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Effects of protein and carbohydrate on glycogen resynthesis post exercise

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Abstract
Post exercise nutrition is paramount in order to replenish depleted exercise stores, mitigate muscle protein degradation, rebuild damaged muscle tissue, and have improved subsequent performance. The purpose of this study was to collect literature that investigated how post exercise ingestion of carbohydrates and proteins affected glycogen resynthesis post exercise. Collection involved only peer-reviewed literature that examined glycogen resynthesis from protein and carbohydrate ingestion post exercise, depleted glycogen stores via exercise, involved subjects who were adults and deemed healthy by preliminary health screenings. This entire review covers a total of 82 subjects with age range of 19-28, most who were trained cyclists (n = 44). Not all literature mentioned the gender number, but there were at least 64 males and 8 females. Those who were not trained cyclists were described as "endurance trained" (n=6). Literature came from by searching databases such as Researchgate, EBSCOhost, sport Discus, Google Scholar, Medscape, and Pubmed. Searches were conducted with key words such as protein synthesis, nutrient timing, anabolic window, insulin effect on protein synthesis, and nutrition effect on insulin. The results of the literature suggests that the addition of protein to a carbohydrate supplement post exercise creates greater increases in glycogen resynthesis compared to carbohydrate of equal caloric value alone when 2 hour feeding intervals. All studies that used frequent intervals (15-30 min) always resulted in greater or equal glycogen resynthesis in the carbohydrate (CHO) only group compared to carbohydrate plus protein (CHO + Pro) group.

Keywords: dietary protein, carbohydrate intake, recovery, muscle glycogen synthesis

1. Introduction

Athletes of all ages, abilities, and skill levels should adopt some form of post-exercise nutrition to improve performance and enhance the body's recovery processes following exercise, as they are highly susceptible to the detriments of heavy training regimens. Consuming the right nutrients is paramount in order to replenish glycogen stores, repair muscle tissue and have peak performance. Considering this, in the last few decades, the timing of these nutrients has been a focus to many researchers in the field of exercise science. The nutrients that have drawn much attention, in reference to the recovery phase of exercise, are protein and carbohydrates (Poole, Wilborn, Taylor, & Kerksick, 2010).

Candow and Chilibeck (2008) suggest that the early post exercise period, described as the anabolic window, is the most critical period to ingest nutrients. After a workout, stored fuels such as glycogen are depleted and muscle tissue is damaged. Carbohydrate intake during recovery has been shown to replenish depleted glycogen after intense or exhaustive exercise (Ivy *et al.* 2002) [9]. Consuming the right amount of nutrients immediately post exercise is necessary in order to replenish lost glycogen stores and rebuild damaged muscle tissue. However, carbohydrates play a limited role in protein synthesis, and thus are probably not necessary to prompt hypertrophy training effects. Evidence suggests that consuming carbohydrate and protein together work synergistically together for enhancing glycogen synthesis and subsequent performance improvements (Miller *et al.* 2003). According to Ivy *et al.* (2004) [8], this will also promote protein synthesis and reduce protein degradation, thus having the added benefit of stimulating muscle tissue repair and adaptation more than carbohydrate ingestion alone. Other research examining the addition of protein to carbohydrate consumption in the post-exercise period has led to mixed results (van Loon, Saris, Kruijshoop, & Wagenmakers, 2000) [19].

Replenishing fuel stores after an exercise session is required in order to minimize muscle damage and recover effectively. Muscle glycogen is the primary fuel source used during an exercise bout. However, macronutrients such as protein and fat will become fuel for enabling muscle contraction, especially when muscle glycogen stores are depleted from exercise. This occurs in concert with muscle tissue damage from the exercise. Because of this, one can see the significance of proper nutrition intake in order to replenish glycogen stores and mitigate muscle degradation. Evidence has suggested that the consumption of the right macronutrients in the post exercise anabolic window can enhance the anabolic environment and blunt catabolism (Ivy, Goforth, & Damon, 2002) [9]. While the consumption of carbohydrates would replenish the depleted glycogen stores, it would also prevent the catabolic breakdown of muscle protein to be used as a fuel source. Much research has been done to investigate the synergistic capabilities of protein and carbohydrates consumed together post exercise, and the results have shown increases in muscle glycogen resynthesis, protein synthesis,

and even increased performance of subsequent exercise (Williams, Raven, Fogt, & Ivy, 2003) [20].
Several factors play a significant role in determining the effectiveness of protein and carbohydrate supplementation on post-exercise protein and glycogen synthesis. Improper application of these factors can limit the body's ability to reach an optimal anabolic status. Although past research has clearly defined that timing of ingestion, GI value of food, amount ingested, and nutrient composition of the food are all important factors in determining the effectiveness of glycogen synthesis rates (Poole, Wilborn, Taylor, & Kerksick, 2010). Further research is needed to clarify the role of post-exercise protein and carbohydrate administration. Thus, the purpose of this review is to discuss research that has investigated the relationship of dietary protein and carbohydrate intake during recovery on glycogen synthesis. Does the addition of protein to carbohydrate consumption in the post-exercise period impact muscle glycogen resynthesis?

2. Literature Review

Carbohydrates are a significant nutrient that provides fuel for exercise as well as replenish depleted glycogen stores from an intense exercise session. In fact, the Institute of medicine recommends 45%-65% of daily calories should come from carbohydrates. While proteins can contribute as fuel for exercise, especially when carbohydrate intake is low, their most notable function is the rebuilding of broken down muscle tissue from an exercise session. Evidence has suggested that both carbohydrates and proteins have insulinotropic properties that cause a rise in insulin so that they can be transported to muscle fibers (Spiller *et al.* 1987) [15]. Considering this, this literature review investigates how consuming these macronutrients post exercise may affect glycogen resynthesis.
First, the combined effects of protein and carbohydrate on muscle glycogen synthesis post exercise was first done by researchers Zawadzki, Yaspelkis, & Ivy (1992) (although Roy and Tarnopolsky (1985) [13, 16] tested carbohydrate and protein effect glycogen resynthesis in 1980, they used 5g of fat in their studies). They evaluated the effect of 112 g of carbohydrate in a 21% w/v mixture and 112 g of carbohydrate with 40.7 g of protein provided immediately after and 2-hours after exercise. The authors discovered that the addition of protein to the carbohydrate supplement increased the rate of glycogen storage by approximately 38% over the first 4-hours of recovery. They found that the plasma insulin response of the carbohydrate (CHO) plus protein (Pro) treatment was significantly greater than that of the CHO treatment. Both the CHO and CHO + Pro treatments produced plasma glucose and insulin responses that were greater than those produced by the Pro treatment ($P < .05$). The rate of muscle glycogen storage during the CHO + Pro treatment (35.5 ∓ 3.3 (SE) mumol.g protein-1.h-1) was significantly faster than during the CHO treatment (25.6 ∓ 2.3 mumol.g protein-1.h-1), which was also significantly faster than the PRO treatment (7.6 ∓ 1.4 mumol.g protein-1.h-1) (Zawadzki, Yaspelkis, & Ivy, 1992). Considering that there were concomitant rises insulin and glycogen storage rates, the authors noted that the mechanism responsible for this was the increase in the insulin response to carbohydrate and protein consumed together, as it was higher than carbohydrate consumed by itself.

