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REVIEW ARTICLE

A meta-analytic comparison of the effects of consuming carbohydrate with and without protein on postexercise plasma insulin and glucagon responses in healthy, trained males

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ABSTRACT

This meta-analysis evaluates how hydrolysed protein and carbohydrate (CHO) mixtures compare with intact protein and CHO mixtures regarding post-exercise plasma insulin and glucagon responses in healthy endurance trained males. Studies measuring insulin and/or glucagon following an exercise bout with ingestion of CHO vs. CHO+ protein were included. Random-effects meta-analyses were conducted on the insulin peaks over time. Overall, 33 trials from 20 articles were included. The ingestion of CHO+ protein induced significantly higher insulin peaks than ingestion of CHO only from 30 to 240 minutes postexercise (30-180 min: $p < .001$, 210-240 min: $p < .01$), higher insulin area under the curve ($p < .001$), and greater muscle FSR ($p < .001$). No statistically significant differences on insulin peaks over time were found between the ingestion of CHO+ intact protein and CHO+ hydrolysed protein or differences in muscle glycogen synthesis rate or glycogen peaks. Findings provide evidence the co-ingestion of CHO+ protein is a better strategy for recovery for endurance-type male athletes than the ingestion of CHO only. However, more research is warranted to understand whether there are differences between the ingestion of intact protein and its hydrolysed counterpart with CHO, and the impact on glucagon responses.

Keywords: Plasma glucose; Muscle FSR; Muscle glycogen synthesis; Sports nutrition.

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INTRODUCTION

Muscle glycogen is the most important fuel source during moderate- to high-intensity exercise, the amount of which can decrease rapidly during prolonged endurance-type exercise or high-intensity exercise of relatively short duration (Burke, 2004; van Loon *et al.*, 2000; Ivy *et al.*, 2003; Jentjens *et al.*, 2003; Kaastra *et al.*, 2006). Carbohydrate (CHO) ingestion plays a decisive factor of the rate of muscle glycogen synthesis (Burke *et al.*, 1993; Burke *et al.*, 2004; Costill *et al.*, 1981), and can inhibit muscle protein breakdown to further spare muscle protein from consumption after exercise (Beelen *et al.*, 2008; Børsheim *et al.*, 2004). Therefore, it is essential to ingest adequate amount of CHO to optimize glycogen synthesis rates after exercise (Ivy *et al.*, 1988a). However, how different factors, such as the type of CHO and administration frequency, moderate the muscle glycogen repletion rate remains unclear.

Previous studies discovered that co-ingestion of small amounts of protein and/or amino acids (AAs) with CHO may further accelerate muscle glycogen repletion and reduce muscle damage by increasing net muscle protein availability (Koopman *et al.*, 2005; Miller *et al.*, 2003; Van Loon *et al.*, 2000b; Hausswirth *et al.*, 2011). This is considered to be led by the synergistic effect of the ingestion of carbohydrate and protein mixtures, which promotes a greater insulin response (Nuttall et al., 1984; van Loon et al., 2000b). Such increase in insulin responses has been suggested to accelerate plasma glucose metabolism and thus increase the efficiency of glycogen repletion by increasing the activity of glycogen synthase (Bak *et al.*, 1991; Ivy *et al.*, 1998). Specifically, Kaastra (2006) found that co-ingestion of a casein protein hydrolysate (0.4 $g \cdot kg^{-1} \cdot h^{-1}$) with CHO (0.8 $g \cdot kg^{-1} \cdot h^{-1}$) induced a more than two-fold insulin response compared to CHO ingestion alone during postexercise recovery in endurance-trained cyclists. The insulin response could be further stimulated by the addition of free leucine. Hence, it is generally recommended to ingest both CHO and protein for postexercise recovery. However, the exact amount and type of protein source and the desired timing for protein administration are still under considerable debate.

Another potential aspect of protein ingestion is that protein may play an important role in weight management, although no meta-analyses or reviews have been conducted to address this question. Studies have shown that protein induces a higher thermic effect of food (TEF) (Calcagno *et al.*, 2019). Although TEF accounts for only 10% of total energy expenditure (TEE), having protein in the diet has been demonstrated to be a feasible long-term strategy for weight management (Calcagno et al., 2019). Furthermore, TEF may contribute to a higher percentage of TEE in subjects with higher aerobic capacity (Hill *et al.*, 1984). In other words, this dietary strategy may be more effective for endurance-type athletes such as cyclists or marathon runners.

A reasonable explanation to the protein-induced TEF is that the glucagon concentrations will increase with the elevated plasma AA levels after protein ingestion (Farnfield *et al.,* 2009; Gannon *et al.,* 2010; Ohneda *et al.,* 1968; Unger *et al.*, 1969; van Loon *et al.*, 2000a) in order to metabolize hepatic AA (Holst *et al.,* 2017). Insulin and glucagon have to interact synergistically to promote plasma AA clearance and postprandial AA disposal (Ang *et al.*, 2019). Meanwhile, protein-induced hyperglucagonemia can effectively prevent a decline in blood glucose concentration secondary to aminogenic insulin secretion during CHO-free protein meals (Ohneda *et al.*, 1968; Unger *et al.*, 1969). Therefore, glucagon secretion should be expected for the maintenance of normal plasma glucose levels (i.e., euglycemia) following protein or AA ingestion. More importantly, by measuring the glucagon responses after protein ingestion, it is possible to understand how different types of proteins could be helpful for weight management in endurance-typed athletes.

