

# Micronutrient complexes support glucose metabolism in skeletal muscle cells

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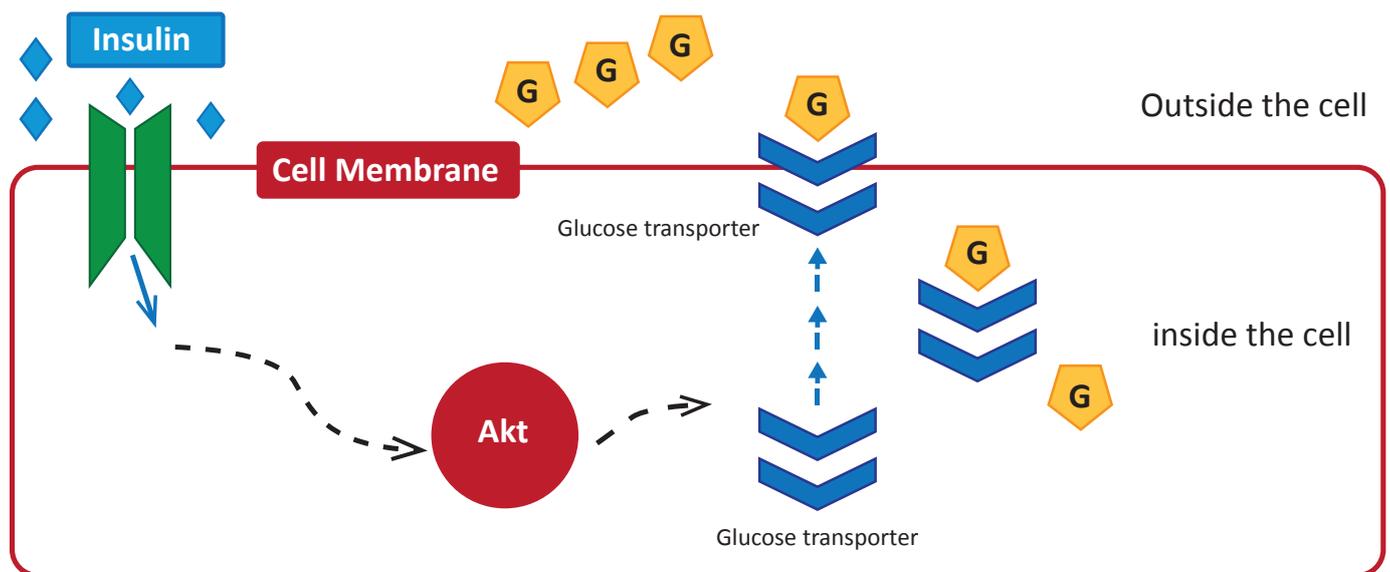
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## Introduction

As a key substrate for generating biological energy in all of the body's cells, glucose is vital for sustaining life. Numerous other metabolic processes and bodily functions also depend on a steady supply of glucose. In all aspects, optimum intracellular transport of glucose is essential for a healthy metabolism and for maintaining healthy blood glucose levels.

Glucose usage (uptake) by cells of the various organs is regulated by insulin and proper functioning of insulin receptors in the cell membranes. It has been known that insulin action is mediated by protein kinase known as Akt, which has to be phosphorylated in order to promote glucose transport inside the cells. This phosphorylated form of Akt relocates glucose transporters towards the cell's outer membrane which draws glucose into the cell and away from the bloodstream. Figure 1 provides a brief explanation of how Akt mediates insulin activity via glucose transporters. Impaired Akt phosphorylation is implicated in diabetes and metabolic syndrome.<sup>1</sup>



**G** Glucose

Fig 1: Insulin (blue diamonds) binds to its receptor (green) and activates Akt (red circle) inside the cells. Akt then sends glucose transporter to the cell membrane where glucose (yellow pentagons) circulating in the bloodstream is captured and taken inside the cells.

It is known that various micronutrients are involved in many steps of glucose metabolism and facilitate its intracellular transport (i.e. vitamin C). It is known that certain micronutrients, like chromium and B vitamins are required for efficient glucose metabolism.<sup>2-4</sup> Most of these nutrients were studied individually; however, in cellular metabolism they cannot act in isolation and must interact with each other.