3. Carbohydrate Effects on Insulin

A notable characteristic that muscle fibers undergo after an exercise session is insulin sensitivity. Because of insulin's ability to transport macronutrients to muscle fibers, this sensitivity enables important nutrients such as protein and carbohydrates to be transported into working skeletal muscle fibers in order to replenish glycogen stores and rebuild broken down muscle tissue (Cartee *et al.* 1989) [5]. Therefore, it is understandable that rises in insulin would occur upon the ingestion of carbohydrates, especially during the time when when muscle fibers are most sensitive to insulin with subsequent membrane permeability to substrates. In order to understand the insulinotropic effects of carbohydrate and sugar consumption, Blom, Høstmark, Vaage, Kardel, & Mæhlum, (1987) [2] investigated the effect of different post-exercise sugar diets on the rate of muscle glycogen resynthesis. They did this by studying the effect of repeated ingestions of fructose, sucrose, and various amounts of glucose on muscle glycogen synthesis during the first 6 hours after an exhaustive bicycle exercise session. The subjects were administered three different amounts of glucose orally at 0, 2, and 4 h after exercise. The amounts were 0.35 (low glucose: N = 5), 0.70 (medium glucose: N = 5), or 1.40 (high glucose: N = 5) g.kg-1 body weight of glucose. The rates of glycogen resynthesis were (mean ∓ SE) 2.1 ∓ 0.5, 5.8 ∓ 1.0, and 5.7 ∓ 0.9 mmol·kg-1·h-1, respectively. When higher amounts of glucose were administered to patients, greater amounts of glycogen resynthesis occurred. Plasma insulin levels were concomitantly increased as well, revealing the corresponding effects of insulin levels and synthesis of glycogen post exercise (Blom, Høstmark, Vaage, Kardel, & Mæhlum, 1987) [2]. Research by Ivy, Katz, Cutler, Sherman, Coyle (1988) has revealed that immediately after exercise is the time of peak muscle sensitivity to insulin, and therefore the ideal to consume nutrients as close to this time as possible, as delayed ingestion of nutrients causes lesser amounts of glucose to be taken into depleted muscle fibers (Ivy, Katz, Cutler, Sherman, & Coyle, 1988). These same authors did a research experiment where they had clients exercise into a glycogen depleted state. After the exercise regime, clients who were divided into two groups consumed either a 25% carbohydrate solution (2 g·kg body weight) immediately postexercise (P-EX), or 2 h postexercise (2P-EX). Muscle glycogen immediately postexercise was not significantly different for the P-EX and 2P-EX treatments. During the first 2 h postexercise, the rate of muscle glycogen storage was 7.7 mumol.g wet wt-1.h-1 for the P-EX treatment, but only 2.5 mumol.g wet wt-1.h-1 for the 2P-EX treatment. During the second 2 h of recovery (3rd and 4th hour), the rate of glycogen storage slowed to 4.3 mumol.g wet wt-1.h-1 during treatment P-EX, whereas, the 2P-EX increased to 4.1 mumol.g wet wt-1.h-1. Despite a gradual slowing from the P-EX group, the 2P-EX group was still 45% slower ($P < .05$) compared to the P-EX treatment during the first 2 h of recovery, suggesting that delaying the ingestion of a carbohydrate supplement post-exercise will result in a reduced rate of muscle glycogen storage (Ivy, Katz, Cutler, Sherman, & Coyle, 1988).

4. Protein Effects on Insulin

Similar to the observation that carbohydrate raises insulin, protein is shown to raise insulin as well, serving as the basis for the synergistic capabilities for both macronutrients being consumed together. Despite this observation, it may not be the case, as there are conflicting results in regards to rising insulin levels from protein/amino acid mixtures (Spiller *et al.* 1987 [15]; Zhang *et al.* 2011 [23]; Gannan & Nuttall, 2010). Researchers Spiller *et al.* (1987) [15] investigated how the ingestion of protein would influence glucose metabolism. The authors found that when increasing protein dosage, mean areas of glucose curves above fasting decreased, revealing the possibility that protein ingestion caused subsequent increases in insulin levels that would then transport glucose throughout the body (i.e the glucose curve decreasing). Further, protein-containing meals produced significantly lower ($p <. 01$) areas than the protein-free meal. In addition to the observation that protein caused glucose curves above fasting to decrease, protein-containing meals also produced significantly greater ($p <. 01$) insulin areas compared with the protein-free meal. These results further implicate that increasing the ingestion of protein may act as an insulin secretagogue which may increase glycogen storage (Spiller *et al.* 1987) [15]. Further, Zhang *et al.* (2011) [23] investigated changes in insulin levels from amino acid ingestion by having subjects participate in an overnight fast and consume 1 gram of BCAAs and 5 grams of BCAAs 5 days later. On both days, insulin levels rose, with peak levels being reached 15 minutes into the trial. This is an interesting finding considering that consuming 5 grams of BCAA actually caused a lesser amount of insulin response than the group that ingested only 1 gram. Considering that amino acids are the building blocks of protein, the idea that lesser amounts of protein being consumed would cause a lower spike in insulin and subsequently lowered glycogen resynthesis seems trivial. However, certain amino acids, like Leucine, are shown to spike insulin levels greater than others. In fact, Zhang *et al*. (2011) [23] noted that leucine may have been the reason for the insulin spike. Proteins of high biological value include all 9 essential amino acids, which includes leucine. Proteins that are missing any essential amino acid are considered of low biological value. It is not mentioned in these studies which kind of biological value the proteins are, or the variations of amino acid content within the proteins, thus making the insulin response of ingested protein, either by itself or with carbohydrate, seem variable. Nonetheless, proteins are shown to be stimulants of insulin. Researchers Gannan & Nuttall (2010) investigated the insulin response from the ingestion of most essential amino acids and a few non-essential amino acids on subjects. They found that the individual ingestion of almost all of the amino acids resulted in an increase in circulating insulin concentration, with histidine and tyrosine being the only two amino acids that did not. Each of the amino acids that did stimulate insulin levels varied, but phenylalanine and glycine stimulated the greatest response. The authors also had these same amino acids consumed concomitant with glucose. The effect on the insulin response of an individual amino acid ingested with glucose is relatively small, with the exception of leucine, which elevated insulin levels significantly more. Alanine, tyrosine, methionine, arginine, histidine, and valine raised insulin levels but not greater than glucose alone. Further, leucine, isoleucine, proline, glutamine, aspartic acid, serine, lysine, phenylalanine, and glycine raised insulin levels greater than glucose alone. Although histidine and tyrosine may not have raised insulin, none of the literature used to measure the central question of this systematic review specifically used these two amino acids alone, making their inability to show elevated insulin levels irrelevant (Gannan & Nuttall, 2010).