It is worth noting that proteins can be further categorized into intact and hydrolysed protein, also named protein hydrolysate. Protein hydrolysate contains mostly di- and tripeptides, and is suggested to be absorbed

more rapidly than free AA and intact proteins, which may lead to a faster increase in plasma AA (Claessens *et al.*, 2008; Koopman *et al.*, 2009; Manninen *et al.*, 2004). Protein hydrolysate has gained success and been widely used for patients with gastrointestinal tract disorder as a great protein source due to this higher digestibility than normal intact proteins (Potier *et al*., 2008). However, such advantage seems inconspicuous in healthy individuals. Few studies have addressed to whether there are meaningful differences between the ingestion of intact protein and its hydrolysed counterpart for healthy individuals.

Objectives

The aim of the present study included two interests: 1) to evaluate whether there is a difference between the ingestion of protein with CHO and the ingestion of CHO alone; and 2) to conduct a meta-analysis to evaluate how hydrolysed protein with CHO mixtures compare with intact protein with CHO mixtures in regards to post-exercise plasma insulin and glucagon responses in healthy endurance trained male subjects. Plasma insulin responses were used as a measurement of postexercise recovery quality due to a positive correlation between insulin responses and muscle glycogen repletion and protein synthesis, and glucagon responses were used to examine the potential influences of protein ingestion on weight management. The primary outcomes evaluated were the insulin peaks over time and area under curve (AUC), and glucagon peaks over time. Other secondary outcomes included muscle glycogen storage, glycogen synthesis rate, muscle fractional synthesis rate (FSR), and plasma glucose peaks over time.

METHODS

Eligibility criteria

Studies published before August 2021 were reviewed. Eligible studies were English-language reports of insulin and/or glucagon response after post-exercise interventions conducted with healthy trained male subjects. Only peer reviewed, randomized controlled trials (RCTs) were included. Studies had to have been conducted with healthy trained male subjects aged 18-65. Since the definition of a trained individual is not consistent among different articles, for the purpose of this meta-analysis, anytime authors described their sample as trained, or included professional or college-level athletes, they were considered trained individuals. If a sample was only described as healthy adults or subjects, or as adults who were obese or insulin-resistant, they were excluded. The supplementation type was liquid only, and the control trial was the ingestion of CHO alone. No restrictions on the concentration and frequency of each macronutrient intake were made. All units were integrated to be effectively measured and compared.

Study selection

Studies for potential inclusion were found by searching the following electronic databases: PubMed, Google scholar, Cochrane, and MDPI. Two search strategies were employed. Information sources included electronic databases and author searches. Search terms included protein*, insulin*, glucagon*, and athlete terms (athlete*, trained*). The star symbol (*) was used to capture derivatives (by suffixation) of the search terms. Computerized author searches were completed for corresponding authors of eligible studies.

A staged eligibility determination process was used to identify eligible studies for this meta-analysis in order to ensure all eligible studies for any part of the parent project reached the coding phase. First, title and abstracts were reviewed for visual heralds suggesting a potentially eligible study. Second, full reports were examined to determine whether the study included a post-exercise (usually involved cycling until depletion) protein intervention in healthy male participants. Third, potential primary studies were examined for any eligible primary outcomes for the parent study. Fourth, potential primary studies were evaluated to calculate insulin and glucagon response over time and AUC to identify the optimal type of post-exercise protein mixture.

Finally, studies that included secondary outcomes were evaluated to compare with the results of primary outcomes.

Data collection process

Data was extracted from studies by the lead author, with oversight second author. Raw data was mainly obtained from the research articles. For eligible studies without complete data presented in tables/narratives, the corresponding author was contacted via e-mail or the data was extracted from graphs using WebPlotDigitizer (Rohatgi, 2020). All included papers were subject to an assessment of risk of bias based on guidelines outlined by the Cochrane Handbook for Systematic Reviews of Interventions (Cochrane Collaboration, 2007). To assess for risk of publication bias, study sample size and effect sizes were evaluated using a funnel plot (Borenstein *et al.*, 2009).

Statistical analysis

A random-effects mean-comparison meta-analysis was conducted to assess if differences exist on the post-exercise insulin and glucagon responses by the ingestion of protein with CHO as compared to the ingestion of CHO alone across the studies retrieved in RStudio. As part of analyses, an overall effect size and confidence intervals were calculated in order to determine how the post-exercise insulin and glucagon responses varies across independent variables in the studies retrieved. The missing standard deviations (SD) in any trials were replaced by the average score of all the available SDs at the indicated time. Data analyses were conducted using RStudio 2021.09.0 build 351.

RESULTS

Overview of included studies

Overall, 286 potential articles were identified, of which 28 articles were duplicated. After excluding the duplicated articles, 258 were screened, and all abstracts were reviewed. Initial review resulted in 194 articles excluded, and the remaining 63 articles were reviewed. A total of 44 studies were finally excluded because their study design did not meet with the eligibility criteria of this study, such as female participants (or inability to distinguish findings by participant sex), before and/or during exercise supplementation, supplements containing lipids, untrained participants, or not reporting on the outcomes of interest. Ultimately, 33 trials derived from 20 articles were included in this meta-analysis (Table 1) with 32 trials reporting insulin peaks over time and subgroup analyses, 9 insulin AUC, 9 muscle FSR, 30 trials were included for plasma glucose peaks over time, 6 with muscle glycogen synthesis rate, 8 for muscle glycogen storage, and 5 trials were included for plasma glucagon peaks over time. No publication reported glucagon AUC (Figure 1).

