

## Post Exercise Glycogen in Activity Related to Ingestion of Carbohydrates and Protein Mixture through Gymnastics Training Program.

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### Abstract

**Background:** Regular intensive gymnastic exercise activities causes muscle glycogen and protein depletion. Post-exercise recovery helps re-synthesis of glycogen and proteins. Ingestion of carbohydrates alone or with protein after exercise stimulates re-synthesis. All previous studies investigated tissue biopsies for estimating glycogen and protein resynthesis. The initiation of glycogen synthesis is provided by a self-glucosylation of a protein self extracting enzyme glycogenin under the control of insulin and blood glucose levels. Plasma glycogenin could be used as a good marker for glycogen resynthesis.

**Methods:** Sixteen male students from the faculty of physical education for boys, Menufia University, grade four were constituted subjects of this study. They were subgrouped in two equal groups, subjected to a gymnastic training program for three months. The first group (control) ingested sucrose at the end of every training session while the second (experimental) ingested a mixture of CHO/protein beverage. Their anthropometric measurements and blood glycogenin-1, insulin and glucose were assayed and correlated.

**Results:** All variables were revealed significant improvement in all physical measurements with increased response of post exercise blood levels in glycogenin-1 and insulin with administration of post-exercise sucrose or CHO/protein drinks with most higher results of the mixture .

**Conclusions:** It is concluded that gymnastic training program with post exercise carbohydrate or CHO/protein intake increase the physical efficiency with improved high performance.

**Key words:** Plasma glycogenin-1, insulin, plasma glucose levels, CHO/protein, gymnastic training program, physical measurements.

### Introduction:

Prolonged sub-maximal exercise is dramatically reduce the muscle and liver glycogen. Mode of exercise and the volume, intensity, and frequency of the training sessions induce muscle adaptations, causing marked change in the storage profile of

skeletal muscles glycogen (Hawley et al., 2011). Also, muscle protein synthesis is decreased during an acute bout of resistance exercise; however, during post-exercise recovery the rate of glycogen and muscle protein synthesis is increased under control of insulin. Furthermore, ingesting of

protein/carbohydrate in close to the end of exercise enhances the metabolic response of post-exercise recovery (**Breen et al., 2011**).

Glycogen -the storage form of carbohydrate- is stored in many tissues with the major depots located in muscle and liver. Muscle glycogen is degraded to generate ATP during increased energy demand, whereas hepatic glycogen is broken down, releasing glucose into the bloodstream to supply other tissues. During recovery, glycogen re-synthesis starts at strictly ordered process, resulting in a complex structure (**Melendez et al., 1999 and Roach (2002)**). Post-exercise muscle glycogen synthesis is an important factor in determining the time needed to recover from prolonged exercise (**van Loon et al., 2000**).

Glycogenin is a glycogen re-synthesizing enzyme initiates granule formation by the addition of about 7–11 glucose residues to a single tyrosine residue on its apo-protein. Then, glycogen synthase utilizes UDP-glucose to transfer glucose molecules by 1–4 linkages to glycogen, which is the rate-controlling step of glycogen synthesis (**Greenberg et al., 2006**). Although it is appreciated that glycogenin is essential for the formation of a glycogen granule, its regulation has rarely been studied in human plasma. Glycogenin-1 (42 kDa) content is abundant in skeletal muscle, responsible for muscle and tissue glycogen re-synthesis (**Shearer et al., 2005**).

Protein ingestion after a single bout of resistance-type exercise stimulates net muscle protein accretion during post-exercise recovery. Consequently, it is generally accepted that protein supplementation is required to maximize the adaptive response of the skeletal muscle to prolonged resistance-type exercise training (**Cermak et al., 2012**). Also, Reidy et al. (2013) found that protein ingestion following resistance

exercise promotes human muscle protein synthesis.