5. Carbohydrate Plus Protein Effects on Insulin and Glycogen Resynthesis

Research has shown that the consumption of carbohydrates alone can replenish glycogen stores in muscle fibers (Ivy, Katz, Cutler, Sherman, Coyle, 1988). The consumption of carbohydrates will cause the rising of insulin levels to transport these substrates throughout the body, especially to exercised muscle fibers. Ivy *et al.* (2002) [9] has expressed that the greater amounts of glycogen resynthesis from the co-ingestion of protein and carbohydrates is attributed to the greater rises in insulin. It seems inviting to assume that in order to maximize the synthesis of glycogen in muscle fibers, that raising insulin further would create a greater amount of resynthesis. However, not all literature has found this to be true. For example, Ivy *et al.* (2002) [9] found that although there was an increase in glycogen resynthesis in the CHO + Pro compared to the high calorie carbohydrate group (HCHO) group and low-calorie carbohydrate group (LCHO), plasma insulin levels did not differ at any time among treatments (Ivy *et al.* 2002) [9]. In the CHO + Pro group, subjects consumed 80 g CHO, 28 g Pro, and 6 g fat, totaling 486 calories. This was greater than the low-calorie carbohydrate which consumed 80 g CHO, 6 g fat, totaling 374 calories, and equal to the high-calorie carbohydrate group which consumed 108 g CHO, 6 g fat, totaling 486 calories. Because insulin levels were equal throughout the trial, and the caloric value was equal between the CHO + Pro and HCHO trials, it suggest that insulin may not always have to be higher to cause greater resynthesis of glycogen, considering that the CHO + Pro group caused greater glycogen resynthesis. Considering this, it is necessary to investigate the various literature that investigated how the caloric intake from either CHO or CHO + Pro would influence insulin dynamics as it relates to the resynthesis of glycogen into muscle fibers in order to gain more understanding into this matter.

First, authors Zawadzki, Yaspelkis, and Ivy (1992), who had their clients consume CHO (112.0 g) + Pro (40.7 g) or CHO (112 g) alone, found that CHO + Pro creates greater glycogen resynthesis than the CHO group. However, they found different insulin responses compared to Ivy *et al.* (2002) [9]. During recovery, the authors found that the plasma glucose response of the CHO treatment was significantly greater than that of the CHO + Pro treatment, but the plasma insulin response of the CHO + Pro treatment was significantly greater than that of the CHO treatment. However, this combined CHO + Pro supplement was the individual CHO supplement added to the Pro supplement, thus resulting in a supplement with a 42% greater energy content, implicating that a greater amount of calories would have an advantage over CHO only. Of interest is the observation that there was greater glucose response in the CHO group compared to the CHO + Pro group. While there may be greater insulin rises from protein and carbohydrates together, other literature has found that adding protein to carbohydrate reduces glycemic responses (Stanstrup, Schou, Holmer-Jensen, Hermansen, Dragsted, 2014). This namely happens because protein can delay the speed with which food empties from the stomach and enters the small intestine, particularly the jejunum region, the main site for nutrient absorption, a process known as gastric emptying. While this may be a reason for reduced glucose response, the authors in this literature review did not measure the gastric emptying in clients. It is still necessary to evaluate subsequent literature and differing energy intakes to gain more understanding of how macronutrients and energy differences affects insulin dynamics as well as the resynthesis of glycogen.

Moreover, supplementing at 30 minute intervals, van Loon, Saris, Kruijshoop, Wagenmakers (2000) [19] did an interesting study in which they added highly insulinotropic amino acids leucine and phenylalanine into their CHO (.8 g·kg) + Pro (.4 g·kg amino acid plus protein hydrolysate) group. In the high CHO trial (CHO + CHO), they used an isoenergetic amount of 1.2 g·kg (50% glucose and 50% maltodextrin). Finally, they had low CHO trial (0.8 g carbohydrate·kg 50% glucose and 50% maltodextrin) in the control group. The authors noted that the addition of the amino acid and protein hydrolysate mixture resulted in an insulin response that was 87.5 ± 17% higher than in the control trial. Further, the ingestion of an isoenergetic amount of carbohydrate (1.2 g·kg) resulted in an insulin response that was 45.8 ± 18% higher than the control trial. This was concomitant with the observation that muscle glycogen resynthesis was significantly higher in both the CHO + Pro and CHO + CHO trials than in the control trial (35.4 ± 5.1 and 44.8 ± 6.8 compared with 16.6 ± 7.8 μmol glycosol units·g dry muscle $wt^{-1}·h^{-1}$)(van Loon, Saris, Kruijshoop, Wagenmakers & 2000) [19]. This study shows that when highly insulinotropic amino acids were added into the CHO + Pro mixture, glycogen resynthesis will be accelerated, compared to a control trial. However, an isocaloric carbohydrate supplement can also accelerate glycogen resynthesis, even with lower amounts insulin levels.