Plasma insulin peaks over time

The included trials reported insulin peaks from 30 to 240 minutes, respectively. The insulin data of 3 trials (Churchward-Venne et al., 2020) at 40 minutes were used as and compared with other 30-minute data. In order to make all data consistent in having only one peak over the testing periods, the data of 2 trials (Cogan *et al.*, 2018) were streamlined, and only the data from the first two hours during recovery were utilized. The results indicated that ingestion of protein/and AA with CHO induced significantly higher insulin responses than the ingestion of CHO alone (from 30 - 180 min: $p < .001$; $I^2 = 82.88\%$ - 88.78%; from 210 - 240 min: $p < .01$; $I^2 = 71.16\%$ - 86.89%; $SMD_{30\ min} = 1.7379$, $SMD_{60\ min} = 1.6611$, $SMD_{90\ min} = 1.54$, $SMD_{120\ min} = 1.54$, $SMD_{150\ min} = 2.00$, $SMD_{180\ min} = 1.34$, $SMD_{210\ min} = 1.25$, $SMD_{240\ min} = 0.81$). The most representative results were insulin peaks at 60 minutes, which included all 32 trials (the standard mean difference was 1.6611, 95% CI 1.1864–2.1357, $p < .001$; $I^2 = 84.18\%$) (Figure 2).

Table 1. Characteristics of all included trials.

Author	Year	Age (yr.)	Weight (kg)	Hydro	Protein Type	Protein (g·kg-1·h-1)	AA Type(s)	AA Ratio	AA (g·kg-1·h-1)	CHO Type	CHO Ratio	CHO (g·kg-1·h-1)
Zawadzki	1992	-	73.1 ± 3.1	No	Milk	0.28			0	Glucose/ Maltodextrin	-	0.77
van Loon	2000	24 ± 0.6	70 ± 0.1	Yes	Wheat	0.2			0	Glucose/ Maltodextrin	4:6	1.2
	2000	24 ± 0.6	70 ± 0.1	Yes	Wheat	0.4			0	Glucose/ Maltodextrin	4:6	1.2
	2000	24 ± 0.6	70 ± 0.1	Yes	Wheat	0.1	Leu/ Phe	1:1	0.05 (Each)	Glucose/ Maltodextrin	4:6	1.2
	2000	24 ± 0.6	70 ± 0.1	Yes	Wheat	0.2	Leu/ Phe	1:1	0.1 (Each)	Glucose/ Maltodextrin	4:6	1.2
van Loon-2	2000	24 ± 0.6	70 ± 0.1	Yes	Wheat	0.2	Leu/ Phe	1:1	0.1 (Each)	Glucose/ Maltodextrin	1:1	0.8
van Hall	2000	25 ± 3	72 ± 3	Yes	Wheat	0.3			0	Glucose	1	0.8
	2000	25 ± 3	72 ± 3	Yes	Whey	0.3			0	Glucose	1	0.8
van Hall-2	2000	26 ± 2	74 ± 2	Yes	Whey	0.3			0	Sucrose	1	1
Jentjens	2001	27.1 ± 2.6	69.6 ± 2.9	Yes	Wheat	0.2	Leu/ Phe	1:1	0.1 (Each)	Glucose/ Maltodextrin	1:1	1.2
Betts	2005	21 ± 1	79.6 ± 11.2	Yes	Wheat	0.2			0	Glucose/ Fructose		1.2
	2005	22 ± 0.5	83.5 ± 11.8	Yes	Wheat	0.13			0	Glucose/ Fructose	2:1	0.8
Kaastra	2006	24.3 ± 0.8	73 ± 1.8	Yes	Casein	0.4			0	Maltose/ Maltodextrin	-	0.8
	2006	24.3 ± 0.8	73 ± 1.8	Yes	Casein	0.4	Leu	1	0.1	Maltose/ Maltodextrin	-	0.8
Betts	2007	21 ± 3	72.6 ± 8.4	No	Whey	0.3			0	Sucrose	1	0.8
Howarth	2009	22 ± 1	90 ± 5	Yes	Whey	0.4			0	Maltodextrin	1	1.2
Cepero	2010	39 ± 9.8	74.4 ± 7.2	Yes	Whey	0.13			0	-	-	0.47
	2010	39 ± 9.8	74.4 ± 7.2	Yes	Casein	0.13			0	-	-	0.47
Breen	2011	29 ± 6	77.2 ± 6.5	No	Whey	0.26			0	-	-	0.66
Bagato	2014	21 ± 1.1	63 ± 3.7	No	Milk	0.2			0	Sucrose	1	0.8
Rahbek	2014	23.9 ± 0.8	78.1 ± 1.8	Yes	Whey	0.3			0	-	-	0.3
Rustad	2016	24 ± 0.4	75 ± 3	No	Whey	0.4			0	Glucose/ Maltodextrin	1:1	0.8

Table 1. Characteristics of all included trials (Cont.).

Author	Year	Age (yr.)	Weight (kg)	Hydro	Protein Type	Protein ($g \cdot kg^{-1} \cdot h^{-1}$)	AA Type(s)	AA Ratio	AA ($g \cdot kg^{-1} \cdot h^{-1}$)	CHO Type	CHO Ratio	CHO ($g \cdot kg^{-1} \cdot h^{-1}$)
Cogan	2018	28.8 ± 2.3	75 ± 2.3	No	Casein	0.16			0	Glucose/ Maltodextrin	1:1	1.04
	2018	28.8 ± 2.4	75 ± 2.3	Yes	Casein	0.16			0	Glucose/ Maltodextrin	1:1	1.04
Sollie	2018	22.9 ± 1.2	79.5 ± 3	No	Whey	0.4			0	Glucose/ Maltodextrin	1:1	0.8
Churchward-Venne	2019	23 ± 0.3	74.2 ± 1.1	No	Whey	0.27			0	Dextrose/ Maltodextrin	-	0.61
	2019	23 ± 0.3	74.2 ± 1.1	No	Casein	0.27			0	Dextrose/ Maltodextrin	-	0.61
	2019	23 ± 0.3	74.2 ± 1.1	No	Milk	0.27			0	Dextrose/ Maltodextrin	-	0.61
Dahl	2020	26.7 ± 1.7	76.4 ± 3.2	No	Whey	0.8			0	Glucose/ Maltodextrin	1:1	0.4
Churchward-Venne	2020	27 ± 1	74.9 ± 0.9	No	Milk	0.2			0	Dextrose/ Maltodextrin	-	0.6
	2020	27 ± 1	74.9 ± 0.9	No	Milk	0.4			0	Dextrose/ Maltodextrin	-	0.6
	2020	27 ± 1	74.9 ± 0.9	No	Milk	0.6			0	Dextrose/ Maltodextrin	-	0.6