Recent work has shown that the ingestion of whole proteins such as whey or casein with carbohydrate (CHO) following resistance exercise accelerate muscle protein synthesis following an acute bout of resistance exercise (**Elliot et al., 2006, Koopman et al., 2005 & Koopman et al., 2007**) or when ingested chronically following 3 months of resistance exercise training (**Fujita et al., 2007**). **Zawadzki et al. (1992)** reported that addition of an intact protein to a carbohydrate-containing solution resulted in higher glycogen synthesis rates in subjects after exercise than did ingestion of carbohydrate only.

However, there is much discrepancy in the literature regarding the proposed benefits of regular protein/carbohydrate supplementation during recovery from exercise through training program. Also, all previous studies interested in muscle biopsy for assessing glycogen re-synthesis. Very rare studies concerned plasma constituents for assaying glycogen re-synthesis in the recovery period. The aim of this study was to evaluate the effect of chronic post-exercise ingestion of carbohydrate/protein mixture on the adaptation process and glycogen recovery rate after gymnastics training program in a three months period.

Plasma insulin, glycogenin-1 and glucose concentrations were measured pre-exercise, 1 hour post-exercise and 3 hours later to determine glycogen synthesis activity and muscle glycogen content. Anthropometric measurements including circumferences of chest, neck, upper arm, thigh and calf were assayed before and after the training program.

## Material and Methods:

### Subjects:

Sixteen male students from the faculty of physical education for boys, Menofia University, grade four were volunteered for this study. They were recruited for the study after completing a medical questionnaire and giving their informed, written consent. All experimental procedures were approved by the Helwan University Human Research Ethics. Participants were divided into two groups as control who received sucrose beverage and second experimental group received CHO/Protein mixture beverage at the end of every training session.

### Study design:

In the present study, subjects were subjected to prolonged gradual increasing intensity to be sub-maximal gymnastics exercise (~50% to 90 of VO<sub>2</sub>max) to reduce the muscle

glycogen and then sucrose or sucrose/protein mixture were administrated to discover the rate of glycogen re-synthesis during recovery and muscle adaptations after training program for three months. We started at intensity of 50% of VO<sub>2</sub>max and increased by 10% every two weeks for two months, then by 5% every two weeks in the third month. We tested for changes in plasma glycogenin-1, insulin and levels of plasma glucose levels under these conditions. Also, anthropometric adaptation including circumferences of chest, neck, arm, thigh and calf muscles before and after the training program.

### Program design:

Subjects were subjected to the intense upgraded gymnastic program for 12 weeks for 3hrs/ 3 days/ week. The gymnastics exercise session was partitioned as prescribed in the following table.

Exercises	Warming Up	Flexibility	Anaerobic	Aerobic	Technique	Resistance	Flexibility	Sum
minutes	20	15	20	20	70	20	15	180

### CHO/Protein supplementation:

Subjects received a beverage volume of 2 ml/kg 15 min after exercise to ensure a given dose of 1.0 g/kg carbohydrate only (CHO) for control group, or a dose of 0.8 g/kg carbohydrate with 0.2 g/kg protein (CHO/PRO) for experimental group.

This supplementation regimen has been proven effective to allow a continuous supply of glucose and amino acids from the gut and, as such, to minimize perturbations in plasma glucose, amino acid, and circulating insulin concentrations during recovery (**Beelen et al., 2011**). Sucrose was used as the carbohydrate supplement while milk casein

protein was used as the protein portion of the mixture.

### Blood analysis:

Blood samples at rest were collected under aseptic conditions from the anti-cubital vein. Post-exercise blood sampling was performed immediately after the exercise and three hours later. Samples were collected in tubes containing coagulation activator, centrifuged and kept at -20 C° until tested. Plasma glycogenin-1 and insulin levels were determined using a commercial ELISA kits provided by Teco medical group, Germany. Glucose was measured colorimetrically using kits provided by Randox, England.