Arguably, it is evident from the literature provided thus far that certain beverages can cause increases in insulin levels but not always cause greater glycogen resynthesis. However, authors that had their clients ingest carbohydrate and protein beverages post exercise either had the intake not eucaloric, or at least eucaloric but with great great caloric differences. To simplify as well as gain a more in depth insight into these matters, researchers Tarnopolsky *et al.* (1997) [16], and Alghannam *et al.* (2016) [1] used eucaloric and isoenergetic supplementation post early exercise**.** Tarnopolsky *et al.* (1997) [16] had subjects consume CHO (1 g·kg) and CHO (0.75 g·kg) + Pro (0.1 g·kg) + Fat (0.02 g·kg) immediately and 1 hour post exercise. The authors found that insulin levels in both groups were peaked close to the half hour mark. After that, CHO + Pro + Fat levels started to decrease below CHO levels and remained below until the next supplementation at the 1 hour mark, causing almost equal rises in insulin levels as well as similar levels throughout the rest of the trial. These similar plasma insulin levels were concomitant with similar greater glycogen resynthesis in the CHO group after resistance exercise (CHO 23.0 ± 4.5 and CHO + Pro + Fat 19.3 ± 6.1 mmol · kg dry muscle−1 · h−1, respectively). This study suggest that with a larger CHO intake, insulin levels rise similarly and resynthesis of glycogen was similar as well (Tarnopolsky *et al.* 1997) [16].

On the other hand, Alghannam *et al.* (2016) [1] had clients consume CHO (0.8 g·kg) + Pro (0.4 g·kg) or isocaloric CHO (1.2 g·kg) beverages at 30 min intervals during recovery. Insulin levels rose similarly until the 1 h mark, reaching values of about 160 pmol · -1. However, the insulin in the CHO + Pro values continued to rise above the CHO group, reaching values of about 280 pmol · -1 at the 3 h mark compared the CHO group, which had insulin values remaining at the 160 mark. Immediately after exercise, glycogen concentrations were similar between the two treatment groups (99 ± 3 mmol·kg dry mass [dm-1]). During recovery, muscle glycogen concentrations increased to 252 ± 45 mmol·kg dm-1 in CHO and 266 ± 30 mmol·kg dm-1 in CHO + Pro. These results show that CHO + Pro ingestion equally accelerates muscle glycogen resynthesis compared to CHO (1.2 g·kg) post exercise despite insulin levels being higher in the CHO + Pro trial. A key difference from Tarnopolsky *et al.* (1997) [16] is that Alghannam *et al.* (2016) [1] used equal amounts of CHO and Pro in both trials and had more calories. Nonetheless, the results still showed similar glycogen resynthesis despite differing insulin levels.

Furthermore, when authors had their clients consume beverages that were eucaloric, but not isocaloric, rises in insulin levels were not observed to concomitantly increase glycogen resynthesis, as was expressed by Zawadzki, Yaspelkis, & Ivy (1985) [21]. For example, after having client's receive beverages post early exercise, Tarnopolsky *et al.* (1997) [16] observed that by the 40 min mark, insulin levels that rose from the CHO + Pro + Fat beverage had peaked close to 40 μIU·L, but declined sharply, close to 25 μIU·L. However, by time the 60 minute mark arrived, CHO insulin levels passed CHO + Pro and continued to rise until it peaked just over 40 μIU·L. Considering this, CHO insulin levels stayed well ahead of the CHO + Pro + Fat group until the end of the trial. There was ultimately greater glycogen resynthesis in the CHO compared to CHO + Pro + Fat (37.2 vs. 24. 6 mmol · kg dm-1 · h-1, respectively). An important characteristic in this design is that the subjects in the CHO + Pro + Fat group consumed 0.75 g·kg CHO, 0.1 g·kg Pro, and 0.02 g·kg Fat, while the CHO group consumed only 1 g·kg carbohydrates. That being said, the caloric intake is higher in the CHO group, a possibility in why there was greater insulin levels as well as resynthesis of glycogen.

Further, researchers Jentjens *et al.* (2001) [8] had their clients consume either CHO (1.2 g · kg-1) or CHO (1.2 g·kg) + Pro (.4 g·kg) in 30 minute intervals. At no point did the insulin levels from the carbohydrate pass levels stimulated by CHO + Pro group. At the 150 min mark, insulin levels from the CHO group peaked just over 60 μU·ml. Whereas, in the CHO + Pro group, insulin levels peaked at levels close to 140 μU·ml. Because this trial was not isocaloric, it's important to realize that a possible reason why the insulin levels were consistently greater in the CHO + Pro group is that the calories were greater than the CHO group. Nonetheless, there were no differences in glycogen resynthesis between the groups

(Jentjens *et al.* 2001) [8].

Further, Van Hall, Shirreffs, and Calbet (2000) [18] had subjects consume a supplement that was also eucaloric but not isocaloric. The CHO + Pro group consumed 1.67 g·kg of sucrose and 0.5 g·kg of a whey protein hydrolysate, and the CHO group contained 1.67 g·kg of sucrose. By the 60 minute mark, insulin levels in the CHO + Pro group peaked close to 80 mU·I -1 compared to CHO, which was just below 50 and eventually peaked at 50 at the 120 minute mark. Further, throughout the trial the insulin levels in the CHO + Pro group remained higher than the CHO group (Van Hall, Shirreffs, and Calbet, 2000) [18]. Similar to Jentjens *et al.* (2001) [8], calories were higher in the CHO + Pro group, an indication of why the insulin levels were consistently greater. Again, there were no differences in glycogen resynthesis between the groups.

