately contrasted for efficacy. This is especially true given that sugar (i.e., glucose, fructose) might stimulate the immune response in prolonged and intensive exercise (Bishop et al., 2002; Niemann et al., 2005; Hajizadeh-Maleki et al., 2016).

The immunological effects of chronic honey supplementation have been studied in an early investigation with contrasting results. No attenuations were

demonstrated when honey (i.e., 3 x 10 $ml \cdot d^{-1}$) containing plasmolysed yeast, malt, and orange juice, was consumed over 31 days (Gmünder et al., 1990). Specifically, serum globulins, complement factor b and c, IL-2, lymphocytes, beta cells and T-cell immune biomarkers were similar between the honey and placebo trials, perhaps due to low doses of honey. That said, T-cell effects were reported in another study among participants ingesting 20 $g \cdot d^{-1}$ for eight weeks (Rahim et al., 2017). T-cytotoxic (CD8), T-helper (CD4), and lymphocytes were all higher when honey was supplemented with aerobic dance, with no exercise, or exercise alone. The latter study was conducted among sedentary females at varying intensities, however, given that physical fitness and exercise intensity are purported to moderate immune response (Niemann and Wentz, 2019), the results do not represent athlete populations. Furthermore, researchers have suggested that excessive exercise of longer duration, training, and short recovery may induce pro-inflammatory cytokines, oxidative activity (Fischer, 2006; Kawamura and Muraoka, 2018), and influence physical performance declines (da Rocha et al., 2019). Further studies that take these variables into account will need to be undertaken before ascertaining the physiological (e.g. immunological, oxidative stress) and physical performance correlations amongst athletic populations when honey is consumed.

Despite the obvious benefits of exercise, exposure to prolonged and high-intensity exercise and regular physical activity are known to accentuate biomarkers of oxidative stress (Kawamura and Muraoka, 2018). However, discrepant results have been observed when chronic honey supplementation and exercise have been assessed (Daneghian et al., 2019). Notably, markers of lipid peroxidation (i.e., malondialdehyde) increased following exhaustive exercise

regardless of whether honey or placebo was consumed, while protein stabilisers (i.e., HSP-70) were more abundant when honey was ingested. Total antioxidant capacity (TAC), superoxide dismutase and glutathione peroxidases were also reported by the authors, albeit without time or trial interactions. Unfortunately, while nutritional ingredients were described, no other information was provided (e.g., dose, volumes). Therefore, replicating the interventions would be challenging. Moreover, since the type of honey was undisclosed, the carbohydrate or antioxidant effects/relationships can only be theorised. Indeed, the authors conceded the ingredients utilised in the formula had pro-oxidant effects, which counteracted TAC. Since the polyphenol, flavonoid, vitamin and mineral content of honey provide bioavailable antioxidants, for example buckwheat honey is antioxidant rich (Dżugan et al., 2018), manipulating honey (e.g., type, dose) may yield interesting results regarding antioxidative efficacy, which researchers may wish to explore.

Several additional studies have reported physiological measures specific to oxidative stress when honey was ingested chronically, however there is some heterogeneity between outcomes reported. For example, plasma ROS, DNA damage (Hajizadeh-Maleki et al., 2016), and malondialdehyde (Denaghian et al., 2019) have only been reported in individual studies, while antioxidant capacity/status, catalase, and superoxide dismutase have been reported in two studies (Hajizadeh-Maleki et al., 2016; Deneghian et al., 2019). Whilst it is accepted that a number of these are hard to report with accuracy due to relatively short half-lives (e.g., ROS), researchers conducting chronic investigations may wish to present full biomolecule profiles (e.g., DNA, protein, lipid) to assess oxidative damage and antioxidant status, for example, enzymatic, non-enzymatic

and TAC (see advice from Katerji et al., 2019). Moreover, as only one acute investigation measured an outcome specific to oxidative stress (Abbey and Rankin, 2009), more well-controlled research is needed pertaining to the antioxidant efficacy of acute honey supplementation within sports or exercise settings which are physically demanding.

4.2. Effect of honey supplementation on physical or skilled performance

A number of attempts have been made to quantify the physical performance effects of acute honey ingestion. Indeed, post-exercise ingestion of a 6.8 % honey solution (i.e., Acacia honey) in a volume equivalent to ~150 % of weight lost (i.e., three boluses of 30, 50, and 40 %) during a 60 min run, improved subsequent 20 min running performance when compared with water (Ahmad et al., 2015). Specifically, participants covered more distance (3420 ± 350 m or 3120 ± 340 m) when honey was consumed. Given that exogenous carbohydrates are recommended for consumption before exercise to ensure glucose availability and physical performance (Thomas et al., 2016), and immediately after exercise (i.e., for muscle glycogen repletion), such observations are not unexpected as water is void of energy (Ivy, 2004; Jeukendrup, 2014). Moreover, it could be argued that the study design is not ecologically valid in relation to practical settings (i.e., 2 h rehydration period prior to another run), while MTC solutions consumed up to four hours post exercise may stimulate liver glycogen resynthesis (Gonzalez et al., 2016; Maunder et al., 2018). Thus, in order to better determine the efficacy of low GI honey on subsequent running performance, it may be of interest to investigate sport-specific practical strategies, for example soccer players may play up to

three games per week under conditions of fixture congestion (Ranchordas et al., 2017).

