Dietary Carnitine Supplementation as a potential modulator of insulin sensitivity.

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Special praise must go to Dr Stuart Galloway and the support he has given me throughout my academic and working career. He has fully understood the position I was in with regards to working situations and combining study whilst trying to gain secure and enjoyable employment. Whether it be last to drop on a Christmas night out, dropping into my office to pester me where I was at, or always being there whatever and when ever my question, Stuart has been inspiration and I have no doubt with any other supervisor in charge, I would have struggled immensely.

Abstract

L- Carnitine is a vitamin like compound synthesised endogenously from the essential amino acids methionine and lysine and plays an essential role in the metabolism of fatty acids. Beta-oxidation, the major process by which long chain fatty acids are oxidised in mitochondria, depends on carnitine. In theory, increased carnitine availability could increase the ability to transport long chain fatty acids into the mitochondria and thus increase their oxidation.

Recent work has however shown that it is unlikely that carnitine supplementation will increase fat oxidation and may instead promote carbohydrate metabolism. Researched elements have included the role carnitine may play in muscle glycogen storage, carnitine as a potential preventative mechanism for clinical populations at risk from metabolic disorders and "insulin like" effects observed in carnitine trials.

This study examined the effects of 14 days L-Carnitine L-Tartrate (LC) supplementation on blood glucose and insulin response to an oral glucose tolerance (OGTT) test and submaximal exercise. Sixteen male participants were recruited and assigned to two groups (lean (n=8) and overweight/obese (n=8)). After completing a first visit for a submaximal predictive exercise test, participants attended on three further occasions, in the morning after an overnight fast, for fasting blood samples and 2hr OGTT tests followed by an exercise bout (20 min at 40% of predicted VO_{2peak}).

The first test was a familiarisation trial and the final two tests were conducted two weeks apart following 14 days of ingestion of placebo (PL, 3g glucose/day) or LC

(3g/day) ingested as 2 capsules 3x/day with meals. Blood was drawn at rest and at 15, 30, 45, 60, 90, and 120 minutes of OGTT and at 10 and 20 min of exercise for analysis of glucose and insulin. Data obtained were then used for determination of usual insulin sensitivity indices (HOMA-IR, Area Under Curve (AUC) glucose, AUC insulin, 1st phase and 2nd phase beta cell function, estimated insulin sensitivity index, and estimated metabolic clearance rate).

There was a significant difference between groups for body mass, % fat and BMI with no significant difference in age and height. Mean fasting glucose (5.4(0.2) Placebo (PL); 5.5(0.2) L-Carnitine (LC); mmol/L), insulin (13.7(3.5) PL; 13.7(4.2) LC; muU/ml) and HOMA-IR (2.6(0.6) PL; 2.7(0.4) LC) were not different between trials, and no significant differences were observed between groups prior to supplementation. Analysis of the blood glucose response during the OGTT revealed no group effect but there was a time effect ($P<0.01$) and a trial x time interaction ($p<0.01$).

Highest plasma glucose concentration was observed at 30 minutes in both trials but was significantly lower ($p<0.05$) at 30 min in the trial after LC (8.55(0.44) PL; 7.34(0.36) LC; mmol/L). AUC glucose was not different between trials but AUC insulin tended to be higher following LC (5029(889) PL; 5845(1032) LC, $p=0.09$). Estimated 1st phase (281(64) PL; 419(38) LC; muU/ml) and 2nd phase (106(12) PL; 132(8) LC; muU/ml) beta-cell function were both significantly ($p<0.05$) greater following LC supplementation.

No treatment differences were observed in glucose and insulin response to exercise but a trial by group interaction revealed that plasma glucose was lower after 10 minutes in the lean group ($p<0.05$) and plasma insulin higher at 20 minutes of exercise in the overweight/obese group ($p<0.05$). No effects of LC supplementation were observed on heart rate, VO_2 or Respiratory Exchange Ration (RER) during the exercise task.

It is concluded that LC appears to induce changes in blood glucose regulation during an OGTT, that this may be driven by changes in beta-cell function or in incretin levels, with Glucagon Like Peptide-1 (GLP-1) of particular interest, and that this response to LC supplementation is not different between lean and overweight/obese but may be related to aerobic capacity. Muscle glycogen levels focusing on an elite athlete population are also of interest.

Therefore, further investigation of carnitine action on beta-cell function, incretin and muscle glycogen level is warranted.

Declaration

I hereby declare that this thesis has been composed by myself, and the work of which it is a record has been done by myself except where assistance has been acknowledged, that it has not been submitted in any previous application for a higher degree and that all sources of information have been specifically acknowledged by means of reference.