In our study we investigated how different multivitamin/nutrient formulas can affect glucose uptake and its regulatory mechanisms (Akt phosphorylation) in skeletal muscle cells. We tested 3 nutritional supplements: supplement A containing a comprehensive combination of essential vitamins and other components to support general cell metabolism, supplement B containing select micronutrients important for glucose metabolism in cells and supplement C comprising B Vitamins. We demonstrated that nutritional supplement complexes play an important role in regulating uptake of glucose by the cells and that their efficacy depends on both ingredient selection and dosage.

## Methods

### Reagent Preparation and Assays

All test formulations—A, B and C were dissolved in accordance with the protocol recommended by United States Pharmacopeia.<sup>5</sup> Three recommended daily doses of each supplement were powdered (tablets were crushed using ceramic pestle and mortar; capsules were cut open and powder poured out), placed into glass container with 900 ml of 0.1N hydrochloric acid and incubated for 1 hour at 37°C in a shaking incubator set with rotation speed of 75 rpm. Resulting solutions were filter-sterilized using 0.2 micrometer pore size filters, aliquoted and kept frozen at -20°C until analyses. Vitamin B complex was dissolved in DMSO. For the glucose uptake assay Formula A was applied at 0.13 micrograms/ml, Formula B was applied at 0.51 micrograms/ml and Formula C which is Vitamin B complex was applied at 0.01 micrograms/ml. For the Akt Phosphorylation assay

Formula A was applied at 0.13 micrograms/ml, Formula B was applied at 0.26 micrograms/ml and Formula C was applied at 0.01 micrograms/ml. Basic compositions of the supplements used in our experiments are shown in Table 1.

### Culture of Rat Myoblasts

Rat Myoblasts (L6) were obtained from ATCC (American Type Culture Collection, Rockville, MD, USA) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS, 100U/ml penicillin and 100U/ml streptomycin. In the experiments, the cells were grown to confluency in a 96 well plate.

### Glucose Uptake Assay

Myoblast L6 cells were treated with various nutritional formulas for 24 hours in serum-free media followed by 1 hour starvation by incubating in Hanks' Balanced Salt solution from Sigma Aldrich (St. Louis, MO). Subsequently, glucose uptake by the cells was measured using Glucose Uptake kit from Sigma. In principle, glucose-deprived cells were incubated with 2-deoxyglucose (2DG) which is taken into the cells in the same way as glucose. Inside the cells 2DG is converted to 2DG6P: a compound which cannot be metabolized further. 2DG6P is then converted to NADPH, used to produce glutathione, which—in turn— reacts with substrate provided in the assay kit to produce a yellow color whose optical density is measured at 412 nm.

### Akt Phosphorylation Assay

Myoblast L6 cells were treated with various nutrient mixtures for 24 hours and the amount of phosphorylated Akt protein was measured in cell lysates by Akt (pS473) ELISA kit from Abcam. All cells were treated with various nutrients for 24 hours, then lysed and total Akt was trapped in the provided assay plate. Out of this trapped Akt, phosphorylated Akt was detected by binding to the specific antibody 'phospho-Akt (Ser473)' provided in the kit. The amount of bound phospho-Akt antibody bound to the plate was measured at 450 nm.

## Results

The nutrient composition of the formulas tested given in Table 1 shows that all 3 tested formulas contain vitamins, but in different numbers. Compared to two

other formulations, Formula A is enriched with amino acids, plant extracts and bioactive compounds.

Table 1: Basic composition of tested nutritional supplement formulas

Ingredient	Formulas		
	A	B	C
<b>Vitamins</b>			
Beta Carotene	X		
Vitamin B1 Thiamine	X	X	X
Vitamin B2	X	X	X
Vitamin B3		X	X
Vitamin B6	X	X	X
Vitamin B12 -cyanocobalamin	X	X	X
Panathotic acid	X	X	X
Biotin	X	X	X
Folic acid	X	X	
Vitamin C (Various Forms)	X	X	
Vitamin D3	X		
Vitamin E	X	X	
<b>Minerals</b>			
Magnesium	X		
Calcium	X	X	
Potassium	X		
Zinc	X		
Selenium	X		
Managnese	X		
Copper	X		
Chromium	X		
Molybdenum	X		
Phosphorus	X		
<b>Amino Acids</b>			
Arginine	X		
Cysteine	X		
Lysine	X		
Proline	X		
Carnitine	X		
<b>Active Compounds</b>			
Coenzyme Q10	X		
Inositol	X		
<b>Plant Extracts</b>			
Citrus Bioflavonoids	X		
Pycnogenol	X		
Carotenoid Complex	X		
Tocopherol mix	X		