The only study to disclose GI and report a corresponding measure of physical performance, compared 60 g gel solutions of honey (GI=35) with dextrose (GI=100) and a placebo ingested continuously (i.e., 15 g) during a 64 km cycling time-trial (i.e., at 0, 16, 32, 48 km intervals). Earnest et al. (2004) reported that the time taken to complete the trial was similar between honey and dextrose, being 128.0 versus 128.3 min respectively, while both were significantly quicker than the placebo (131.3 min). However, it should be noted that the aforementioned study withheld presentation of the nutritional information of either carbohydrate solution. As such, making comparative assumptions of the effect of carbohydrate in this case could be problematic. Using another low GI honey as a benchmark, for example, 98.2 g of Acacia Honey (Rowse; Wallingford: UK) provides ~80 g carbohydrate, however when weights are translated to the above investigation, 60 g of acacia honey contributes only 48.9 g carbohydrate. Given that oxidation of multiple transportable carbohydrates is known to extend beyond 60 $g \cdot h^{-1}$ (Jentjens et al., 2004; Triplett et al., 2010), it may a prudent approach to obtain more empirical evidence of the physical performance effects of manipulated honey doses, GI and timing (e.g., continuous as above, or when feeding is significantly restricted).

Only one study in this review investigated the physical performance effects of honey ingested when feeding restrictions apply (Abbey and Rankin, 2009). Notably, acute honey ingestion (6 %) had no significant effect on high-intensity running performance (i.e., 55 m covered at 120 % $\dot{V}O_{2max}$) versus a sucrose

sports drink and water ingested prior to (0.5 $g \cdot kg^{-1}$), and at half-time (0.5 $g \cdot kg^{-1}$), during soccer-specific exercise. However, whilst at the time of publication the simulated match protocol was appropriate (Kingsley et al., 2005), its half-time phase (10 min) and running stimulus (5 x 15 min blocks and 55 m sprints) are not representative of soccer matches. That said, when ecologically valid exercise protocols have been implemented to investigate low GI (GI=32) isomaltulose versus maltodextrin (GI=90-100) carbohydrates during 120 min soccer-specific exercise, sprint velocities (i.e., 15 and 20 m) were also similar between interventions (Stevenson et al., 2017). As soccer players run between 9 and 12 km, perform over 300 accelerations and decelerations, and execute ~40 technical actions per match, it is both aerobically and anaerobically taxing (Rampinini et al., 2009; Russell et al., 2016); although physical performance may also be behaviour-related and modulated by perception of effort (Sgherza et al., 2002). Thus, more well-controlled research adopting such variables would be welcomed to help determine the physical performance and physiological effects of acute honey supplementation.

4.3. Effect of honey supplementation on perceptual responses

Several studies have reported subjective perceptions of effort recorded when honey was ingested acutely (Earnest et al., 2004; Kreider et al., 2007; Abbey and Rankin, 2009; Ahmad et al., 2015; Łagowska et al., 2017). However, no between-study differences are evidenced when honey was compared with carbohydrate or placebo solutions. However, it should also be noted that due to inconsistencies in studies reporting specific doses (e.g. carbohydrate consistency and volume) of carbohydrates within this review (Table 2), and feeding procedures (i.e.,

continuous or restricted), generalising this data should be discouraged until more research is available. Nevertheless, concomitant increases in subjective perceptions of effort and time to exhaustion have been reported when mental fatigue has been deliberately induced via a ~90 min working memory task, albeit without carbohydrate (Marcora et al., 2009). However, given that evidence on the effects of cognitive fatigue on physical performance is generally equivocal (McMorris et al., 2018), an interesting opportunity exists for researchers to implement studies with demanding cognitive activity (i.e., manipulating motivation), so that perceptual responses can be analysed alongside physical and mental performance effects of acute honey supplementation.

Indeed, when perceptual responses to honey ingestion have been reported, discrepant results pertain to fluid acceptance/feasibility (e.g., flavour, texture, gut fullness). Notably, fluid consistency was reported as an issue (Łagowska et al., 2017), whilst acacia honey served cool at 8°C was perceived sweeter than water, and did not cause any palatability/acceptability issues regarding perceptions of thirst nausea, gut fullness and stomach upset (Ahmad et al., 2015). It should be noted that the latter study did not investigate an alternative solution, only water, so feasibility comparisons cannot be made in this instance. Similarly, powdered honey (i.e., 120 g with 40 g whey) returned perceptual responses in line with maltodextrin and sucrose carbohydrate gel solutions (Kreider et al., 2007). That said, the honey solution utilised by Łagowska et al. (2017) was high in fibre due to its fruit content (i.e., banana, pineapple juice, lemon juice) likely contributing to the reported fluid consistency issue and disrupting gastrointestinal comfort. Interestingly, commercially available carbohydrate solutions (6 %) have previously been reported to be palatable and encourage voluntary uptake,

possibly due to their sweetness and low serving temperature (Passe et al., 2000; Ahmad et al., 2015). Given that honey may have potentially beneficial effects on reducing perceptions of effort, as similar results have been demonstrated when MTC solutions ~90 $g \cdot h^{-1}$ have been ingested (Jeukendrup, 2013), researchers should consider reporting broad perceptual responses if assessing the physical performance effects of honey consumed in such doses. This would also clarify feasible doses.