Chapter 1 – Introduction

L- Carnitine (molecular weight 472.49) is a vitamin like compound synthesised

endogenously from the essential amino acids methionine and lysine and plays an

essential role in the metabolism of fatty acids. Beta-oxidation, the major process by

which long chain fatty acids are oxidised in mitochondria, depends on carnitine (Reda

et al., 2003; Karlic and Lohninger 2004). More than 95% of the body's total carnitine

store exists within skeletal muscle tissue (Brass, 1995). The best described function

of L-carnitine is in its role as a cofactor of Carnitine, acyltransferases transporting

long-chain fatty acids across the mitochondrial membrane (Karlic and Lohninger,

2004). In the absence of L-Carnitine, the inner mitochondrial membrane would be

impermeable to long-chain fatty acids and fatty acyl-CoA esters. Thus there are

reports suggesting that Carnitine supplementation can thus increase the contribution

of fatty acids to oxidative metabolism (Maughan et al., 2004) and indeed this has been

shown in normal (Muller *et al.*, 2002) and obese subjects (Wutzke and Lorenz, 2004)

at rest.

Due to the findings that Carnitine supplementation may not in fact promote fatty acid

oxidation and may promote carbohydrate metabolism (Hultman *et al.*, 1991;

Broderick *et al.* 1992; Millington *et al.*, 1993; Brass *et al.*, 1994; Hawley *et al.*, 1998;

Karlic and Lohninger, 2004; Maughan *et al.*, 2004, Abramowicz and Galloway,

2005), Carnitine supplementation may be beneficial to those at risk of developing

metabolic disorders such as impaired glucose tolerance and type 2 diabetes.

Carnitine has been observed to increase non-oxidative glucose disposal during hyperinsulinaemic euglycaemic clamps in controls and type II diabetics (Mingrone *et al*., 1999; Giancaterini *et al*., 2000) and increase glucose disposal during intravenous glucose tolerance testing(De Gaetano *et al*., 1999). Whole body glucose metabolism in subjects with type II diabetes is significantly increased after carnitine infusion (Capaldo *et al*., 1991) and similar results were shown to be independent of body weight or the degree of insulin resistance (Ferrannini *et al*., 1988). In other clinical situations such as renal failure (Gunal, 1999) peripheral vascular disease (Brevetti *et al*., 1988) and chronic fatigue syndrome (Plyioplys and Plyioplys, 1997), carnitine has been shown to have a positive effect on insulin sensitivity (Gunal, 1999) and exercise capacity (Brevetti *et al*., 1988; Plyioplys and Plyioplys, 1997).

Lean subjects have lower insulin levels whether they are normal, Impaired Glucose Tolerant (IGT) IGT or diabetic (Matsumoto *et al*., 1997) and insulin resistance is a major characteristic of obesity and the development of type II diabetes (Stephens *et al*., 2005). Obesity is a risk factor associated with IGT, dyslipidemia, insulin resistanc, hypertension, hyperurecemia and Impaired Fasting Glucose (IFG) (Vega, 2001) and nutritional interventions including L-Carnitine may play a role in the reversal of insulin resistance (Kelly, 2000). This provides the basis of the study supplementing to adult males with a BMI which would class them as being at risk from developing such metabolic conditions.

In line with the observations above, a direct insulin like effect of carnitine has also been suggested. A 15% increase in skeletal muscle carnitine content resulted in an overnight increase in muscle glycogen content (Stephens *et al*., 2006) after

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hyperinsulinemic clamp conditions and Rodgers *et al*. (2001) observed insulin like actions of carnitine on rat cardiac muscle. This could suggest either a direct effect of intracellular carnitine on glycogen synthase activity and therefore rates of glycogen synthesis, or effects of extracellular carnitine concentration on insulin sensitivity of tissues.

Furthermore, if carnitine has an impact upon insulin sensitivity or insulin release in response to an oral glucose load it could result in suppressed mobilization of non-esterified fatty acids (NEFA; Stumvoll *et al*., 2000) and thus impact upon substrate use during low intensity exercise during which plasma derived NEFA's are a main contributor to skeletal muscle oxidation. This provides the basis for measuring substrate use at low intensity exercise in subjects. Therefore the aim of the present study was to investigate insulin like actions of carnitine on healthy active males and sedentary obese subjects.

Chapter 2 – Literature Review

2.1 Structure and sources of Carnitine

Carnitine is a small polar compound that is found is all mammals (figure2.1.1). L-Carnitine is a vitamin like compound synthesised endogenously from the essential amino acids methionine and lysine and plays an essential role in the metabolism of fatty acids. More than 95% of the body's total carnitine store exists within skeletal muscle tissue (Brass, 1995; Maughan *et al*., 2004). The remaining carnitine is divided between extracellular fluids and the liver and kidney (Newsholme and Start, 1973). Carnitine homeostasis is maintained through a combination of dietary intake as well as endogenous synthesis combined with renal excretion (Borum, 1983).

Carnitine intake in the diet is highly variable and depends largely upon red meat intake (Galloway and Broad, 2005). Dietary intake has been estimated to be between 20 and 300mg per day for healthy non-vegetarian adults (Rebouche and Chenard, 1991). And will be in the region of 1-3 mg per day for strict vegetarians (Rebouche *et al*., 1993). It might be thought that individuals who follow a vegan lifestyle might be at increased risk of deficiency, but carnitine can also be synthesized from lysine and methionine in the liver and kidney (Maughan *et al*., 2004) and therefore carnitine is classed as a non-essential nutrient (Bremner, 1983).

