AMERICAN UNIVERSITY OF BEIRUT

THE ROLE OF PHOSPHORUS IN DIET INDUCED THERMOGENESIS OF BOTH LEAN AND OBESE SUBJECTS

by
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A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Science
to the Department of Nutrition and Food Sciences
of the Faculty of Agricultural and Food Sciences
at the American University of Beirut

Beirut, Lebanon
April 2016

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ACKNOWLEDGEMENTS

Sincerely, it was a pleasure working with my advisor Dr. Omar Obeid on this interesting topic. I appreciate his inspiration, support, and guidance.

I would like to thank both Dr. Hala Ghattas and Dr. Ammar Olabi for their support and for being members of the thesis committee.

I take this opportunity to express gratitude to all my colleagues and faculty members for their help and support.

Finally, I would like to thank all my adorable family members and my lovely husband for their ceaseless love, care and support.

AN ABSTRACT OF THE THESIS OF

Mariam Albert Assaad for Master of Science
Major: Nutrition

Title: The Role of Phosphorus in Diet Induced Thermogenesis of both Lean and Obese Subjects

The problem of both overweight and obesity is becoming globally spread and it is one of the main health issues nowadays. Even though genetics certainly plays a role, weight gain is eventually the result of a failure in the balance between energy expenditure and energy intake. We can get energy expenditure by summation of the resting metabolic rate, thermogenesis and energy expended on physical activity. Diet induced thermogenesis (DIT) represents the main form of thermogenesis in human, it accounts for 5-15% of total energy expenditure and is primarily related to ATP production that is utilized for the digestion, absorption, transport and storage of food. The macronutrients have variable thermic effect (fat 0-3%, carbohydrate 5%-10%, and protein 20%-30%). Moreover, this thermic effect is valued to account for 5% to 15% of total energy expenditure. The production of ATP mainly depends on hormonal (mainly insulin) factors and on phosphorus availability, which is affected by dietary intake.

ATP production in the human body depends upon adequate sources of phosphorus (P) that can be distorted by the digestion and absorption of high-carbohydrate–low P food. Mainly due to the increase in insulin release, that simultaneously stimulates peripheral uptake of P and the phosphorylation of many compounds. Actually, this creates a competition for P that compromises its availability for ATP production, which is thought to be translated into low DIT.

Studies have shown that diet induced thermogenesis is low among obese subjects as compared to lean subjects.
Our objectives are to measure:
Experiment 1. The involvement of phosphorus in DIT among lean subjects
Experiment 2. The involvement of phosphorus in DIT among obese subjects.

Subjects will be given oral high carbohydrate meal with or without Phosphorus (potassium phosphate). Postprandial thermogenesis will be measured. The primary outcome variables are thermogenesis. We hypothesized that phosphorus ingestion will increase thermogenesis of subjects.

A cross over study was conducted on 14 male subjects (8 lean, 6obese). Phosphorus effect on diet induced thermogenesis of all subjects was studied after they received a 500 Kcal high carbohydrate meal with (500 mg of P) or without P. Energy expenditure was measured at baseline and at 30 minute intervals for 4 hours following meal ingestion using a ventilated hood and canopy system COSMED QUARK CPET unit.

The results showed that postprandial energy expenditure of the meal containing P was significantly higher than that of placebo among lean and obese males (p=0.021, p= 0.000) respectively. This increase was associated with a significant rise in fat oxidation (%) among lean male subjects (p= 0.020), while carbohydrate oxidation (%) was significantly decreased (p= 0.020). As for obese subjects, the increase in postprandial energy expenditure was not accompanied with a significant increase in fat oxidation (p= 0.850) or a significant decrease in CHO oxidation (p= 0.052).
In conclusion, P was able to increase postprandial energy expenditure. This data may have promising effects for the management of obesity.

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ILLUSTRATIONS

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ABBREVIATIONS

%	Per Cent
&	And
/	Per
<	Less Than
=	Equal To
>	Greater Than
±	Plus or Minus
AI	Adequate Intake
ATP	Adenosine Triphosphate
AUB	American University of Beirut
AUBMC	American University of Beirut Medical Center
BMI	Body mass index
CO2	Carbon Dioxide
CHO O	Carbohydrate Oxidation
DRI	Dietary Reference Intake
DIT	Diet Induced Thermogenesis
EE	Energy Expenditure
FBG	Fasting blood glucose
FO	Fat Oxidation
FI	Food Intake
G	Gram

GFR	Glomerular filtration rate
Kcal	Kilocalorie
Kg	Kilogram
LDL	Low Density Lipoprotein
Mg	Milligram
mL	Milliliter
N	Number
P	P-value
P	Phosphorus
PO_4	Phosphate
RDA	Recommended Dietary Allowance
RQ	Respiratory Quotient
REE	Resting Energy Expenditure
SD	Standard Deviation
TEE	Total Energy Expenditure
WHO	World Health Organization
Wt	Weight

CHAPTER I

LITERATURE REVIEW

A. Diet Induced Thermogenesis DIT

Energy expenditure of human's body consists of three components: basal metabolic rate, diet induced Thermogenesis (DIT) and the energy cost of physical activity. Diet induced thermogenesis is a component of the energy expenditure. DIT is the increase in the energy expenditure above the basal fasting level, and when this increase is divided by the energy content of the food that was ingested; we will have the DIT percentage (Westerterp, 2004).

Diet-induced thermogenesis (DIT) is the energy used following the consumption of a meal and reveals the energy needed for the digestion, absorption, transport and storage of the ingested food and drinks. It is commonly stated that the DIT contributes approximately 10% of the total daily energy (Donaho et al., 2004; Scott & Devore, 2004). This percentage has been noted to differ significantly between individuals or within individuals due to the changes in energy balance. Yet, different studies have not revealed any reliable variation in the DIT among individuals (Granata &Brandon, 2002; Schutz et al., 1984). Actually, some factors might contribute to the differences of DIT among different studies. Some of these factors could be meal size, duration of the post-meal measurement period, and the methods used to calculate DIT (Ruddick et al., 2013).

Total DIT response might take as long as 8–10 hours following the ingestion of larger meals (≤4180 kJ; 1000 kcal) (Donahoo et al., 2004, D'Alessio et al., 1988). Nevertheless, the majority of studies used meals with different energy contents fluctuating between 1670 and 4180 kJ (400 and 1000 kcal), and DIT has been measured between 3 and 6 h and it has been frequently incomplete at the end of the measurement period (Ruddick et al.,2013). Other factors might affect the duration of the DIT response among different individuals. Such factors could be the individual differences in each of the frequency of gastric emptying, nutrient digestion, and nutrients storage (Scott et al., 2007). Other factors could be the differences in the meal contents.For example, a larger meal size and a larger ratio of fat: protein and protein: carbohydrate, in addition to larger adiposity, might lead to a variable DIT response and cause a delay in the peak in energy expenditure (Melanson et al., 1998, Tentolouris et al., 2008, Reed & Hill, 1996, Karst et al., 1984). On the other hand, extended measurement durations place an important burden on the participant; therefore it is essential to decide whether shorter measurement durations can precisely reveal the total DIT response in the same individuals after doing repetitive tests on different days (Ruddick et al., 2013).

As explained earlier, the diet induced thermogenesis (DIT) is the increase that takes place in energy expenditure after the ingestion of a food component; noting that this increase is above the basal (resting) rate. Actually, this increase can be largely related to the increase production of ATP which is used to cover the cost of digestion, absorption, transport and storage of the ingested food.

According to theory, the DIT differs from nutrient to another according to the amount of ATP needed for the initial steps in both metabolism and storage. Several studies have reported that different DIT values for separate nutrients (fat, carbohydrate,

and protein). Fat DIT is 0 to 3%, carbohydrate DIT is 5 to 10%, the protein DIT is 20 to 30% (Acheson, 1993) and DIT for alcohol is 10 to 30% (Kimutai et al., 2009). Healthy subjects consuming a mixed diet will have a DIT of 10% of the total quantity of energy ingested over 24 h. DIT is 10% of the daily energy expenditure among subjects who are energy balanced, where their energy intake equals their energy expenditure (Westerterp, 2004). A review by De Jonge et al reported that out of 29 studies measuring DIT in subjects that were age matched and sufficiently obese, 22 reported a reduced DIT in obesity (De Jonge et al., 2012). Another study described that there is an inverse correlation between the percentage body fat and the diet-induced thermogenesis ($p=0.001$) (Y Schutz et al., 1984). It was reported that this overall thermogenic response to feeding may be the main factor contributing for energy storage in some obese subjects; a blunted response of the sympathetic nervous system could explain this low thermogenic response (Y Schutz et al., 1984).

Results from this study, may help in explaining the controversy over DIT of obese subjects and may prove to be of vital importance for future work on the potential use of phosphorus supplements for weight reduction.

B. Phosphorus P

Phosphorus is rarely found in a free form.Naturally it is found as a multivalent non-metal element; therefore phosphorus is regularly present in the anion form PO_4 (Kalantar et al., 2010).

Phosphorus is considered one of the most important minerals in the human body, where it plays an essential role in cellular metabolism, by being a major constituent of all key enzymes involved in phosphorylation, and by integrating with

adenosine triphosphate (ATP). Furthermore, phosphorus is important for the normal bone development, blood cell function, nervous system, muscles (Kimutai et al., 2009; Freiman et al., 1982). Phosphorus also plays a role in maintaining acid-base homeostasis (urinary buffering) (Amanzadeh & Reilly, 2006).

The average total body phosphorus content is 700g; where 85% is found in bones and teeth, 14% in soft tissues, and 1% in the extracellular fluid. The usual average dietary intake of phosphorus is around 800-1400 mg/day, and the normal serum phosphorus concentration must be between 2.5-4.5 mg/dl or 0.8 – 1.45 mm/l (Amanzadeh & Reilly, 2006). The Food and Nutrition Board of the Institute of Medicine reported that the recommended daily allowance for phosphorus for both male and female adults over the age of 19 years should be 700 mg/day. (Table 1)

Naturally, phosphorus is highly found in foods that are rich in protein. Animal products are protein rich foods which contain high amounts of organic phosphorus that is hydrolyzed in the intestine and then absorbed in its inorganic form PO_4. Normally, between 40 to 60% of the dietary phosphorus is absorbed in the small intestine.

The inorganic phosphorus found in supplements, medications, and foods that contain P additives, is highly absorbed >90% absorbed by the intestine. This is due to its free and unbound form (Kalantar et al., 2010). Phosphorus absorption primarily takes place at the level of the jejunum and ileum. A huge amount is absorbed mainly through concentration-dependent passive transport, whereas a smaller portion is absorbed by active transport, which is assisted by 1,25-dihydroxyvitamin D (Kalantar et al., 2010; Davis et al., 1983).

Digestibility of P from animal-based foods, such as dairy products, meat,

poultry, and fish is higher than P from the plant proteins. Table 2summarizes several animal and plant dietary sources of phosphorus (Kalantar et al., 2010).

Table 1. Recommended Dietary Allowance (RDA) in mg/day for P by Age Group (National Academy of Science, 1997)

Age Group	RDA (mg/day)	UL (g/day)
1 through 3 years	460	3
4 through 8 years	500	3
9 through 18 years	1250	4
19 through 70 years	700	4
>70 years	700	3

Table 2. Dietary P and protein content and P-to-protein ratio in selected food items (Kalantar et. al 2010)

Food Item	**P (mg/100g)**	**Protein (g/100g)**	**P-to-Protein ratio (mg/g)**
Animal-based food			
Egg White	2.5	9	1.4
Lamb	00	32	6.3
Tuna (canned in water)	64	25.5	6.4
Chicken Breast	31	31	7.5
Ground Beef	94	25.8	7.5
Whole Egg	40	10.5	13.3
Dairy Products			
Mozzarella cheese	26	26	20.1
Milk, low fat (2%)	3.5	3.3	28.3
Cottage Cheese	31	12.2	10.7
Plant-based food			
Soy beans	11	14.3	14.7
Peanuts	56	23.6	15.1
Edamame	61	10.3	15.6
Black beans	40	9	15.8
Lentils	78	8.9	20.0

Table 3. Total and phytate phosphorus content of selected plant-based food items (Ravindran et al., 1994&Eeckhout et al., 1994))

Food Item	Phosphorus (g/100g)		Phytate-P % of Total
	Total	Phytate-P	
Maize	0.26	0.22	84.6
Oats	0.36	0.21	59
Rice Brown (unpolished)	0.38	0.28	73.7
Rice (polished)	0.31	0.17	54.8
Wheat	0.33	0.18	55
Wheat Bran	1.16	0.97	84
Wheat feed flour	0.56	0.39	70
Rye	0.36	0.22	61
Triticale	0.37	0.25	67
Barley	0.37	0.22	60
Peas	0.38	0.17	45
Sorghum, dark colored seeds	0.41	0.27	65.9
Sorghum, light colored seeds	0.36	0.23	63.9
Soy beans	0.6	0.37	61.7
Groundnut	0.49	0.40	81.6
Potato	0.24	0.05	20.8
Soy Bean	0.6	0.37	61.7
Lentils	0.31	0.2	64.5
Chick peas	0.41	0.21	51.2

Many developing countries rely hugely on the plant-based foods such as cereals and legumes, which are rich in P but in the form of phytate which in turn renders it unavailable for absorption. (Table 3). Unrefined whole grain cereals (whole wheat or brown rice) contain high amounts of P that is bound in the form of phytate,Phytate is formed during the maturation stage of the seed; it represents the main storage form of phosphorus in plants (60-90% of the total phosphate). Humans and monogastric animals lack the enzyme phytase which hydrolyzes phytate; that is why it is impossible for them to hydrolyze and absorb phytate (Kumar et al. 2010). Alternatively, refined cereals, and cereal products contain less phytate since they has been subjected to several forms of processing (milling, extraction, and fermentation). For example, the polished rice contains about 20% less phytate than unpolished brown rice (Table 4). Nevertheless, studies have shown that processing and refining cereals also leads to approximately 70% reduction in several essential minerals, such as potassium, phosphorus, and magnesium (Obeid et al., 2014). In conclusion, plant-based food, whole grains, and refined cereals products are comparatively insufficient sources of phosphorus when compared to animal-based food.

C. Adenosine triphosphate ATP

During the past few decades, obesity was paralleled with numerous variations in human dietary habits that favored low phosphorus consumption. Several studies have reported a lot of evidence revealing the association between phosphorus and both glycemic control and lipid profile (Obeid et al., 2014). For example, studies done on non-diabetic subjects have reported that the increase in phosphorus intake and in serum

phosphate has led to an improvement in both glucose tolerance and insulin sensitivity (Obeid, 2013). Possible mechanisms behind these findings could be related to Adenosine triphosphate (ATP) production. ATP production depends primarily on phosphorus availability and is essential for several metabolic processes (Solomon & Kirby, 1990; Morris et al., 1978). It is claimed that insulin release in the body stimulates the phosphorylation of several compounds; and this might lead to limited phosphorus availability for ATP production, as a result, this will possibly be translated into low diet-induced thermogenesis. Consequently, an increase in the insulin release along with a low phosphorus diet might exacerbate the situation. Additionally, impaired glucose tolerance causes a reduction in the phosphorus peripheral uptake thus affecting thermogenesis (Obeid, 2013).Additionally, decreased hepatic ATP production is assumed to be transmitted to the central nervous system through neural afferents, causing an increase in food intake. In contrast, a positive relation is found between both phosphorus and red blood cell 2, 3-diphosphoglycerate, which decreases oxygen affinity to hemoglobin (Obeid et al., 2013). This positive relation between phosphorus and 2, 3-diphosphoglycerate would be estimated to lessen the ability for physical activity (Obeid et al., 2013). In line with that, several studies have reported that plasma phosphorus status is inversely related to body weight. That is why it is important to have an adequate phosphorus intake that could have a potentially protective pathway against widespread obesity which is rising across the globe (Obeid et al., 2013).

A crossover study done on healthy male subjects reported an improvement in insulin sensitivity after the addition of phosphorus to an oral glucose load (Khattab et al., 2015).A possible mechanism is that insulin capacity to trap glucose within the cells increases as a result of its phosphorylation (Obeid et al., 2014).

In addition, a study done on rats to address the direct effect of high amounts of dietary phosphorus added to gelatinized potato on lipid metabolism revealed that rats on higher phosphorus diet had a lower hepatic triglyceride level. The mechanism behind this lipid lowering effect might be attributed to the slowly digestible starches (Kanazawa et al., 2007). A study that examined the effect of dietary P consumption on cholesterol metabolism reported that dietary phosphorous restriction significantly increased hepatic lipid-accumulation stimulated by a high-cholesterol diet (Tanaka et al., 2013). Another study was done to investigate the effect of phosphorus on glycogen synthesis and lipogenesis of rats feeding on high fructose diet. Rats were fed a high-fructose diet with different P content (0.15%, 0.165%, 0.30%, and 1.65%) for 4 days (Mattar et al., 2010).After measuring the in vivo rates of glycogen, lipid synthesis and hepatic glycogen levels, it was shown that an increase in dietary phosphorus was significantly associated with an increase in postprandial hepatic lipogenesis ($p = .029$), and epididymal fat pad ($p = .007$) as well as glycogenesis ($p = .024$) (Mattar et al., 2010).

D. Phosphorus, ATP, Food intake

In human body, the physiological regulation of food intake acts mainly at the central level. This regulation is partially governed by signals produced originally from the liver via hepatic postprandial metabolism involving ATP production (Friedman, 2007, Langhans & Scharrer, 1992). A lot of evidence has supported a relationship between declining hepatic ATP levels and increasing food intake. This decline in hepatic ATP production is thought to transduce changes in hepatic energy status into neural signals or hepatic vagal afferent activity that is transmitted to the central nervous

system (Freidman, 2007, Hong et al., 2000, Oberhaensli at al., 1986, Rwason & Freidman, 1994, Riquelme et al., 1984). In relation with this information and to support the mentioned hepatic ATP production and obesity, several studies which were conducted on animals and humans reported several abnormalities in hepatic ATP of obese individuals (Chavin et al., 1999, Cortez et al., 1999). In addition, in human liver, ATP status was reported to vary, with hepatic ATP store (Nair et al., 2003) and recovery from hepatic ATP depletion (using fructose infusion) being inversely related to body mass index (BMI) (Cortez et al., 1999). Moreover, an analysis of the metabolic data using the Knowledge Discovery in Databases has revealed that decreased energy levels or ATP deficiency were strongly linked to the development of obesity. This is due to driving of overeating and conserving energy (Wlodek & Gonzales, 2003). In line with that, recently we have found that if we add 500 mg P to different preloads, this will lead to substantial reduction in *ad libitum* subsequent energy intake (27-33%) (Obeid et al., 2010).

E. Factors affecting ATP production

The production of ATP, including hepatic ATP, depends on the adequate sources of phosphorus (P) (Morris et al., 1978, Solomon & Kirby, 1990), along with two other factors. First, there are only limited quantities of free phosphate which are stored within cells, and the metabolic phosphate of most tissues depends on the extracellular fluid (ECF) inorganic phosphate (P_i). When ECF P_i levels are low, this will be followed by cellular dysfunction. Second, there is almost constant phosphorus absorption across a wide range of intakes (Food and Nutrition Board, 1997).This

suggests a lack of the adaptive mechanism that usually improves the phosphorus absorption during low intakes and this can occur with other micronutrients as well.

Fructose affects ATP production in the body since it has “phosphate-sequestering” capacity. Unlike the glucose feedback mechanism, fructose lacks a feedback mechanism for fructose phosphorylation; so as a result, fructose 1-phosphate (F 1-P) will accumulate inside the liver. Due to this, phosphorus will be unavailable to take part in other important metabolic reactions such as the regeneration of ATP (Bizeau et al., 2005, Karczmar et al., 1989, Morris et al., 1978, Oberhaensli et al., 1968). Moreover, the insulin release which takes place after carbohydrate “glucose” ingestion increases the intracellular uptake of glucose along with phosphorus and other several electrolytes, in addition to the phosphorylation of different compounds. Therefore, in a condition where there is high fructose or high-glucose/low-phosphorus condition, a competition for phosphorus will occur between the phosphorylation of some compounds and ATP production. Furthermore, phosphorus might provide a relation between different metabolic situations related to increased body weight or energy intake. Several studies have revealed a relation between the consumption of high-fructose corn syrup (HFCS) and obesity (Bray et al., 2004). In addition, the increased protein intake, which is high in phosphorus, led to a decrease in energy intake (Halton & Hu, 2004, Latner & Schwartz, 1999). Studies revealed a converse association between dairy products (high in phosphorus) and energy intake and body weight, where calcium failed to elucidate such an association (Teegarden, 2005, Wgner et al., 2007, Yanovski et al., 2009). In contrast, an increase in the calcium intake from either diet or supplements can compromise the availability of phosphorus due to the negative impact

calcium has on the phosphorus absorption (Heaney & Nordin, 2002), not forgetting to mention that high doses of calcium carbonate will be highly used as phosphate binder.

One of the obesity characters is insulin resistance which in turn predisposes to the development of impaired glucose tolerance which is known to decrease the peripheral uptake of both glucose and phosphorus (Campillo et al., 1982).Consequently, this will lead to the reduction in the capacity of ATP production thus altering the diet induced thermogenesis (Felig, 1984).

F. Phosphorus and body weight

Increased obesity during the past few decades was coupled with several changes in dietary habits and nutrient intake. These variations are mainly correlated to the increase in the consumption of sugar, oils, and sweeteners such as HFCS which contain small amounts of phosphorus. Moreover, refined cereals have small amount of phosphorus due to the refinement process which reduces phosphorus content by about 70%. The Food Balance Sheet of the FAO (FAO, 2010) reveals that these commodities contributed to approximately 56% and 59% of the food supply (kcal/capita/day) in the USA and Lebanon, respectively. Nevertheless, it is important to know that starchy foods such as potatoes are low in phosphorus. The socioeconomic status has a strong effect on the consumption of these commodities: people of low socioeconomic status highly consume starchy food, the main reason behind that is because these foods have high energy density (kcal/g food) and low energy cost (US$/1000 kcal). This was strongly proposed to be the source of the high prevalence of overweight and obesity among people of low socioeconomic status (Drewnowski, 2009). Since we have a strong correlation among people who consume high quantities of commodities which contain

low levels of phosphorus and the increased prevalence of overweight obesity among those people, it is reasonable to assume that phosphorus intake and body weight are inversely related to each other's.

In fact, both the ancestral and the current intakes of phosphorus are lower than the upper intake limit which is 4 g/day (Food and Nutrition Board, 1997).