4.4. Limitations and future research recommendations

Whilst the review is comprehensive and the first to solely explore human studies, the review does have its limitations. Notably, measured outcomes lack homogeneity, while research designs (e.g., acute and chronic), exercise stimuli and interventions (e.g., supplementation dose) also varied. Thus, it is largely unclear how results of honey supplementation studies compare or translate to other sports, under different physical demands. Moreover, Honey supplementation has been disproportionately investigated amongst male (n=59) versus female (n=21) participants in acute settings, while the opposite is true in chronic supplementation studies, when females (n=98) were better represented than males (n=71). As such, ensuring homogeneity and gender representation should be a priority for researchers. More specifically, when assessing oxidative variables, full biomolecule profiles (e.g., DNA, protein, lipid, enzymatic, non enzymatic and TAC) should be considered for comparability. Similarly, there is scope for researchers to explore multiple variables under a physical performance umbrella, and at a range of intensities (e.g., running, sprint, skill and mental/cognitive performance), which should be tested concomitantly alongside

blood glucose measurements throughout exercise. Researchers are guided to the work of Stevenson and colleagues (2017), for ecological validity (i.e., carbohydrate and exercise stimulus), which apply continuous outcome measures and robust scientific controls.

Indeed, the absence of scientific controls (e.g., crossover designs, comparator interventions) identified in some included studies should also be addressed. The honey solution utilised by Abbey and Rankin (2009) poses an interesting intervention option (i.e., 1 $g \cdot kg^{-1}$) as it is best weighted to current carbohydrate recommendations for exercise. Comparator interventions identified in this review as part of a randomised controlled trial is additionally advocated (e.g., sucrose, maltodextrin, or glucose and an energy free placebo). Additionally, the unclear risk of bias inherent in the Cochrane judgement presented regarding randomisation, allocation sequence and blinding suggest that observational studies would benefit from fully disclosing procedures to minimise selection, performance and/or detection bias. As such, it is possible that the findings presented are influenced by such limitations and subjective interpretation of risk thresholds. However, this review utilised a supporting quality assessment scale to help define the quality of overall research data. Therefore, the review presents useful information into the physiological and performance responses when honey supplementation is ingested as a primary carbohydrate source.

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4.5. Conclusions

This review has demonstrated an ergogenic benefit of acute honey ingestion in line with high GI carbohydrates (i.e., maltodextrin). Specifically, when 6.8 % honey solutions consumed continuously during exercise (i.e., cycling), or 6 %

ingested prior to and at half-time in soccer-specific exercise when feeding is restricted (~1 $g \cdot kg^{-1}$), power output and high-intensity running performance were similar to comparator carbohydrates. When ingested chronically (i.e., daily over several weeks), or before moderate-intensive exercise, honey has demonstrated effectiveness in attenuating biomarkers of oxidative stress including DNA damage, cytokines and peroxidative biomarkers, while antioxidant status is likely to be improved. Although research contributions are small, it appears that ingesting 70 g, ~90 min before intensive activity will benefit oxidative physiology, while immunology (i.e., CD cells) is likely to be improved following 20 $g \cdot d^{-1}$ doses. From this perspective, the data presented supports the use of honey supplementation to facilitate physical performance, while honey may have an advantage over other carbohydrates on some physiological outcomes.

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Appendices

Appendix 1. MEDLINE search strategy

Key word search list	Foodstuff	MeSH terms search list
Exercise* OR	Honey	Racquet Sports Or Sports
Physical activity OR		Athletic performance
Sport* OR		Swimming
Endurance* OR		Gymnastics or Gym
Aerobic* OR		Volleyball
Athlet$ or sport$ OR		Water Sports
Athlete* OR		Track and Field
Football OR		Resistance Training
Soccer OR		Baseball
Rugby OR		Hockey
Basketball OR		Running
Hockey OR		Football
Baseball OR		Soccer
Cricket OR		Athletes
Lacrosse OR		Exercise
Netball OR		Physical Exertion or Physical
Runn* OR		Endurance
Weightlift* OR		Sports Nutritional Sciences
Weight training or resistance		
training or strength training		
OR		
Olympic* OR		
Triathlon* OR		
Track and field OR		
Row* OR		
Volleyball OR		
Cycling* OR		
Cross country* OR		
Crossfit OR		
Swim* OR		
NCAA OR		
Anaerobic* OR		
Performance OR		
Physiological OR		
Physical OR		

MEDLINE search strategy was developed first due to MeSH term search. Later the keyword search was adapted to PubMed and SPORTDiscus.