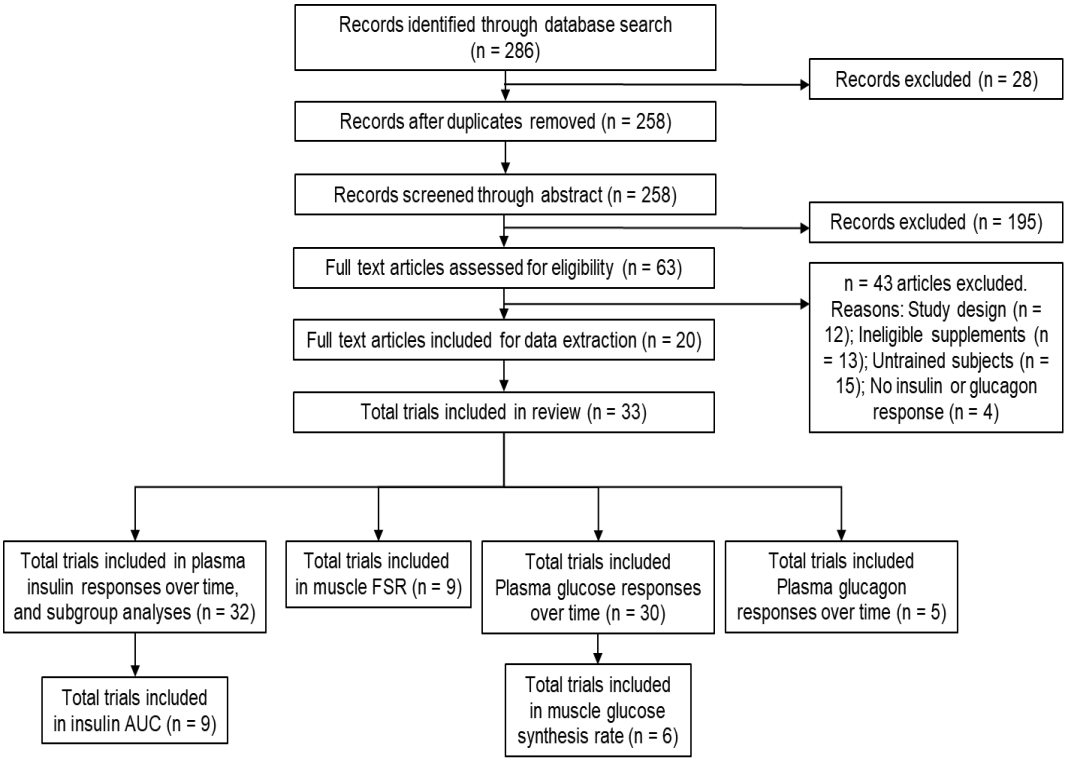
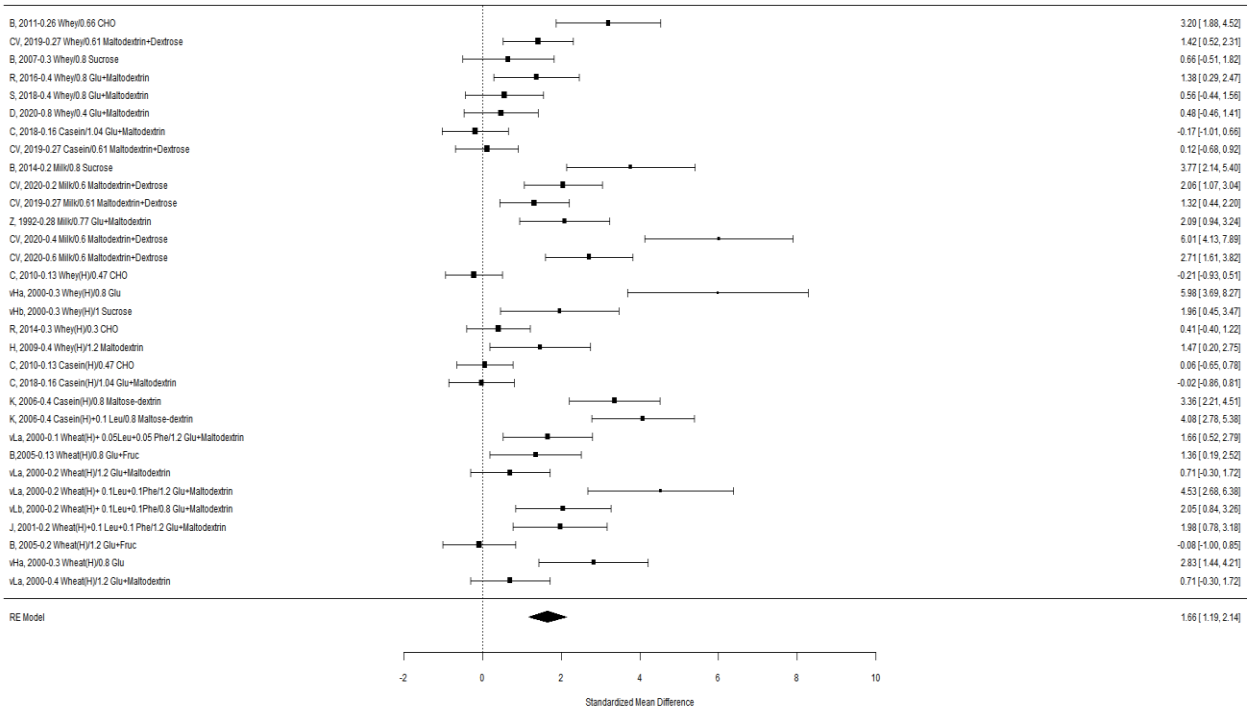