Several studies have observed the relation between phosphorus intake and body weight but conflicted findings were reported (Alonso at al., 2010, Beydoun et al., 2008, Elliott et al., 2008). While other studies have reported that serum phosphate is inversely related to body weight (Haap et al., 2006, Haglin et al., 2001, Kalaitzidis et al, 2005, Lind et al., 1993, Lindgarde and Trell, 1977), and hypophosphatemia was assumed to be involved in the development of metabolic syndrome, including increased BMI (Haglin, 2001). Since there is a similarity in the phosphate fractional excretion rate between lean, overweight, and obese subjects, this indicates that the reduction in serum phosphate is mainly due to a reduction in dietary intake rather than problems with excretion (Kalaitzidis, 2005).

G. Obesity, Thermogenesis and Phosphorus

Till now, the difference in the DIT between lean and obese subjects is still debatable but there are large number of studies that have revealed a reduction in DIT among obese that appears to be normalized after losing weight (D'Alessio et al., 1988, Ravussin et al., 1996, Segal et al., 1985, Segal et al., 1990, Segal et al., 1990, Tentolouris et al., 2008, Thorne et al., 1989, Thorne et al., 1990). Nevertheless, the reduced DIT among diabetic as compared to DIT among non-diabetic obese subjects (Golay et al., 1982) indicates that insulin might have played a role in the debate (Felig,

1984). DIT was reduced in diabetics who have reduction in insulin response; this implicates a reduction in the peripheral uptake of both glucose and phosphorus leading to the progression of the insulin resistance to an impaired glucose tolerance and diabetes (Campillo et al., 1982). A study has reported that the addition of phosphorus to orange juice led to an increase in the postprandial thermogenesis among obese but not among lean subjects (Jaedig et al., 1994). It was shown that obese subjects taking phosphorus supplementation in a weight reducing program had an increase in their resting metabolic rate (Kaciuba et al., 1993, Nazar et al., 1996). Therefore, the possible mechanism is that phosphorus supplementation might have increased the peripheral phosphorus (as well glucose) uptake, especially that P is able to stimulate insulin sensitivity (Campillo et al., 1982,Haap et al., 2006, Nowicki et al., 1996). However, more studies are needed to be done in order to determine precisely whether the magnitude of insulin resistance can play part in the phosphorus induced thermogenesis. Additionally, glucose intolerance was described to increase under phosphate depletion through stimulating the hepatic glucose production and a reduction in insulin level (Xie et al., 2000). In line with this, the non-diabetic subjects who were taking phosphorus and having an increased serum phosphate were reported to have an improvement in glucose tolerance and insulin sensitivity (Campillo et al., 1982, Haap et al., 2006, Nowocki et al., 1996); as well as improvement in exercise performance (Clarkson & Haymes, 1995). The above information indicates that phosphorus usage may have related effects on energy metabolism of: normal, impaired glucose tolerance and diabetic obese subjects (figure 1 & 2). In addition, the observed high DIT of protein may partially be attributed to the high phosphorus content of proteins.

In summary, ATP production dependents strongly on the phosphorus availability, and at the same time it is important for many processes including eating behavior and energy expenditure (figure 1 & 2).Increase in the consumption of refined cereals, sugars (fructose) , potatoes, and oils will negatively influence the phosphorus availability in our body. Upon the release of insulin, stimulation of the phosphorylation of many compounds will take place and as a result, this may compromise the phosphorus needed for ATP production, particularly given that ATP can act as phosphate donor (Karczmar et al., 1989). Therefore, when there is an increase in insulin release under a low phosphorus diet, this will cause impairment in the situation. Furthermore, a reduction in the phosphorus peripheral uptake will take place due to the impaired glucose tolerance (Campillo et al., 1982) and this will affect thermogenesis (Felig, 1984). In contrast, the high DIT of proteins may be related to their high content of phosphorus. Still, it is not clear whether phosphorus ingestion can affect the postprandial energy expenditure among obese subjects according to their degree of glucose tolerance.

Figure 1. Proposed interaction among phosphorus, ATP production and obesity

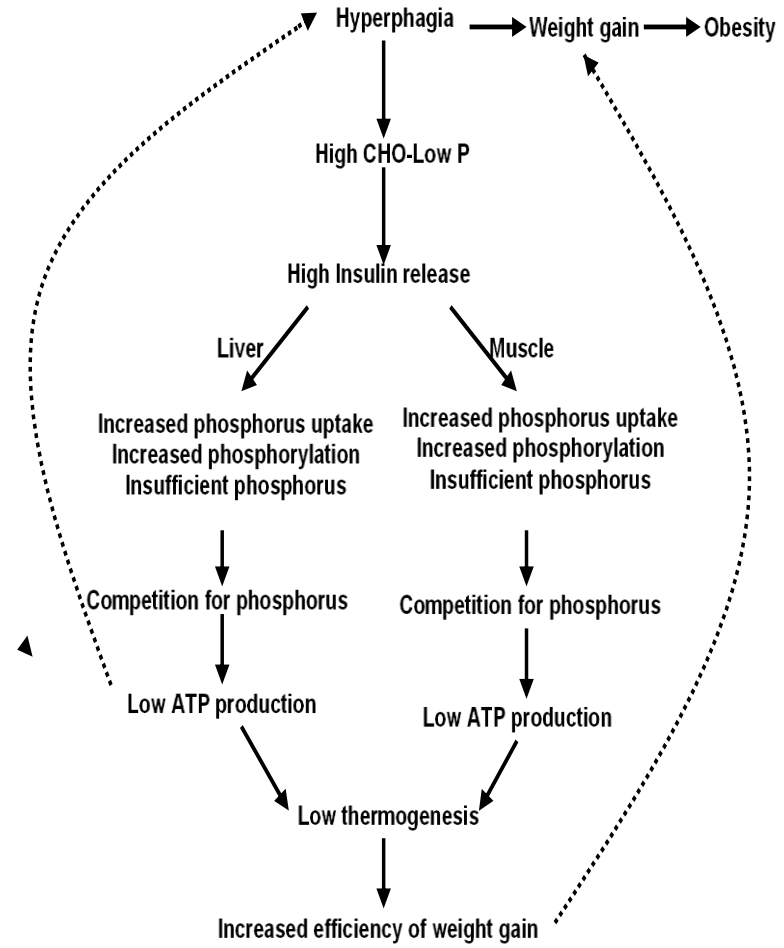
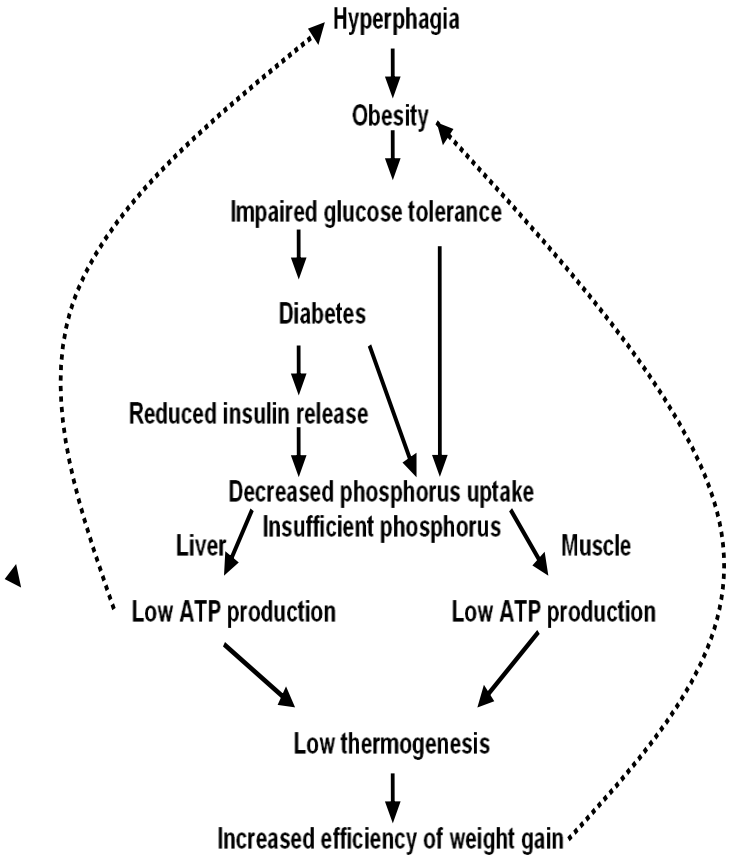


Figure 2. Proposed interaction among phosphorus, impaired glucose tolerance, thermogenesis and obesity.



AIM OF THE THESIS

Both obesity and body adiposity are becoming global health problems, with an increased prevalence in the USA and the Middle East. While genetics certainly play a major role in obesity, also weight gain can be the result of a failure to balance the energy expenditure and the energy intake (food intake). Energy expenditure is reported to be related to 3 major components: resting (basal) metabolic rate (RMR), energy expended on physical activity and the diet induced thermogenesis (DIT). As mentioned earlier, DIT is mainly related to ATP production needed to cover the cost of digestion, absorption, transport and storage of food, and contributes to 5% - 15% of total energy expenditure. Actually, many studies have reported a reduced DIT among obese subjects compared to lean subjects (Segal et al., 1990, Thorne et al., 1989). Some studies showed no difference (D'Alessio et al., 1988, Ravussin & Swinburn, 1996, Tentolouris et al., 2008). Moreover, these differences in DIT seem to diminish following weight loss (Thorne et al., 1989, Thorne et al., 1990) indicating that DIT is not a causal factor for obesity.

On the other hand, there is a suggestion that phosphorus status is inversely related to body weight due to the fact that all food sources that cause weight gain are low in phosphorus (such food are refined cereals, sweeteners, and oils). On the other hand, high-protein foods that are high in phosphorus have a major and successful role in weight loss. Finally, several studies have reported a correlation between high dairy consumption and decreased body weight that could not be explained by calcium (Wagner et al., 2007, Yanovski et al., 2009). Since dairy products contain high amount of phosphorus, the decrease in body weight reported is due to P. In line with that, it was

reported that body weight and plasma phosphorus are inversely related to each other (Haap et al., 2006, Haglin et al., 2001, Kalaitzidis et al., 2005, Lind et al., 1993, Lindgarde & Trell, 1977). In addition, thereis strong evidence in animals that supportsan inverse relationship between eating behavior and hepatic ATP levels. In these studies, a decrease in hepatic ATP is transmitted by the neural afferents to the central nervous system, and this resulted in an increase in food intake (Friedman, 2007). While the fundamental evidence for this mechanism has been tested primarily in animals, human studies indirectly support a potential role for hepatic ATP in energy and body weight regulation (Cortez-Pinto et al., 1999, Nair et al., 2003). Recent data from our laboratory showed that the ingestion of phosphorus preload was associated with a 30% decrease in energy intake at subsequent meal (Obeid, 2010).

Furthermore, the ingestion of a high carbohydrate-low phosphorus meal is recognized to induce a noticeable decrease in plasma inorganic phosphate. An explanation for this decrease is that carbohydrate stimulates the release of insulin, which is known to increase phosphorus uptake by peripheral tissues (mainly muscles and liver). Moreover, insulin activates the phosphorylation of many compounds (e.g. protein, carbohydrate etc.). As a result, this creates a competition for phosphorus between ATP production and the phosphorylation of other compounds. Therefore, ATP production decreases and this is thought to eventually influence thermogenesis. On the other hand, it was reported that phosphorus supplementation causes an increase in RMR (Kaciuba et al., 1993, Nazar et al., 1996) and postprandial thermogenesis (Jaedig & Henningsen, 1991, Jaedig et al., 1994) of obese subjects. The reduction in DIT among obese diabetic subjects, along with both increased and reduced insulin response (Golay et al., 1982), suggests that this reduction was not related to insulin *per se*, but rather to

its function. The fact that insulin resistance is inversely correlated to peripheral uptake of glucose (impaired glucose tolerance) and phosphorus implicates phosphorus in this process. Therefore, it is essential to explore the role of phosphorus on diet induced thermogenesis among obese subjects with varied glucose tolerance levels.

The overall hypothesis of this proposal is that phosphorus ingestion by lean and obese (normal glucose tolerance) subjects would be associated with an increase in diet induced thermogenesis, the proposed study will clarify the effect of phosphorus supplementation on DIT of both lean and obese subjects. Our specific aims are as follows:

Specific Aim: The impact of phosphorus ingestion on DIT of lean and obese subjects consuming high carbohydrate meal (low phosphorus).

This will be divided into two experiments:

Experiment 1: The impact of phosphorus ingestion on DIT of lean subjects consuming high carbohydrate meal.

Experiment 2: The impact of phosphorus ingestion on DIT of obese subjects consuming high carbohydrate meal.

CHAPTER II

MATERIALS AND METHODS

A. Participants

The study received approval from the Institutional Review board (IRB) at the American University of Beirut.

1. Inclusion and Exclusion criteria for Experiment 1.

Inclusion criteria: age range between 18 and 60 years and normal BMI between 18.5 and 24.9 kg/m^2.

Exclusion criteria: Subjects with BMI > 30 $kg/m^{2,}$ diabetes, cardiovascular, cerebrovascular, pulmonary, hepatic, renal, endocrinological (PTH), or any significant medical disease. Glomerular filtration rate < 60 ml min^1 per $1.73m^2$. In addition, subjects on a regular use of medications that affect body weight and/or having a weight loss of 3% or more in the preceding 3 months were excluded.

2. Inclusion and Exclusion criteria for Experiment 2.

Inclusion criteria: age range between 18 and 60 years and BMI > 30 kg/m^2.

Exclusion criteria: Subjects with normal BMI between 18.5 and 24.9 kg/m^2 ,diabetes, cardiovascular, cerebrovascular, pulmonary, hepatic, renal, endocrinological (PTH), or any significant medical disease. Glomerular filtration rate < 60 ml min^1 per $1.73m^2$. In addition, subjectson a regular use of medications that affect body weight and/or having a weight loss of 3% or more in the preceding 3 months were excluded.

Subject's Recruitment: For the purpose of recruiting subjects, we used poster advertisements (American University Medical Center including Obesity related clinics) and personal contacts as well. The posters included all the contact information of the principal investigator and research assistant (phone, e-mail, address).

Interested subjects who fulfilled the general entrance requirement (see exclusion and inclusion criteria) were asked to fill and sign the IRB consent form with a copy kept with the subjects.

After that, every subject was asked to come to the American University of Beirut medical center (Department of Pathology and Laboratory Medicine) after 12 hours of night fasting and give blood, in order to make sure none of them have diabetes or suffers from kidney disease.

The following blood tests were done:

- Blood glucose
- Blood creatinine
- Glomerular Filtration Rate

After receiving the lab test results and making sure that all results were normal, we arranged with every subject a suitable date for a visit to the Department of Nutrition and Food Sciences.All subjects were asked to come after an overnight fast (which is a minimum of a 12-hour fast).

B. Experimental design

This is a cross over study where every subject served as his own control.

We randomized the experimental sessions in a random order over 2 different days separated by a minimum washout period of one week.

We asked every subject to: take a weight maintenance diet 3 days preceding the test day, avoid any intense physical activity and nutritional supplements one day before the test. Overnight fasted subjects (> 8 hours) attended the testing room at the AUB around 8:00 am.

1. Anthropometry measurements& health questionnaire

Upon arrival to the department, every subjectwas met by the research assistant who was responsible for asking him to fill a health questionnaire regarding his medications, health status, smoking habits and sport habits.

After that,the following anthropometric measurements were measured:

- Weight (kg) using digital scale.
- Height (cm) using standiometer.
- Waist and hip circumference (cm) measurements were taken using tape measure; where waist circumference (WC) was measured at the narrowest part between the last rib and iliac crest after a gentle expiration and the hip circumference at the maximum posterior extension of behind.

2. Measurement of Resting Energy Expenditure

Familiarization session (First 30 min of session day 1 and 2): Upon arrival of the participant to the laboratory, anthropometric measurements were followed by 30 minutes measurement of baseline resting energy expenditure (REE) including VO2 and VCO2. This was done using a ventilated hood and canopy system (COSMED QUARK CPET UNIT) for indirect calorimetry. This REE measurement worked as the ‘baseline’ energy expenditure for the calculation of DIT.

Resting and postprandial energy expenditure was measured using a ventilated hood and canopy system (COSMED QUARK CPET UNIT) for indirect calorimetric measurement. The rate of flow of air pumped through the hood is maintained by keeping CO2 levels constant in the hood, between 1.00 and 1.20 %. O2 concentration, as measured by the machine oxygen analyzer and CO2 concentration was measured with the machine's own CO2 analyzer. The machine automatically measures the O2 consumption and CO2 production and calculates the difference between the expired air in the hood and room air. Energy expenditure, respiratory quotient, percentage fat oxidation, percentage carbohydrate oxidation, and percentage protein oxidation were calculated automatically by (COSMED QUARK CPET UNIT) for indirect calorimetric measurement. The respiratory quotient (RQ) is the ratio:

RQ = CO2 eliminated / O2 consumed.

RQ of carbohydrate is 1.0 and for fat 0.7. This is because carbohydrates have the same 2:1 ratio of hydrogen to oxygen as water, whereas fats require extra oxygen for the formation of H2O. Protein has an approximate ratio of 0.82, but is complex to calculate as protein is incompletely oxidized in vivo.

Table 4. Meal composition & phosphorus content

	Experimental Meal
	High carbohydrate diet (low in P)
Toast *P: 103 mg/100 g*	60 grams
Orange Juice *P: 17mg/100g*	3 fruits (1.5cup) 180ml
Jam *P: 19 mg/100g*	2 Tbsp) 28.3 grams
Butter *P: 24 mg/100g*	6 tsp – 2tbsp 30 grams
CHO - grams - Calories - percentage	 - 105 grams - 420 Calories - 65%
PROTEIN - grams - Calories - percentage	 - 6 grams - 24 Calories - 3.73 %
FAT - grams - Calories - percentage	 - 22 grams - 198 Calories - 30.84 %
Total calories	642 Calories
P (mg)	135.77g

Table 5. Tablets composition

Placebo Tabs Ingredients	Unit weight (mg)	Phosphorus Tabs Ingredients	Unit Weight (mg)
Micro Crystalline Cellulose	300	Phosphorus	125
		As Potassium Phosphate Monobasic (Kh2po4) 22.76%	189.4
		As Potassium Phosphaye Dibasic	349.5
		As Dicalcium Phosphate 19%	108
Calcium Carbonate	200	Micro Crystalline Cellulose	50
Stearic Acid	160	Stearic Acid	50
Magnesium Stearate	15	Magnesium Stearate	10
Croscarmellose Sodium	20	Croscarmellose Sodium	10
Silicon Di Oxide	5	Silicon Di Oxide	5
Coating Ingredients		Coating Ingredients	
Pharmaceutical Glaze	QS	Pharmaceutical Glaze	QS
Total Theoretical Wt	700	Total Theoretical Wt	772

3. Experimental Meal

Directly after baseline REE, subjects were asked to consume the meal with appropriate tablets within 10 min. In both sessions, subjects consumed the same standard meal. The standard meal was all prepared using the same ingredients. The meal used was a high carbohydrate meal. The standard fixed breakfast meal on both test days 1 and 2 was a typical breakfast which consisted of 60 grams toast with 30 grams butter and 30 grams jam + one and a half cup of orange juice(180ml) with either 4 tablets of placebo or phosphorous supplement (500 mg P) (Tables 4 & 5).The subjects were blinded on the content of the tablets. The meal provided 642 kcal and contained 22 grams of fat, 105 grams of carbohydrate and 6 grams of protein. The percentage of energy from:

- Fat:30.84 %
- Carbohydrate 65 %
- Protein 3.73 %

4. Measurement of Diet Induced Thermogenesis DIT

Diet-induced thermogenesis measurements (Test days 1 and 2): after the meal consumption with the tablets, the metabolic measurement was then carried on, and measurement of the postprandial energy expenditure was done for a total of 4 hours on a 15 min interval, with a 15 break between each interval (Figure 3).

Those breaks were obligatory, and all the participants were asked to walk alongside the corridor to the restrooms during both test days 1 and 2. During the metabolic measurements, the participants were seated on a lounge chair in a consistent position during all sessions.

Data collected from the total 4 h postprandial measurement period were averaged over a time of 15 min intervals and then plotted against time. For the purpose of minimizing the effect of movement on the energy expenditure measurements, we excluded from the calculations the first 5 min of the postprandial measurement that immediately followed the breakfast and every 5 min periods following each of the given breaks. The TEE percentage difference from the baseline energy expenditure was calculated by subtracting every 15 postprandial min data points from the average RMR and getting the percentage difference out of it. The same process was done to calculate the percentage difference of fat and carbohydrate oxidation of every subject on both visits. Subjects were allowed bathroom access and provided with drinking water, when needed.

5. Calculations

- All baseline and postprandial data measurements of: EE, RQ, % Fat oxidation, and % CHO oxidation were collected.

- Data collected during the 30 minutes baseline (two 15 minutes intervals) and 4 postprandial hours (eight 15 minutes intervals).

- We calculated the average: TEE, RQ, % fat oxidation and % CHO oxidation of the two 15minutes baseline measurements.

- Calculation of difference (Δ) in total Energy Expenditure (TEE), RQ, %Fat oxidation, & % CHO oxidation: for every subject, we subtracted every postprandial TEE value from the average baseline values to get the difference at every time period. The same thing was done to calculate the (Δ)in RQ, (Δ) in % Fat oxidation, and (Δ) in % CHO oxidation.