Appendix 1. Cont. (SPORTDiscus Search strategy)

Key word search	Foodstuff
exercise	**Honey**
physical activity	
physical activity	
*sport	
endurance	
aerobic	
athlet$ or sport$	
football	
soccer	
rugby	
basketball	
*hockey	
baseball	
cricket	
lacrosse	
netball	
run*	
weightlifting OR weight lifting OR strength training	
weight training	
resistance training	
resistance exercise	
olympic	
triathl*	
track and field	
row*	
rower*	
rowing*	
volleyball	
cycli*	
cross country*	
crossfit	
NCAA	
anaerobic*	
*performance	

Retrieved 264 papers in SPORTDiscus database.

Appendix 1. Cont. (PubMed search strategy)

Keyword Search	Foodstuff	MeSH TERMS
performance exercise* physical activity physical exertion sport* endurance* aerobic* anaerobic* athlet$ or sport$ athlet* football soccer rugby basketball hockey baseball lacrosse netball runn* resistance exercise strength training resistance training weight lift* weightlift* weight training olympic* triathl* track and field row* volleyball cycli* cycle* cross country* crossfit swim* racquet* racket* tennis gymn* NCAA	Honey	Automatically searches MeSH terms

Retrieved 1282 papers in PubMed database. These searches have been developed using keywords from scoped lit searching.

Appendix 2.Screening and selection tool for included studies

REVIEW QUESTION: What is the evidence for determining the efficacy of using honey as a supplement in ergogenic strategies in sports performance?			
Inclusion criteria based on PICO Population – male or female or both if comparison group similar, no age specification. Intervention- honey in any format consumed over time or on day of testing. Comparator- treatment vs. placebo or treatment vs. comparator and placebo. Outcomes- physiological OR physical performance data. Study design- experimental (ideally RCT as is the gold standard)			
SYSTEMATIC REVIEW SCREENING AND SELECTION TOOL			
Reviewer name:		**date:**	
Author name/ study id:			
Title: The Physiological and Performance Effects of Honey Consumption in Sport and Exercise: A Systematic Review			
Patient population	**Include** Human Age between 16 and 60.	**Exclude** Animal	
Interventions	**Include** Must include an exercise stimulus. Must represent physical exertion.	**Exclude** Any studies which avoid significant exercise stimulus in their study designs.	
Comparators	**Include** Placebo, alternative treatments. White blood cell counts.	**Exclude** No comparator outcomes	
Outcomes	**Must include** Outcomes relative to physical endurance should be considered. This should include, although not limited to: HR, glucose measurements, force, lactate, power output, physical performance.	**Exclude**	
	May include Some studies outcomes present data on body fluids (e.g., urine, blood). This is ok. Other health related outcomes.		
Study design	**Include** RCT	**Exclude** Not an RCT? or systematic review/meta-analysis	

Example of PICO table and search and selection tool used to define suitable research articles.

Appendix 3. PEDro quality assessment table

Reference	Eligibility criteria explained	Random allocation	Concealed allocation	Baseline measures similar between	Key outcomes	Subjects	Researcher	Assessor	85 % participants completed	Intention to treat	Between group statistical comparisons made	Variability presented	Total Pedro score
Guta et al. (2018)	✓	✓	✗	✗	✗	✗	✗	✗	✗	✓	✓	✓	5/10
Kreider et al. 2002	✗	✗	✗	✗	✗	✓	✓	✗	✓	✓	✓	✗	5/10
Ovchinnikov et al.(2016)	✗	✗	✗	✓	✓	✗	✗	✗	✓	✗	✓	✓	5/10
Abbey and Rankin (2009)	✓	✓	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	10/10
Ahmad et al. (2015)	✓	✓	✓	✗	✓	✗	✓	✓	✓	✓	✓	✓	9/10
Earnest et al. (2004)	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	9/10
Kreider et al. (2007)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	10/10
Łagowska et al (2017)	✓	✓	✓	✗	✓	✗	✗	✗	✗	✓	✓	✓	7/10
Deneghian et al. (2019)	✓	✓	✓	✗	✓	✓	✓	✗	✗	✓	✓	✓	8/10
Gmünder et al. (1990)	✗	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	8/10
Hajizadeh-Maleki et al. (2016)	✓	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✗	9/10
Ooi et al. (2011)	✗	✗	✗	✓	✓	✗	✗	✗	✓	✓	✓	✓	7/10
Rahim et al. (2017)	✓	✓	✗	✓	✓	✗	✗	✗	✓	✓	✓	✓	8/10
Tartiban and Hajizadeh-Maleki (2012).	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✗	8/10
Criterion	**1**	**2**	**3**	**4**	**4, 7-11**		**5-7**		**8**	**9**	**10**	**11**	