**Statistical analysis:**

All data are expressed as means  $\pm$  SD. The plasma insulin, glucose, and glycogenin levels in control group were compared to that obtained in experimental group by T. test. A two-factor repeated-measures analysis of variance (ANOVA) was used to compare differences between treatments within the same group. Statistical significance was set at  $P \leq 0.05$ . All calculations were performed using SPSS Statistics 17.0 (SPSS, Chicago, IL).

**Results:**

Table (1) postulate anthropometric measurements obtained from the students before and after the training program in both groups. There are significant differences of arithmetic means of all anthropometric data between initial measurements of the experimental group students compared to the post-training program results. Control group data was also significant except for calf muscle and weight.

(Table 1)  
Anthropometric data obtained for control and experimental groups  
(Before compared to after program)

Control (Mean $\pm$ SD)					
	Before	After	T	p	Sig.
Chest (circum. Cm)	91.21 $\pm$ 1.17	92.97 $\pm$ 1.36	-7.47	0.00	S
Neck (circum. Cm)	36.28 $\pm$ 1.48	37.43 $\pm$ 1.64	-4.97	0.00	S
Arm (circum. Cm)	30.13 $\pm$ 1.34	32.1 $\pm$ 0.99	-5.66	0.00	S
Thigh (circum. Cm)	46.81 $\pm$ 1.46	47.77 $\pm$ 1.45	-2.63	0.03	S
Calf (circum. Cm)	32.35 $\pm$ 0.74	33.11 $\pm$ 1.69	-1.35	0.22	NS
Height (cm)	168.12 $\pm$ 4.26	xxx	xxx	xxx	
Weight (kg)	63.75 $\pm$ 2.23	64.25 $\pm$ 3.28	-1.12	0.30	NS
Age (years)	21.125 $\pm$ 0.83	xxx	xxx	xxx	
Experimental (Mean $\pm$ SD)					
	Before	After	T	p	
Chest (circum. Cm)	91.36 $\pm$ 1.23	93.75 $\pm$ 1.28	-11.10	0.00	S
Neck (circum. Cm)	36.31 $\pm$ 1.37	37.87 $\pm$ 1.64	-10.59	0.00	S
Arm (circum. Cm)	30.16 $\pm$ 1.39	31.62 $\pm$ 1.19	-12.10	0.00	S
Thigh (circum. Cm)	46.38 $\pm$ 1.41	48.12 $\pm$ 1.64	-13.28	0.00	S
Calf (circum. Cm)	32 $\pm$ 1.78	33.75 $\pm$ 1.67	-17.50	0.00	S
Height (cm)	168.5 $\pm$ 4.44	xxx	xxx	xxx	
Weight (kg)	63 $\pm$ 3.66	64.25 $\pm$ 3.28	-5.00	0.00	S
Age (years)	21 $\pm$ 1.07	xxx	xxx	xxx	

(Table 2)  
Anthropometric data obtained for control compared to experimental group  
(Before and after program)

Control compared to experimental groups before program			
	t	p	Sig.
Chest (circum. Cm)	-0.46	0.66	NS
neck (circum. Cm)	-0.13	0.90	NS
arm (circum. Cm)	-0.07	0.94	NS
thigh (circum. Cm)	1.41	0.20	NS
calf (circum. Cm)	0.65	0.54	NS
height (cm)	-0.46	0.66	NS
weight (kg)	1.25	0.25	NS
age (years)	0.36	0.73	NS
Control compared to experimental groups after program			
Chest (circum. Cm)	-3.31	0.01	S
neck (circum. Cm)	-3.92	0.01	S
arm (circum. Cm)	1.70	0.03	S
thigh (circum. Cm)	-1.78	0.12	NS
calf (circum. Cm)	-3.28	0.01	S

There is no statistically significant difference in any of the anthropometric parameters obtained data between the two groups at the start of the study (Table 2). All physical parameters were found to be differed after the program.