Figure 1. Flow diagram.



*Note. *Dotted lines separate the type of proteins within each group (Intact protein or hydrolysed protein). *Bolded line separates intact and hydrolysed proteins. *The data were sorted by the order: Intact or Hydrolysate > Protein types (Whey > Casein > Wheat > Milk) > Protein & Amino acid amounts.*

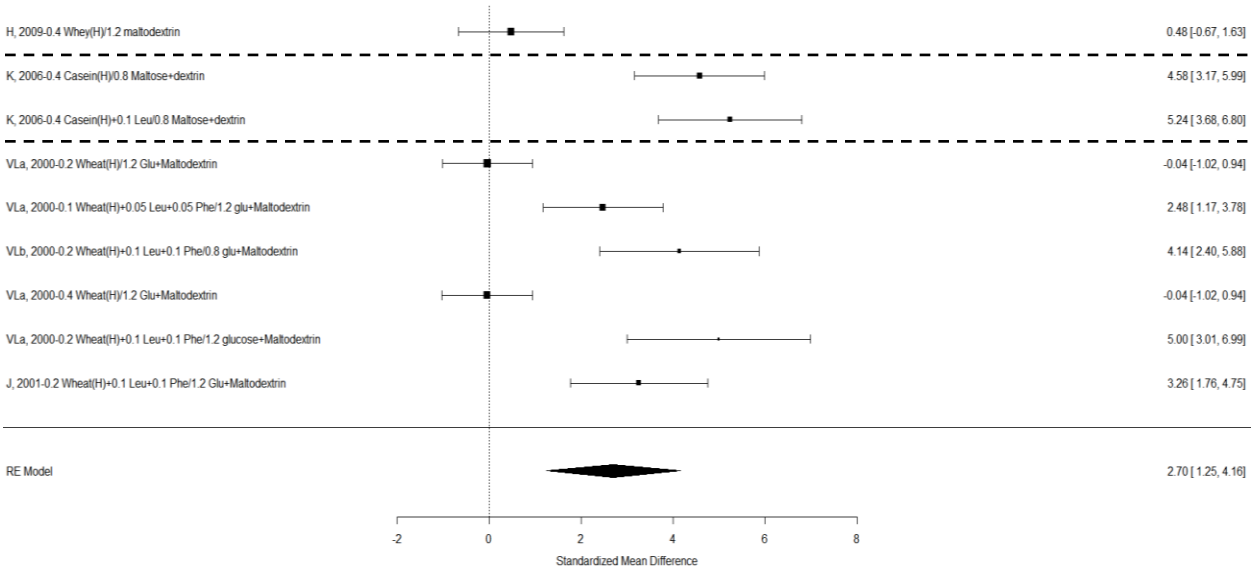
Figure 2. Insulin peaks at 60 minutes.

Plasma insulin peak subgroup tests

Subgroup analyses was applied in order to assess the difference between the ingestion of intact and hydrolysed proteins for postexercise insulin responses at 30, 60, 90, and 120 minutes. No significant difference was found on plasma insulin responses between the ingestion of intact and hydrolysed proteins (from 30 to 120 min: $\chi_{1,30}^2 = 0.08$; $\chi_{1,60}^2 = 0.01$; $\chi_{1,90}^2 = 0.01$; $\chi_{1,120}^2 = 2.71$; $df_{30\text{-}120} = 1$, $p_{30} = .77$, $p_{60} = .94$, $p_{90} = .4$, $p_{120} = .1$, $p_{30\text{-}120} > .05$).

Insulin AUC

Plasma insulin AUC after the ingestion of protein hydrolysate with CHO mixture was measured in 9 trials derived from 5 publications. No intact protein was included. The result indicated that the ingestion of protein and AA with CHO induced a significantly higher overall insulin response than the ingestion of CHO alone (the standard mean difference was 2.70, 95% CI 1.25–4.16, $p < .001$; $I^2 = 90.82\%$). (Figure 3)



*Note. * Dotted lines separate the type of proteins within each group (intact protein or hydrolysed protein). *The data were sorted by the order: protein type (whey > casein > wheat > milk) > protein & AA amounts > CHO amount. Muscle Fractional Synthesis Rate (Muscle FSR).*