Appendix 4. Cochrane risk of bias assessment

Entry	Judgement	Support for judgement
Abbey and Rankin 2009		
Random sequence generation (selection bias)	Unclear risk	Quote: "Ten experienced male soccer players randomly performed 3 trials" Comment: Probably done as randomisation declared albeit not sufficient to classify as low risk as per Cochrane bias guidelines
Allocation concealment (selection bias)	Low risk	Quote: "The participants were not informed about the formulation of the beverages to remove any bias in performance" Comment: Completed given the attention focused towards obtaining an exit survey with focused questioning
Blinding of participants and personnel (performance bias)	Low risk	Quote: "In an exit survey administered after completion of all performance tests, the percentages of participants who correctly identified the H, S, and P treatments were 50 %, 20 %, and 20 %, respectively" Comment: Probably done
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	Unclear risk	No mention of detection bias Comment: only 20 % assumed placebo and sucrose solutions. While no procedures noted, it is unclear what shielding contributed to the aforementioned statistic.
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	3 weeks: 12 recruited 10 completed all outcomes. 2 did not due to health or scheduling conflicts. ~83 %
Incomplete outcome data addressed (attrition bias) (Longer-term outcomes (>6 weeks))	Low risk	Crossover study design (3 trial days) – not perceived relevant in this case
Selective reporting (reporting bias)	High risk	Results reflect methods used Statistical methods appear to be suitable Several cytokine results (1 hr post-test) significantly lower than immediate post-test - expected given relatively short half-lives Mostly done but high risk for some outcomes
Other bias	Low risk	No anomalies regarding participant characteristics, intervention (volumes) Sample size in line with several other studies of this type. External validity appropriate given the age of the study
Ahmad et al. (2015)		
Random sequence generation (selection bias)	Unclear risk	Quote: "The drinks were given to the subjects in a random order." Comment: Randomisation methods not sufficient to categorise as low risk although as per description, we cannot assume not done either
Allocation concealment (selection bias)	Low risk	Quote: "This randomization and distribution was done by a laboratory technologist" Comment: Probably done as completed by a third party, which Cochrane defines as the most appropriate method
Blinding of participants and personnel (performance bias)	Unclear (as medium classification)	Quote: "The present study was a single blind study, thus the researcher did not know about the type of drink the subjects were prescribed during each experimental trial " Comment: Partially achieved
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	Unclear (as medium classification)	Quote: "single blind". Comment: May affect participant reported outcomes (fluid sensation) but not researcher assessed outcomes. Consider a 'medium' risk but for primary outcomes low.
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	2 weeks: 10 participants were recruited and completed outcome assessments Comment: Achieved

Appendix 4. Cont.

Entry	Judgement	Support for judgement
Incomplete outcome data addressed (attrition bias) (Longer-term outcomes (>6 weeks))	Low risk	As above. Comment: 100 % participants completed the study although this category is not appropriate As such, low risk
Selective reporting (reporting bias)	Low risk	Appears to be similar (methods vs. results) Comment: probably achieved
Other bias	High risk	No comparator carbohydrate provided (water vs. honey) Comment: assume high risk given favourable outcomes skewed toward intervention.
Earnest et al. (2004)		
Random sequence generation (selection bias)	Unclear risk	Comment: Reference of randomisation refers only to treatment administration creating doubt as to how randomisation achieved
Allocation concealment (selection bias)	Unclear risk	Comment: In line with Cochrane guidance, implementation of a specific allocation sequence not described, only that randomisation existed so remains unclear.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Double blind, counter-balanced" Comment: Probably done
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	Low risk	Quote: "double blind" Comment: Probably achieved
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	Comment: 3 week crossover study with all 9 recruited participants contributing to outcomes
Incomplete outcome data addressed (attrition bias) (Longer-term outcomes (>6 weeks))	Low risk	Comment: 3 week study only
Selective reporting (reporting bias)	Low risk	Comment: While statistical methods are similar to other studies, the method – results seem to be well linked.
Other bias	Low risk	Comment: No obvious anomalies
Kreider et al. (2007)		
Random sequence generation (selection bias)	Unclear risk	"subjects received in a double blind and randomized manner" Comment: Probably done but no reference to sequence generation so in-sufficient information provided to categorise as low risk in line with Cochrane guidance
Allocation concealment (selection bias)	Unclear risk	"Consequent to randomization procedures, total lifting volume, fasting glucose and insulin concentrations were different between groups" Comment: Safe to assume procedures implemented based on above declaration, however allocation concealment/randomisation procedures not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "subjects received in a double blind and randomized manner a CHO and PRO supplement containing 40 g of whey PRO with 120 g of sucrose (S), powdered honey (H), or maltodextrin (M)" Comment: Probably achieved
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	Low risk	Quote: "double blind" Comment: Probably done

Appendix4. Cont.