L-Carnitine

Acetyl-L-Carnitine

Propionyl-L-Carnitine
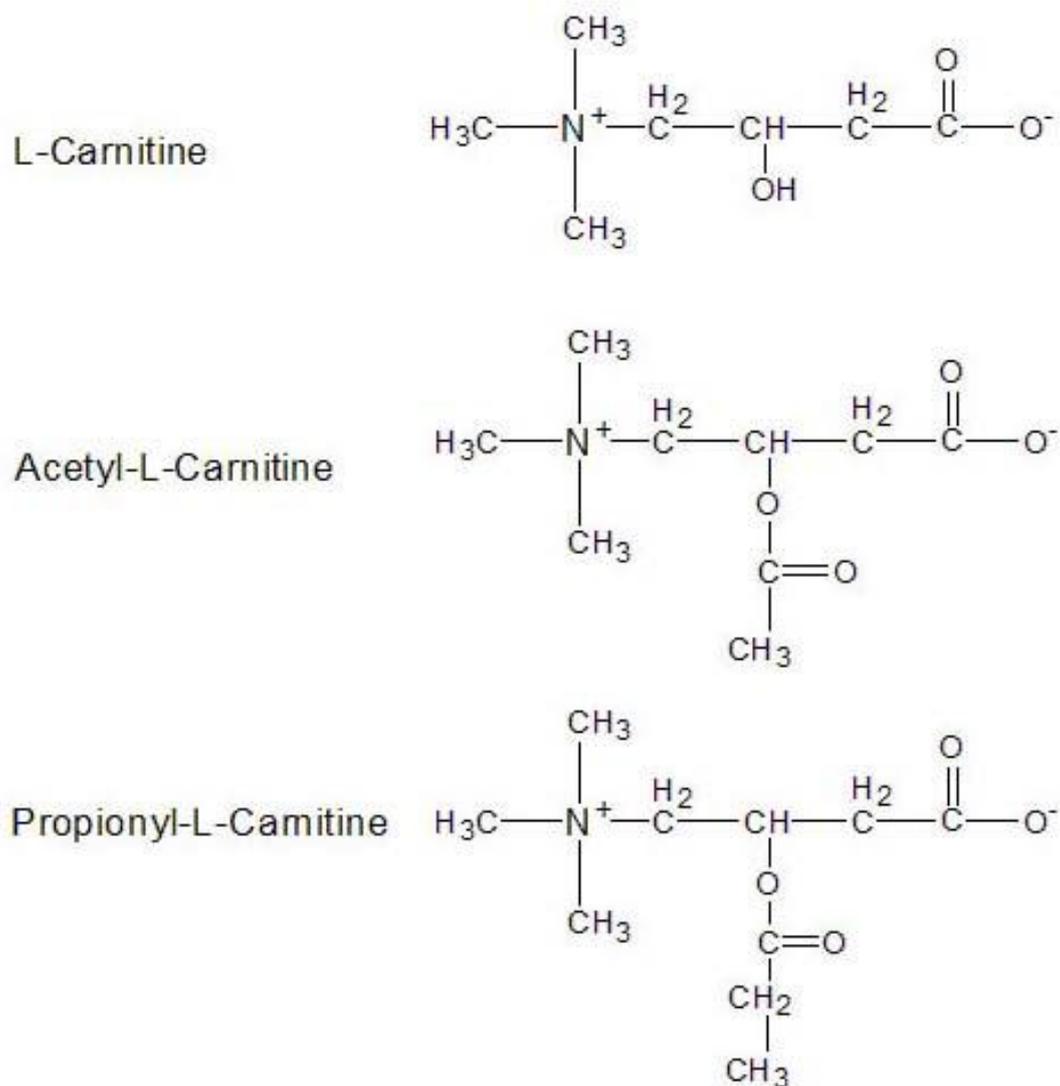
Figure 2.1.1 – Chemical structures of L-Carnitine (molecular weight 472.49), Acetyl-L-Carnitine and Propionyl-L-Carnitine

Beta-oxidation, the major process by which long chain fatty acids are oxidised in mitochondria, depends on carnitine (Reda *et al*., 2003; Karlic and Lohninger 2004). Besides fatty acid oxidation, carnitine may also play a role in maintaining the cellular coenzyme A (CoA):acyl-CoA ratio by acting as an acceptor for the acyl group (Jogl *et al*., 2004). Along with carnitine deficiency, acquired abnormalities in carnitine metabolism exist in diverse pathological conditions causing carnitine insufficiency

(e.g. dialysis, diabetes, thyroid disease, obesity and cancer; Reda *et al*., 2003). In such circumstances, the carnitine insufficiency leaves the subject unable to metabolise fat efficiently. Thus there are reports suggesting that Carnitine supplementation can thus increase the contribution of fatty acids to oxidative metabolism (Maughan *et al*., 2004). Again, for this reason, Carnitine is commonly marketed as a "fat burner" by sports nutrition manufacturers.