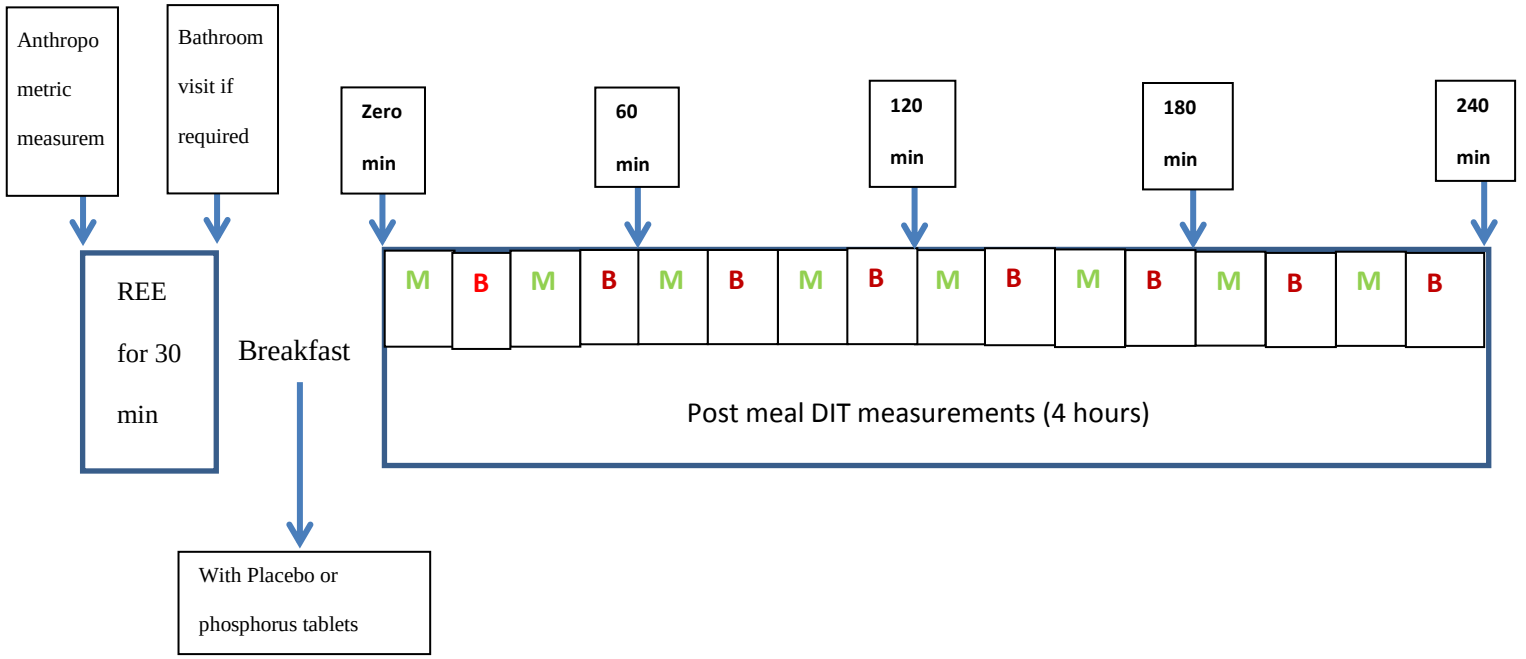


Figure 3. Procedure for Diet-induced thermogenesis (DIT) test days. M represents the measurements for 15 minutes on the COSMED machine; and B represents the break for 15 minutes.

C. Statistical analysis

Data are expressed as means ± SE of all values. At the level of analysis, we adjusted for two variables; treatment (P or placebo) and time of assessment. Two way ANOVA test was done to compare phosphorus treatment with control and to identify the changes from baseline within each treatment type. The level of significance was fixed < 0.05.

CHAPTER III

RESULTS

EXPERIMENT 1

A. Subject characteristics

Characteristics of the eight lean healthy male participants, blood test results, and the baseline measurements of their resting (energy expenditure (EE), fat oxidation, and CHO oxidation) are shown in Tables 6, 7 and 8 respectively. All 8 participants had normal: glucose blood test, blood creatinine and glomerular filtration rate (Table 7). Their average baseline resting energy expenditure (EE) was 1818.8 kcal (±61.6) (Table 8).

Table6. Experiment 1: Characteristics of participants

	Lean Males
Number of subjects	8
Average Age (yrs), (SE[b])	23.5 (± 1.38)
Anthropometric measurements, average	
Weight (kg), (SE[b])	77.51 (± 2.29)
Height (cm), (SE[b])	182.63 (± 2.1)
BMI [a](kg/m^2), (SE[b])	23.229 (± 0.506)
Abbreviations: BMI: body mass index, (SE[b]): standard error	

Table7. Experiment 1: Blood Test results of participants

Blood Test levels	*8 Lean Males*	*Normal Levels*
FBG (mg/dl)	94.25	[76-110]
Creatinine (mg/dl)	0.93	[0.6-1.2]
GFR(mL/min/1.73mý)	111.38	Decreased kidney function if <60 mL/min/1.73mý
FBG: fasting blood glucose, GFR: glomerular filtration rate		

Table8. Experiment 1: Baseline Energy Expenditure of participants

Lean Subjects Energy Expenditure	
REE (kcal/day), (SE[b])	1818.8 (± 61.6)
Resting RQ, (SE[b])	0.84 (± 0.045)
Resting fat oxidation (%),(SE[b])	61.54 (± 4.43)
Resting CHO oxidation (%),(SE[b])	22.1 (± 4.5)
REE: Resting energy expenditure, SE: standard error, RQ: respiratory quotient, CHO: carbohydrate, (SE[b]): standard error	

B. Resting EE, RQ, fat oxidation %, & carbohydrate oxidation %

After measuring for 30 minutes the baseline Resting EE and RQ using CPET COSMED machine, the average EE, RQ, Fat oxidation and carbohydrate oxidation were calculated for both lean and obese males.

Lean males had an average EE of 1818.8 kcal/ day, average RQ ratio of 0.8469, average fat oxidation of 61.54%, and an average CHO oxidation 22.1%.(See Table 8)

C. Postprandial EE

Following meal ingestion, we noticed a similar increase in postprandial EE during the two sessions (meal+P-tablets, meal+placebo-tablets) during the first 60 minutes. This increase continued until 120 minutes after phosphorus ingestion, while for control it starts to decrease gradually after 60 minutes. The maximum increase of EE after P tablets ingestion was (6.3 kcal/ min) at 120 min, while the maximum increase of EE of control was (5.02kcal/min)at 90 min. In both cases, the EE level failed to go back to baseline but the phosphorus treatment maintained a higher level till the end of the experiment and this was significant according to time ($p= 0.000$) and treatment ($p=0.021$)(Fig.4)

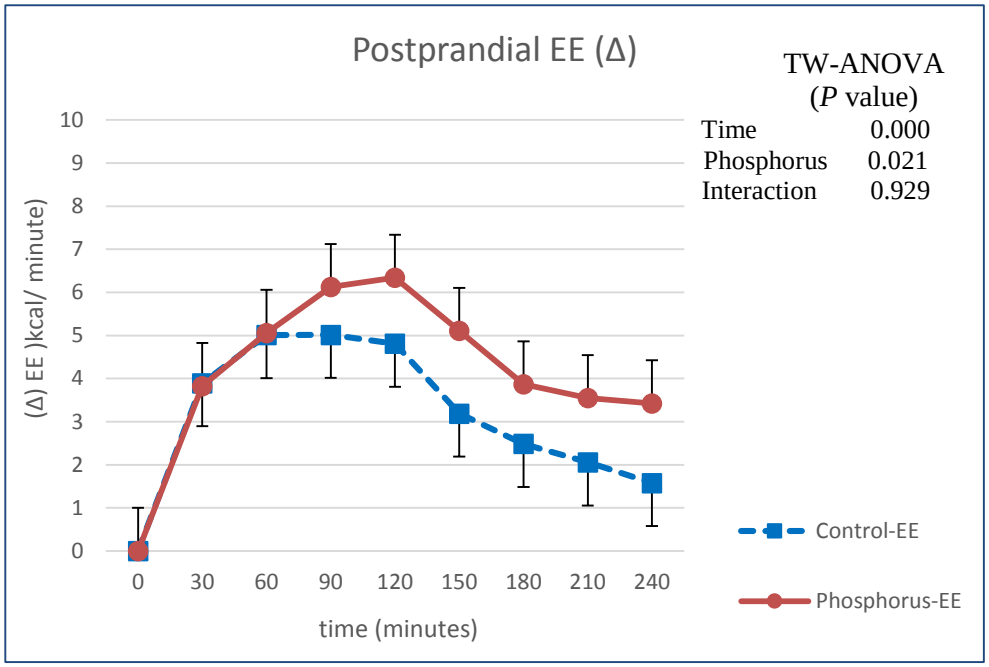


Figure 4. Change in Postprandial EE among lean males (SE: standard error)

Table 9. Changes in postprandial EE among lean male (SE: standard error)

Time (min)	Control-EE	SE-C	Phosphorus-EE	SE-P
Baseline EE	1818.8	61.6	1818.8	61.6
0	0.00	0.00	0.00	0.00
30	3.89	0.50	3.82	0.14
60	5.01	0.98	5.06	0.76
90	5.02	0.92	6.12	0.85
120	4.81	1.09	6.34	0.99
150	3.19	1.14	5.11	1.07
180	2.49	1.10	3.87	1.20
210	2.06	0.88	3.55	1.19
240	1.58	1.01	3.43	1.34

D. Postprandial Respiratory Quotient RQ

After both meals, postprandial difference in RQ increased slightly during the first 120 minutes. After 120 minutes, both RQ started to decrease and continued on decreasing until 240 minutes. We noticed that the decrease in RQ after phosphorus ingestion was stronger than that of placebo; this difference was significant according to time ($p=0.000$) but it was not significant according to treatment ($p=0.055$). (Figure 5)

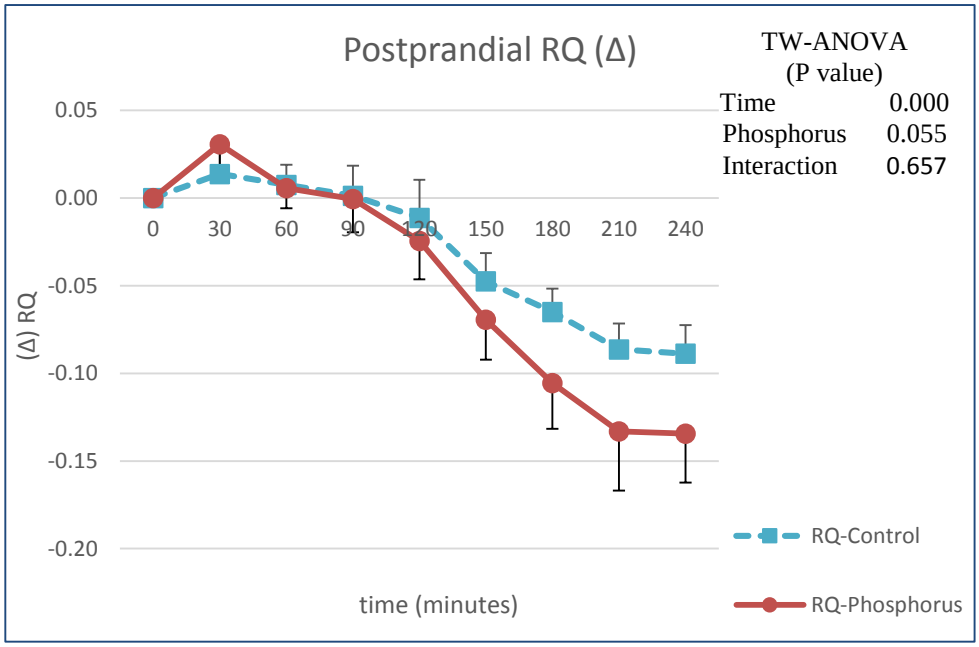


Figure5. Change in postprandial RQ among lean males

Table10. Changes in postprandial RQ among lean males (SE: standard error)

time (min)	RQ-Control	SE-C	RQ-Phosphorus	SE-P
Baseline RQ	0.84	0.045	0.84	0.045
0	0.00	0.00	0.00	0.00
30	0.01	0.02	0.03	0.01
60	0.01	0.01	0.01	0.01
90	0.00	0.02	0.00	0.02
120	-0.01	0.02	-0.02	0.02
150	-0.05	0.02	-0.07	0.02
180	-0.07	0.01	-0.11	0.03
210	-0.09	0.01	-0.13	0.03
240	-0.09	0.02	-0.13	0.03

E. Postprandial fat oxidation

Among lean males, we noticed a decrease in fat oxidation during the first 90 minutes after both meals. After 90 minutes, fat oxidation of both (meal+P- tablets, meal+ placebo- tablets) started to increase slowly but the increase from meal+P- tablets was higher than that of meal+ placebo, and this was significant according to time ($p=0.000$) and treatment ($p=0.020$). This increase continued to reach a maximum value of 28.96% at time=210 minutes. (Figure 6)

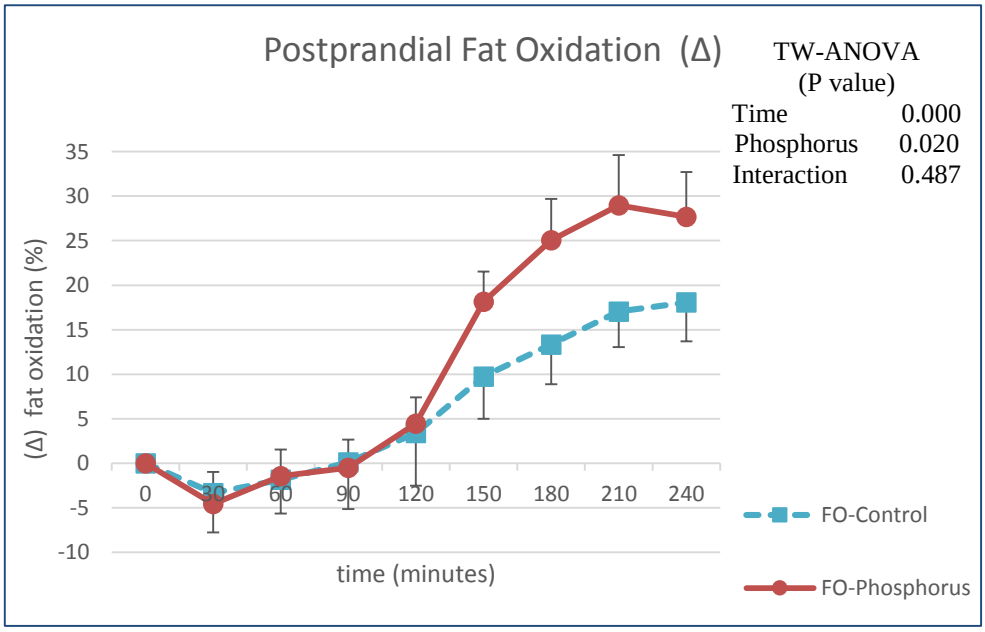


Figure 6. Change in fat oxidation among lean males

Table 11. Changes in postprandial fat oxidation (%) among lean males (SE: standard error)

time (min)	FO-Control	SE-C	FO-Phosphorus	SE-P
Baseline fat oxidation %	61.54	4.43	61.54	4.43
0	0.00	0.00	0.00	0.00
30	-3.34	4.43	-4.59	3.61
60	-1.85	3.82	-1.44	2.99
90	0.11	5.25	-0.51	3.17
120	3.39	5.91	4.46	2.95
150	9.75	4.78	18.14	3.40
180	13.34	4.48	25.04	4.66
210	17.05	4.00	28.96	5.66
240	18.06	4.36	27.67	5.06

F. Postprandial carbohydrate oxidation

Among lean male subjects, after meals (with P-tablets or placebo-tablets), the postprandial carbohydrate oxidation increased between time 0 and time 120, where it reached its peak at 30 minutes. At time 120minutes, both started to decrease but we observed stronger decrease in postprandial carbohydrate oxidation after P-tablets ingestion which was significant according to time ($p=0.000$) and treatment ($p= 0.020$). (Figure 7)

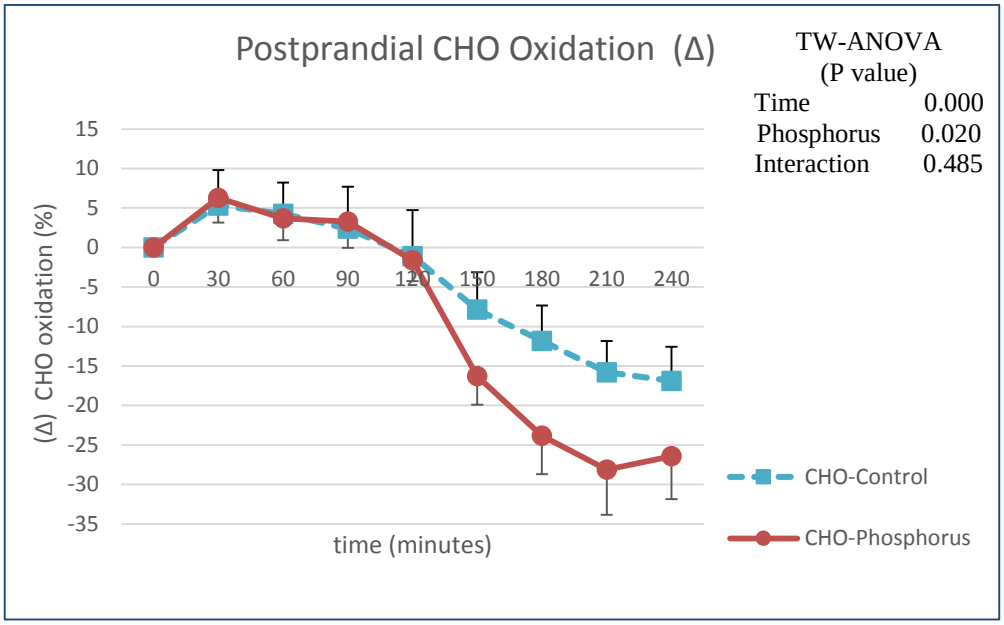


Figure7. Change in carbohydrate oxidation among lean males

Table 12. Changes in postprandial CHO oxidation (%) among lean males (SE: standard error)

time (min)	CHO-Control	SE-C	CHO-Phosphorus	SE-P
Baseline CHO oxidation %	22.1	4.5	22.1	4.5
0	0.00	0.00	0.00	0.00
30	5.32	4.48	6.26	3.12
60	4.26	3.95	3.68	2.74
90	2.38	5.31	3.27	3.31
120	-1.10	5.84	-1.63	2.63
150	-7.88	4.76	-16.31	3.58
180	-11.82	4.46	-23.83	4.88
210	-15.80	3.96	-28.11	5.74
240	-16.89	4.32	-26.44	5.42

EXPERIMENT 2

A. Subject characteristics

Characteristics of the 6 obese healthy male participants, their blood test results, and the baseline measurements (Resting energy expenditure, RQ, fat oxidation%, and CHO oxidation) are shown in Tables 13, 14 and 15 respectively. All 6 participants had normal: glucose blood test, blood creatinine and glomerular filtration rate (Table 14). Their average baseline resting energy expenditure (EE) was 2153 kcal (±112) (Table 15).

Table 13. Experiment 2: Characteristics of participants

	Obese Males
Number of subjects	6
Average Age (yrs),(SEb)	21.667 (± 0.667)
Anthropometric measurements, average	
Weight (kg),(SEb)	119.27 (± 5.19)
Height (cm),(SEb)	182.83 (± 2.8)
BMI a(kg/m2),(SEb)	35.63 (± 1.01)
Abbreviations: yrs: years, BMI: body mass index	

Table14. Experiment 2: Blood test results of participants

Blood Test levels	6 Obese Males	Normal Levels
FBG (mg/dl)	95.83	[76-110]
Creatinine (mg/dl)	0.92	[0.6-1.2]
GFR(mL/min/1.73mý)	116	Decreased kidney function if <60 mL/min/1.73mý
Abbreviations: FBG: fasting blood glucose, GFR: glomerular filtration rate		

Table15. Experiment 2: Baseline Energy Expenditure of subjects

Obese Subjects Energy Expenditure	
REE (kcal/day), (SEb)	2153 (± 112)
Resting RQ, (SEb)	0.78 (± 0.0325)
Resting fat oxidation (%),(SEb)	61.77 (± 9.4)
Resting CHO oxidation (%),(SEb)	30.47 (± 8.11)
Abbreviations: REE: Resting energy expenditure, SE: standard error, RQ: respiratory quotient, CHO: carbohydrate, (SEb): standard error	

B. Resting EE, RQ, fat oxidation %, & carbohydrate oxidation %

The average baseline measurements of obese males showed that they have average REE of 2153(± 112) kcal, average RQ 0.78, average fat oxidation of 61.77%, and an average CHO oxidation 30.47% (Table 15). The average CHO oxidation % among obese males was higher than that of the lean males (30.47% vs 22.1%).