Additionally, Tarnopolsky *et al.* (1997) [16] had greater amounts of carbohydrates that subsequently caused similar amounts of insulin levels, resulting in similar glycogen resynthesis compared to carbohydrates with an added protein supplement. Considering this, there were lesser amounts of CHO by van Hall, Shirreffs, Calbet (2000) [18] and Jentjens *et al.* (2001) [8], with subsequent lower insulin response compared to the CHO + Pro trial. Ultimately, there were no observable significant differences in glycogen resynthesis. Because Tarnopolsky *et al.* (1997) [16] had similar amounts of insulin levels and glycogen resynthesis in the CHO group (which had greater calories) compared to CHO + Pro, and van Hall, Shirreffs, Calbet (2000) [18] and Jentjens *et al.* (2001) [8] observed no difference in glycogen resynthesis with lesser amounts calories and lower levels of insulin in the CHO group, it seems that only a certain amount of insulin is necessary to elicit the maximal amount of glucose uptake in muscle fibers and going beyond this value may not increase glycogen resynthesis. This was also observed when researchers had clients supplement with beverages that were isocaloric and eucaloric. For example, Roy and Tarnopolsky (1998) [13] found similar insulin levels and no difference in glycogen resynthesis, while Alghannam *et al.* (2016) [1] found that CHO + Pro caused a greater insulin response, but still no difference in the resynthesis of glycogen.

Moreover, although authors such as Ivy *et al.* (2002) [9] has found greater insulin rises from protein and carbohydrates together, literature has also found that adding protein to carbohydrate reduces glycemic responses (Stanstrup, Schou, Holmer-Jensen, Hermansen & Dragsted, 2014). This namely happens because this macronutrient delays the speed with which food empties from the stomach and enters the small intestine, particularly the jejunum region, the main site for nutrient absorption. This process is known as gastric emptying. Because of this delayed gastric emptying, it seems inviting to assume that stimulation of insulin secretion may subsequently decrease, as these nutrients would enter the bloodstream later, but that is not necessarily true, as the literature in this section has shown multiple times that the addition of protein to a carbohydrate supplement caused greater increases insulin levels compared to carbohydrate alone, regardless if rates of glucose appearance were delayed. It has been observed, however, that there were lower blood glucose appearances in the CHO + Pro group. Authors van Hall, Shirreffs, and Calbet (2000) [18] expressed that this reduced glucose appearance from the gastrointestinal tract is from protein's ability to delay gastric emptying. Not only has protein been shown to delay gastric emptying, energy density has an effect as well (Vist & Maughan, 1995). For example, In the study by van Hall, Shirreffs, and Calbet (2000**)** [18]**,** the energy density of the CHO + Pro drink was higher than that of the CHO drink, an indication of why gastric emptying may have been slowed down, thus slowing glucose appearance. Regardless, the authors expressed that this had no influence on glucose uptake because it was not different with CHO + Pro group despite the lower glucose concentrations.

Furthermore, some authors used a small amount of fat in their trials (5g/kg). Because fat has also been shown to delay gastric emptying, potentially affecting glucose appearance or influence insulin secretion, researchers Owen and Wolever (2003) sought to investigate this subject matter. They did this by having 12 healthy subjects complete an overnight-fast and consume 50g available carbohydrate (white bread) plus 0, 5, 10, 20, or 40g fat (non-hydrogenated-fat margarine) on 5 separate days. Blood glucose peak rise (PR) and incremental area under the curve (iAUC) were reduced after 40g fat by 38 and 30%, respectively ($p <. 05$). However, more than half these effects were seen after 5g fat (Owen & Wolever, 2003). Thus, the results support the hypothesis that fat reduces glycemic responses in a dose-dependent, but non-linear fashion. Variation of fat intake across the normal range of intakes (17–44% energy) did not significantly affect glycemic responses. Considering this, authors that did use fat in their in their trials only used 6g or much less, which seems to not cause any significant effects on the glycemic responses. Additionally, no authors that used fat in their trials expressed that it would influence glycogen resynthesis compared to a trial that would not have used any fat.

6. Frequency of Supplementation

The frequency of supplementation may have influenced the magnitude glycogen resynthesis, as many researchers have found that having more frequent feeding intervals (i.e. every 15-30 minutes) of carbohydrates post exercise can increase muscle glycogen resynthesis compared to ingesting less frequent intervals (Alghannam *et al.* 2016[1]; Wagenmakers, 2000; van Hall, Shirreffs & Calbet, 2000) [19]. Greater frequency could promote a steady insulin secretion, enabling glycogen to continuously be transported to working skeletal muscle fibers. Authors who found greater or equal glycogen resynthesis in the carbohydrate group compared to the carbohydrate plus protein group all had their clients supplement in either 15 minute, 30 minute, or 1 hour intervals, after immediate supplementation. On the contrary, all authors who had their clients supplement in intervals beyond 1 hour (i.e 2 hours) after immediate supplementation found greater glycogen resynthesis in the carbohydrate plus protein group than that of the carbohydrate only group. Zawadzki, Yaspelkis, & Ivy (1985) [21] did a study in which they had subjects consume a supplement immediately and 2 hour after a glycogen depleting exercise bout. The supplement contained 112.0 g CHO, 40.7 g Pro, or 112.0 g CHO and 40.7 g Pro. The authors found that the rate of muscle glycogen storage during the CHO + Pro treatment (35.5 ∓ 3.3 mumol·g protein-1·h-1] was significantly faster than the CHO treatment (25.6 ∓ 2.3

mumol.g protein-1·h-1), which was significantly faster than during the PRO treatment (7.6 ∓ 1.4 mumol·g protein-1·h-1). Of interest was the fact that the CHO + Pro group had a greater amount of calories than the group that consumed CHO alone. Because of this, the extra calories in the CHO + PRO group may have influenced the greater magnitude of resynthesis. A subsequent study by Ivy *et al.* (2002) [9] used a similar methodology by including a low calorie carbohydrate group (LCHO) and a high calorie carbohydrate group (HCHO) in order to compare how equal caloric values would affect glycogen resynthesis. Supplements contained CHO + Pro (80 g CHO, 28 g Pro, 6 g fat), LCHO (80 g CHO, 6 g fat), or HCHO (108 g CHO, 6 g fat) on subjects immediately after exercise (10 min) and 2 h postexercise. The results showed that after 240 min of recovery, muscle glycogen was significantly greater for the CHO-Pro treatment (88.8 ∓ 4.4 mmol·l) when compared with the LCHO (70.0 ∓ 4.0 mmol·l; P = 0.004) and HCHO (75.5 ∓ 2.8 mmol·l; P = 0.013) treatments. The CHO + PRO group had more calories than the lower calorie carbohydrate group, which could be a possible implication for greater glycogen resynthesis. However, because the authors compared the resynthesis amount of a high calorie carbohydrate group of equal caloric value to the CHO + PRO group as well, it becomes evident that the extra calories of an added protein supplement to carbohydrate may not be the rationale for the extra increase in glycogen resynthesis.