Entry	Judgement	Support for judgement
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	40 participants started and 40 participants completed the study
Incomplete outcome data addressed (attrition bias) (Longer-term outcomes (>6 weeks))	Low risk	12 weeks: N/A study over 1 experimental session
Selective reporting (reporting bias)	Low risk	Methods and results appear well linked and statistics appear fine
Other bias	Unclear (as medium classification)	Maltodextrin group appear to be slightly different than other comparator groups given prognostic indicators
Łagowska et al. (2017)		
Random sequence generation (selection bias)	Unclear risk	Comment: Randomisation identified but no further evidence of random allocation
Allocation concealment (selection bias)	Unclear risk	Comment: Although central randomisation procedures not described, hard to ascertain whether done or not done from publication
Blinding of participants and personnel (performance bias)	High risk	Comment: No declaration of blinding so hard to ascertain who was blinded increasing risk of performance and detection bias
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	High risk	Comment: As above
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	Data complete
Incomplete outcome data addressed (attrition bias) (Longer-term outcomes (>6 weeks))	Low risk	As above
Selective reporting (reporting bias)	Low risk	Suggested methods and reports appear well linked
Other bias	Unclear risk (as medium classification – not high or low)	Intervention fluid high in osmolality and likely consistency affecting sensory evaluation No other anomalies observed
Deneghian et al. (2019).		
Random sequence generation (selection bias)	Low risk	Quote: "The training coach, who was not aware of random sequences, assigned the participants to the numbered boxes of supplements" Comment: Probably done, given the above admission
Allocation concealment (selection bias)	Low risk	Quote: "Randomization was done using Excel (random number generation), using Bernoulli distribution with $P = 0.5$". Comment: Completed to good standard given explanation of technique used as per Cochrane recommendations
Blinding of participants and personnel (performance bias)	Low risk	Quote: "double blind" Comment: assume criteria satisfied

Appendix 4. Cont.

Entry	Judgement	Support for judgement
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	Low risk	Quote: "The training coach, who was not aware of random sequences, assigned the participants to the numbered boxes of supplements" Comment: Safe to assume risk is low given the procedures in place to minimise risk of bias regarding individuals measuring/reporting outcomes
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	4 weeks: 24/24 allocated and received the intervention 12/12 – placebo, 12/12 intervention
Incomplete outcome data addressed (attrition bias) (Longer-term outcomes (>6 weeks))	High risk	Follow up: 4/12 missing from intervention group; 2/12 missing from control group 75 % total recruited
Selective reporting (reporting bias)	Low risk	Statistics appear suitable while methods and results are aligned
Other bias	Unclear risk (as medium classification)	Questionable whether 3 x 38 sessions p/w at 60-65 % over 4 weeks truly provides evidence of training adaptions
Gmünder et al. (1990)		
Random sequence generation (selection bias)	Unclear risk	Quote: "They were randomly assigned…" Comment: In line with Cochrane guidelines, an incompletely defined approach exists here As such, unclear risk of bias must be assumed
Allocation concealment (selection bias)	Unclear risk	As above, incompletely defined Comment: Ideally, a third party would perform central randomisation to be considered safe, but without a declaration this cannot be ascertained. That said, wrong to assume not done either.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "The effect of a food supplement on immunological parametersof16long-distance runners was tested in a randomized, double-blind and placebo-controlled trial" Comment: Probably done
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	Low risk	As above Comment: Probably done
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	27 days: No problems reported
Incomplete outcome data addressed (attrition bias) (Longer-term outcomes (>6 weeks))	Low risk	As above
Selective reporting (reporting bias)	Low risk	Methods clearly reported and described how achieved
Other bias	Low risk	Mix of male and females in each group, which likely affects baseline values (although similar between groups) while physiology different (e.g., cortisol production)
Hajizadeh-Maleki et al. (2016)		
Random sequence generation (selection bias)	Low risk	Quote: "Randomization was performed by random number generation, and group assignment was placed in a sealed envelope, which was opened by the study coordinator at the time of randomization" Comment: Criteria sufficiently satisfied

Appendix 4. Cont.