C. Postprandial EE

Among obese males, there was an increase in postprandial EE after both meals ingestion, and this increase failed to return back to the baseline value after 4 hours measurement. At time = 30 minutes, postprandial EE after P-tablets started to increase more than that of control and continued to increase without returning to its baseline level. This increase upon P-tablets ingestion reached its peak at 90 min (7.2 kcal/min) while it reached only a maximum of (5.8kcal/ min) at 120 min upon placebo- tablets. The increase in the postprandial EE after P-tablets was significantly higher than that of placebo-tablets when adjusting for time and treatment (p=0.000, p=0.000) respectively. (Fig.8)

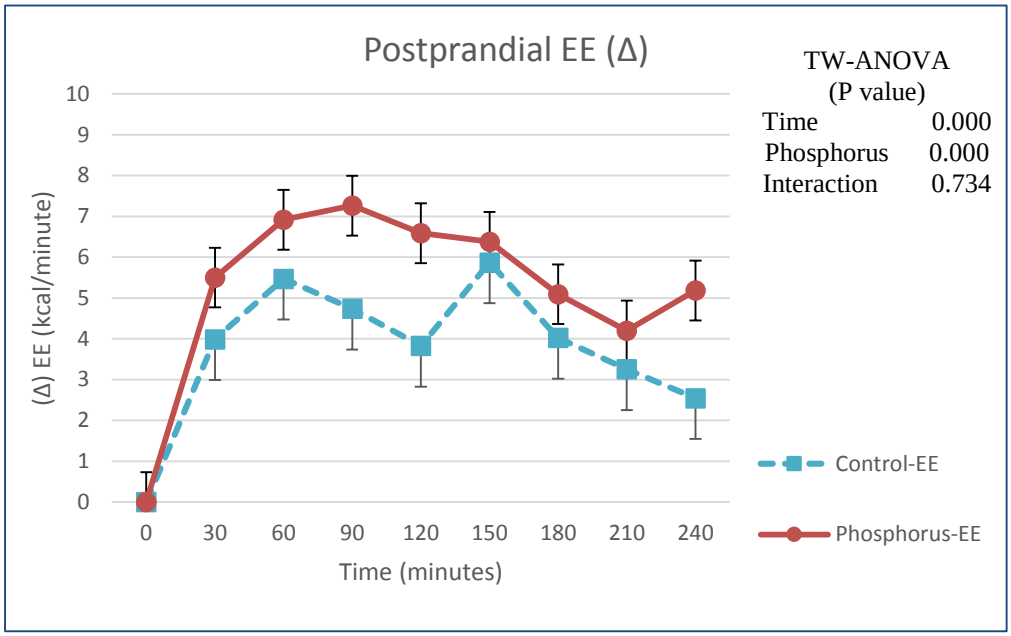


Figure 8. Change in postprandial EE among obese males

Table16. Changes in postprandial EE among obese males (SE: standard error)

Time (min)	Control-EE	SE-Control	Phosphorus-EE	SE-Phosphorus
Baseline EE	2153	112	2153	112
0	0	0	0	0
30	3.99	0.46	5.50	0.64
60	5.47	0.71	6.92	1.33
90	4.74	0.91	7.26	1.38
120	3.83	0.74	6.59	1.27
150	5.87	0.63	6.38	0.85
180	4.03	0.57	5.09	0.58
210	3.25	0.91	4.20	1.21
240	2.55	0.80	5.18	0.88

D. Postprandial Respiratory Quotient RQ

After both meals, there was a slight increase in postprandial RQ during the first 120 minutes. After 120 minutes, both RQ started to decrease until reaching 240 minutes. When comparing the difference between phosphorus and control, there was no significant difference according to treatment ($p=0.305$). (Figure 9)

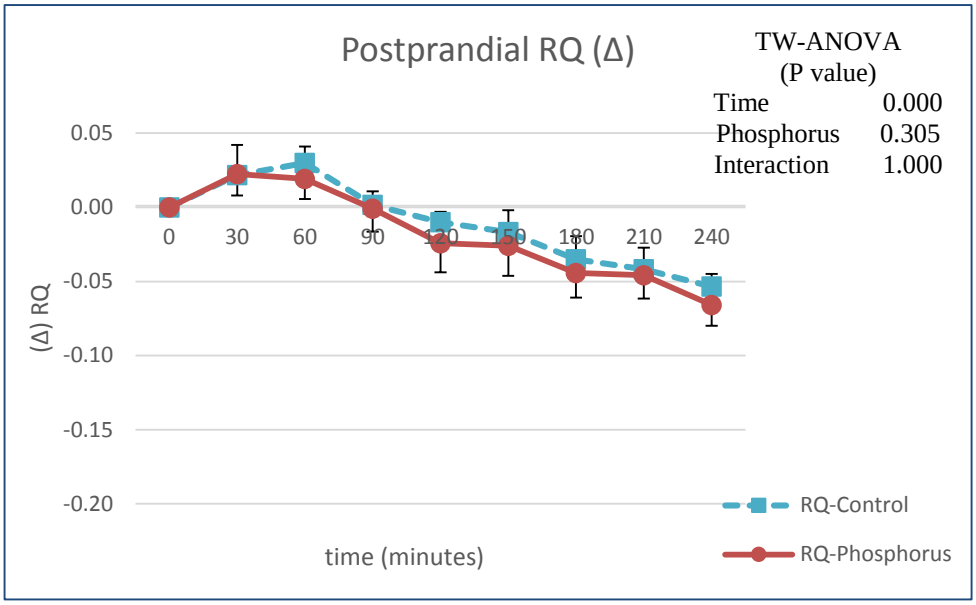


Figure 9. Change in postprandial RQ among obese males

Table 17. Changes in postprandial RQ among obese males (SE: standard error)

time (min)	RQ-Control	SE-C	RQ-Phosphorus	SE-P
Baseline RQ	0.78	0.0325	0.78	0.0325
0	0.0000	0.0000	0.0000	0.0000
30	0.0217	0.0205	0.0225	0.0146
60	0.0300	0.0110	0.0192	0.0135
90	0.0017	0.0092	-0.0008	0.0155
120	-0.0100	0.0070	-0.0242	0.0196
150	-0.0167	0.0148	-0.0258	0.0203
180	-0.0350	0.0156	-0.0442	0.0166
210	-0.0417	0.0145	-0.0458	0.0157
240	-0.0533	0.0083	-0.0658	0.0141

E. Postprandial fat oxidation

Among obese males, postprandial fat oxidation after meal+ P-tablets decreased during the first 30 minutes (-7%) while in control it decreased for 60 minutes (-8 %). After that, an increase started to take place at 90 minutes after both meals. The fat oxidation continued to increase by time until it reached a maximum increase by 14.38 % on the 240 minutes. We noticed that between 90 and 150 minutes this increase was higher after phosphorus ingestion when compared to that of control. There was no significance in the difference between phosphorus and control according to treatment ($p= 0.850$), but there was significance according to time only ($p=0.000$). (Figure 10)

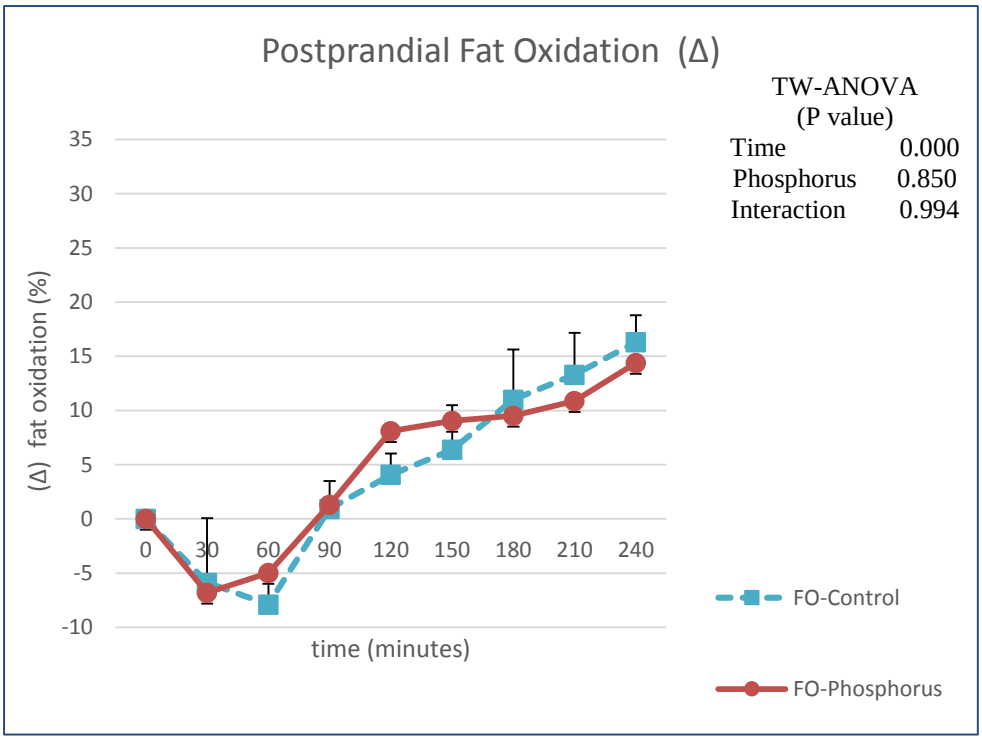


Figure 10. Change in fat oxidation % among obese males

Table 18. Changes in postprandial fat oxidation among obese males (SE: standard error)

time (min)	FO-Control	SE-C	FO-Phosphorus	SE-P
Baseline fat oxidation %	61.77	9.4	61.77	9.4
0	0	0.00	0.00	0.00
30	-5.88	5.96	-6.80	3.38
60	-7.89	3.18	-4.98	3.86
90	0.94	2.56	1.30	4.37
120	4.08	1.95	8.10	5.37
150	6.38	4.13	9.05	4.98
180	11.01	4.62	9.52	4.58
210	13.31	3.86	10.87	4.61
240	16.31	2.48	14.38	4.87

F. Postprandial CHO oxidation

Among obese subjects, measurements of postprandial CHO oxidation upon placebo tablets showed an increase for 90 minutes duration (peak of 9.9% at 30-60 min) and started to decrease after that. While after the P-tablets, this increase remained for 60 minutes (peak of 8% at 30 min), and started to decrease after that time. After 30 min, a decline was noticed in the postprandial CHO oxidation and it was stronger after phosphorus ingestion. Statistical analysis showed no significant difference between the decrease between postprandial CHO oxidation of phosphorus and control according to treatment ($p=0.052$), but there was significance according to time only ($p=0.000$) (Figure 11)

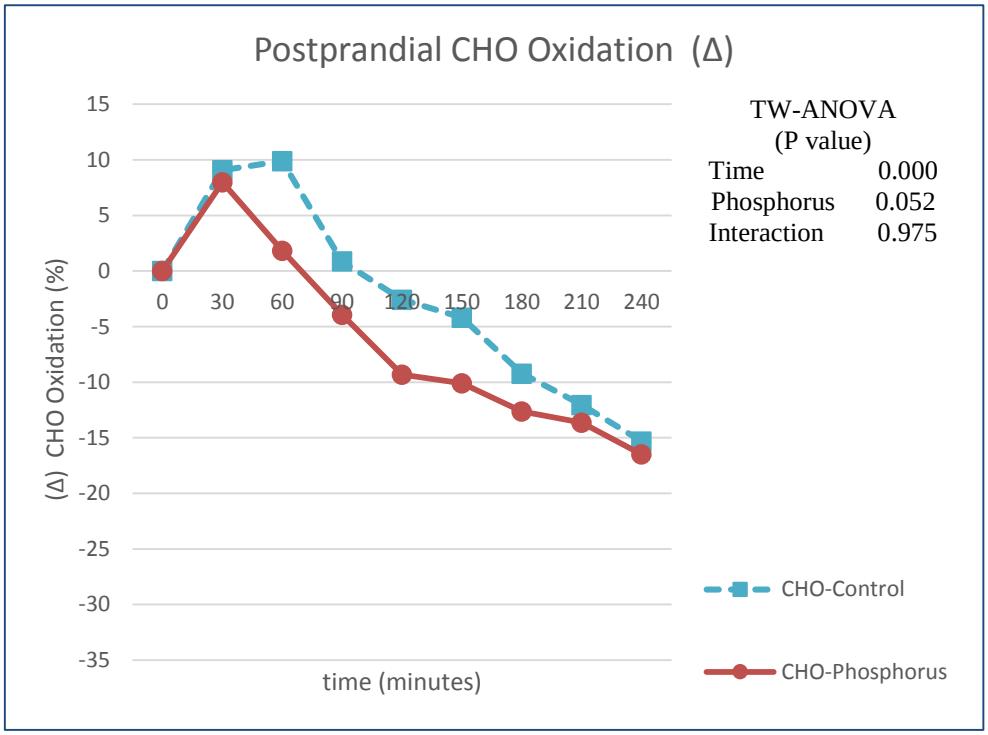


Figure 11. Postprandial CHO oxidation decrease among obese males

Table 19. Changes in postprandial CHO oxidation among obese males (SE: standard error)

time (min)	CHO-Control	SE-C	CHO-Phosphorus	SE-P
Baseline CHO oxidation%	30.47	8.11	30.47	8.11
0	0	0.00	0.00	0
30	9.08	6.82	7.99	3.36
60	9.9	3.18	1.84	2.24
90	0.87	2.54	-3.93	3.07
120	-2.57	1.90	-9.31	5.63
150	-4.18	4.33	-10.08	5.28
180	-9.22	4.77	-12.63	4.51
210	-12.05	3.97	-13.63	4.21
240	-15.33	2.52	-16.48	4.71

CHAPTER IV

DISCUSSION

Studies have revealed that ATP production in the human body depends upon adequate sources of phosphorus (P) that can be distorted by the digestion and absorption of high-carbohydrate–low P food mainly due to the increase in insulin release, that simultaneously stimulates peripheral uptake of P and the phosphorylation of many compounds (Obeid et al., 2013). Actually, this creates a competition for P that compromises its availability for ATP production, which is thought to be translated into low DIT (Obeid et al., 2013). ATP production is essential for several metabolic processes (Solomon & Kirby, 1990; Morris et al., 1978). Consequently, an increase in the insulin release along with a low phosphorus diet might exacerbate the situation. Additionally, impaired glucose tolerance causes a reduction in the phosphorus peripheral uptake thus affecting thermogenesis (Obeid, 2013). Decreased hepatic ATP production is assumed to be transmitted to the central nervous system through neural afferents, causing an increase in food intake.

Results of our study showed a strong association between phosphorus and diet induced thermogenesis (DIT). In both lean and obese male subjects, we observed a significant increase in the DIT upon phosphorus ingestion (500mg) meaning that an increase in ATP production took place. For lean subjects, the maximum DIT increase from baseline upon P ingestion was 1.53 kcal higher than that reached upon placebo ingestion. While for obese subjects, the maximum DIT increase from baseline was 1.39 kcal higher upon P ingestion than that of placebo ingestion. This gradual increase upon

P ingestion was maintained throughout the experiment without decreasing back to baseline. This increase in DIT is definitely associated with an increase in ATP production that depended primarily on the availability of the ingested P.

During fasting conditions, skeletal muscle relies on lipid oxidation for the majority of resting energy production, this was clearly revealed by our baseline measurements, where percentage fat oxidation was high among both lean and obese (61.54% and 61.77 % respectively). A study by Chevalier et al (2006) revealed that the fractional and absolute contribution of gluconeogenesis to glucose production is increased among obese subjects, and this is associated with an increased rate of protein turnover and insulin resistance of glucose, lipid, and protein metabolism. This comes in line with our study results, where both measurements of resting protein and CHO oxidation percentage were higher among obese subjects (30.47% and 16.13 % respectively) when compared to lean subjects (22.1% and 7.99% respectively). Moreover, Boden et al (2001) revealed that after overnight fasting, obese subjects have an increase in gluconeogenesis, glycogenolysis, and hyperglycaemia that lead to hepatic insulin resistance and cause an increased fasting serum insulin. Thus, the insulin-induced suppression of both gluconeogenesis and glycogenolysis was impaired among obese subject. Also, it has been shown that an increase in FFA in the fasted state among obese nondiabetic subjects impairs insulin-induced suppression of glycogenolysis (Boden et al., 2001). All mentioned mechanisms explain the high percentage of CHO oxidation (30.47%) among obese subjects after overnight fasting in our study.

Study done by Khattab et al (2015) reported that a lower magnitude of increase in the postprandial serum glucose along with a lower insulin concentration level and higher insulin sensitivity took place after glucose meal + P ingestion as compared to

glucose ingestion alone. The maximum increase of both serum insulin and serum glucose was reached at 30 min after glucose+ P ingestion (Khattab et al., 2015). This explains the mechanism behind the results of the postprandial CHO oxidation percentage in our study, where an increase from baseline occurred after P ingestion and reached a maximum level at 30 min in both lean and obese. Despite the fact that P has stimulated insulin activity and improved its sensitivity, it was reported by Bogardus et al (1984) that obese subjects have 20% lower mean maximal insulin-stimulated glucose disposal rate and 30% lower maximal insulin-stimulated glucose transport rate when compared to lean subjects. This explains the difference duration in the increase of percentage CHO oxidation from baseline between lean and obese (120 min and 60 min respectively).

In obese, the increase in the postprandial CHO oxidation percentage from baseline took less time after P-tablets (60 min) as compared to placebo-tablets (90 min). This difference is attributed to the phosphorus availability which enhanced insulin mediated peripheral phosphorylation (Khattab et al., 2015). This increase in CHO oxidation percentage from baseline during the first 60 minutes was similar to that revealed by Khattab et al (2015) study; where measured levels of insulin after (glucose meal+ p-tablets) were significantly lower than that of the glucose treatment alone at time 60 min ($P = 0.002$) (Khattab et al., 2015). The mechanism is that an increase in intracellular glucose trapping (phosphorylation) took place due to the insulin secretion and higher P availability; where ATP production increased. This process of fast CHO oxidation might be due to an improvement in insulin sensitivity (Khattab et al., 2015). In details; CHO meal +P-tablets ingestion had led to the uptake of peripheral P by insulin, which in turn increases cellular uptake and utilization of glucose, and activates

the phosphorylation of several compounds including carbohydrate, protein and fat (Karczmar et al., 1989, Obeid et al., 2014). Since there is enough amount of P availability, this led to an increase in hepatic ATP production and was translated into a high DIT. Furthermore, the interaction of glucose and phosphorus in the proximal part of the small intestine may have been involved in insulin sensitivity through incretin hormones secretion such as glucagon like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP). Those incretin hormones are normally secreted after meal ingestion, especially after protein rich meal ingestion (rich in P) (Veldhorst et al., 2008 &Becker et al., 1996). Such hormones were described to affect insulin status and were reported to have essential role in insulin activity and in regulating postprandial blood glucose (Veldhorst et al., 2008, Elliott et al., 1993, Soltani et al., 2007).

The improvement in insulin sensitivity and P availability among the subjects might have caused an increase in glycogen synthesis and protein synthesis; which explains the source of increase in DIT during the first 1.5 hour (90 min) among both obese and lean. Protein synthesis is known to be energy expensive with a minimal cost of 4 X ATP per amino acid incorporated or some 2·8 kJ per gram of protein synthesized (Bender et al.,2012). The cost of protein synthesis was shown to be 11·7% of TEE after the high carbohydrate meals (Bender et al., 2012). This explains the mechanism behind the significant increase of DIT following the CHO meal +P ingestion where P availability caused this increase in the protein synthesis.

Upon P ingestion, obese males utilized glucose for the first hour and then shifted to glycogen synthesis in the liver and muscles. As for lean males, they spent 2 hours on glucose utilization (120 min) which started to decrease significantly after that. The peak of serum phosphorus concentration is reached at 60 min, and 60 % of the

ingested P was translocated from the extracellular compartment to the intracellular ones, primarily in skeletal muscles (Karczmar et al., 1989 and Khattab et al., 2015). Phosphorus ingestion caused an increase in the peripheral glucose uptake, especially in skeletal muscles, which is triggered by insulin dependent Glut 4 stimulation (Huang et al., 2007). In both liver and skeletal muscle cells, glycogen can be synthesized from glucose through the direct route or it may also be synthesized by an indirect pathway which includes prior degradation of glucose to trioses, followed by gluconeogenesis to resynthesize hexose phosphates, which are then committed to glycogen formation (Prelleraet al., 2007). When the resultant glucose 6-phosphate is isomerized to glucose 1-phosphate and then used for glycogen synthesis in the liver, an additional requirement of 1 X UTP (equivalent to ATP) per mol is needed (Bender et al., 2012). But if the glucose is released from the liver and then used for glycogen synthesis in muscle cells, an additional requirement of 2 X ATP equivalents per mol of glucose incorporated is needed. This mechanism explains the increase in DIT that started to show at 60 min after P ingestion in lean and obese.

Increase in fat oxidation percentage from the baseline started taking place at 90 min in both lean and obese, it was significantly higher after P ingestion among lean subjects only. RQ reached 0.7 at time 240; this reveals that the energy expenditure shifted from CHO oxidation to fat oxidation; where ATP produced was used for glycogen and protein synthesis. Studies have reported that adiposity is negatively correlated to adiponectin (also known as ACRP30), a hormone that decreases hepatic gluconeogenesis and increases lipid oxidation in muscle (Arita et al., 1999, Tomas et al., 2002). This explains the reason why the postprandial percentage fat oxidation increase from baseline reached a maximal increase of 14.38% which is lower than that

of lean subjects 28.96%. This difference could be due to the low production of adiponectin among obese subjects. Another mechanism that may further explain the low fat oxidation among obese subjects is the reduced oxidative enzyme capacity and the diminished activity of carnitine palmitoyl transferase (CPT) in their skeletal muscle (Kelley etal., 1999, Lithell et al., 1981). The significant increase in fat oxidation (%) among the lean subjects after P ingestion is mainly attributed to an increase in the activation of glucagon hormone, hormone sensitive lipase, and CPT after 2 hours from the meal+ P ingestion. It is worth noting that P ingestion in our study stimulated the activation cascade of glucagon, hormone sensitive lipase, and CPT that enhanced lipolysis; resulting in ATP production . Meanwhile, the P ingestion prolonged the release of ApoB48 into circulation until the end of the experiment (Hazim et al., 2014). This comes in line with the results of an earlier study where phosphorus has been proven to be beneficial to lipid metabolism; high level of phosphate might reduce free fatty acids and triglycerides in serum and hepatic triglyceride (Kanazawa et al., 2008). Additionally, this significant increase in postprandial fat oxidation from baseline explains the mechanism of an earlier study done by Ayoub et al (2015) where P ingestion for 12 weeks caused a significant waist circumference decrease.

The observed significant increase in the DIT following meal+ P might be partly involved in the described synergic relationship between the consumption of whole grains and glucose tolerance especially that the dietary fiber and whole grains are rich sources of phosphorus (YE et al., 2012). Moreover, our observation may partially explain the observed parallel rise in metabolic syndrome with global urbanization and westernization of dietary habits, which favor low P intake. Results of our experiments can reveal the possible mechanism of previous study results, where P ingestion among

overweight subjects led to a significant weight loss and waist circumference (Ayoub et al., 2015). This took place due to the phosphorus effect on increasing fat oxidation among subjects, thus decreasing weight and waist circumference.

Further future studies should be done to have an advanced understanding of the mechanism behind phosphorus effect on DIT. Insulin sensitivity test along with other blood hormonal tests could be done throughout the experimental process in order to investigate the exact metabolism that might be taking place.

Limitations of Research

The limitations of this study lies in its small number of subjects who were enrolled in the experimental design. Moreover, upon the measurement of the energy expenditure, some subjects fell asleep for few minutes, which explains the sudden decrease in the DIT revealed at 120 min among some obese subjects. For the sake of precision, we will include all the graphs of the postprandial EE, fat and CHO oxidation (%), and RQ for all subjects in the appendices section.

CHAPTER V

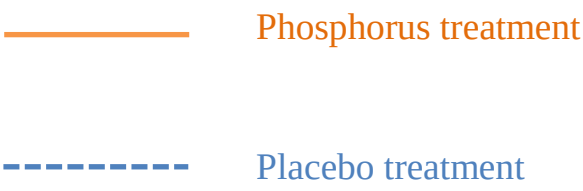
CONCLUSION AND RECOMMENDATIONS

In our study, lean and obese male subjects had a significant increased diet induced thermogenesis after 500 mg of phosphorus with a high CHO meal. Results are parallel to that of an earlier study which reported a significant weight loss and significant decrease in waist circumference of overweight and obese subjects after phosphorus ingestion (375 mg per main meal) for 12 weeks (Ayoub et al., 2015). Phosphorus could be the key for weight loss mainly through its effect on increasing the diet induced thermogenesis; this is either through the ATP production from postprandial fat oxidation or through ATP production due to protein synthesis. Dietary phosphorus intake can play beneficial role in preventing the deleterious effect of depleting intracellular phosphorus. Phosphorus ingestion enhances the insulin mediated peripheral phosphorylation, which strongly depends on extracellular phosphorus availability or exogenous (dietary) phosphorus supply. Moreover, P ingestion might have promising effect on glucose and lipid metabolism as it showed to improve glucose oxidation especially among obese who have an improvement in insulin sensitivity upon P ingestion. Furthermore, the increase in fat oxidation among lean males in our experimental study opens the door to a lot of further studies that could investigate the exact mechanism behind this improvement. Further research should be done to examine the exact mechanism of phosphorus in the diet-induced thermogenesis (DIT) in the human body.