2.2 Role of L-Carnitine

L- Carnitine is a vitamin like compound synthesised endogenously from the essential amino acids methionine and lysine and plays an essential role in the metabolism of fatty acids. Beta-oxidation, the major process by which long chain fatty acids are oxidised in mitochondria, depends on carnitine as transport of activated fatty acids across the mitochondrial membrane is a carnitine dependant process (Reda *et al*., 2003; Karlic and Lohninger 2004). It has thus been hypothesised that increased availability of L-Carnitine will increase the capacity to transport fatty acids into mitochondria as well as subsequent oxidation (Hawley *et al*. 1998). This beta-oxidation of fatty acids in the mitochondrial matrix is a major source of cellular energy, especially in the heart and muscle. Besides fatty acid oxidation, carnitine may also play a role in maintaining the cellular coenzyme A (CoA):acyl-CoA ratio by acting as a receptor for the acyl group (Jogl, *et al*., 2004; Karlic and Lohninger 2004).
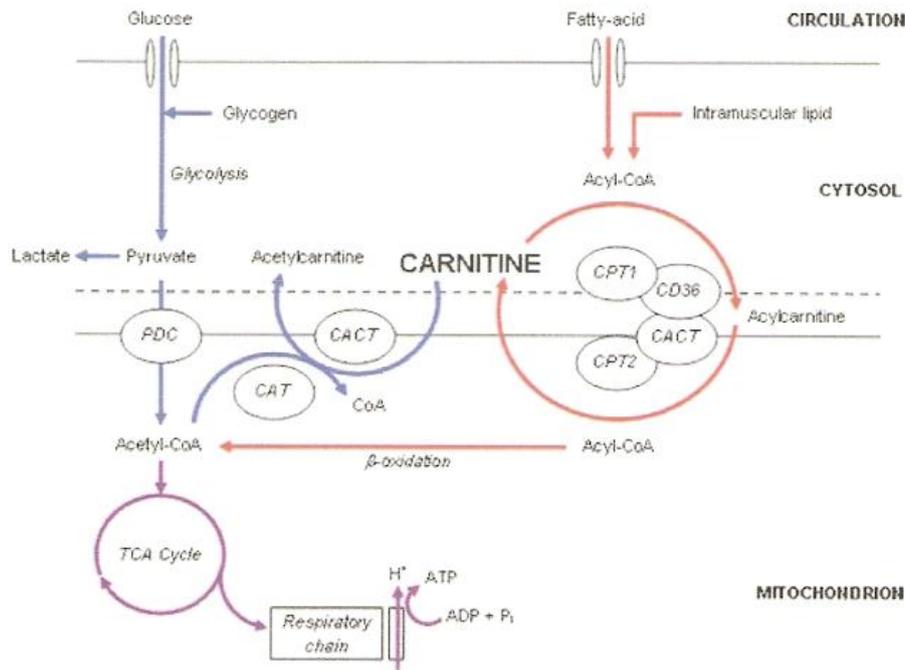
Figure 2.2.1 – A schematic diagram of the metabolic roles of carnitine in skeletal muscle (Stephens *et al*., 2007c)

Carnitine's most important physiological function is the transport of fatty acids across the mitochondrial matrix. Long chain fatty acids are unable to penetrate the inner mitochondrial matrix where β–oxidation takes place. Carnitine acts as the cofactor for penetrating the inner mitochondrial membrane. In the absence of L-Carnitine, the inner mitochondrial membrane would be impermeable to long-chain fatty acids and fatty acyl-CoA esters. However, carnitine itself is not the limiting factor in this transport process but carnitine palmitoyltransferase I (CPT I) and carnitine palmitoyltransferase II (CPT II) activity (McGarry and Brown, 1997).

Control of fatty acid oxidation is vested in the carnitine palmitoyltransferase system, which consists of three enzymes: CPT I, CPT II, and carnitine:acylcarnitine translocase (CACT). CPT I exists as three isoforms: CPT IA (liver form), CPT IB

(muscle form) and CPT IC (brain form). No isoforms exist for CPT II or CACT: they are the same proteins through out the body (Foster 2004).

In order for the CoA esters of fatty acids to be transported to the inner mitochondrial matrix, they must first be converted to carnitine esters (acylcarnitine). This reaction is catalyzed by CPT I which is located in the outer membrane of the mitochondria. The carnitine esters are transported across the inner membrane by the enzyme CACT and converted back to CoA esters by CPT II (Jogl, *et al*., 2004) which is situated on matrix side of the inner mitochondrial membrane (Stephens *et al*., 2007c). The CoA esters then enter the β–oxidation pathway in the mitochondrial matrix. The carnitine used for transport is freed for exchange by CT. Thus this reaction is fully reversible with no need for energy input. CPT I is considered to be the rate limiting enzyme for long-chain fatty acid entry into the mitochondria and oxidation (McGarry and Brown, 1997; Jeukendrup 2002).