Entry	Judgement	Support for judgement
Allocation concealment (selection bias)	Low risk	Quote: As above Comment: Probably done
Blinding of participants and personnel (performance bias)	Unclear (as medium risk)	Quote: "…as well as including a double-blinded placebo controlled setup would have strengthened our conclusions" Comment: Safe to assume double-blinding not done in this case as above, however, it is still unclear whether single blinding took place which was attained in an earlier study (Tartiban and Maleki, 2012).
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	Unclear (as medium risk)	As above Comment: unclear
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	4 weeks (protocol): n=29 randomised to study protocols
Incomplete outcome data addressed (attrition bias) (Longer-term outcomes (>6 weeks))	High risk	16 weeks (analysis): 3/15 missing from intervention group; 2/14 missing from control group Lost to exercise adherence (n=1); dietary adherence (n=3); injury (n=1)
Selective reporting (reporting bias)	Low risk	Statistics similar to others and indicative of research group while methods link well with results
Other bias	Low risk	Prognostic characteristics similar between groups
Ooi et al. (2011)		
Random sequence generation (selection bias)	High risk	No mention of how randomisation/sequences generated
Allocation concealment (selection bias)	High risk	In line with Cochrane guidance, specific methods (e.g., third party randomisation) not disclosed in this case
Blinding of participants and personnel (performance bias)	High risk	Blinding not disclosed
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	Low risk	Although no mention of blinding, no subjective measures recorded
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	6 weeks: 3/40 missing across groups due to other commitments. (92.5 %)
Incomplete outcome data addressed (attrition bias) (Longer-term outcomes (>6 weeks))	Low risk	As above
Selective reporting (reporting bias)	Low risk	Links between methods and results well linked, while statistics appear appropriate
Other bias	Low risk	No anomalies
Rahim et al. (2017).		
Random sequence generation (selection bias)	Unclear risk	Quote: "The participants were matched in age, body mass, body height and body fat before they were randomly assigned into the experimental groups". Comment: According to Cochrane guidance, this information in useful, although without details on how it was achieved, the adequacy of sequence generation remains unclear

Appendix 4. Cont.

Entry	Judgement	Support for judgement
Allocation concealment (selection bias)	Unclear risk	No mention of how sequences generated. Comment: Randomisation occurred, as such unsafe to suggest sequences did not exist.
Blinding of participants and personnel (performance bias)	High risk	Quote: no declaration of blinding or measures to achieve it Comment: Probably not done
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	High risk	As above Comment: Probably not done
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	40/44 completed the study (90 %) – 2/11 (honey group) and 2/11 (honey with exercise) Drop outs due to pregnancy or personal circumstance - (19 % per group) – within the 20 % drop out rate expected.
Incomplete outcome data addressed (attrition bias) (Longer-term outcomes (>6 weeks))	Low risk	8 weeks: as above for final analysis
Selective reporting (reporting bias)	Low risk	Methods and results well aligned
Other bias	Low risk	No clear anomalies
Tartiban and Hajizadeh-Maleki (2012).		
Random sequence generation (selection bias)	Low risk.	Quote: "subjects were randomly assigned" … "using a table of random numbers" Comment: Low risk as per Cochrane guidelines
Allocation concealment (selection bias)	Unclear risk	As per data from above, procedures of randomisation carefully considered and although concealment not describe, we cannot ascertain whether complete or incomplete Comment: categorise as unclear accordingly
Blinding of participants and personnel (performance bias)	Low risk	Quote: "double blind" Comment: Probably done
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	Low risk	Quote: "double blind" Comment: Probably done
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	4 weeks: 39 participated in study – unclear on recruitment vs. completion Comment – assume zero drop out rate
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))	Low risk	As above at 8 weeks
Selective reporting (reporting bias)	Low risk	Study appears well linked regarding methods and results
Other bias	Low risk	No other anomalies regarding study design and baseline prognostic indicators appear similar between groups

Appendix 5. Measured physiological biomarkers.

Physiological measurement	Supporting study	Physiological category
Blood/Serum samples		
Cortisol	Abbey and Rankin (2009); Kreider, et al (2007); Gmünder, et al (1990).	Hormone Response
Testosterone	Kreider, et al (2007).	Hormone Response
Cytokines - (IL-2, IL-6, IL-10, IL-1ra).	Abbey and Rankin (2009); Gmünder, et al (1990); Hajizadeh-Maleki, et al (2016).	Oxidative Stress
MDA – Malondialdehyde	Deneghian, et al (2019).	Oxidative stress
DNA damage	Hajizadeh-Maleki, et al (2016).	Oxidative stress
Lipid Peroxidation	Hajizadeh-Maleki, et al (2016); Deneghian, et al (2019).	Oxidative stress
ROS	Hajizadeh-Maleki, et al (2016).	Oxidative stress
SOD	Deneghian, et al (2019); Hajizadeh-Maleki, et al (2016).	Antioxidant physiology – breakdown oxygen molecules
GPx	Deneghian, et al (2019).	Antioxidant physiology – oxidative protection
HSP-70	Deneghian, et al (2019).	Antioxidant physiology
TAC/TAS – Total Antioxidant Capacity OR Status	Deneghian, et al (2019); Hajizadeh-Maleki, et al (2016).	Antioxidant physiology - metabolites
Plasma Antioxidant Capacity	Abbey and Rankin (2009).	Antioxidant physiology - metabolites
FRAP	Łagowska, et al (2017).	Antioxidant physiology
ORAC	Abbey and Rankin (2009).	Antioxidant physiology - capacity
Serum Lactate	Łagowska, et al (2017).	Blood serum physiology
Plasma Volume	Abbey and Rankin (2009).	Blood serum physiology
Glucose	Abbey and Rankin (2009); Ahmad, et al (2015); Earnest, et al (2004); Łagowska, et al (2017).	Blood Serum Physiology-metabolites
Insulin	Abbey and Rankin (2009); Ahmad, et al (2015); Earnest, et al (2004); Kreider, et al (2007).	Blood Serum Physiology-metabolites
Heamoglobin and/or Heamatocrit	Abbey and Rankin (2009); Ahmad, et al (2015); Deneghian, et al (2019); Łagowska, et al (2017).	Blood serum physiology - red blood cells and immunology.
Immunoglobulins – IgG, IgG subclass 1, IgG subclass 2, B-2 microglobulin	Gmünder, et al (1990).	Blood serum physiology - red blood cells and immunology.
White Blood Cells – lymphoytes, monocytes, granulocytes	Łagowska, et al (2017); Gmünder, et al (1990); Hajizadeh-Maleki, et al (2016); Rahim, et al (2017).	Blood serum physiology - white Blood cells
T cells – CD3, CD4, CD8	Gmünder, et al (1990); Rahim, et al (2017).	Blood serum physiology -red blood cells and immunology.
Serum Osmolality	Ahmad, et al (2015).	Blood serum physiology - electrolyte water balance
Hepatorenal – creatinine and BUN	Kreider, et al (2007).	Blood serum physiology - kidney, urea
Muscle and Liver Enzymes - lactate dehydrogenase, creatine Kinase, aspartate aminotransaminase, alanine aminotransaminase	Kreider, et al (2007); Łagowska, et al (2017).	Blood serum physiology - enzyme metabolites
Bone formation biomarkers – ALP	Ooi, et al (2011).	Blood serum physiology - enzyme metabolites
Bone Resorption Biomarkers- 1CTP	Ooi, et al (2011).	Blood serum physiology - enzyme metabolites