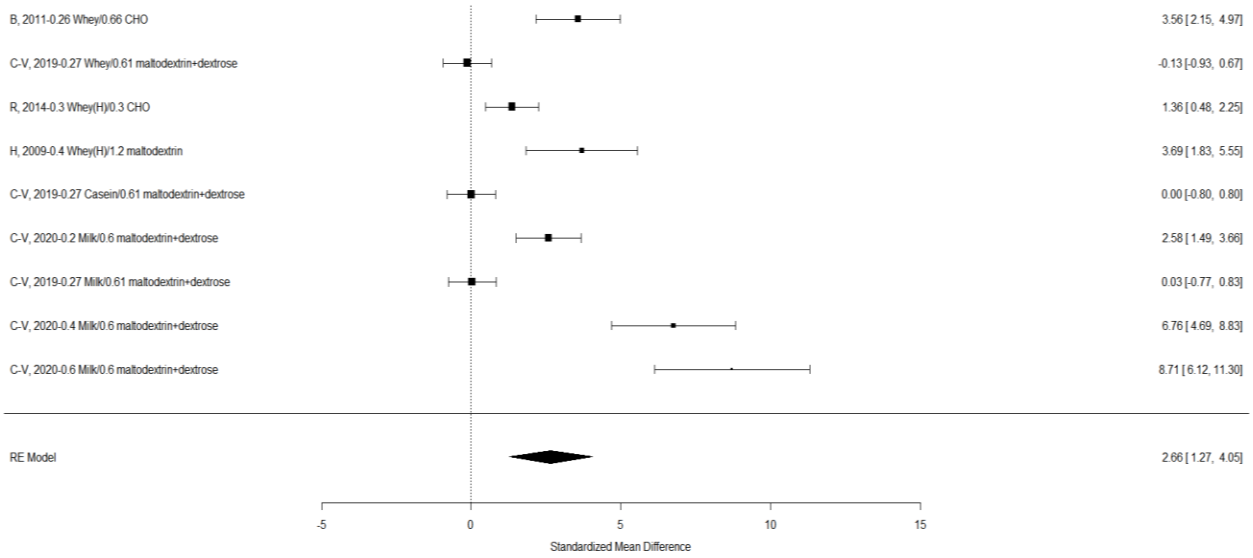
Figure 3. Forest plot: Insulin Area Under Curve (AUC).

Muscle Fractional Synthesis Rate (Muscle FSR)

FSR following ingestion of intact protein was measured in 9 trials derived from 5 publications. The result indicated that the ingestion of protein and AAs with CHO induced a significantly higher muscle FSR than the ingestion of CHO alone (the standard mean difference was 2.66, 95% CI 1.27–4.05, $p < .001$; $I^2 = 92.82\%$) (Figure 4).

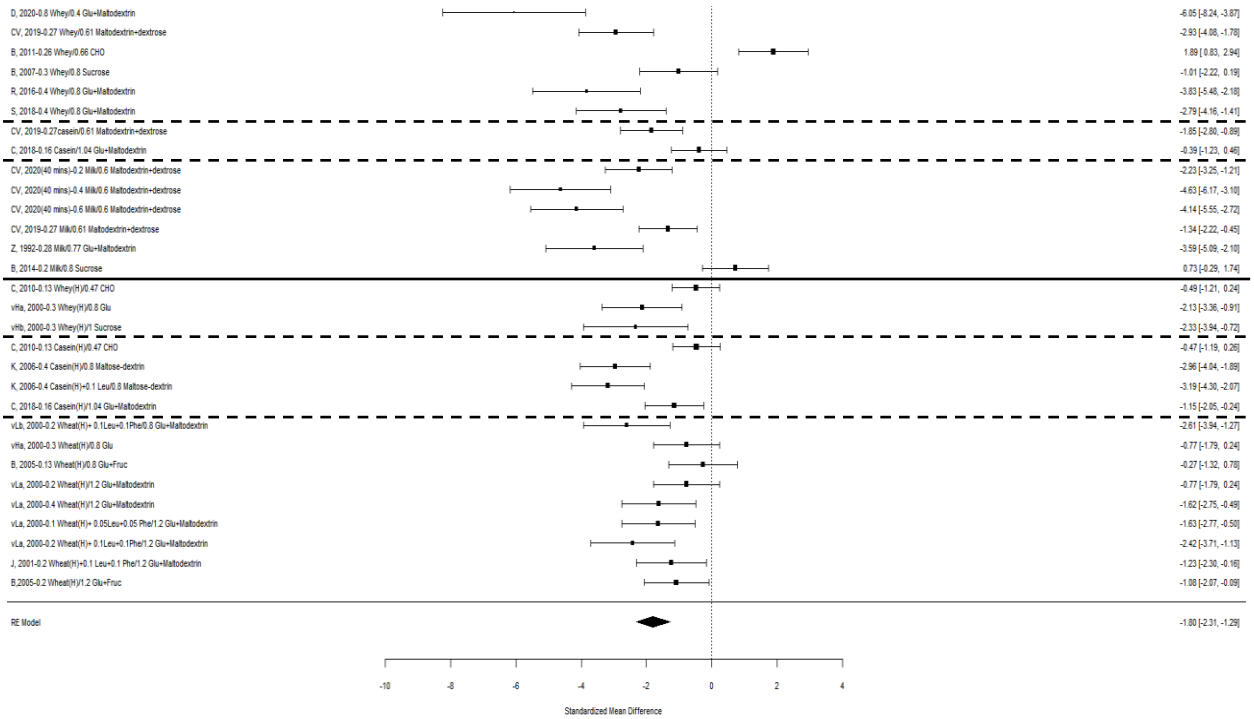
Plasma Glucose Peaks

Overall, 30 trials derived from 18 publications investigated the plasma insulin responses over time after the ingestion of intact/hydrolysed protein with CHO mixture. The glucose data of 3 trials (Churchward-Venne et al., 2020) at 40 minutes were used as and compared with other 30-minute data. In order to make all data consistent in having only one peak over the testing periods, the data of 3 trials (2 trials from Cogan *et al.*, 2018, and 1 trial was from Zawadzki *et al.*, 1992) were streamlined, and only the data from the first two hours during recovery were adopted in this study.