Several regulators of CPT I activity have been proposed, including malonyl-CoA concentration, hydrogen ion accumulation in the sarcoplasm, and reduced free carnitine availability. Malonyl-CoA is a potent inhibitor of CPT I and is thus a potential candidate for the regulation of fat metabolism. Malonyl-CoA is formed from acetyl-CoA, and malonyl-CoA levels decrease in rodent skeletal muscle from rest to moderate intensity exercise, when energy production from fat increases (Winder *et al*., 1989). It is believed that the resting concentrations of malonyl-CoA are sufficiently high to inhibit CPT I, and a decrease in the malonyl-CoA concentration would therefore result in a relief of the inhibition of CPT I and increased long chain fatty acid transport into the mitochondria (Jeukendrup, 2002).

Other factors may be interacting with malonyl-CoA and CPT I in regulating long chain fatty acid transport into the mitochondria and the accumulation of hydrogen ions has also been proposed.

Starritt *et al* (2000) studied CPT I activity in isolated mitochondria from resting human skeletal muscle and it was found that small changes in pH from 7.0 to 6.8 inhibited CPT I activity by 50%. Thus, the relative large decrease in pH during intense exercise (>80% VO_2max) could explain the reduced fat oxidation rate from moderate to high intensity exercise as a result of inhibited CPT I activity.

Another mediator which may be responsible for the decrease in fat oxidation at high exercise intensities is decreased free carnitine concentration. During low intensity exercise, the flux through the Pyruvate Dehydroganese (PDH) is lower than the flux through the TCA cycle (Constantin-Teodosiu *et al*., 1991). This would result in minimal acetylation of the carnitine pool. Relatively low acetylcarnitine concentrations and high free carnitine concentrations have been observed (Constantin-Teodosiu *et al*., 1991). With increasing exercise intensity, the flux through PDH may increase more rapidly than the flux through the TCA cycle. This would result in an accumulation of acetyl-CoA. In order to free up the CoA, the acetyl units are bound to free carnitine. This acetylation of the carnitine pool will result in a decrease of the free carnitine concentration. It is possible that the reduced rates of fat oxidation at high intensity exercise (Constantin-Teodosiu *et al*., 1991) are caused by a reduced transport of FA into the mitochondria because the availability of free carnitine becomes rate limiting (Jeukendrup 2002). Constantin-Teodosiu *et al* (1991) showed that, at very high intensities (90% VO_2max), a large percentage of the carnitine was

bound to acetyl-CoA and the concentration of the free carnitine pool was reduced to very low levels to support this hypothesis.

Carnitine relationship with acetyl-CoA also plays a key role as a buffer in the mitochondrial matrix by buffering excess acetyl-CoA to form acetylcarnitine (Roepstorff *et al*. 2005; Karlic and Lohninger, 2004). This has been confirmed in contracting frog (Alkonyi *et al*. 1975) and rat (Carter *et al*. 1981) skeletal muscle, and has also been established in equine and human skeletal muscle during exercise (Stephens *et al*. 2007c). Acetyl-CoA is an important intermediate in glucose and fatty acid metabolism and normally undergoes complete oxidation in the Krebs (TCA) cycle in the skeletal muscle. In some metabolic conditions (e.g. exercise, fasting or acute stress) increased pyruvate dehydrogenase (PDH) activity and fatty acid supply from activated lipolysis exceed the rate of acetyl-CoA oxidation, which leads to an accumulation of acetyl-CoA and of short-chain acyl-CoA esters (Stephens *et al*. 2005; Stephens *et al*. 2007b; Stephens *et al*. 2007c; Sahlin, 1990; Constantin-Teodosiu *et al*. 1991) which are mainly degradation products of branched chain amino acids (Karlic and Lohninger 2004). These metabolic changes may limit the working capacity of the skeletal muscle since the accumulated acetyl-CoA inhibits the activity of the PDH. Under these conditions, the acetyl-CoA/CoASH ratio shows a linear correlation with the acetylcarnitine/carnitine ratio (Karlic and Lohninger 2004). The acetyl-CoA/CoASH ratio is an important regulating factor of the oxidation of pyruvate, α-ketoglutarate and fatty acids. All of them depend on the availability of a common mitochondrial CoASH pool.