CHAPTER VI

APPENDIX

1. **Graphs of the changes in the postprandial EE, fat oxidation and CHO oxidation for every lean male subject**



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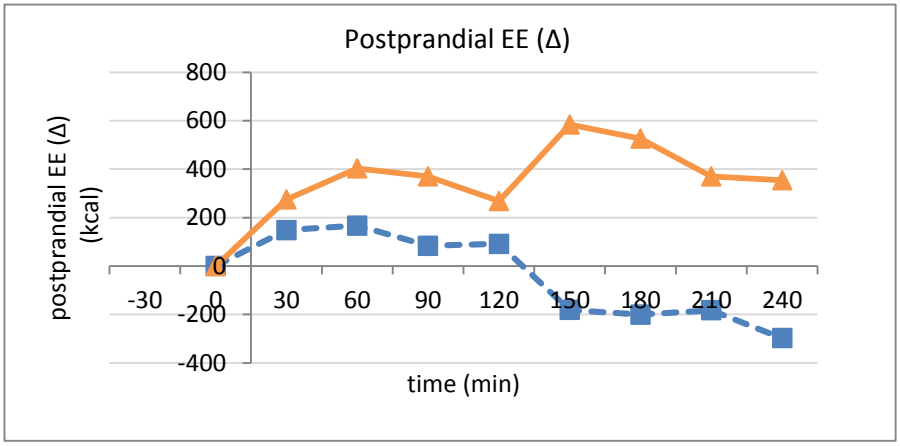
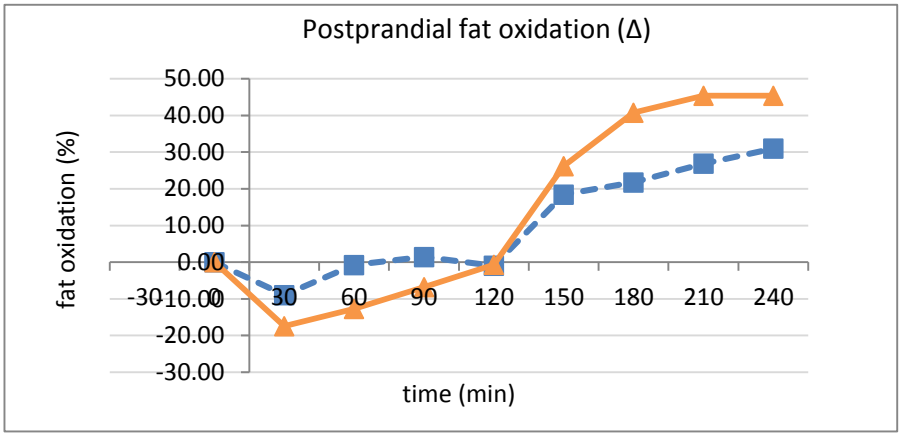
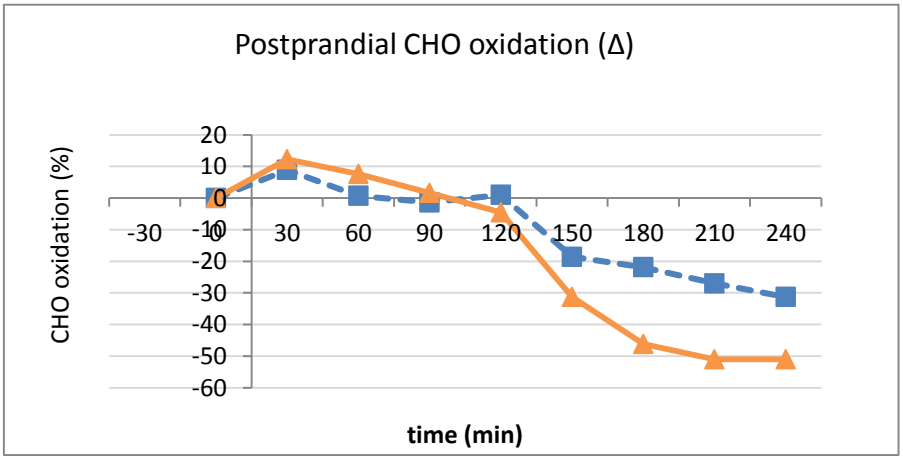
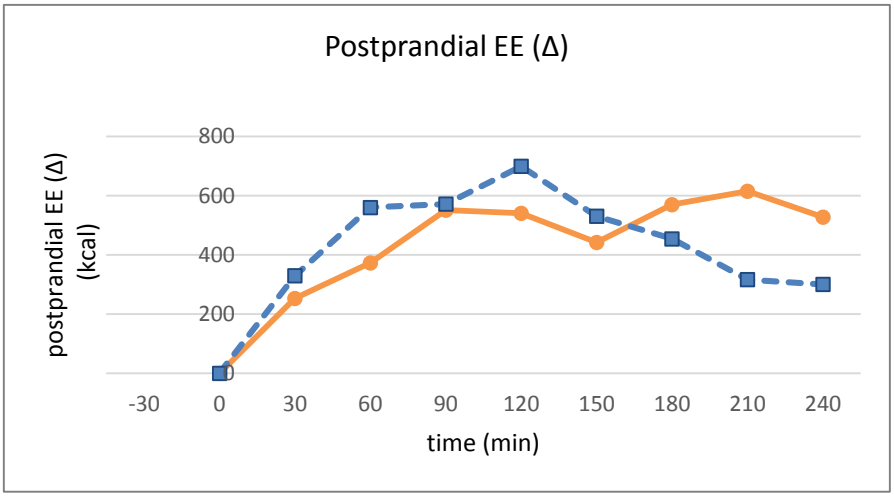
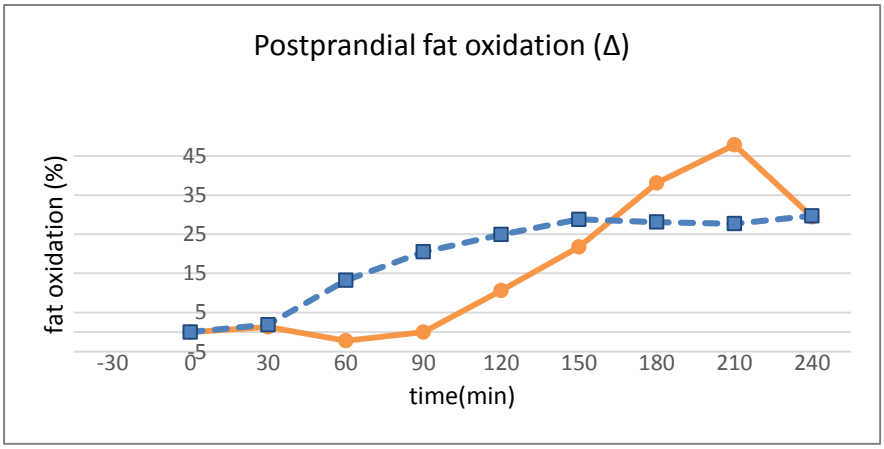

Postprandial EE (Δ)
postprandial EE (Δ) (kcal)
800
600
400
200
0
-200
-400
-30
0
30
60
90
120
150
180
210
240
time (min)

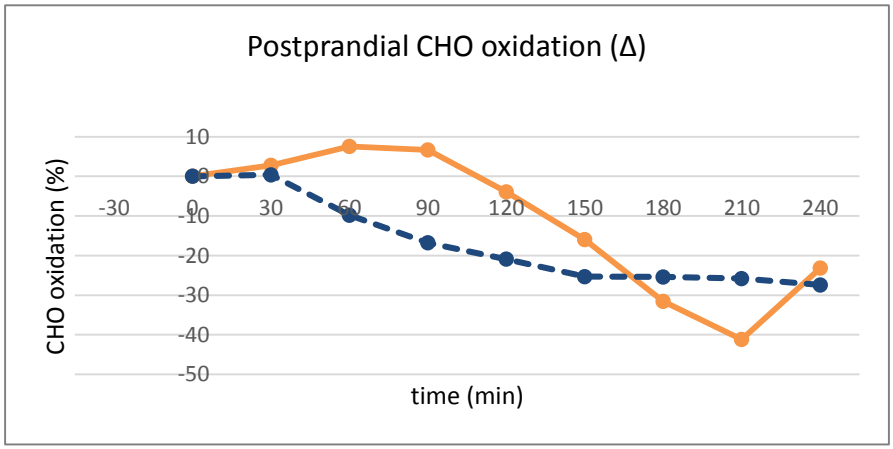

Postprandial fat oxidation (Δ)
fat oxidation (%)
50.00
40.00
30.00
20.00
10.00
0.00
-10.00
-20.00
-30.00
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial CHO oxidation (Δ)
CHO oxidation (%)
20
10
0
-10
-20
-30
-40
-50
-60
-30
0
30
60
90
120
150
180
210
240
time (min)

Subject 2:


Postprandial EE (Δ)
postprandial EE (Δ) (kcal)
800
600
400
200
0
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial fat oxidation (Δ)
fat oxidation (%)
45
35
25
15
5
-5
-30
0
30
60
90
120
150
180
210
240
time(min)


Postprandial CHO oxidation (Δ)
CHO oxidation (%)
10
0
-10
-20
-30
-40
-50
-30
0
30
60
90
120
150
180
210
240
time (min)

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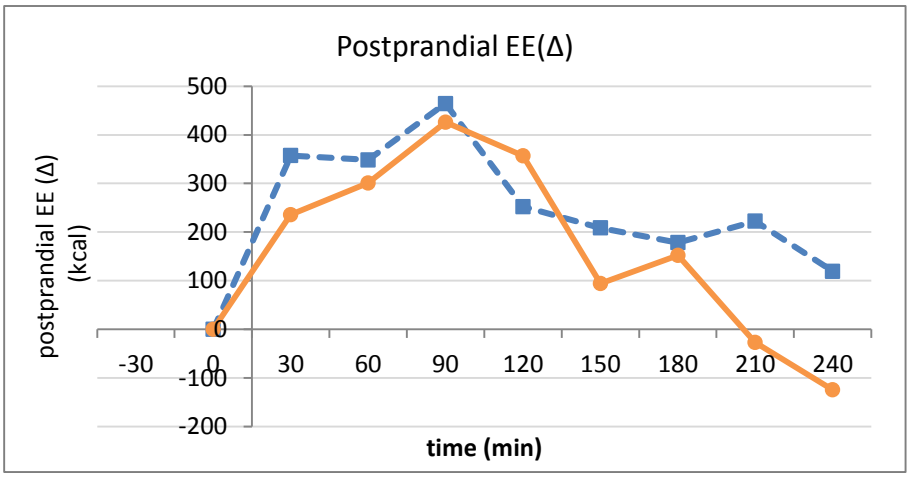
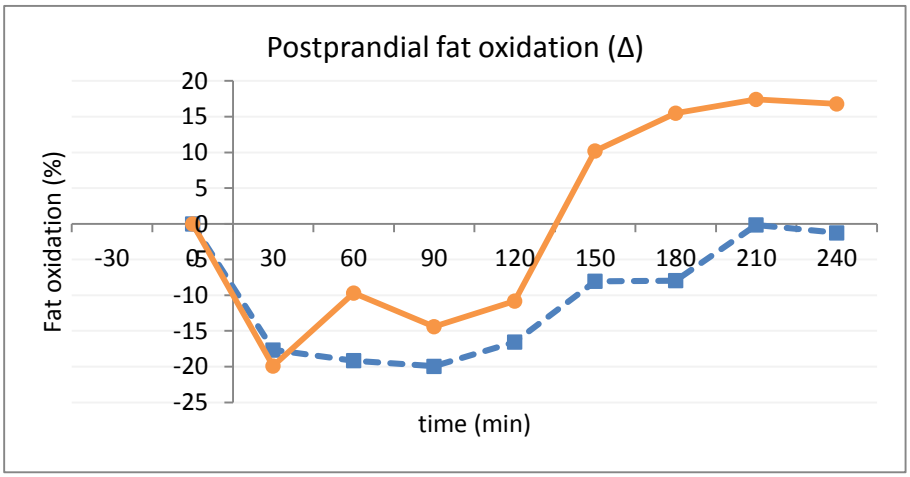
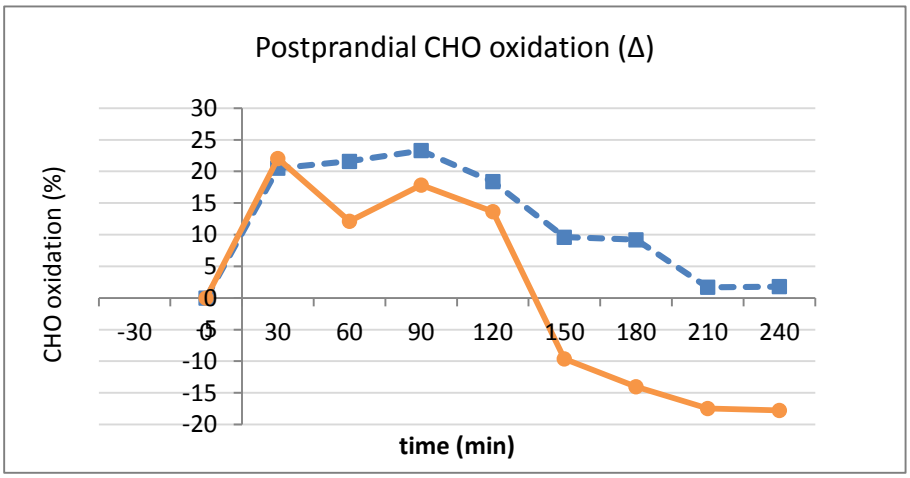

Postprandial EE(Δ)
postprandial EE (Δ) (kcal)
500
400
300
200
100
0
-100
-200
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial fat oxidation (Δ)
Fat oxidation (%)
20
15
10
5
0
-5
-10
-15
-20
-25
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial CHO oxidation (Δ)
CHO oxidation (%)
30
25
20
15
10
5
0
-5
-10
-15
-20
-30
0
30
60
90
120
150
180
210
240
time (min)

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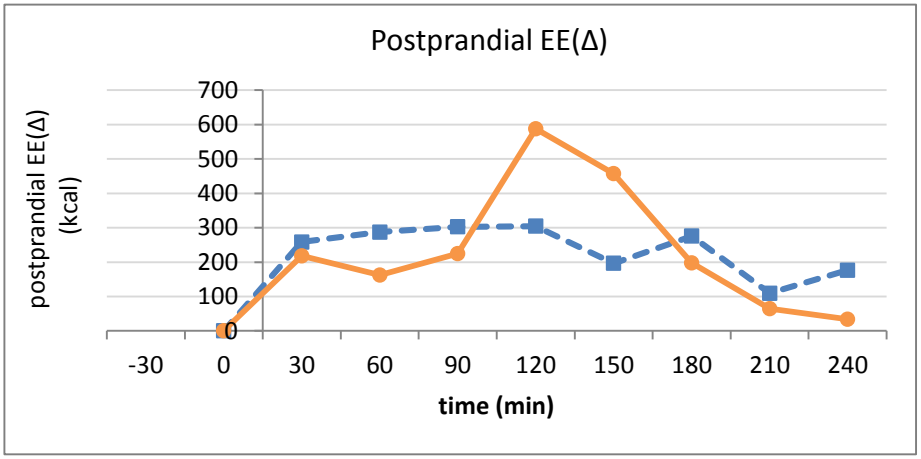
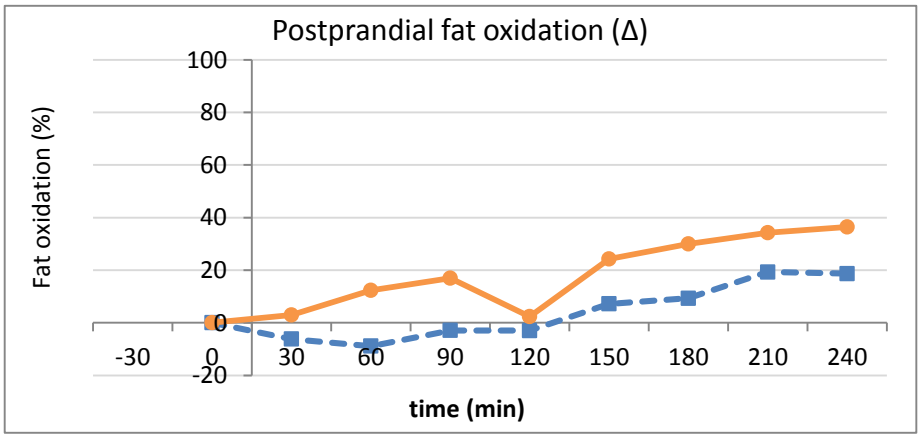
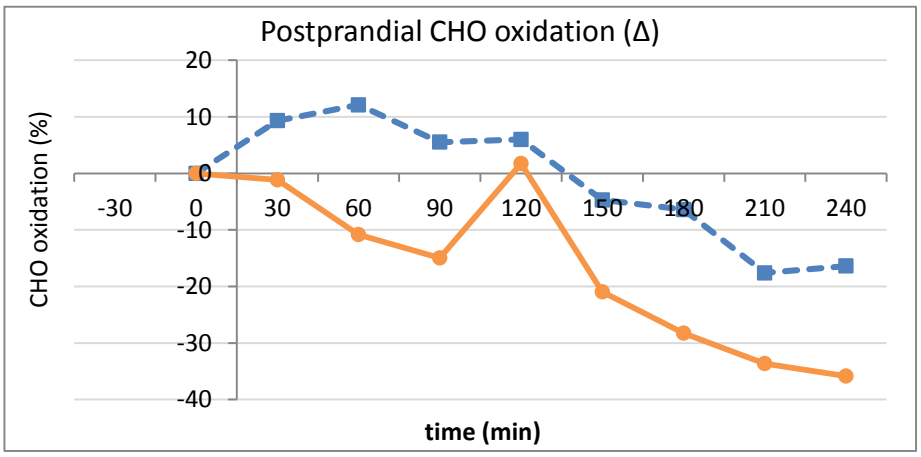
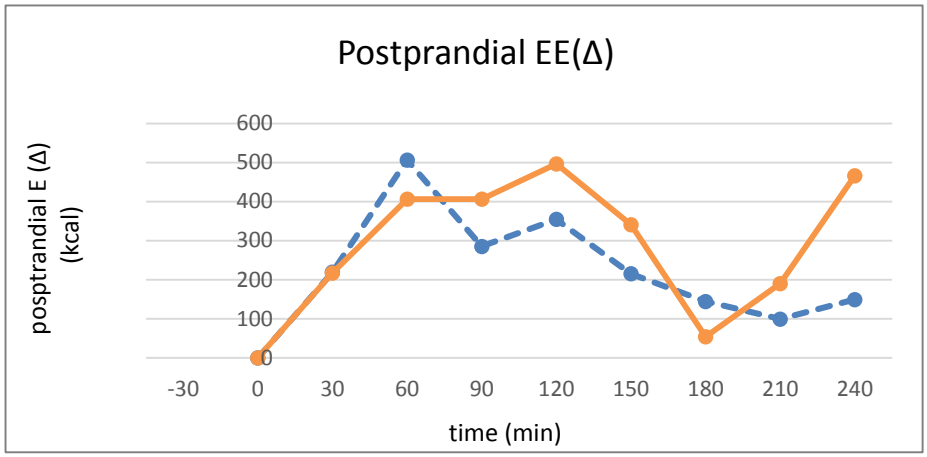
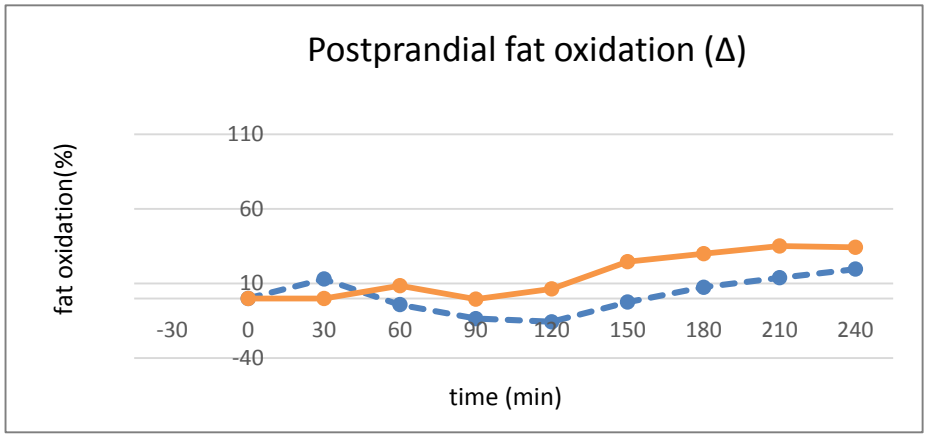

Postprandial EE(Δ)
postprandial EE(Δ) (kcal)
700
600
500
400
300
200
100
0
-30
0
30
60
90
120
150
180
210
240
time (min)

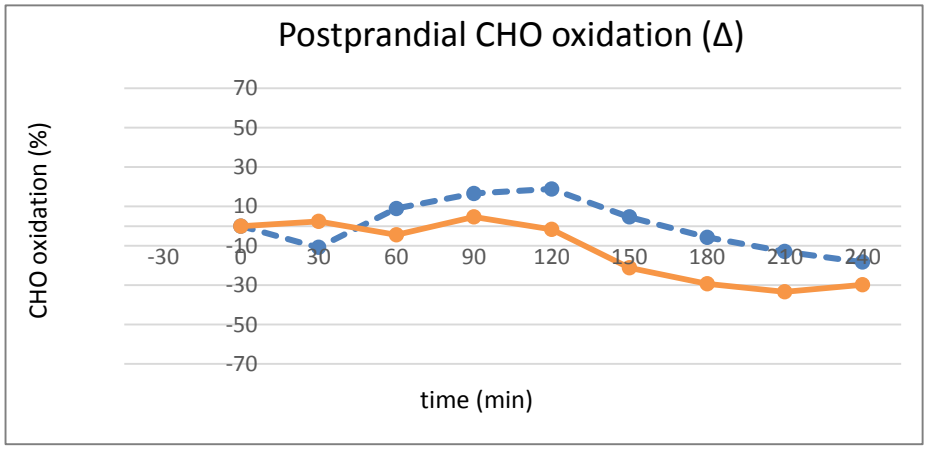

Postprandial fat oxidation (Δ)
Fat oxidation (%)
100
80
60
40
20
0
-20
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial CHO oxidation (Δ)
CHO oxidation (%)
20
10
0
-10
-20
-30
-40
-30
0
30
60
90
120
150
180
210
240
time (min)