On the contrary, literature that involved supplementation at 1 hour intervals had differing results. Berardi, Price & Lemon (2006), who found that the carbohydrate plus protein group had higher glycogen resynthesis compared to carbohydrate alone when supplementing Immediately, 1 h, and 2 h postexercise. The authors found that during the 6-h recovery, that there was a 67% recovery of the glycogen utilized in the carbohydrate plus protein group, and a 57% recovery of glycogen utilized in the carbohydrate group. A significant characteristic in this study showed that the calories between CHO + Pro and CHO only group were equal, suggesting that when caloric intake is equal, carbohydrates and proteins have greater synergistic capabilities compared to carbohydrates of equal caloric value alone, with the use of 1h intervals anyways.

Further, in a similar study design, Roy & Tarnopolsky (1998) [13] had clients consume a CHO (1 g·kg) supplement, or an isoenergetic supplement (66% CHO, 23% Pro, 11% fat) supplement immediately after and 1 hour after exercise. While, the rate of glycogen resynthesis was significantly greater (P <. 05) for both CHO+Pro+Fat and CHO (23.0 ∓ 4.5 and 19.3 ∓ 6.1 mmol · kg dry muscle-1 · h-1), the group that contained CHO + Pro had a greater resynthesis amount as compared to CHO alone (Roy & Tarnopolsky, 1998) [13]. However, in a similar study design, Tarnopolsky *et al.* (1997) [16] found differing results. After having clients finish a glycogen depleting exercise, clients were split into groups to consume a beverage containing CHO (0.75 g/kg) + Pro (0.1 g/kg) + Fat (0.02 g/kg), CHO (1 g/kg), or a placebo (artificial sweetener) immediately and 1h post exercise. The authors found that there was greater glycogen resynthesis in the CHO alone compared to the CHO + Pro + Fat (37.2 vs. 24. 6 mmol · kg dry muscle-1. h-1) (Tarnopolsky *et al.* 1997) [16]. Of interest is the fact that the supplements were given immediately and 1 hour after exercise, as opposed to the previous study by authors Berardi, Price & Lemon (2006), which the client’s consumed a supplement immediately, 1 hour, and 2 hours after exercise and found that carbohydrates plus protein yielded greater glycogen resynthesis compared to carbohydrate alone. From the studies done by Roy & Tarnopolsky (1998) [13] and Tarnopolsky *et al.* (1997) [16], it seems that supplementation immediately and 1 hour after exercise can show variable results in glycogen resynthesis. Whereas, literature consistently suggests that when supplementing in 1 hour intervals that go beyond 1 hour, carbohydrates plus protein will enable greater glycogen resynthesis compared to carbohydrates alone. These results change dramatically when intervals go below 1 hour, as results consistently revealed that glycogen resynthesis was always greater in the carbohydrate only group. In a most recent study by Alghannam *et al.* (2016) [1] had clients consume a carbohydrate plus protein or carbohydrate only supplement in 30 minute intervals. After a glycogen depleting exercise, clients consumed a CHO (.8 g·kg) + Pro (.4 g·kg) or isocaloric carbohydrate (1.2 g·kg). The authors found that the ingestion of carbohydrates and protein equally accelerates muscle glycogen resynthesis during short-term recovery from exhaustive running. An important characteristic in this study is the fact that there is more carbohydrate in the group that contained carbohydrate alone, suggesting the possibility that the extra 0.4 g of carbohydrate could act a greater insulin secretagogue compared to the the 0.4 g of protein in the carbohydrate plus protein group. Further, van Loon, Saris, Kruijshoop, Wagenmakers (2000) [19] did a study where they used a very similar method to that of Alghannam *et al.* (2016) [1], in which clients would consume a supplement containing CHO (.8 g·kg) + Pro (.4 g·kg), high calorie carbohydrate (1.2 g·kg), or a low calorie carbohydrate (0.8 g·kg) in 30 minute intervals. The authors noted that in this study, the CHO + Pro group also contained free leucine and phenylalanine, two amino acids that are known to be highly insulinotropic. The authors found that muscle glycogen resynthesis was higher in both trials than in the low calorie carb trial (35.4 ∓ 5.1 and 44.8 ∓ 6.8 compared with 16.6 ∓ 7.8 micromol glycosol units·g dry wt-1·h-1, respectively; P < 0.05). While CHO + Pro with insulinotropic amino acids had greater glycogen resynthesis compared to the carbohydrates of lower caloric value, it did not equal the high carbohydrate group, which had equal calories to the CHO + Pro group. These results have further revealed that despite containing highly insulinotropic free leucine and phenylalanine, supplementation in greater intervals with carbohydrates of equal caloric content will enable greater glycogen resynthesis post exercise compared to carbohydrates with an additive protein supplement. In another study, van Hall, Shirreffs, Calbet (2000) [18] did a similar study with more frequent intervals at 15 minutes. After having subjects complete a glycogen depleting exercise, they consumed either a CHO (1.6 g·kg sucrose) + Pro (0.5g·kg whey protein hydrolysate) supplement, or a CHO (1.67 g·kg sucrose). The authors found that there were no differences in glycogen resynthesis rates in CHO group compared to CHO +

Pro group (van Hall, Shirreffs, Calbet, 2000) [18].

7. Discussion

The dimension of time as it relates to nutrition has become a subject of interest for various researchers. This subject is often encountered in many academic nutrition and physiology text books, such as Nutrient Timing: The Future of Sports Nutrition, which explains how to implement a nutritional program in and around exercise sessions in order to maximize glycogen resynthesis, reduce muscle damage, maximize protein synthesis, and have enhanced subsequent performance. Glycogen, being a vital component to fuel exercise, can become depleted during intense strength and conditioning that is involved with sports or other activities (Hermansen *et al.* 1965). Further, many sports may involve training sessions that would last multiple times throughout the day causing depletion of muscle glycogen and muscle damage. A nutritional intervention that would quickly and effectively refill glycogen stores, and rebuild and preserve damaged muscle tissue is paramount in order for all athletes to effectively recover and continue to build progress in their training. Considering that the consumption of carbohydrates and proteins are vital macronutrients in order to carry out these aforementioned benefits, the central point of the research question was to investigate how the co-ingestion of these macronutrients would affect glycogen resynthesis post exercise compared to carbohydrates alone. Considering that carbohydrates and proteins are both known to be insulinotropic, it would seem rational to assume that co-ingestion would cause greater amounts of insulin response, increasing the transportation of these macronutrients to working skeletal muscle fibers. The literature that investigated these topics did find relationships between the rising in insulin levels concomitantly with carbohydrate and protein ingestion, with greater rises from co-ingestion. However, the most prevalent barrier to glycogen resynthesis was co-ingestion frequency.