*Note. *The data were sorted by the order: Protein type (Whey > Casein > Wheat > Milk) > Protein & Amino acid amounts > Carbohydrate amount. Plasma Glucose Peaks Over Time.*

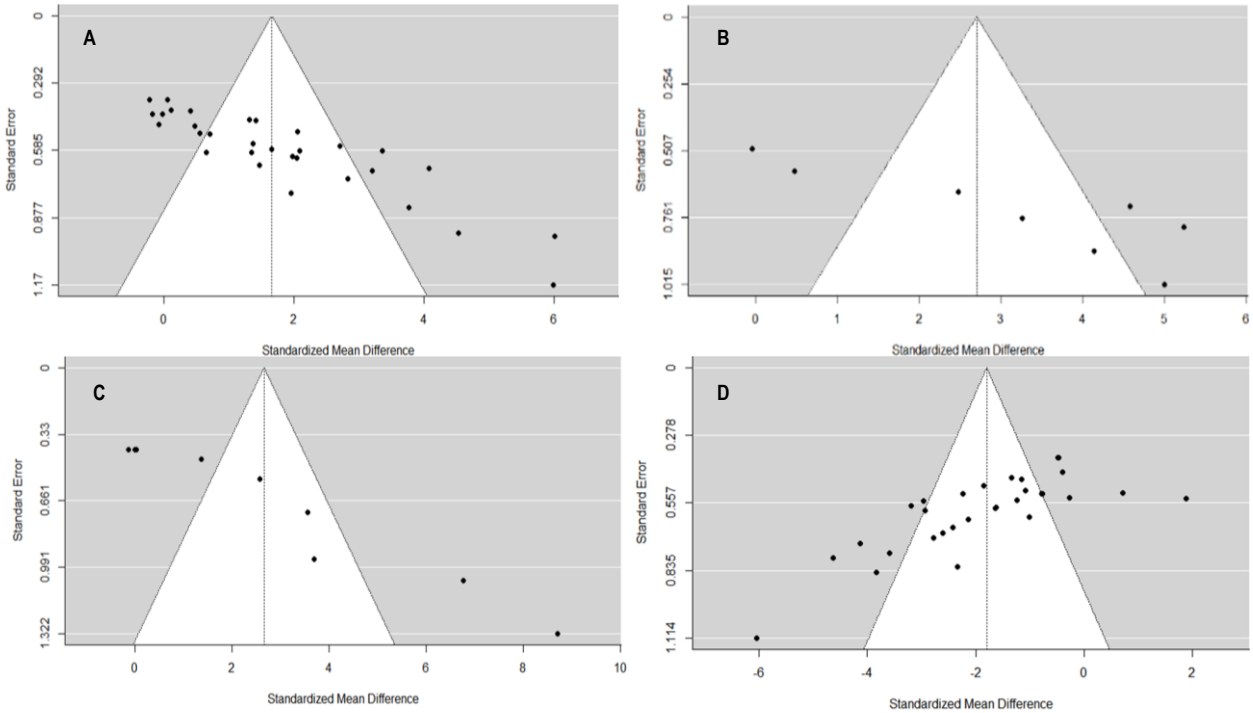
Figure 4. Muscle Fractional Synthesis Rate (Muscle FSR).



*Note. * Dotted lines separate the type of proteins within each group (Intact protein or hydrolysed protein). * Bolded line separates intact and hydrolysed proteins. *The data were sorted by the order: Intact or Hydrolysate > Protein type (Whey > Casein > Wheat > Milk) > Carbohydrate amount.*

Figure 5. Plasma glucose peaks at 60 minutes.

The results indicated that the ingestion of CHO alone induced a significantly higher plasma glucose response than the ingestion of protein/and AA with CHO (from 30 - 90 min: $p < .001$; $I^2 = 81.21\%$ - 87.97%; at 120 min: $p < .01$; $I^2 = 78.59\%$; $SMD_{30\ min} = (-1.42)$, $SMD_{60\ min} = (-1.80)$, $SMD_{90\ min} = (-1.35)$, $SMD_{120\ min} = (-0.58)$). The most representative results were glucose peaks at 60 minutes, which included all 30 trials (the standard mean difference was (-1.80), 95% CI (-2.31) - (-1.29), $p < .001$; $I^2 = 84.48\%$). (Figure 5)



Note. ***6a.*** *Funnel Plot – Insulin Peak at 60 Minutes.* ***6b.*** *Funnel Plot - Insulin Area Under Curve (AUC).* ***6c.*** *Funnel Plot - Muscle Fractional Synthesis Rate (Muscle FSR).* ***6d.*** *Funnel Plot - Plasma Glucose Peaks at 60 Minutes.*

Figure 6. Funnel plots.

Muscle glycogen synthesis rate

No significant difference on muscle glycogen synthesis rate between the ingestion of protein and AAs with CHO and CHO alone was found within the 6 trials from 5 publications (The standard mean difference was 0.82, 95% CI (-0.41)–2.05, $p > .05$; $I^2 = 85.53\%$).

Plasma glucagon peaks over time

No significant difference on plasma glucagon responses between the ingestion of protein and AAs with CHO was detected among the 5 trials derived from 4 publications (from 30 - 60 min: $p_{30} = .99$ & $p_{60} = .85 > .05$; $I^2 = 78.57\%$ - 84.56%).