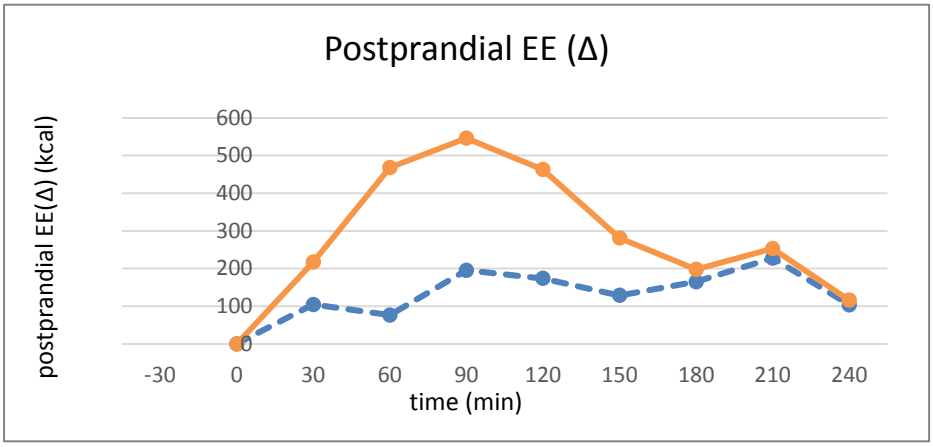
Subject 5:

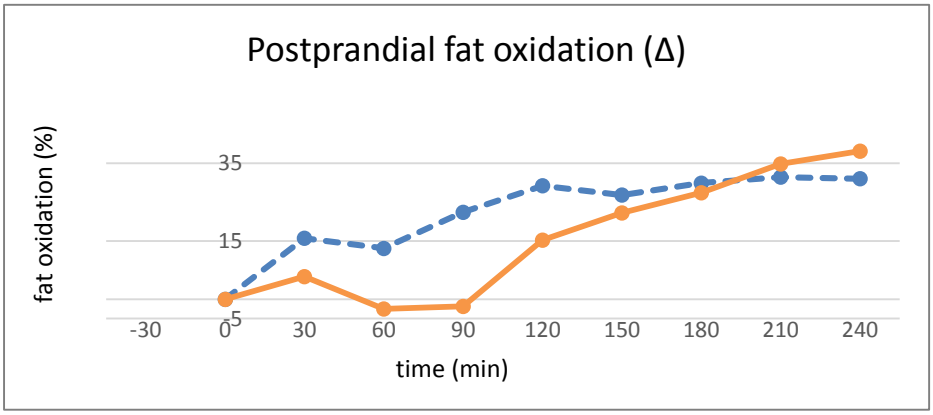

Postprandial EE(Δ)
posptrandial E (Δ) (kcal)
600
500
400
300
200
100
0
-30
0
30
60
90
120
150
180
210
240
time (min)

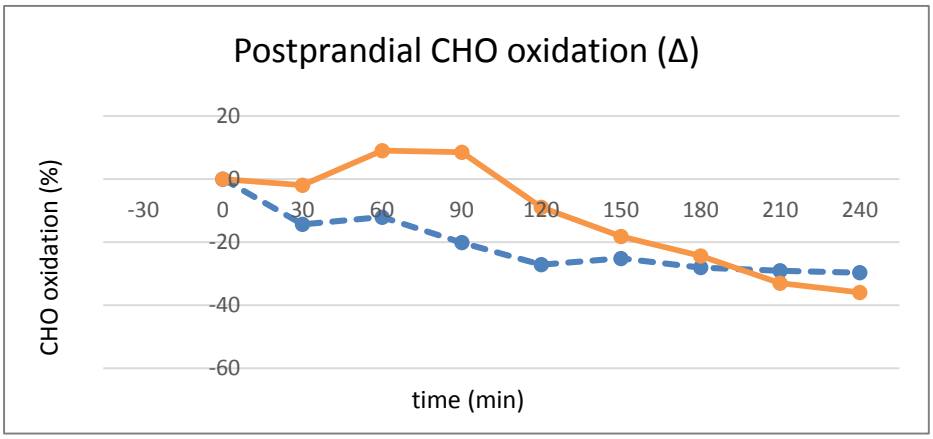

Postprandial fat oxidation (Δ)
fat oxidation(%)
110
60
10
-40
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial CHO oxidation (Δ)
CHO oxidation (%)
70
50
30
10
-10
-30
-50
-70
-30
0
30
60
90
120
150
180
210
240
time (min)

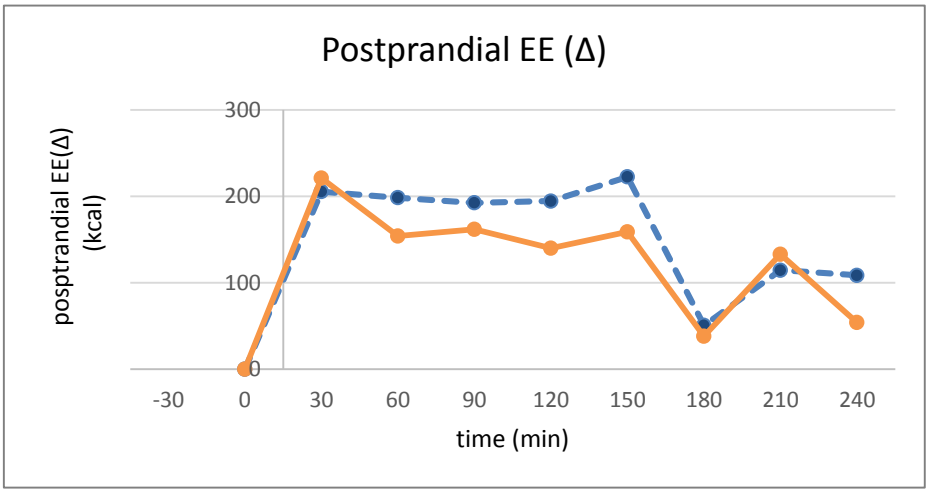
Subject 6:

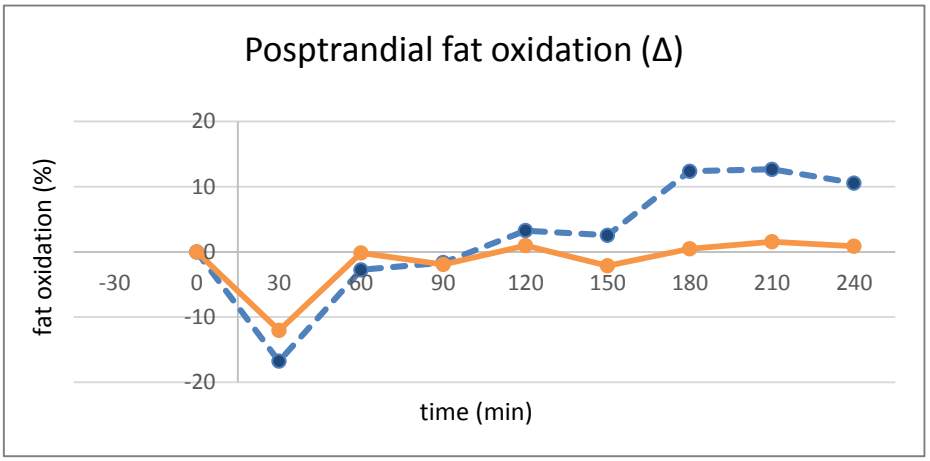

Postprandial EE (Δ)
postprandial EE(Δ) (kcal)
600
500
400
300
200
100
0
-30
0
30
60
90
120
150
180
210
240
time (min)

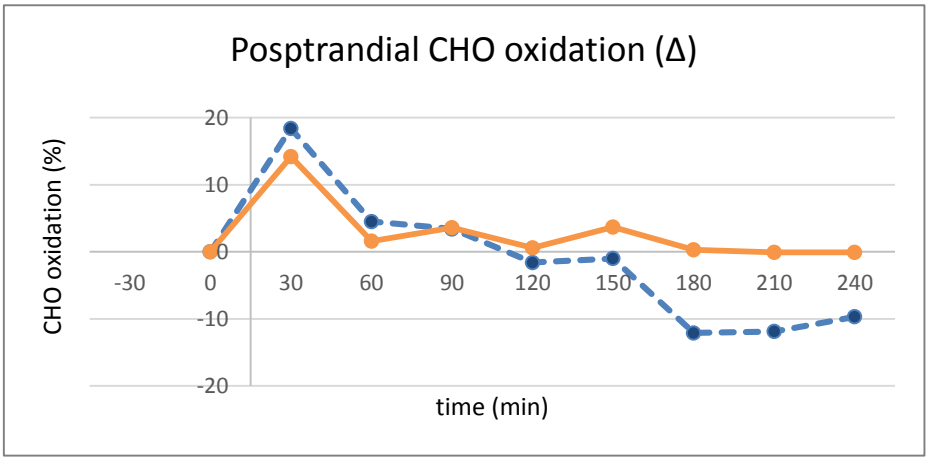

Postprandial fat oxidation (Δ)
fat oxidation (%)
35
15
-5
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial CHO oxidation (Δ)
CHO oxidation (%)
20
0
-20
-40
-60
-30
0
30
60
90
120
150
180
210
240
time (min)

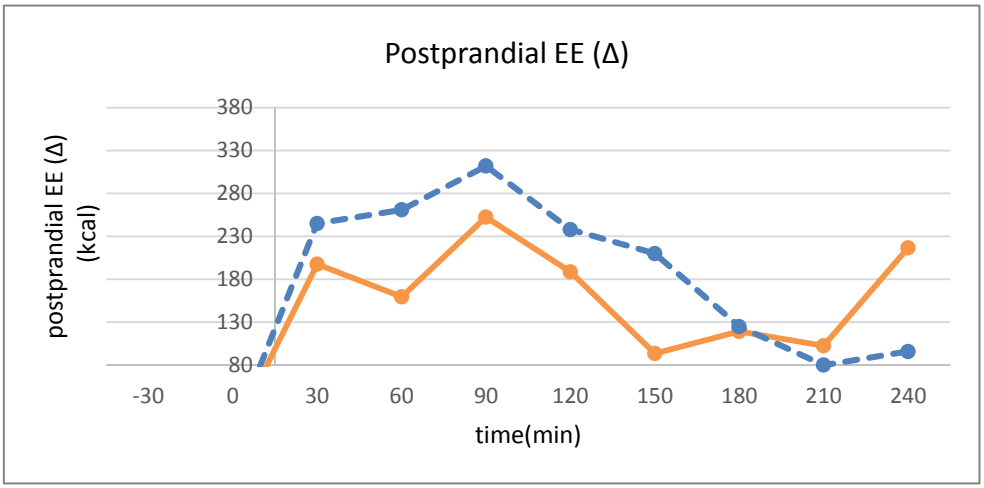
Subject 7:

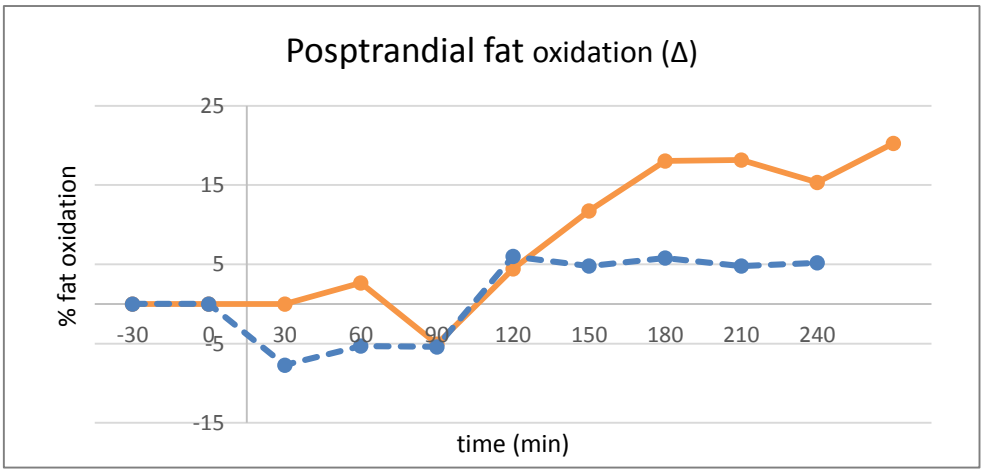

Postprandial EE (Δ)
posptrandial EE(Δ) (kcal)
300
200
100
0
-30
0
30
60
90
120
150
180
210
240
time (min)

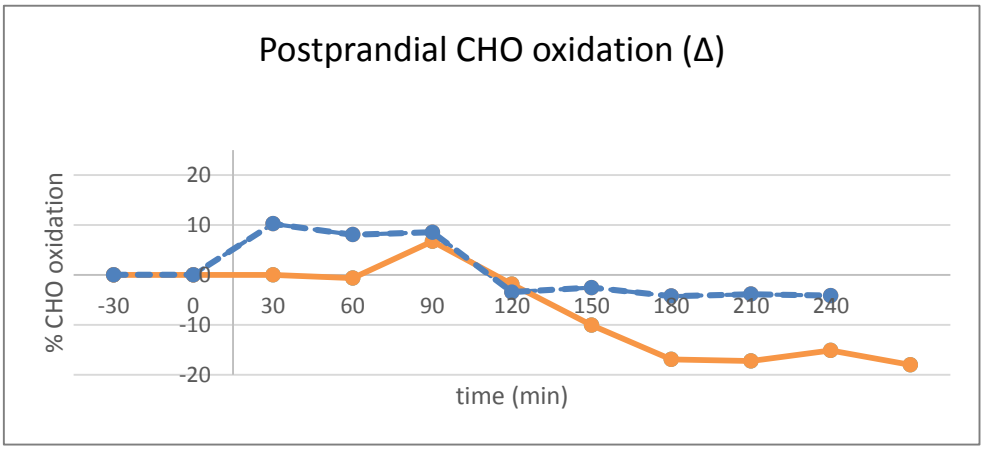

Posptrandial fat oxidation (Δ)
fat oxidation (%)
20
10
0
-10
-20
-30
0
30
60
90
120
150
180
210
240
time (min)


Posptrandial CHO oxidation (Δ)
CHO oxidation (%)
20
10
0
-10
-20
-30
0
30
60
90
120
150
180
210
240
time (min)

Subject 8:

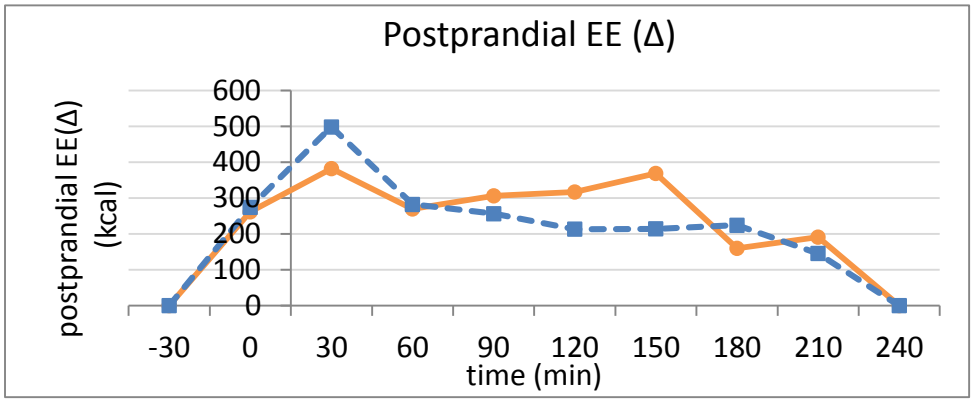

Postprandial EE (Δ)
postprandial EE (Δ) (kcal)
380
330
280
230
180
130
80
-30
0
30
60
90
120
150
180
210
240
time(min)

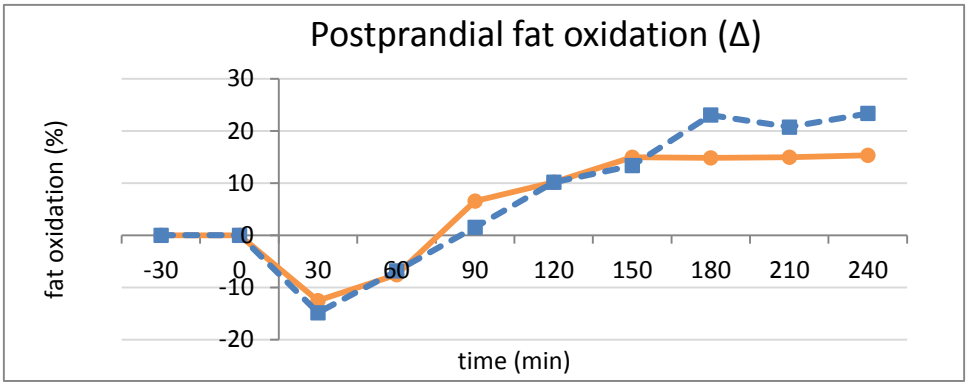

Posptrandial fat oxidation (Δ)
% fat oxidation
25
15
5
-5
-15
-30
0
30
60
90
120
150
180
210
240
time (min)

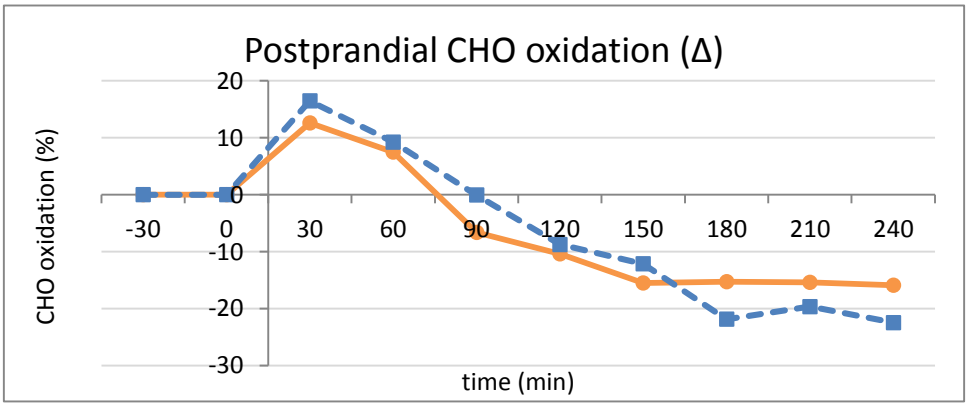

Postprandial CHO oxidation (Δ)
% CHO oxidation
20
10
0
-10
-20
-30
0
30
60
90
120
150
180
210
240
time (min)

2. Graphs of the changes in the postprandial EE, fat oxidation and CHO oxidation for every obese male subject

Subject 1:







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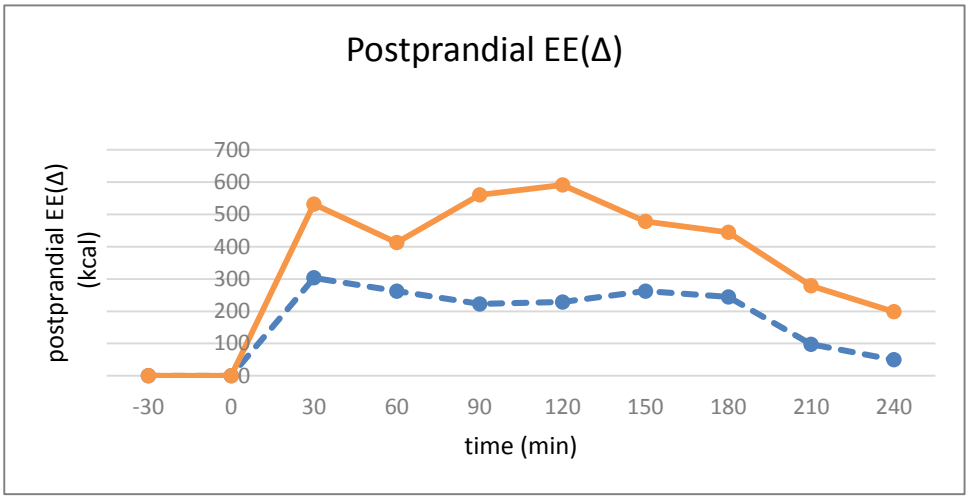
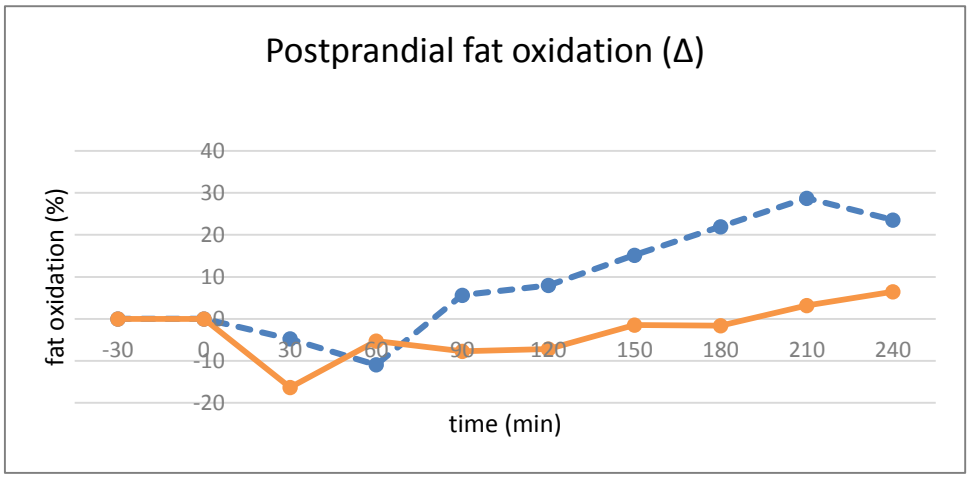
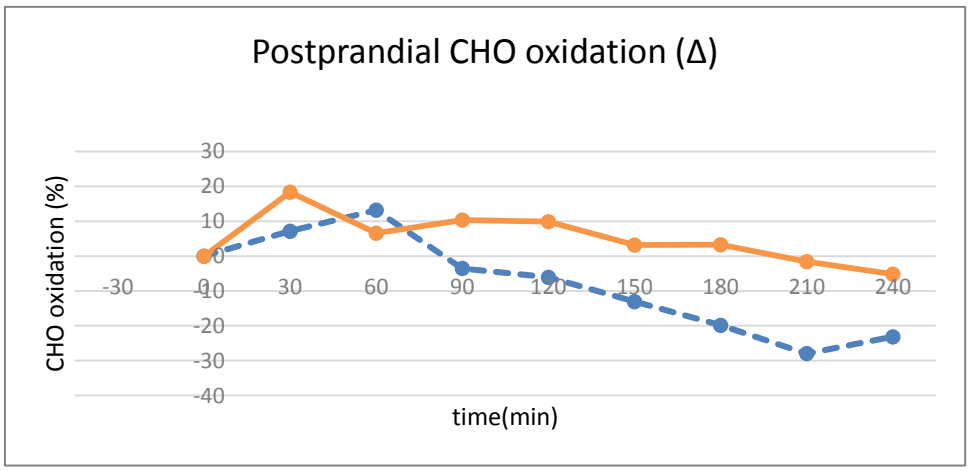
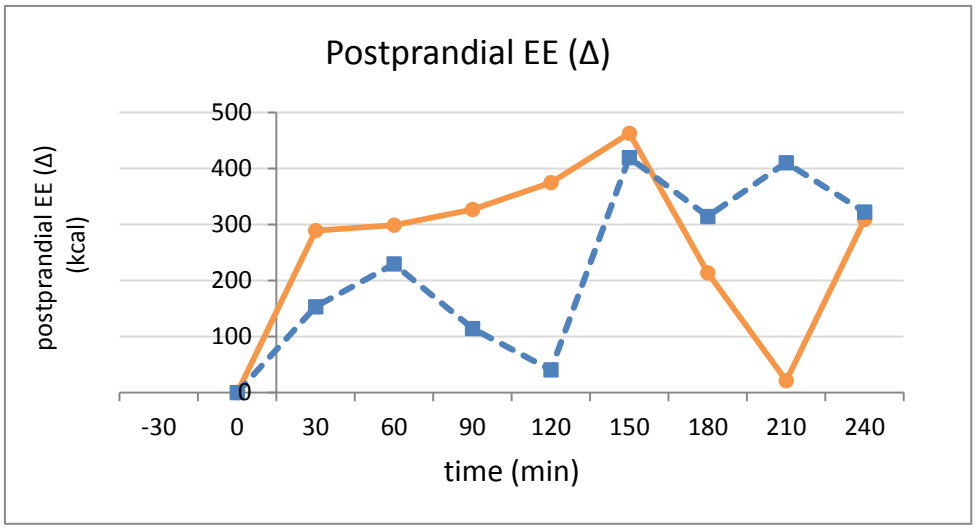
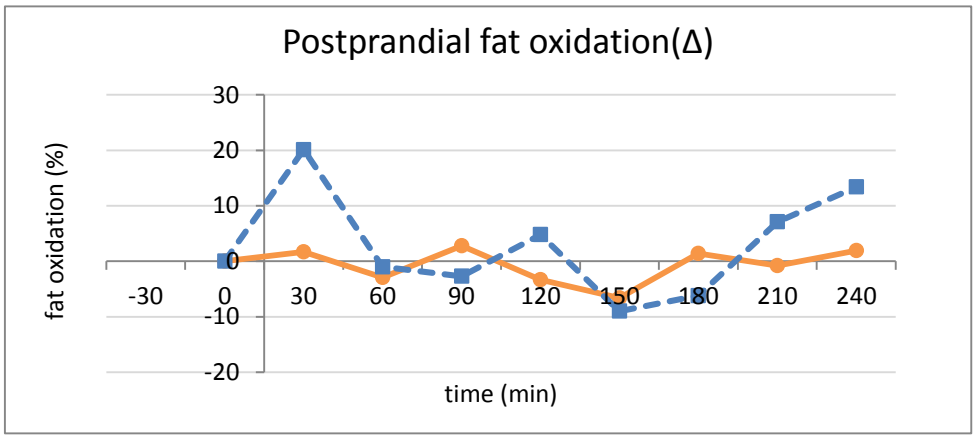