(Table 3)  
Difference between experimental and control groups biochemical parameters (t test) (Mean  $\pm$  SD)

	Before program				
	Control	experimental	t	p	Sig.
	Pre exercise				
GLU (mg/dl)	82.6 $\pm$ 6.17	81.6 $\pm$ 3.1	.419	.685	NS
Insulin ( umol/ml)	16.6 $\pm$ 3.39	17.37 $\pm$ 3.62	-.788	.451	NS
Glycogenin-1 (pg/ml)	35.35 $\pm$ 7.07	40.85 $\pm$ 7.98	-1.637	.136	NS
	After 1 hour				
GLU (mg/dl)	82.7 $\pm$ 3.86	81.4 $\pm$ 4.33	.652	.531	NS
Insulin ( umol/ml)	26.2 $\pm$ 6.83	37.72 $\pm$ 4.97	-3.604	.006	S
Glycogenin-1 (pg/ml)	65.11 $\pm$ 9.47	63.27 $\pm$ 11.45	.435	.673	NS
	After 3 hours				
GLU (mg/dl)	82.9 $\pm$ 4.95	81.2 $\pm$ 4.05	.953	.365	NS
Insulin ( umol/ml)	21.07 $\pm$ 3.31	39.92 $\pm$ 9.12	-6.722	.000	S

	Before program				
	Control	experimental	t	p	Sig.
	Pre exercise				
Glycogenin-1 (pg/ml)	42.26 ± 9.1	41.94 ± 4.73	.103	.920	NS
	After program				
	Control	experimental	t	p	Significance
	Pre exercise				
GLU (mg/dl)	82.00 ± 3.83	82.00 ± 3.33	.000	1.000	NS
Insulin ( umol/ml)	18.95 ± 6.45	17.24 ± 2.84	.631	.544	NS
Glycogenin-1 (pg/ml)	37.87 ± 12.47	44.00 ± 7.2.	-1.159	.276	NS
	After 1 hour				
GLU (mg/dl)	81.4 ± 2.91	84.6 ± 5.27	-1.489	.171	NS
Insulin ( umol/ml)	34.21 ± 6.20	64.4 ± 8.73	-13.352	.000	S
Glycogenin-1 (pg/ml)	75.96 ± 8.94	80.34 ± 6.35	-1.277	.234	NS
	After 3 hours				
GLU (mg/dl)	83.1 ± 4.53	81.7 ± 3.23	.924	.379	NS
Insulin ( umol/ml)	28.58 ± 7.29	54.6 ± 4.97	-8.567	.000	S
Glycogenin-1 (pg/ml)	50.66 ± 8.31	53.52 ± 6.87	-.947	.369	NS

Biochemical results obtained for both groups were compared in table (3). Both groups were equivalent at rest before exercise either before or after the program. After exercise with an hour or three hours, only insulin

levels were elevated either before or after the program indicating that CHO/Protein beverage has an acute and chronic effects on the recovery state.

(Table 4)  
Analysis of difference (ANOVA) for control and experimental group biochemical parameters  
(Mean  $\pm$  SD)