During this critical period, carnitine acetyltransferase converts excess acetyl groups into acetylcarnitine (Brass and Hoppel, 1978). Carnitine forms esters with a wide range of acyl groups. The acylation state of the mobile carnitine pool is linked to that of the limited and compartmentalized CoA pools, and the carnitine translocase allows the transfer of these excess acyl groups as carnitine esters to the cytoplasm. Hence, more CoASH is available in the mitochondrial matrix.
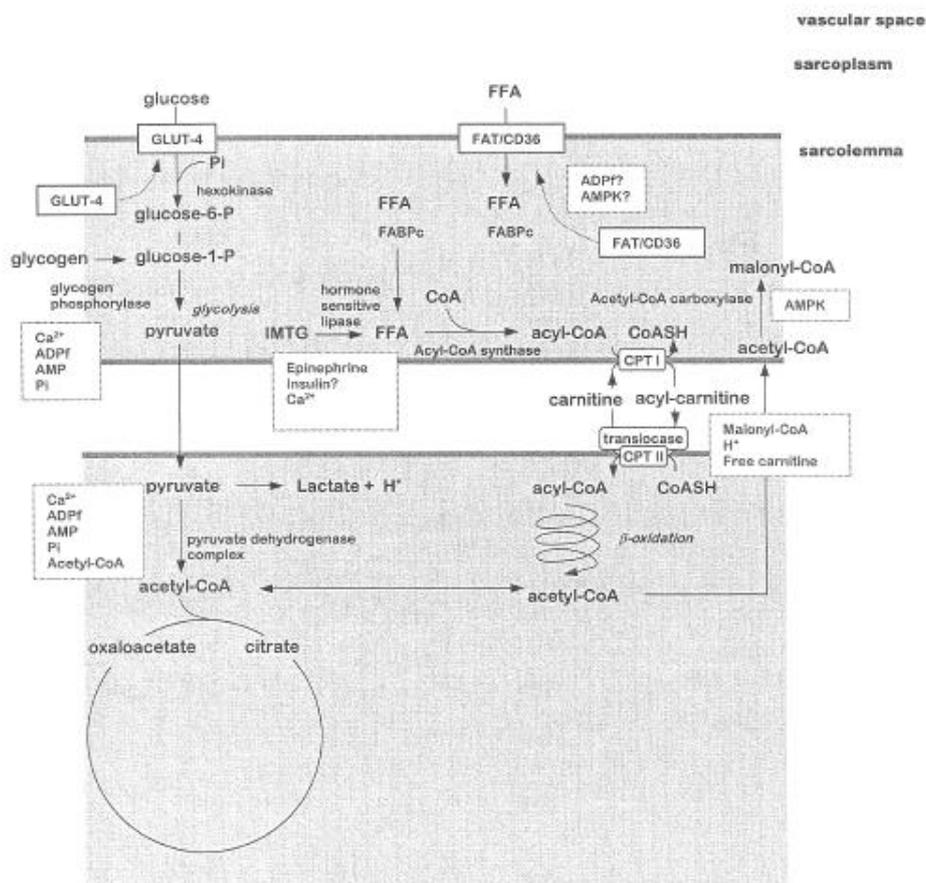
Figure 2.2.2 – Potential regulators between carbohydrate and fat metabolism. Particular attention is drawn to carnitine's role buffering acetyl-CoA, resulting in a higher availability of CoASH in the mitochondrial matrix (Spriet and Watt, 2003).

In essence, the accumulation of acetyl-CoA is due to an enhanced production rate, higher than the rate the acetyl-CoA can be used in the TCA cycle such as at exercise above the lactate threshold (Wachter *et al*. 2002). The removal of this excess acetyl CoA in the mitochondria is important as it can potentially inhibit cell function as the accumulated acetyl-CoA/COASH inhibits the activity of the pyruvate dehydrogenase (Bremner, 1969). If the skeletal muscle free carnitine pool could be increased, the CoASH pool should also rise, potentially resulting in an increased working capacity (Wachter *et al*. 2002) due to enhanced regulation of substrate oxidation in skeletal muscle. This is one such mechanism by which carnitine supplementation could potentially enhance regulation / integration of skeletal muscle metabolism.

This acetylation depletes the free carnitine pool however and it has been hypothesised that the resulting reduction in the availability of free carnitine to CPT I might be limiting to long-chain fatty acid oxidation in human skeletal muscle during exercise (McGarry and Brown, 1997; Stephens *et al*. 2006; Stephens *et al*. 2007b; Stephens *et al*. 2007c; Roepstorff *et al*. 2005). It has been demonstrated that as exercise intensity increase to > 70% VO_2max, muscle free carnitine falls to ~ 30% (5.6 mmol/kg dm) of the total carnitine store and is paralleled by a 35% decrease in the rate of fat oxidation (van Loon *et al*., 2001). But changes in cytosolic and intramitochondrial carnitine content are unknown.

Van Loon *et al*., (2001) showed that once exercise intensity was increased to 75% VO_2max, substrate utilisation changed markedly from resting values and intensities up to 55% VO_2max (there was no measurement between 55% and 75% VO_2max). The 35% decrease in the rate of fat oxidation, compared with 55% VO_2max, was not

due to a reduction in the FFA availability and Van Loon *et al*., (2001) concluded that the "glucose-FFA cycle" does not operate during high intensity exercise. During the high intensity exercise, flux through the glycolytic pathway and the PDC reaction would greatly exceed flux through the TCA cycle, thereby markedly increasing muscle lactate and acetylcarnitine concentrations and reducing free carnitine availability. The study clearly showed marked increases in glycolytic and PDC flux with increasing exercise intensity, which were paralleled by a marked decline in muscle free carnitine availability in support of the hypothesis that a decrease in free carnitine availability could possibly by directly responsible for the decreased fat oxidation. Whether or not this directly affects CPT I activity is unknown but the possibility cannot be excluded that during high intensity exercise the free carnitine concentration declines to a value that could limit CPT I activity and reduce long-chain fatty acid oxidation (Van Loon *et al*., 2001).