Postprandial EE(Δ)
postprandial EE(Δ) (kcal)
700
600
500
400
300
200
100
0
-30
0
30
60
90
120
150
180
210
240
time (min)

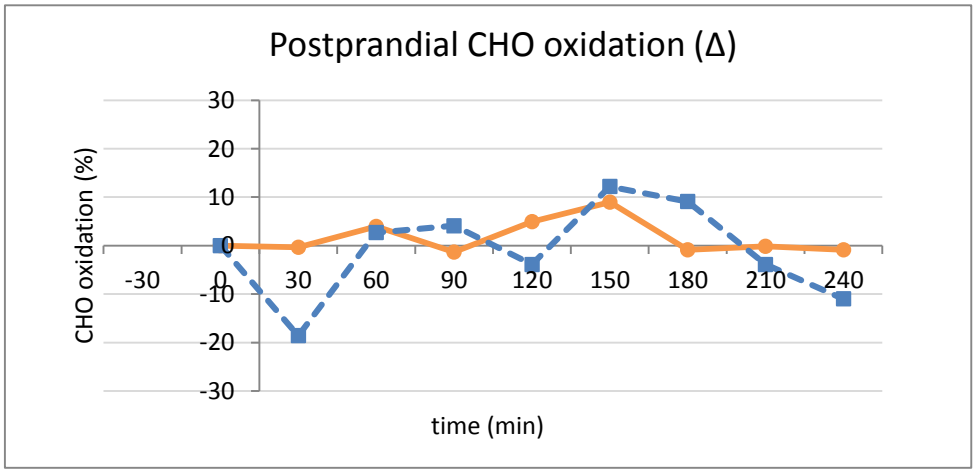

Postprandial fat oxidation (Δ)
fat oxidation (%)
40
30
20
10
0
-10
-20
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial CHO oxidation (Δ)
CHO oxidation (%)
30
20
10
0
-10
-20
-30
-40
-30
0
30
60
90
120
150
180
210
240
time(min)

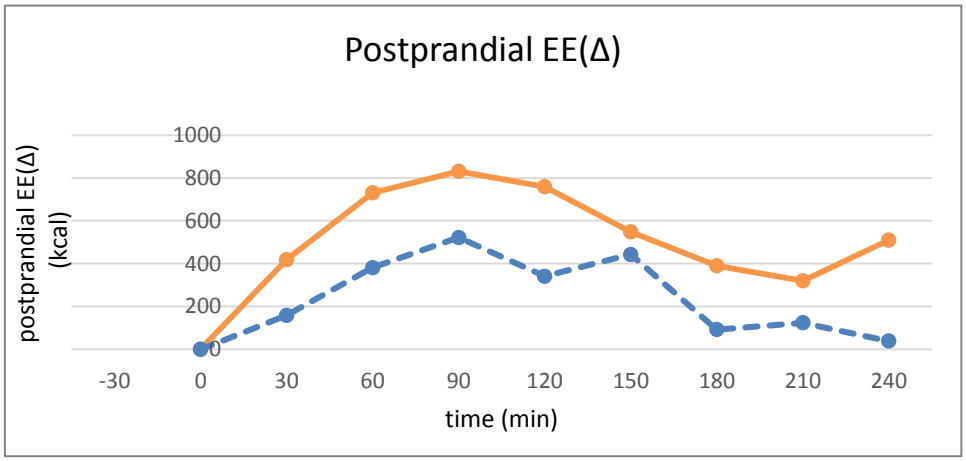
Subject 3:

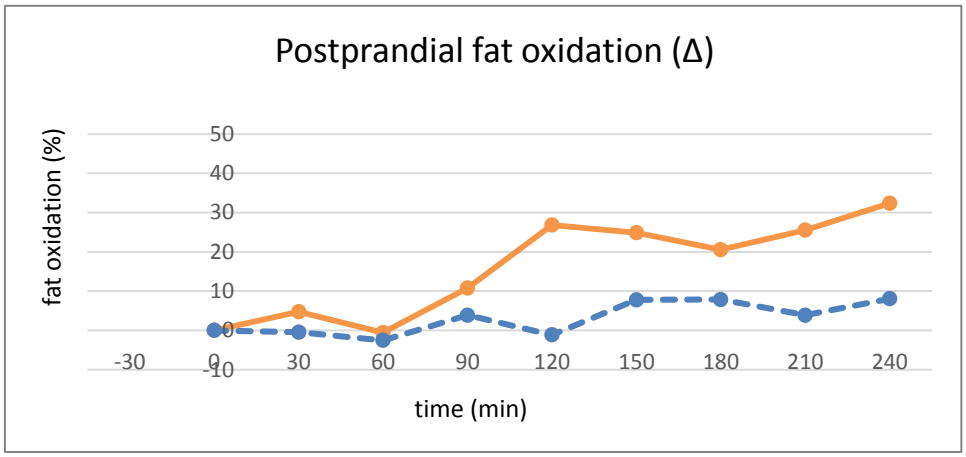

Postprandial EE (Δ)
postprandial EE (Δ) (kcal)
500
400
300
200
100
0
-30
0
30
60
90
120
150
180
210
240
time (min)

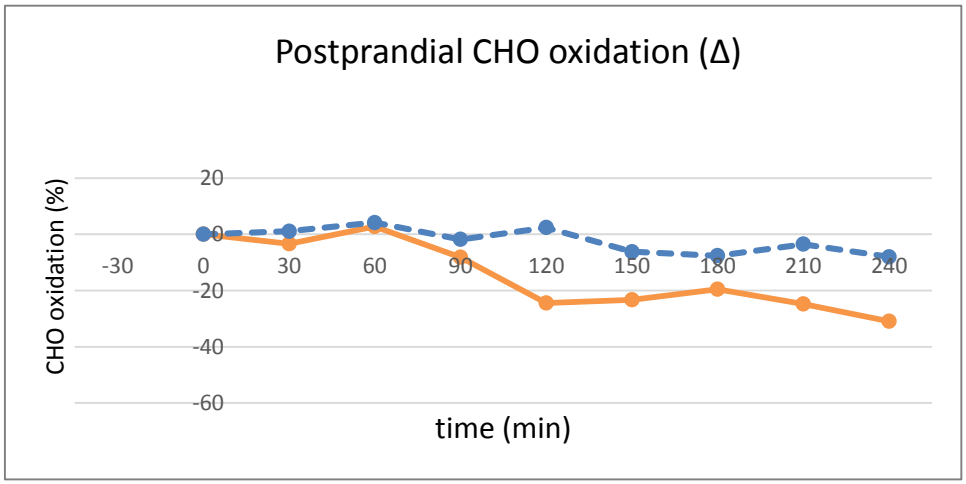

Postprandial fat oxidation(Δ)
fat oxidation (%)
30
20
10
0
-10
-20
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial CHO oxidation (Δ)
CHO oxidation (%)
30
20
10
0
-10
-20
-30
-30
0
30
60
90
120
150
180
210
240
time (min)

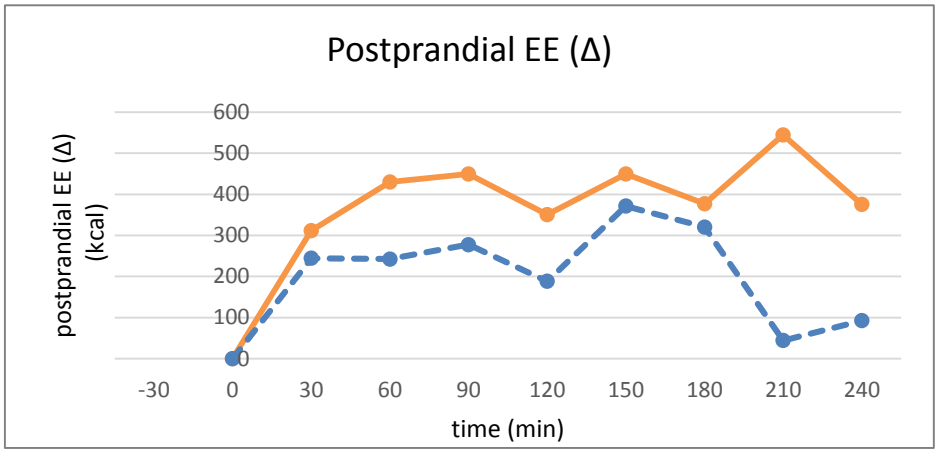
Subject 4:

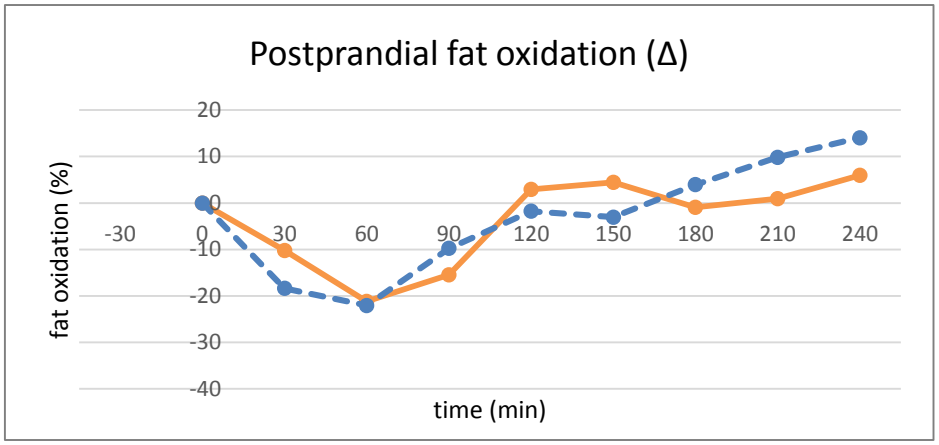

Postprandial EE(Δ)
postprandial EE(Δ) (kcal)
1000
800
600
400
200
0
-30
0
30
60
90
120
150
180
210
240
time (min)

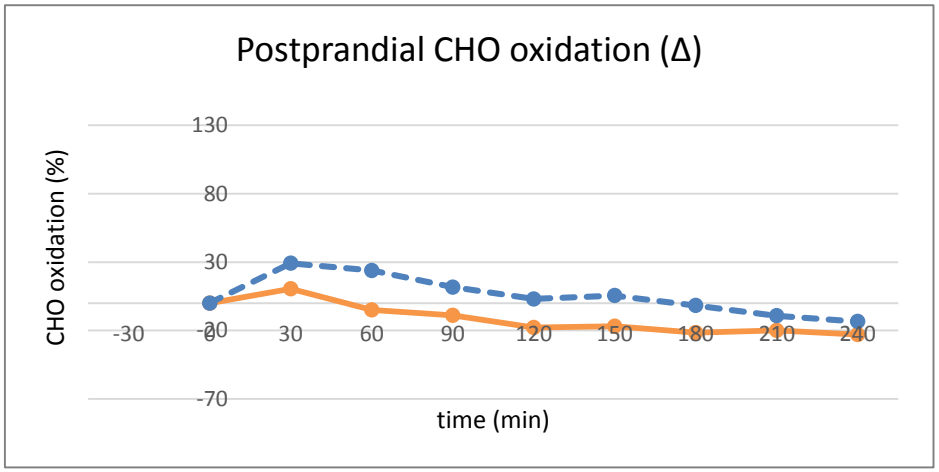

Postprandial fat oxidation (Δ)
fat oxidation (%)
50
40
30
20
10
0
-10
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial CHO oxidation (Δ)
CHO oxidation (%)
20
0
-20
-40
-60
-30
0
30
60
90
120
150
180
210
240
time (min)

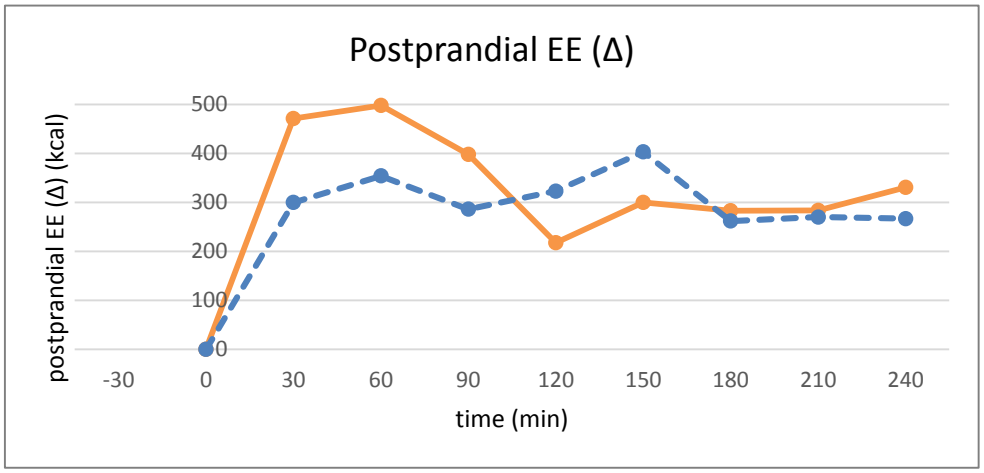
Subject 5:

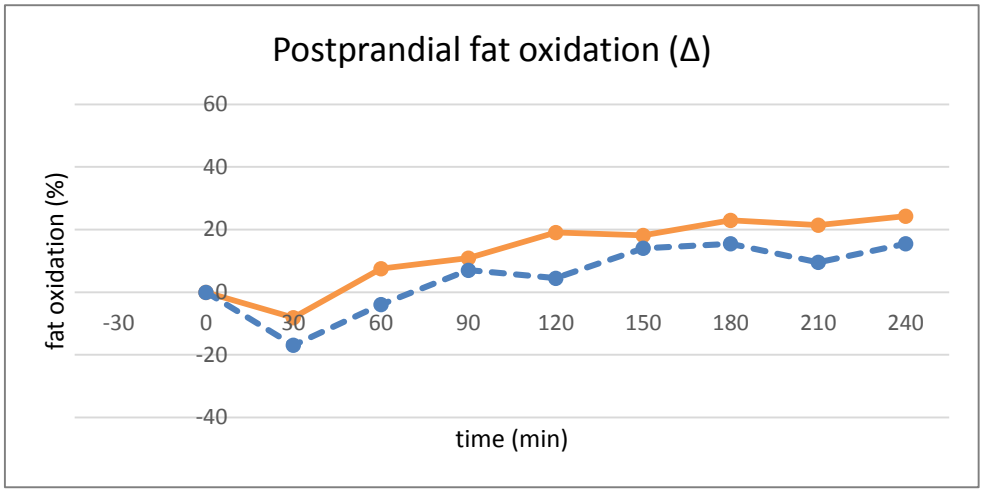

Postprandial EE (Δ)
postprandial EE (Δ) (kcal)
600
500
400
300
200
100
0
-30
0
30
60
90
120
150
180
210
240
time (min)

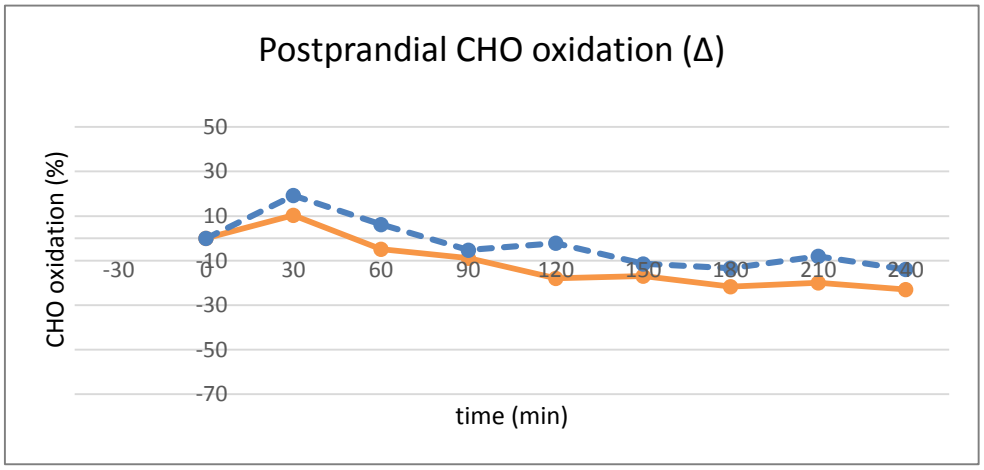

Postprandial fat oxidation (Δ)
fat oxidation (%)
20
10
0
-10
-20
-30
-40
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial CHO oxidation (Δ)
CHO oxidation (%)
130
80
30
-20
-70
-30
0
30
60
90
120
150
180
210
240
time (min)

Subject 6:


Postprandial EE (Δ)
postprandial EE (Δ) (kcal)
500
400
300
200
100
0
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial fat oxidation (Δ)
fat oxidation (%)
60
40
20
0
-20
-40
-30
0
30
60
90
120
150
180
210
240
time (min)


Postprandial CHO oxidation (Δ)
CHO oxidation (%)
50
30
10
-10
-30
-50
-70
-30
0
30
60
90
120
150
180
210
240
time (min)

BULK

S NO	RM CODE	CLAIM	UNIT	UNIT WT	UNIT	INGREDIENTS	OV	KGS	ACTUAL WT	R M LOT #	WEIGHED	CHECKED
		/1TAB									BY/DT	BY/DT
1	EXNCN0000030			300	MG	MICRO CRYSTALLINE CELLULOSE		0.00				
2	MINCA00000V4			200	MG	CALCIUM CARBONATE		0.00				
3	EXNCN0000093			160	MG	STEARIC ACID		0.00				
4	EXNCN000002D			15	MG	MAGNESIUM STEARATE		0.00				
5	EXART0000043			20	MG	CROSCARMELLOSE SODIUM		0.00				
6	TMNCN00003N3			5	MG	SILICON DI OXIDE		0.00				
						COATING INGREDIENTS						
	APNCN00000XD			QS	MG	PHARMACEUTICAL GLAZE						
				700	MG	TOTAL THEORITICAL WT		0.00				

APPROVED BY/DT:

BULK

S NO	RM CODE	CLAIM	UNIT	UNIT WT	UNIT	INGREDIENTS	OV	KGS	ACTUAL WT	R M LOT #	WEIGHED	CHECKED
		/1TAB									BY/DT	BY/DT
		125	MG			PHOSPHORUS						
1	EXPOW000008E			189.4	MG	AS POTASSIUM PHOSPHATE MONOBASIC (KH2PO4) 22.76%		0.00				
2	CHPOW00000L0			349.5	MG	AS POTASSIUM PHOSPHATE DIBASIC (K2HPO4) 17.78%		0.00				
3	CHNCN0000649			108	MG	AS DICALCIUM PHOSPHATE 19%		0.00				
4	EXNCN0000030			50	MG	MICRO CRYSTALLINE CELLULOSE		0.00				
5	EXNCN0000093			50	MG	STEARIC ACID		0.00				
6	EXNCN000002D			10	MG	MAGNESIUM STEARATE		0.00				
7	EXART0000043			10	MG	CROSCARMELLOSE SODIUM		0.00				
8	TMNCN00003N3			5	MG	SILICON DI OXIDE		0.00				
						COATING INGREDIENTS						
	APNCN00000XD			QS		PHARMACEUTICAL GLAZE						
				772	MG	TOTAL THEORITICAL WT		0.00				

CHAPTER VII

BIBLIOGRAPHY

Acheson, K.J. (1993). Influence of autonomic nervous system on nutrient- induced thermogenesis in humans. *Nutrition*, 9(4):373-80.