control before program						
	Pre-exercise	After 1 hr.	After 3 hrs.	F	p	Sig.
GLU (mg/dl)	82.6 $\pm$ 6.17	82.7 $\pm$ 3.86	82.9 $\pm$ 4.95	0.004	0.99	NS
Insulin ( $\mu$ mol/ml)	16.6 $\pm$ 3.39	26.2 $\pm$ 6.83	21.07 $\pm$ 3.31	7.95	0.002	S
Glycogenin-1 (pg/ml)	35.35 $\pm$ 7.07	65.11 $\pm$ 9.47	42.26 $\pm$ 9.1	29.11	0.001	S
control group after program						
	Pre-exercise	After 1 hr.	After 3 hrs.	F	p	Sig
GLU (mg/dl)	82.00 $\pm$ 3.83	81.4 $\pm$ 2.91	83.1 $\pm$ 4.53	0.51	0.606	NS
Insulin ( $\mu$ mol/ml)	18.95 $\pm$ 6.45	34.21 $\pm$ 6.20	28.58 $\pm$ 7.29	13.41	0.001	S
Glycogenin-1 (pg/ml)	37.87 $\pm$ 12.47	75.96 $\pm$ 8.94	50.66 $\pm$ 8.31	37.05	0.001	S
experimental group before program						
	Pre-exercise	After 1 hr.	After 3 hrs.	F	p	Sig
GLU (mg/dl)	81.6 $\pm$ 3.1	81.4 $\pm$ 4.33	81.2 $\pm$ 4.05	0.02.	0.97	NS
Insulin ( $\mu$ mol/ml)	17.37 $\pm$ 3.62	37.72 $\pm$ 4.97	39.92 $\pm$ 9.12	38.35	0.001	S
Glycogenin-1 (pg/ml)	40.85 $\pm$ 7.98	63.27 $\pm$ 11.45	41.94 $\pm$ 4.73	22.06	0.001	S
experimental group after program						
	Pre-exercise	After 1 hr.	After 3 hrs.	F	p	Sig
GLU (mg/dl)	82.00 $\pm$ 3.33	84.6 $\pm$ 5.27	81.7 $\pm$ 3.23	1.54	0.23	NS
Insulin ( $\mu$ mol/ml)	17.24 $\pm$ 2.84	64.4 $\pm$ 8.73	54.6 $\pm$ 4.97	17.4	0.001	S
Glycogenin-1 (pg/ml)	44.00 $\pm$ 7.2.	80.34 $\pm$ 6.35	53.52 $\pm$ 6.87	76.48	0.001	S

Analysis of variance for every group concerning pre-exercise compared to after 1 hour and three hours post-exercise resulted in significant differences in insulin and glycogenin-1 levels in plasma but not glucose (table 4). These results reveal high anabolic metabolism in the recovery period regardless the type of beverage consumed.

### Discussion

The main cause of this study was investigating plasma insulin, glycogenin-1 and glucose as a precursor for post-exercise resynthesis. Glycogenin-1 was found to be elevated markedly after an hour and three hours of exercise with drinking sucrose or

CHO/protein beverage. Also, it is more elevated at the end of the program up to double fold of resting levels meaning response to the program with CHO/protein drink. Insulin levels were elevated post-exercise either before or after program with highest significant levels after program with CHO/protein beverage compared to sucrose only.

The primary finding from our study was that muscle protein synthesis (expressed by increased muscle circumference) increased post-exercise in both the CHO/Protein group and control (CHO only) group. But performing a program of gymnastic training exercise with CHO/protein ingestion lead to

further increase in muscle protein synthesis during post-exercise recovery compared with the control group.

Protein ingestion after a single bout of resistance-type exercise stimulates net muscle protein accretion during post-exercise recovery. Consequently, it is generally accepted that protein supplementation is required to maximize the adaptive response of the skeletal muscle to prolonged resistance-type exercise training (**Cermak et al., 2012**). Also, **Reidy et al. (2013)** found that protein ingestion following resistance exercise promotes human muscle protein synthesis

These data are in agreement with a large number of studies in which nutrient ingestion (i.e., amino acids, protein, or essential amino acids/ carbohydrates (EAA/CHO) following a bout of resistance exercise has been shown to increase muscle protein synthesis in human subjects (Biolo et al., 1997, Borsheim et al., 2002, Dreyer et al., 2008 & Elliot et al., 2006). In addition, recent work has shown that when subjects ingested fat-free milk (i.e., protein/CHO) following each resistance exercise bout (during 3 months of training), the increase in muscle mass was greater than in either an iso-energetic soy protein group or an iso-energetic CHO control group (Hartman et al., 2007). Fujita et al. (2009). Although all these studies concerned same subject, but all of the investigated muscle biopsies.