2.3 Regulation of Carbohydrate and Fat Metabolism

Fuel metabolism is highly regulated to ensure adequate energy for cellular function. In the fasting state, plasma glucose concentration is determined by the rate the endogenous glucose is released into the circulation and the rate that glucose is taken up by body tissues (Maggs *et al*., 2007). In the fasting state, and during physical activity, glucagon, secreted from pancreatic α-cells, prevents an abnormal fall in plasma glucose by stimulating hepatic glucose production (HGP), while basal rates of insulin secretion from pancreatic β-cells act primarily to suppress HGP and prevent an abnormal rise in plasma glucose.

In the fed state, glucose homeostasis becomes more complex as the gastrointestinal tract becomes a second source of (exogenous) glucose. Marked and rapid changes in glucose flux occur as a result of the considerable inflow of meal-derived glucose into the circulation. Insulin secretion rises sharply during the early meal-time period, stimulates glucose uptake in peripheral tissue and contributes to the suppression of HGP. Through these mechanisms, insulin in a key factor limiting abnormal postprandial glucose excursions (Maggs *et al*., 2007).

Regulation of the activity of the Pyruvate Dehydroganese Complex (PDC) is an important component of glucose homoeostasis (Maggs *et al*., 2007). Activation of PDC promotes glucose disposal, whereas the suppression of PDC activity is crucial to glucose conservation in prolonged starvation, when three-carbon compounds (including pyruvate) are required for gluconeogenesis to maintain glycaemia (Sugden *et al,* 2001). PDC is rendered inactive by phosphorylation by pyruvate dehydrogenase kinases (PDKs) and PDC is a target for substrate competition between glucose and fatty acids. This is achieved both via end-product inhibition by the common products of glucose and fatty acid metabolism (acetyl-CoA and NADH) and by the opposing acute effects of intermediates of the fatty acids and glucose metabolism on the activity of the PDKs. The latter include acute suppression of PDK activity by pyruvate, generated via glycolysis or from circulating lactate, and acute activation but the high concentration ratios of acetyl-CoA to CoA and of NADH to NAD+ in the mitochondria, generated by increased rates of fatty acid β-oxidation (Sugden *et al*., 2001).

In resting conditions and especially after fasting, fatty acids are the predominant fuel used by skeletal muscle. In contrast to carbohydrate metabolism, which increases as a function of the aerobic work rate, fat oxidation is reduced at high intensity exercise. Lipolysis in adipose tissue is mostly dependent on the concentrations of hormones (epinephrine to stimulate lipolysis and insulin to inhibit lipolysis) and one of the important factors determining substrate utilization is the availability of substrates. It was originally thought that that the classical glucose – fatty acid cycle (Randle *et al.*, 1963) could explain this reciprocal relationship between carbohydrate and fat metabolism as shown in figure 2.3.1.
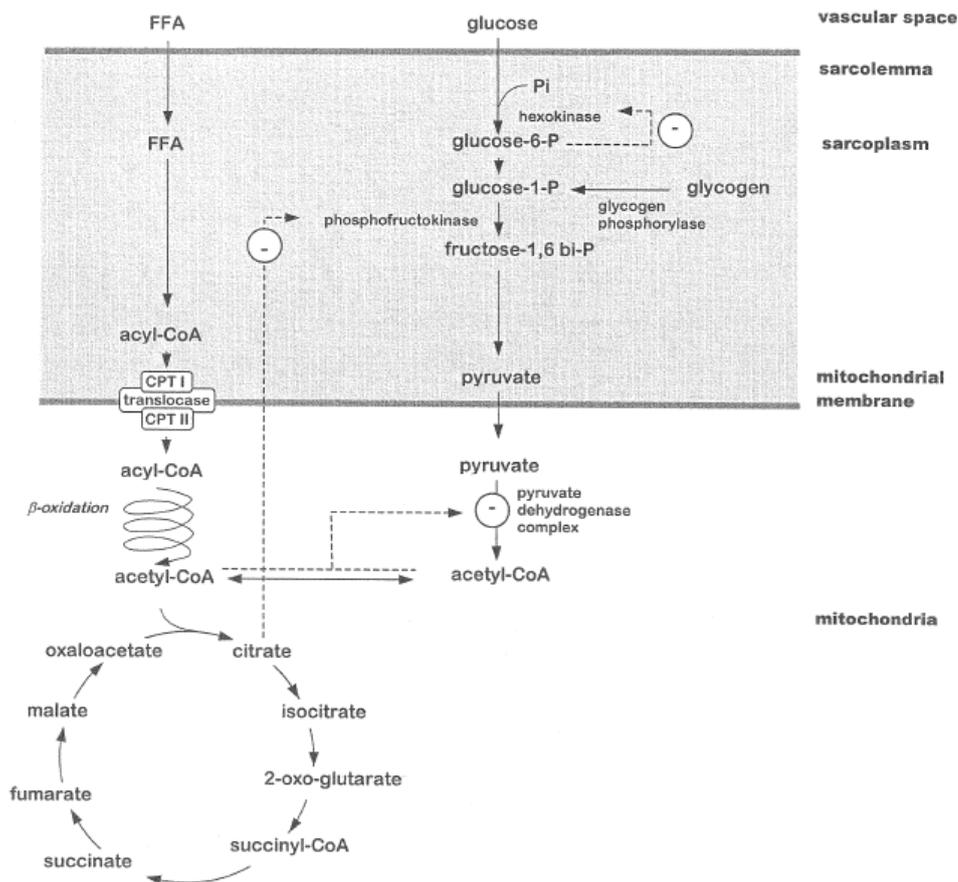
Figure 2.3.1 – The glucose – fatty acid cycle or Randle cycle (Randle *et al.*, 1963).