Alonso, A., Nettleton, J.A., Ix, J.H., De Boer, I.H., Folsom, A.R., Bidulescu, A., Kestenbaum, B.R., Chambless, L.E., Jacobs, D.R. (2010). Dietary Phosphorus, Blood Pressure, and Incidence of Hypertension in the Atherosclerosis Risk in Communities Study and the Multi-Ethnic Study of Atherosclerosis. *Hypertension*, 55: 776-784.)

Arita, Y., et al (1999). Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochemical and Biophysical Research Communications*, 257:79-83.

Amanzadeh, J., & Reilly, R. F. (2006). Hypophosphatemia: an evidence-based approach to its clinical consequences and management. *Nature Clinical Practice Nephrology*, 2(3), 136-148.

Ayoub. J., Samra. M., Hlais. S., Bassil, M. and Obeid. O. (2015). Effect of phosphorus supplementation on weight gain and waist circumference of overweight/obese adults: a randomized clinical trial. *Nutrition & Diabetes*, e189; doi:10.1038/nutd.2015.38.

Basu,R., Chandramouli, V., Dicke, B., Landau,B., and Rizza,R. (2005). Obesity and Type 2 Diabetes Impair Insulin-Induced Suppression of Glycogenolysis as Well as Gluconeogenesis. *Diabetes*, VOL. 54.

Becker, TC.., Noel, R.J., Johnson, J.H., Lynch, R.M., Hirose, H., Tokuyama, Y. (1996). Differential effects of overexpressed glucokianse and hexokianse I in isolated islets. *Journal of Biology Chemistry*, 271:390–4.

Bender,D.A. (2012). The metabolism of "surplus" amino acids. British Journal of Nutrition (2012), 108, S113–S121

Beydoun, M.A., Gary, T.L., Caballero, B.H., Lawrence, R.S., Cheskin, L.J., and Wang, Y. (2008). Ethnic differences in dairy and related nutrient consumption among US adults and their association with obesity, central obesity, and the metabolic syndrome. *American Journal of Clinical Nutrition*, 87:1914 –25.

Bizeau, M.E., Pagliassotti, M.J. (2005). Hepatic adaptations to sucrose and fructose. *Metabolism Clinical and Experimental*, 54: 1189-1201.

Boden, G., Chen, X., Stein, T.P. (2001). Gluconeogenesis in moderately and severely hyperglycemic patients with type 2 diabetes mellitus. *American Journal of Physiology*, 280:E23–E30.

Bogardus,C., Lillioja,S., Mott,D., Reaven,G.R., Kashiwagi,A., and Foley,J.E. (1984). Relationship between obesity and maximal insulin-stimulated glucose uptake in vivo and in vitro in Pima Indians. *Journal Clinical Investigation*, 73(3): 800–805.

Bray, G.A., Nielsen, S.J., Popkin, B.M. (2004). Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *American Journal of Clinical Nutrition*, 79: 537–543.

Campillo, J.E., Aguayo, J., Pages, I., Castillo, M., Osorio, C. (1982). Inorganic phosphate-insulin relationships in normal subjects and in patients with moderate glucose intolerance. *Diabetes and Metabolism*, 8 (4):289-93.

Chapuy, M.C., Arlot, M.E., Duboeuf, F., Brun, J., Crouzet, B., Arnaud, S., Delmas, P.D., Meunier, P.J. (1992). Vitamin D3 and calcium to prevent hip fractures in the elderly women. *New England Journal of Medicine*, 327(23):1637-42.

Chevalier,S., Burgess,S.C., Malloy,C.R., Gougeon,R., Marliss,E.B., and Morais,J.A. (2006).The Greater Contribution of Gluconeogenesis to Glucose Production in Obesity Is Related to Increased Whole-Body Protein Catabolism. *DIABETES*, VOL. 55.

Chavin,K.D., Yang, S., Lin, H.Z. (1999). Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. *Journal of Biology Chemistry*, 274:5692-700.

Clarkson, P.M., Haymes, E.M. (1995). Exercise and mineral status of athletes: calcium, magnesium, phosphorus, and iron. *Medicine and Science in Sports and Exercise*, 27(6):831-43.

Cortez-Pinto, H., Chatham, J., Chacko, V.P., Arnold, C., Rashid, A., Diehl, A.M. (1999). Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. *JAMA*, 282: 1659–1664.

D'Alessio, D.A., Kavle, E.C., Mozzoli, M.A. (1988). Thermic effect of food in lean and obese men. *Journal of Clinical Investigation*, 81:1781-9.

De Jonge, L., Bray, G.A. (1997).The Thermic Effect of Food and Obesity: A Critical Review. *Obesity Research*, Vol. 5 No. 6.

Donahoo, W.T., Levine, J.A. &Melanson, E.L. (2004). Variability in energy expenditure and its components. *Current Opinion in Clinical Nutrition Metabolism Care*, 7, 599–605.

Drewnowski, A. (2009). Obesity, diets, and social inequalities. *Nutrition Reviews*, 67(Suppl. 1):S36–S39.

Edith, G., Michael, L.P., Isabel, C.G., and Terence, P. (2004). Herbert. Glucose-stimulated Protein Synthesis in Pancreatic β-Cells Parallels an Increase in the Availability of the Translational Ternary Complex (eIF2-GTP·Met-tRNAi) and the Dephosphorylation of eIF2α. *The Journal of Biological Chemistry*, Vol. 279, No. 52.

Eeckhout.W, De Paepe, M. (1994). Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Animal Feed Science and Technology*, 47: 19-29.

Elliott, P., Kesteloot, H., Appel, L.J. (2008). Dietary Phosphorus and Blood Pressure International Study of Macro- and Micro-Nutrients and Blood Pressure. *Hypertension*, 51:669-675.

Elliott, R., Morgan, L., Tredger, J., Deacon, S., Wright, J., Marks, V. (1993). Glucagon-like peptide-1 amide and glucose-dependent insulintropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *Journal of Endocrinology*, 138:159–66.

Ervin, R.B., Wang, C.Y., Wright, J.D., Kennedy-Stephenson, J. (2004). Dietary intake of selected minerals for the United States population: 1999-2000. *Advance Data*, 27(341):1-5.

FAO (2010). Food Balance Sheet. www.fao.org.

Felig, P. (1984). Insulin is the mediator of feeding-related thermogenesis: insulin resistance and/or deficiency results in a thermogenic defect which contributes to the pathogenesis of obesity. *Clinical Physiology*, 4(4):267-73.

Flatt, J.P. (1978). The biochemistry of energy expenditure. *Recent advances in obesity research. London*: Newman, p. 211-28.

Food and Nutrition Board (1997), Institute of Medicine: Dietary Reference Intakes for Calcium, Magnesium, Phosphorus, Vitamin D, and Fluoride.Washington, DC. National Academy Press.

Freiman, I., Pettifor, J. M., &Moodley, G. M. (1982). Serum phosphorus in protein energy malnutrition. *Journal of pediatric gastroenterology and nutrition*, 1(4), 547-550.

Friedman, M.I. (2007). Obesity and the hepatic control of feeding behavior. *Drug News Perspect*, 20: 9: 573-578.

Golay, A., Schutz, Y., Meyer, H.U., Thiébaud, D., Curchod, B., Maeder, E., Felber, J.P., Jéquier, E. (1982). Glucose-induced thermogenesis in nondiabetic and diabetic obese subjects. *Diabetes,* 31(11):1023-8.

Granata, G.P. & Brandon, L.J. (2002). The thermic effect of food and obesity: discrepant results and methodological variations. *Nutrition Review*, 60, 223–233.

Haap, M.., E. Heller, C.,Thamer, O.,Tschritter, N.S., , and Fritsche, A.(2006). Association of serum phosphate levels with glucose tolerance, insulin sensitivity and insulin secretion in non-diabetic subjects. *European Journal of Clinical Nutrition*, 60, 734–739.

Håglin, L. (2001). Hypophosphataemia: cause of the disturbed metabolism in the metabolic syndrome. *Medical Hypotheses*, 56(6): 657–663.

Haglin, L., Lindblad, A. and Bygren, L.O. (2001). Hypophosphataemia in the metabolic syndrome. Gender differences in body weight and blood glucose. European *Journal of Clinical Nutrition*, 55: 493-498.

Halton, T.H. and Hu, F.B. (2004). The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *Journal of American College of Nutrition*, 23: 373–385.

Hazim,J., Hlais,S., Ghattas,H., Shatila,D., Bassil,M. and Obeid,O. (2014). Phosphorus supplement alters postprandial lipemia of healthy male subjects: a pilot cross-over trial. *Lipids in Health and Disease*, 13:109.

Heaney, R.P. and Nordin, BE.C. (2002). Calcium Effects on Phosphorus Absorption: Implications for the Prevention and Co-Therapy of Osteoporosis. *Journal of the American College of Nutrition*, 21(3): 239–244.

Hong, J., Graczyk-Milbrandt, G., Friedman, M.I. (2000). Metabolic inhibitors synergistically decrease hepatic energy status and increase food intake. *American Journal of Physiology Regulatory Integrative Comp Physiol*, 278: R1579–R1582.

Huang, S., Czech, M.P. (2007). The GLUT4 glucose transporter. *Cell Metabolism*, 5:237–52.

Jaedig, S., Henningsen, N.C. (1991). Increased metabolic rate in obese women after ingestion of potassium, magnesium- and phosphate-enriched orange juice or injection of ephedrine. *International Journal of Obesity*, 15(6):429-36.

Jaedig, S., Lindgärde, F., Arborelius, M. (1994). Increased postprandial energy expenditure in obese women after peroral K- and Mg-phosphate. *Miner Electrolyte Metabolism*, 20(3):147-52.

Jequier, E., Acheson, K., Schutz, Y. (1987). Assessment of energy expenditure and fuel utilization in man. *Annual Review of Nutrition*, 7:187-208.

Kalantar-Zadeh, K., Gutekunst, L., Mehrotra, R., Kovesdy, C. P., Bross, R., Shinaberger, C. S.&Kopple, J. D. (2010). Understanding sources of dietary phosphorus in the treatment of patients with chronic kidney disease. *Clinical Journal of the American Society of Nephrology*, 5(3), 519-530.

Karst,H., Steiniger, J., Noack, R. (1984). Diet induced thermogenesis in man: thermic effects of single proteins, carbohydrates and fats depending on their energy amount. *Annals of Nutritionand Metabolism*, 28, 245–252.

Kaciuba-Uściłko, H., Nazar, K., Chwalbińska-Moneta,J., Ziemba, A., Kruk, B., Szczepanik, J., Titow-Stupnicka, E., Bicz, B. (1993). Effect of phosphate supplementation on metabolic and neuroendocrine responses to exercise and oral glucose load in obese women during weight reduction. *Journal of Physiology and Pharmacology,* 44(4):425-40.

Kalaitzidis, R., Tsimihodimos, V., Bairaktari, E., Siamopoulos, K.C., and Elisaf, M. (2005). Disturbances of Phosphate Metabolism: Another Feature of Metabolic Syndrome. *American Journal of Kidney Disease*, 45:851-858.

Kanazawa, T., Atsumi, M., Mineo, H., Fukushima, M., Nishimura, N., Noda, T., &Chiji, H. (2007). Ingestion of gelatinized potato starch containing a high level of phosphorus decreases serum and liver lipids in rats. *Journal of oleo science*, 57(6), 335-343.

Karczmar, G.S., Kurtz, T., Tavares, N.J., Weiner, M.W. (1989). Regulation of hepatic inorganic phosphate and ATP in response to fructose loading: an in vivo 31P-NMR study. *Biochimica et Biophysica Acta*, 11; 1012(2):121-7.

Kelley,D.E., Goodpaster,B., Wing,R.R., Simoneau,J.A. (1999). Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *American Journal of Physiology* - Endocrinology and Metabolism, Vol. 277.

Khattab, M., Abi-Rashed, C., Ghattas,H., Hlais, S. and Obeid, O. (2015). Phosphorus ingestion improves oral glucose tolerance of healthy male subjects: a crossover experiment. *Nutrition Journal*, 14:112 DOI 10.1186/s12937-015-0101-5.

Kimutai, D., Maleche-Obimbo, E., Kamenwa, R., &Murila, F. (2009). Hypo-phosphataemia in children under five years with Kwashiorkor and marasmic Kwashiorkor. *East African medical journal*, 86(7).

Klaas, R. W. (2004). Diet induced thermogenesis. *Nutrition & Metabolism*, 1:5.

Kumar, V., Sinha, A. K., Makkar, H. P., & Becker, K. (2010). Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry*, 120(4), 945-959.

Langhans, W., Scharrer, E. (1992). Metabolic control of eating. *World Review*. Nut. Diet, 70: 1-67.

Latner, J.D. and Schwartz, M. (1999). The effects of a high-carbohydrate, high-protein or balanced lunch upon later food intake and hunger ratings. *Appetite,* 33: 119-128.

Ruddick-Collins, L.C., King, N.A., Byrne,N.M., and Rachel, E. (2013). Wood. Methodological considerations for meal-induced thermogenesis: measurement duration and reproducibility. *British Journal of Nutrition*, 110, 1978–1986.

Lind, L., Lithell, H., Hvarfner, A., Pollare, T., Ljunghall, S. (1993). On the relationships between mineral metabolism, obesity and fat distribution. *European Journal of Clinical Investigatio*n, 23(5):307-10.

Lindgärde, F. and Trell, E. (1977). Serum Inorganic Phosphate in Middle-Aged Men. I. Inverse Relation to Body Weight. *Acta Medica Scandinavic*, 202(1-6): 307 – 311.

Lithell, H., F. Lindgarde, K. Hellsing, G. Lundqvist, E. Nygaard, B. Vessby, and B. Saltin (1981). Body weight, skeletal muscle morphology, and enzyme activities in relation to fasting serum insulin concentration and glucose tolerance in 48-year-old men. *Diabetes*, 30: 19–25.

Matthews, D.R., Hosker, J.P., Rudenski, A.S. (1985). Homeostasis model assessment: insulin resistance and b-cell function from fasting plasma glucose and insulin concentrations in man. *Journal of Diabetologia*, 28: 412-9.

Mattar, L. E., Mattar, M. A., Batal, M., Mouneimne, Y., & Obeid, O. A. (2010). Stimulation of postprandial in vivo glycogenesis and lipogenesis of rats fed high fructose diet with varied phosphate content. *Nutrition research*, 30(2), 151-155.

Melanson, K.J., Saltzman, E., Vinken, A.G. (1998). The effects of age on postprandial thermogenesis at four graded energetic challenges: findings in young and older women. *Journal of Gerontology A BiolSci Med Sci*, 53A, B409–B414.

Morris, J. R.., Nigon, K., Reed, E. (1978). Evidence that the severity of depletion of inorganic phosphate determines the severity of the disturbance of adenine nucleotide metabolism in the liver and renal cortex of the fructose-loaded rat. *Journal of Clinical Investigation*, 61: 209–220.

Nair, S., Chacko, V.P., Arnold, C., Diehl, M. (2003). Hepatic ATP reserve and efficiency of replenishing: comparison between obese and nonobese normal individuals. *American Journal of Gastroenterol*, 98:466–470.

Nazar, K., Kaciuba-Uściłko, H., Szczepanik, J., Zemba, A.W., Kruk, B., Chwalbińska-Moneta, J., Titow-Stupnicka, E., Bicz, B., Krotkiewski, M. (1996). Phosphate supplementation prevents a decrease of triiodothyronine and increases resting metabolic rate during low energy diet. *Journal of Physiologyand Pharmacology*, 47(2):373-83.

Nowicki, M., Fliser, D., Fode, P., Ritz, E. (1996). Changes in plasma phosphate levels influence insulin sensitivity under euglycemic conditions. *Journal of Clinical Endocrinology Metabolism*, 81(1):156-9.

Obeid, O., Dimachkie, S. and Hlais, S. (2010). Increased phosphorus content of preload suppresses ad libitum energy intake at subsequent meal. *International Journal of Obesity*, 34, 1446–1448.

Obeid,O.(2013). Low phosphorus status might contribute to the onset of obesity. *Obesity Reviews*, 14, 659–664.

Obeid, O. A., Hachem, D. H., &Ayoub, J. (2014). Refeeding and metabolic syndromes: two sides of the same coin. *Nutrition & diabetes*, 4(6), e120.

Oberhaensli, R.D., Galloway, G.J., Taylor, D.J., Bore, P.J., Radda, G.K. (1986). Assessment of human liver metabolism by phosphorus-31-magnetic resonance spectroscopy. *British Journal Radiology*, 59: 695-699.

Paula, F.J., Plens, A.E. and Foss, M.C. (1998). Effects of Hypophosphatemia on Glucose Tolerance and Insulin Secretion. *Hormone and Metabolic Research*, 30:281-284.

Prellera, A., Kessib, E, Uretaa, T. (2007).Glycogen synthesis by the direct or indirect pathways depends on glucose availability: In vivo studies in frog oocytes. *FEBS Letters*, 581:663–666.

Ravindran, V., Ravindran, G., &Sivalogan, S. (1994). Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. *Food Chemistry*, 50(2).

Ravussin, E., Swinburn, B.A. (1996). Energy expenditure and obesity. *Diabetes Reviews*, 4:403-22.

Rawson, N.E., Freidman, M.I. (1994). Phosphate loading prevents the decrease in ATP and increase in food intake produced by 2, 5-anhydro-D-mannitol. American *Journal of Physiology Regulatory Integrative and ComparativePhysiology*, 266: R1792-6.

Reed, G. & Hill, J. (1996). Measuring the thermic effect of food. *American Journal of Clinical Nutrition*, 63, 164–169.

Reginster, J.Y., Denis, D., Bartsch, V., Deroisy, R., Zegels, B., Franchimont, P. (1993). Acute biochemical variations induced by four different calcium salts in healthy male volunteers. *Osteoporosis International*, 3(5):271-5.

Riquelme, P.T., Wernette-Hammond, M.E., Kneer, N.M., Lardy, H.A. (1984). Mechanism of action of 2, 5-anhydro-D-mannitol in hepatocytes. *The Journal of Biology Chemistry*, 259: 5115-5123.

Schutz, Y., Bessard, T., Jequier, E. (1984). Diet-induced thermogenesis measured over a whole day in obese and nonobese women. *American Journal of Clinical Nutrition*, 40:545-52.

Schutz, Y., Golay, A., Felber, J. (1984). Decreased glucoseinduced thermogenesis after weight loss in obese subjects: a predisposing factor for relapse of obesity? *American Journal of Clinical Nutrition*, 39, 380–387.

Scott, C.B. & Devore, R. (2004). Diet-induced thermogenesis: variations among three isocaloric meal-replacement shakes. *Nutrition*, 21, 874–877.

Scott, C., Fernandes, J. & Lehman, M. (2007). Onset of the thermic effect of feeding (TEF): a randomized cross-over trial. *Journal of the International Society of Sports Nutrition*, 4, 24.

Schutz,Y., Bessard,T., and Eric, J. (1984). Diet-induced thermogenesis measured over a whole day in obese and nonobese women. *The American Journal of Clinical Nutrition*, pp 542-552.

Segal, K.R., Gutin, B., Nyman, A.M., and Pi-Sunyer, F.X. (1985). Thermic Effect of Food at Rest, during Exercise, and after Exercise in Lean and Obese Men of Similar Body Weight. *Journal of Clinical. Investigation*, 76: 1107-1112.

Segal, K.R., Edano, A., Blando, L. (1990). Comparison of thermic effects of constant and relative caloric loads in lean and obese men. *American Journalof Clinical Nutrition*, 51:14-21.

Segal, K.R., Edano, A., Tomas, M.B. (1990). Thermic effect of a meal over 3 and 6 hours in lean and obese men. *Metabolism*, 39:985-92.

Solomon, S.M., Kirby, D.F. (1990). The refeeding syndrome: a review. *Journal Parenteral Enteral Nutrition*, 14:90–7.

Tanaka, S., Yamamoto, H., Nakahashi, O., Kagawa, T., Ishiguro, M., Masuda, M. & Takeda, E. (2013). Dietary phosphate restriction induces hepatic lipid accumulation through dysregulation of cholesterol metabolism in mice. *Nutrition Research*, 33(7), 586-593.

Tomas,E., et al (2002). Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proceedings of the National Academy of Science.* U. S. A, 99:16309-16313.

Soltani, N., Kumar, M., Glinka, Y., Prud'homme, G., Wang, Q. (2007). In vivo expression of GLP-1/IgG-Fc fusion protein enhances beta-cell mass and protects against streptozotocin-induced diabetes. *Gene Therapy*, 14:981–8.

Teegarden, D. (2005). The influence of dairy product consumption on body composition. *Journal of Nutrition*, 135: 2749–2752.

Tentolouris, N., Pavlatos, S., Kokkinos, A., Perrea, D., Pagoni, S., Katsilambros, N. (2008).Diet-induced thermogenesis and substrate oxidation are not different between lean and obese women after two different isocaloric meals, one rich in protein and one rich in fat. *Metabolism*, 57(3):313-20.

Thorne, A., Hallberg, D., Wahren,J.(1989). Meal-induced thermogenesis in obese patients before and after weight reduction. *Clinical Physiology*, 9:481-98.

Thorne, A., Naslund, I., Wahren, J. (1990). Meal-induced thermogenesis in previously obese patients. *Clinical Physiology*, 10:99-109.

Van Baak, M.A. (2008). Meal-induced activation of the sympathetic nervous system and its cardiovascular and thermogenic effects in man. *Physiology & Behavior*, 94; 178 186.

Veldhorst, M., Smeets, A., Soenen, S. (2008). Protein-induced satiety: effects and mechanisms of different proteins. *Physiology Behavior Journal*, 94:300–7.

Wagner, G., Kindrick, S., Hertzler, S., DiSilvestro, R.A. (2007). Effects of Various Forms of Calcium on Body Weight and Bone Turnover Markers in Women Participating in a Weight Loss Program. *Journal of the American College of Nutrition*, 26(5) 456–461.

Wlodek, D., Gonzales, M. (2003). Decreased energy levels can cause and sustain obesity. *Journal Theoretical Biology*, 225: 33–44.

Xie, W., Tran, L., Finegood, D., Werve, G. (2000). Dietary Pi deprivation in rats affects liver CAMP, glycogen, key steps of gluconeogenesis and glucose production. *Biochemical Journal*, 352:227-32

Yanovski, J.A., Parikh, S.J., Yanoff, L.B., Denkinger, B.I., Calis, K.A., Reynolds, J.C., Sebring, N.G., McHugh, T. (2009). Effects of calcium supplementation on body weight and adiposity in overweight and obese adults: a randomized trial. *Annals of Internal Medicine*, 150(12):821-9, W145-6.

Ye, E.Q., Chacko, S.A., Chou, E.L., Kugizaki, M., Liu, S. (2012). Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. *The Journal of Nutrition*, 142:1304–1.