Findings of this study revealed non-significant elevation in plasma glucose in the all investigated stages of the study. Decreasing muscle glycogen content after exercise may play a role, because the activation of AMP kinase (AMPK) and its associated increases in muscle glucose uptake are affected by glycogen content (**Wojtaszewski et al., 2002**). During

exercise, continued muscle contraction lead to glycogen breakdown with increased extracellular glucose uptake and utilization (**Greenberg et al., 2006**). Glucose transport into the muscle is increased, correlated acutely with enhanced glucose protein carrier GLUT4 translocation to the plasma membrane (**Rasmussen et al., 2002**). According to these mechanisms, glucose remains constant in plasma through all investigation periods. This also can be explained by the observed additional increase in plasma insulin concentrations after ingestion of the carbohydrate-protein mixture. Elevated insulin concentrations may lead to increased glucose uptake (**Hartman et al., 2007**) and to an increase in glycogen synthase activity (**Hasten et al., 2000 & Jorfeldt and Wahren 1971**), which forms the major factor in determining the rate of glycogen synthesis when the substrate supply is adequate (**Fujita et al., 2007 & Kim et al., 2005**).

Insulin elevation was found to increase after exercise with or without additives. **Brun et al. (1995)** found insulin to increase after a single bout of exercise at 85% of maximal heart rate (HR), they reported that glucose effectiveness and insulin sensitivity markedly increased at 25 min of recovery after 60-min hard exercise at 85% of estimated maximum HR that corresponded to about 70–80% VO<sub>2</sub> max. **Rheaume et al., (2003)** demonstrated insulin elevation at (70 –80% VO<sub>2</sub> max) and **Mikines et al., (1998)** at 70% VO<sub>2</sub> max and at (65% VO<sub>2</sub> max). Insulin stimulated recruitment of glucose transporter (GLUT-4) to the plasma membrane and activation of glycogen synthase in muscle are the major mechanisms responsible for the enhanced insulin-stimulated glucose transport and metabolism (**Sakamoto and Goodyear, 2002**).

Although this study investigated plasma glycogenin-1, there were very few studies investigated glycogenin in tissue biopsies. Kraniou et al. (2000) and Shearer et al. (2005 [A&B]) have suggested that during recovery from prolonged exercise GN-1 is synthesized to facilitate new granule formation and glycogen (especially pro-glycogen) resynthesis. To date, there have been no comprehensive examinations of this possibility. Intense exercise bout reduce muscular glycogen by 75–85% without GN-1 protein decrease or its mRNA increase even during recovery despite a doubling of glycogen concentration by 2 h of recovery with CHO administration. Within 5 h of recovery, total glycogen levels were re-synthesized to be about 80% of resting levels (Wilson et al., 2007).

Glycogenin-1 (GN-1) catalyzes the addition of glucose units by 1-4 linkage to form an oligosaccharide chain of 7–11 glucose units in length, each attached by  $\alpha$ 1-4 linkages. Once complete, this chain is acted upon by glycogen synthase and phosphorylase and transferred to glycogen by 1-6 linkage. All residues are removed except for the last one attached by Tyr194, whose bond (C-1-O tyrosyl linkage) is not accessible by the enzyme (Shearer et al., 2005 [A]).

It is discussed that elevated plasma GN-1 in exercise training may be due to loss of cell facultative permeability induced by elevated waste products or may be due to increased white blood cells activities during exercise leading to increased GN-1 production and passing to blood stream (Blom et al., 1986 & 1987). These results need more investigations concerning white blood cells glycogenin-1 mRNA and/or re-investigate previous studies hypothesis of muscular glycogenin-1 translational DNA response.

**In conclusion,** chronic CHO/protein ingestion post-exercise appears to be more effective (than ingestion of CHO only) at inducing further increases in the rate of human skeletal muscle protein and glycogen synthesis during early post-exercise recovery.

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