DISCUSSION

The ingestion of protein with CHO was found to have significantly higher insulin responses than the ingestion of CHO alone. The analysis of insulin AUC also confirmed the same overall significantly higher insulin response by the ingestion of protein with CHO. On the other hand, the ingestion of protein with CHO does induce a significantly higher muscle FSR than the ingestion of CHO alone. Both results are in line with the conclusions made by Howarth (2009) that the ingestion of protein with CHO increased muscle FSR and

improved whole body net protein balance. However, no significant difference on plasma insulin responses over time between the ingestion of intact and hydrolysed proteins was found by the heterogeneity and subgroup analysis. Of note, this lack of significant differences could be in part because of the small sample sizes in many of the included trials, the limited number of included studies, or the discrepancies in the intervals of beverage administration. It is worth noting that, in this study, hydrolysed protein with CHO produced more stable and concentrated insulin responses than intact protein with CHO, although there was no statistically significant difference in the absolute values assessed in our analyses. Furthermore, the ingestion of hydrolysed protein with CHO also shows mostly numerically higher, but not significantly different, insulin peaks after two hours (At 150 min, $SMD_{hydrolysed} = 2.11 > SMD_{intact} = 1.41$; At 180 min, $SMD_{hydrolysed} = 1.36 > SMD_{intact} = 1.29$; At 210 min, $SMD_{hydrolysed} = 1.59 > SMD_{intact} = (-0.37)$; At 240 min, $SMD_{hydrolysed} = 0.73 > SMD_{intact} = 1.01$). However, the mechanism remains incompletely elucidated. Perhaps this small difference could be influential to subsequent endurance capacity, or the advantages of ingesting protein hydrolysate would be more pronounced after resistance or high-intensity exercises, where muscle damage typically occurs (Heavens *et al*., 2014) and the AA would be in greater demand.

The plasma glucose responses of the ingestion of CHO alone were as expected and significantly higher than the ingestion of protein with CHO since the plasma glucose concentration directly reflects the overall amount of CHO intake. However, no significant difference on muscle glycogen synthesis rate was found between the ingestion of protein with CHO and CHO alone. This result is in line with the results of previous studies (Cogan *et al.*, 2018; Kaastra *et al.*, 2006; Sollie *et al.*, 2018). The possible explanation is that the increased insulin concentrations induced by the ingestion of protein with CHO may further augment glycogen synthase activity and accelerate the muscle glycogen metabolism (Kaastra *et al.*, 2006; van Loon *et al.*, 2000a; van Hall *et al.*, 2000a). Therefore, possibly due to this increased insulin response, the ingestion of protein with CHO shares a similar post-exercise muscle glycogen amount as the ingestion of CHO alone. Overall, with muscle protein synthesis stimulation being key to attenuation of exercise-induced muscle damage and muscle glycogen concentrations to be decisive in muscle fatigue (Burke *et al.*, 2004; Hausswirth *et al.*, 2011), protein with CHO should be a better strategy for short-term postexercise recovery for endurance-trained athletes or any individual who is working to maintain muscle mass after training.

Unfortunately, there was insufficient data on postexercise plasma glucagon responses with the ingestion of protein with CHO to conduct meaningful meta-analyses. This is likely due to the difficulty and imprecision in measuring *in vivo* glucagon levels. One reason is that circulating glucagon concentrations are in the low picomolar range, which is around 16-17 pmol/L (Holst *et al.*, 2019). Another reason is that many proglucagon substances also contain glucagon-like sequences and can react with reagent chemicals (Wewer Albrechtsen *et al.*, 2016). Both of which make measuring plasma glucagon concentrations more challenging. However, it is still unexpected that the included trials reported no significant difference on plasma glucagon responses between the ingestion of protein with CHO and CHO alone. More research is warranted to determine whether the protein-induced TEF is led by the glucagon response, and if such response is meaningful in long-term weight management.

Finally, the funnel plot analysis (Figure 6) revealed a trend that the studies with high standard errors (which may have been in part a function of lower sample sizes) were more likely to show stronger effects than the studies with lower standard errors. Only the funnel plots of plasma glucose responses presented an opposite trend. The asymmetry detected in these funnel plots may have been due to true heterogeneity with $I^2 > 80\%$ for most cases in this meta-analysis. Though this could also represent evidence of a publication bias for statistically significant results (Borenstein et al., 2009).

This study only included published research, which may introduce published bias. In addition, there other relevant studies published in languages such as Mandarin or French, so some relevant publications may therefore be omitted. There were also diverse units reported resulting in exclusion of some trials from analyses because of the difficulty to convert and unify the units. A limited number of publications in this field also limited the number of analyses. The inability to conduct the analysis of muscle glycogen storage and subgroup tests for glucose or muscle fractional synthesis to determine the differences between the ingestion of intact protein and its hydrolysate are examples of this case. Moreover, the limited numbers of included trials could not minimize the power of individual difference made by the subjects, which could be the reason of no significant difference of some results. On the other hand, bias may still happen due to a lack of report of standard deviations from some included trials as these missing numbers were substituted by the mean value of the sum of the existing standard deviations in this meta-analysis.

CONCLUSION

In conclusion, this meta-analysis demonstrates robust evidence in the existing peer-reviewed literature that, for trained male athletes, the ingestion of protein with CHO is a better post-exercise recover strategy than CHO alone. The varying insulin response induced by different protein ingestion implies a possibility of combined protein ingestion for different recovery purposes and training plans. In addition, it may be possible to further define postexercise food quality by using insulin and glucagon indexes once the mechanism of glucagon is further elucidated.

AUTHOR CONTRIBUTIONS

Conceptualization, T-Y. K., J. L. B., L. R.; Methodology, T-Y. K., J. L. B., K. L., L. R.; Investigation, T-Y. K., L. R.; Writing – Original draft, T-Y. K.; Writing – Review and Editing, T-Y. K., J. L. B., K. L., L. R. All authors approved the final version of this paper.

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