Appendix 5: Cont.

Physiological measurement	Supporting study	Physiological category
Semen samples		
Cytokines – Interleukin (IL).	Tartiban and Hajizadeh-Maleki (2012).	Oxidative Stress
ROS	Tartiban and Hajizadeh-Maleki (2012).	Oxidative Stress
TAC/S	Tartiban and Hajizadeh-Maleki (2012).	Antioxidant physiology - metabolites
Catalase	Tartiban and Hajizadeh-Maleki (2012).	Antioxidant physiology – oxidative protection
SOD	Tartiban and Hajizadeh-Maleki (2012).	Antioxidant physiology – breakdown oxygen molecules

1 CTP = C-terminal telopeptide of type 1 collagen. ALP = Serum Alkaline Phosphatase. BUN = Blood Urea Nitrogen. **FRAP =Ferric Reducing Antioxidant Power. GPx = Glutathione Peroxidases. HSP-70 = Heat Shock Protein**. **IgG = Immunoglobulin. IL = Interleukin. MDA = Malondialdehyde. ORAC = Oxygen Radical Absorbance Capacity. ROS = Reactive Oxygen Species. SOD = Superoxide Dismutase. TAC = Total Antioxidant Capacity. TAS = Total Antioxidant Status. WBC = White blood Cells.**

Appendix 6. Nutritional composition of honey

Component	Average Composition Weight (·100 g^{-1}) Blossom Honey	Range Weight (·100 g^{-1}) Blossom Honey
Water	17.2	15-20
Total Sugars	79.7	
Monosaccharides		
Fructose	38.2	30-45
Glucose	31.3	24-40
Disaccharides		
Sucrose	0.7	0.1-4.8
Others	5	2-8
Trisaccharides		
Melezitose	<0.1	
Erlose	0.8	0.5-.6
Others	0.5	0.5-1
Undetermined oligosaccharides	3.1	
Vitamins		
B1 (mg)	0.01	
B2 (mg)	0.038	
B3 (mg)	0.21	
B5 (mg)	0.068	
B6 (mg)	0.024	
B9 (µg)	2	
C (mg)	0.5	
Minerals	0.2	0.1-0.5
N (g)	0.041	
Fe (mg)	0.42	
K (mg)	52	
Ca (mg)	6	
P (mg)	4	
Mg (mg)	2	
Cu (µg·g^{-1})	1-100	
Zn (mg)	0.22	
Amino Acids-Proteins	0.3	0.2-0.4
pH value	3.9	3.4-4.5

Extracted from Bogdanov, et al (2008) and Ahmed, et al (2018).

Appendix 7. Phenolic compounds in honey

Compound	Honey Varietal
Phenolic acids	
Coumaric	Tualang, Gelam, Acacia
Caffeic	Tualang, Gelam, Acacia
Ferulic	Tualang, Gelam, Acacia
Cinnamic	Tualang, Gelam, Acacia
Chlorogenic	Tualang, Gelam, Acacia
Flavonoids	
Pinobanksin-3-0-propionate	Tualang, Gelam
Pinobanksin-3-0-butyrate	Tualang
Quercetin	Tualang, Gelam, Acacia
Organic acids	
Fumaric	Tualang, Gelam, Acacia
Gluconic	Tualang, Gelam, Acacia

Extracted from Chua, et al (2013).