While authors such as Zawadzki, Yaspelkis, and Ivy (1992) have expressed that the reason there was greater glycogen resynthesis in the CHO + Pro was because of greater rises in insulin levels, this may not be the reason. Despite a substantial insulin difference between the CHO + Pro and CHO trials, authors van Hall, Shirreffs, Calbet (2000) [18] were not able to find a higher glycogen resynthesis rate. There are several possible reasons to explain the apparent difference. First, the glycogen resynthesis rate of ∼41 μmol · kg dry muscle−1 · h−1, as observed by Zawadzki, Ivy, and Ivy (1985) [21] with CHO + Pro intake, is nearly identical to the glycogen resynthesis rate in the study by van Hall, Shirreffs, Calbet (2000) [18], both for CHO + Pro and CHO (39.8 μmol · kg dry muscle−1 · h−1). This may suggest that there is a maximal achievable glycogen resynthesis rate with sufficient and regular carbohydrate loading regardless of higher insulin levels, as a lower amount of insulin may have been a sufficient amount to elicit the maximal positive achievable effect on glycogen resynthesis during recovery from intense exercise (Zawadski, Yaspelkis, & Ivy, 1985) [21]; (Shirreffs and Calbet, 2000) [18].

8. Summary of Results

Authors that investigated the synergistic abilities from the co-ingestion of carbohydrates and proteins had treatments groups consume carbohydrates, or carbohydrates plus protein in intervals that ranged from 15 minutes to 2 hours. The addition of protein to a carbohydrate supplement post exercise has shown greater increases in glycogen resynthesis compared to carbohydrate alone. However, this seems to only occur when 2 hour feeding intervals are implemented. In one study, calories from CHO + Pro group were higher than the CHO only group when supplementing with 2 hour intervals, revealing the possibility that the larger quantity in macronutrients would subsequently have created greater amounts of glycogen resynthesis. However in a subsequent study, caloric values were similar between both groups, suggesting that the greater macronutrient and subsequent caloric content was not responsible for the greater synthesis. When supplementing with 1 hour intervals, differing results occur, as glycogen resynthesis from CHO + Pro was not always greater than CHO alone. When frequencies were below 1 hour, glycogen resynthesis was always higher in the CHO group than the CHO + Pro group. Indeed, protein has been shown to be insulinotropic, however even when authors van Loon, Saris, Kruijshoop, Wagenmakers (2000) [19] added highly insulinotropic amino acids leucine and phenylalanine to the CHO + Pro group, there was not a greater amount of glycogen resynthesis, revealing the superiority of glycogen resynthesis capacities carbohydrates alone when supplementing in frequencies below 1 hour, provided both groups had equal caloric values (van Loon, Saris, Kruijshoop, Wagenmakers 2000) [19]. Regardless of these differing results, ingestion of macronutrients immediately after exercise is essential in order to obtain the greatest results, as literature has suggested that there is an anabolic window in which working skeletal muscle fibers are more sensitive to the effects of insulin. Although supplementing with carbohydrates in 15 to 30 minute intervals has greater effects for glycogen resynthesis compared to the effects of having protein co-ingested, the consumption of proteins is still important considering their benefits for rebuilding and preserving lean muscle tissue. The co-ingestion of carbohydrates and proteins has not been shown to increase glycogen resynthesis post exercise compared to carbohydrates in equal caloric value when supplementing from 15 to 30 minute intervals. However, when supplementation is immediately and 2 hours later, co-ingestion of these macronutrients improves glycogen resynthesis compared to carbohydrates of equal caloric value.

9. Conclusion

The body of literature as it relates to nutrient timing has shown that co-ingestion of carbohydrates and proteins effectively produces the greatest amount of glycogen resynthesis when consumed immediately and 2 hours after. According to Ivy (2004) [8], when both carbohydrate and protein are co-ingested, it is recommended that 0.8 g carbohydrate·kg-1 body wt plus 0.2 g protein·kg-1 body wt be consumed immediately and 2-hours after exercise during a 4-hour recovery period for maximum results (Ivy, 2004) [8]. Under the condition that carbohydrates are the only supplement available for an individual who has completed an exercise bout, supplementing in 15 to 30 minute intervals would be ideal, as literature has shown that this leads to greatest amounts of glycogen resynthesis. Under this circumstance, research suggests that the carbohydrate ingestion should provide about 1.2 to 1.5 g of carbohydrate·kg-1 body wt·h-1 (Ivy, 2002) [9].

10. Recommendation for Further Research

The research has shown carbohydrates and proteins both have effects on insulin levels, as this hormone is stimulated from the ingestion of these nutrients. However, a couple issues arise when considering how these studies can be applied to real life scenarios. One of these issues is the fact that carbohydrates and proteins come in varying forms, as there are dozens of food choices that can contain varying amounts carbohydrates (monosaccharide, disaccharide, and polysaccharides) that have varying types of influences on insulin mechanics. Further, proteins are made up of amino acids, each affecting insulin levels differently (Zhang *et al.* 2011) [23]. Considering this, these studies are all completed using carbohydrate and protein supplements, rather than specific food sources. Comparing and contrasting intake of carbohydrate and protein food sources post early exercise could give insights on what meals are objectively best suited to create the most anabolic environment. For example, an athlete may desire fruits as a source of carbohydrates to ingest, however the fructose can cause gastrointestinal issues that may cause diarrhea and vomiting, an unfavorable circumstance if an athlete had subsequent exercise sessions or performance events within the day.