The results in Figure 2 present the effects of test nutritional formulations on glucose uptake by the skeletal muscle cells (myoblasts). Formula A, which contains a wide selection of micronutrients designed to support basic metabolism of various types of cells in the body, slightly increased glucose influx to the cells when used at the applied dose (based on daily recommendation). Formula B, which contains micronutrients specifically supporting cellular glucose metabolism, was more effective than Formula A in supporting glucose transport inside the cells (about 45% increase compared to control). Interestingly,

Formula B contains some micronutrients also present in Formula A, however their dosages differ as well as these Formulas' daily intake recommendations. In the presence of a combination of Formula A and Formula B the glucose uptake by the muscle cells more than doubled compared to control. Given the known role of B vitamins in different aspects of glucose metabolism, we added the Vitamin B complex to these 2 formulas which further enhanced glucose uptake by these cells. With the combination of these 3 formulas, glucose uptake by the cells increased to 370% .

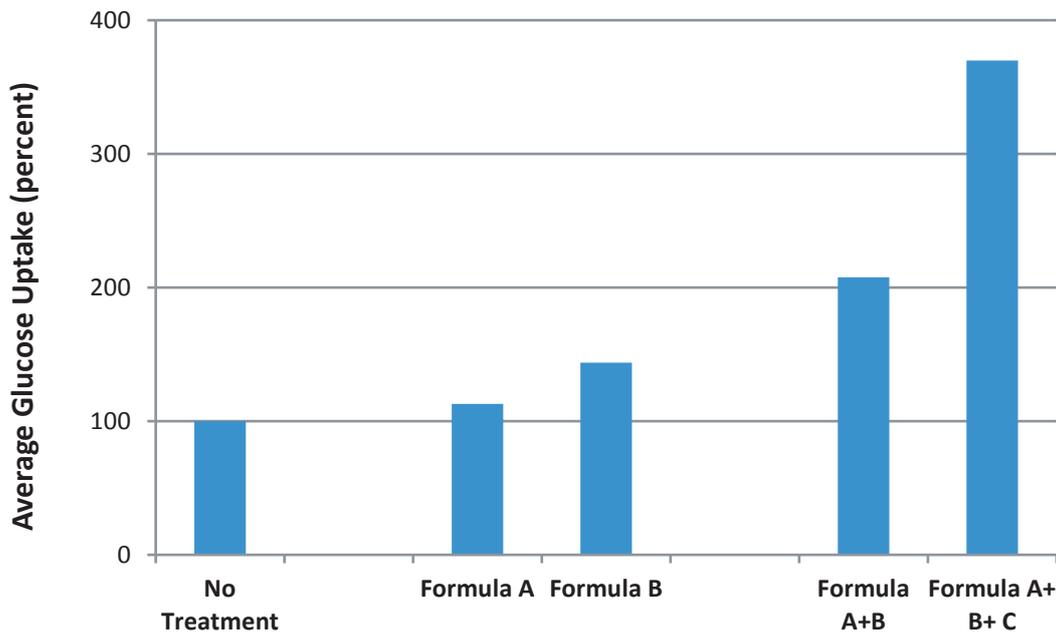


Fig. 2: Glucose uptake by rat muscle cells is expressed as a percentage and compared to glucose uptake by untreated myoblasts cells set at 100%.

As shown in Figure 3, Akt phosphorylation in the muscle cells increased to almost 150% in the presence of Formula A compared to control. Also other formulas tested in this study (Formulas B and C) had a stimulatory effect on Akt phosphorylation. The results show that by combining Formula A with Formula B,

Akt phosphorylation can increase further. However, the highest efficacy was achieved with 3 test formulas combined together which resulted in an increase of Akt phosphorylation to 240%, which is much higher than any of these formulas supplied individually.