Further, in the immediate phases after exercise, GLUT4 will translocate to the surface of the cell membrane as a response to muscle contraction (insulin-independent). Later, however, insulin will be responsible for continuing this process as GLUT4 will become insulin-dependent. It may be possible that only certain amount of insulin is necessary to elicit the maximum achievable amount of glucose uptake into the cell. The specific amount of insulin that is needed is not known at this time, and should be a potential topic for future investigation. Regardless, this may very well be the reason that there are no significant differences in the resynthesis of glycogen between CHO and CHO + Pro despite differing insulin levels.

Moreover, studies that have examined gastric emptying in relation to exogenous CHO oxidation have shown that the rate of gastric emptying is not the limiting step in the oxidation of the oral ingested glucose. It is, therefore, unlikely that the rate of gastric emptying will limit the rate of glycogen synthesis when only CHO is ingested. However, researchers have found that the rate of gastric emptying is affected by the energy density of the food consumed that contains protein and fat. Researchers that had greater energy density in the CHO + Pro trials may have confounded the results by inhibiting gastric emptying. This may have slowed down the rapid delivery of glucose to the muscle and amino acids to stimulate the pancreas for insulin secretion. If gastric emptying would hinder the delivery of glucose to the muscle, it is possible that less glycogen synthesis would have occurred in the first phase of glycogen synthesis when contraction-induced GLUT-4 migration was still present. However, authors in these studies did not measure gastric emptying in order to see if this was the specific cause and would therefore be a recommendation for future research.

11. Summary

Table 1

Study	Population	# of clients	Supplementation	Exercise protocol	Frequency	Glycogen Resynthesis	Instrumentation
Alghannam *et al.* (2016) [1]	Recreationally active runner, 26 ∓ 11 age.	6 (5M, 1F)	CHO (.8g) + Pro (.4g), CHO (1.2g)	Run-to-exhaustion at 70% VO2max	Immediately and 30 min intervals	Similar: 99∓3 mmol/kg	Needle Biopsy
Berardi, Price, Noreen, & Lemon (2006)	6 male cyclists	6	CHO (.8 g/kg) + Pro (.4 g/kg), CHO (1.2g/kg), PLB (no energy)	60-min time trial	Immediately, 1 h, and 2 h postexercise	Greater in CHO + Pro (28.62 ± 2.10 mmol/L), CHO (22.20 ± 1.19 mmol/L ± 0.05)	NMR spectroscopy
Carrithers, Williamson, Gallagher, Godard, Schulze, & Trappe (2000) [4]	7 male collegiate cyclists, 25.6 ± 1.3 age.	7	Based off 1 g/kg carbohydrate: CHO (100% α-d-glucose), CHO (70%) + Pro (20%) + Fat (10%), or a CHO 86% + Amino acid (AA) (14%)	75 min at 70% Vo2 peak on cycle ergometer plus 1-min sprints at 125% of Vo2 peak (1-min rest period between sprints)	Immediately and 30 min intervals	Similar: CHO (231 ± 37 mmol/kg), CHO + Pro (230 ± 29 mmol/kg dm)	Muscle biopsy of vastus lateralis
Ivy *et al.* (2002) [9]	Trained cyclists. 19–26	7	CHO (80g) + Pro (28g) + fat (6g), or	2 h of cycling at 65–75% of his Vo 2 max	Immediately after exercise	Greater CHO + Pro (88.8 ∓ 4.4),	NMR spectrosco

	yr		LCHO (80 g CHO, 6 g fat), or HCHO (108 g CHO, 6 g fat)		and 2 h postexercise	LCHO (70 ∓ 4), HCHO (75.5 ∓ 2.8 mmol/l)	py
Jentjens *et al.* (2001) [8]	Trained cyclists. Male	8	CHO (1.2 g/kg) + Pro (.4g/kg), or CHO (1.2 g/kg).	2-min block periods at alternating workloads of 90 and 50% W˙max	Immediately and 30 min intervals	Similar: CHO (225 ± 22), CHO + Pro (252 ± 48 mmol/kg dm)	Muscle biopsy of vastus lateralis
Roy and Tarnopolsky (1998) [13]	Healthy young men	10	CHO (1 g/kg), or CHO+Pro+Fat (66% CHO, 23% Pro, 11% fat)	3 sets at 80% 1 repetition maximum with unilateral knee extension	Immediately and 1hr after exercise	Similar: CHO+Pro+Fat (23.0 ± 4.5) and CHO (19.3 ± 6.1 mmol/kg dm)	NMR spectroscopy
Tarnopolsky *et al.* (1997) [16]	16 athletes	16 (8M, 8F)	CHO (0.75 g/kg) + Pro (0.1 g/kg) + Fat (0.02 g/kg), or CHO (1 g/kg)	90 min at 65% peak O2 consumption	Immediately and 1h after exercise	Similar: CHO+Pro+Fat (24.6), CHO (37.2 mmol/kg dm)	NMR spectroscopy
van Hall, Shirreffs, Calbet (2000) [18]	Healthy trained. 26 ± 2 yr	5	CHO (1.67) + Pro (.5 g/kg body wt), or CHO (1.67)	2-min block periods at alternating workloads of 90 and 50% W˙max	Immediately and 15 minute intervals	Similar: CHO + Pro / CHO 52 ± 7, 48 ± 5 first 1.5h, 30 ± 6, 36 ± 3 mmol/kg dm between 1.5 and 4h	Automatic Analyzer
van Loon, Saris, Kruijshoop, Wagenmakers (2000) [19]	Healthy trained male cyclists. 24.0 ± 0.6 yr.	8	CHO (.8g/kg) + Pro (.4 g/kg), or CHO (.8 g/kg), or CHO (1.2 g/kg)	2-min block periods at alternating workloads of 90 and 50% W˙max	Immediately and 30 min intervals	Similar: CHO (272 ± 54), CHO + Pro (351 ± 39), and (362 ± 46 µmol glycosol units/g)	Muscle Biopsy
Zawadzki, Yaspelkis, & Ivy (1992)	Healthy male subjects	9	112.0 g carbohydrate (CHO), 40.7 g protein (PRO)	cycled for 2 h on three separate occasions	Immediately and 2 h	Greater CHO + Pro (35.5 ± 3.3), CHO (25.6 ± 2.3 mumol/g)	Muscle Biopsy of vastus lateralis

12. References

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