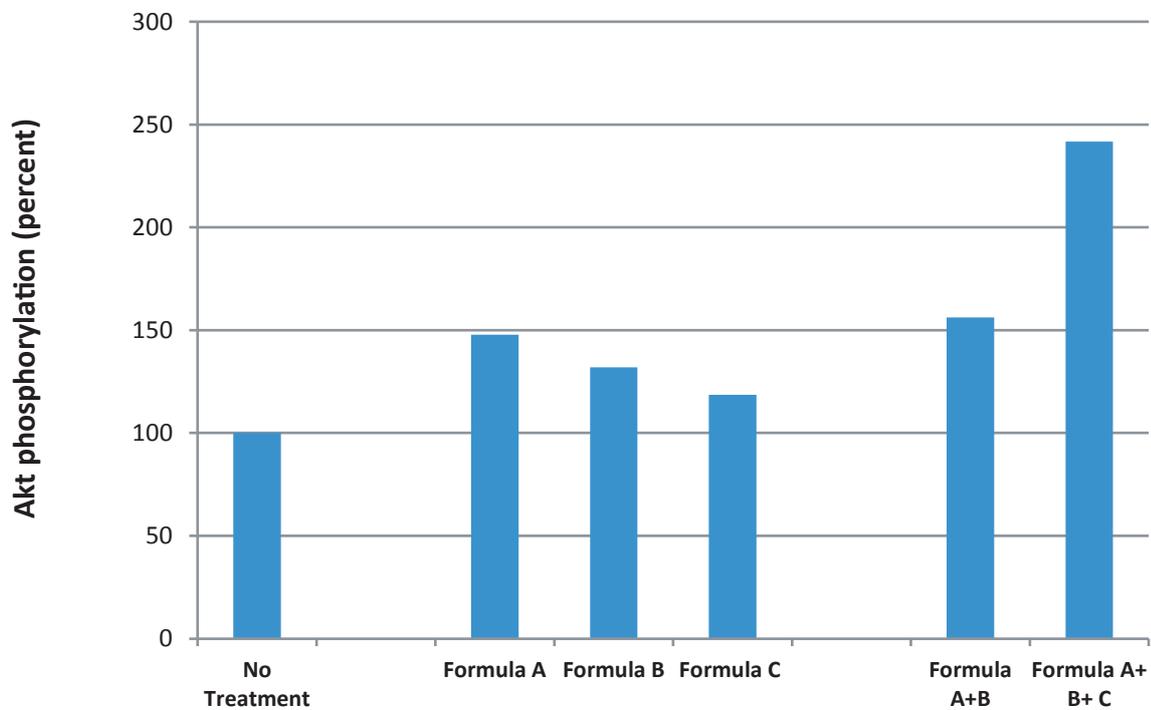


Fig.3: Akt phosphorylation by skeletal muscle cells is expressed as a percentage with untreated myoblasts set at 100%.

## Discussion

More than half the US population takes dietary supplements.<sup>6</sup> Supplementation is an important aspect of nutrition because, unlike many animals, humans have lost the ability to synthesize many vitamins, especially Vitamin C. These vitamins remain vital to life-sustaining metabolic reactions within the body, including glucose metabolism and bioenergy production.<sup>7,8</sup> Therefore it is essential to choose efficacious supplements which are developed on the basis of scientific research and tested for efficacy.

Here we demonstrate two cellular mechanisms by which nutrients combined in different supplement formulations can improve utilization of glucose and its metabolism in the skeletal muscle cells. Firstly, glucose taken up by muscle cells increases muscle metabolic efficiency and bioenergy production and helps manage glucose levels in the blood. Interestingly, the formula B specifically supporting glucose metabolism—was more effective than a general multi-vitamin formulation in increasing glucose uptake by the cells. However, Formula A was more effective in supporting Akt phosphorylation, a mediator of insulin effects on glucose uptake. In both types of tests cellular efficacy of a combination of Formula A and Formula B was superior compared to these formulas tested individually.

In this study we also explored the role of B vitamins in supporting glucose uptake by the cells. Various types of B vitamins serve as cofactors of many essential enzymes involved in glucose metabolism. We observed that both Akt phosphorylation and glucose uptake by the cells increased after combining 2 multi-micronutrient formulations with a vitamin B complex. Given that Formulas A and B also contain B vitamins, the enhanced efficacy observed after the formula was enriched with extra B vitamin complex also highlights the importance of selecting the correct doses of micronutrients.

There is tremendous interest in nutritional supplementation among both the general public and athletes looking for natural ways to boost cellular energy production and increase glucose utilization. The industry has responded with a baffling array of products, but with very limited understanding of their efficacy. This study sheds new light on the way that certain multinutrient formulations can improve the uptake and metabolism of glucose by the skeletal muscle cells and emphasizes the importance of efficacy testing by the manufacturers of nutritional supplements.

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