Figure 2.3.1 describes potential mechanisms involved in the interaction between fat and carbohydrate metabolism. Fatty acid availability seems to be the dominant factor. Increased fatty acid availability would increase acetyl-CoA concentrations: accumulation of acetyl-CoA would result in inhibition of PDH: accumulation of citrate would inhibit phosphofructokinase (PFK): and accumulation of glucose-6-phosphate (G6P) would reduce hexokinase activity. Ultimately, this would inhibit carbohydrate metabolism with increasing availability and oxidation of fatty acids.

Recently, specific carrier proteins have been identified in various tissues including skeletal muscle (figure 2.2.2) for the transport of fatty acids into the muscle (Jeukendrup, 2002). This was previously viewed as a passive process (Jeukendrup, 2002; Holloway *et al*., 2007; Holloway *et al*., 2008). In the sarcolemma, two proteins have been identified that are involved in the transport of fatty acids across the membrane: fatty acid binding protein (FABPpm) and fatty acid translocase protein (FAT/CD36) (Holloway *et al*., 2008). Considerable evidence has accumulated to indicate that FAT/CD36 and FABPpm are important in regulating the uptake of long chain fatty acids in cardiac and skeletal muscle (Holloway *et al*., 2007). Evidence that the transport of fatty acids into the muscle is regulated comes from work showing that after fasting, FABPpm is increased in slow-twitch oxidative muscle (Turcotte *et al*., 1997 reviewed by Jeukendrup 2002) and FABPpm also increases after exercise training (Kiens *et al*., 1997 reviewed by Jeukendrup 2002). It appears that FAT/CD36 works in conjunction with CPTI, as together these two proteins predict rates of mitochondrial fatty acid oxidation , and FAT/CD36 and CPT I are co-localized in mitochondria (Holloway *et al*., 2007).

Whether FABPpm also contributes to regulating mitochondrial long chain fatty acid oxidation is not known (Holloway *et al*., 2007) however it has been shown that that plasma membrane FABPpm contributes to regulating fatty acid transport (Stump *et al*., 1993; Zhou *et al*., 1995 reviewed by Holloway *et al*., 2007) and there is also evidence that FABPpm collaborates with FAT/CD36 to transport long chain fatty acids across the plasma membrane (Holloway *et al*., 2007). Fatty acid transporters are likely to be responsible for most of the transport of fatty acids across the sarcolemma, and these transporters can be regulated both acutely and chronically (Jeukendrup 2002) by altering the rate of long chain fatty acid entry into parenchymal cells (Holloway *et al*., 2007).

Whilst using a combination of exercise protocols in humans and muscle contraction in rats, Holloway *et al.* (2007) showed that unlike FAT/CD36, muscle contraction in rats and exercise in humans does not increase mitochondrial FABPpm/mAspAT, while the upregulation of this protein in muscle stimulates the rate of fatty acid transport, but fails to alter the rate of fatty acid oxidation in isolated mitochondria. They conclude that the FABPpm-induced upregulation of fatty acid oxidation in whole body muscle would seem to be solely related to the increased rate of plasmalemmal fatty acid transport.

2.4 Impaired Glucose Tolerance / Diabetes

The underlying pathophysiologies of type II diabetes are increased insulin resistance, decreased insulin secretion and altered hepatic output of glucose (Maggs *et al*. 2007). Type II diabetes is also associated with β-cell dysfunction as well as insulin resistance (Cerf, 2006; Bergman *et al*., 2002; Cobelli *et al*., 2007). Insulin resistance means, in its simplest sense, that the ability of insulin to dispose of glucose in the liver, skeletal

muscle, and other peripheral tissues is compromised. Skeletal muscle is presumed to have the greatest impact on whole-body glucose disposal, and hence on insulin resistance. Insulin resistance is usually characterised by higher fasting and post-glucose loading insulin levels, and a decreased responsiveness of tissue to the insulin driven clearance of glucose from the bloodstream.

The pre-diabetic phase known as impaired glucose tolerance (IGT) was first proposed by the World Health Organisation (WHO) in 1985. IGT is defined as a normal fasting plasma glucose of <6.0mmol/l and a 2-h post-challenge glucose concentration of 6.0-6.5 mmol/l (WHO, 1985). In 1997, the American Diabetes Association suggested another category of abnormal glucose metabolism known as impaired fasting glucose (IFG) which was defined as a fasting plasma glucose of between 6.1 and 6.9 mmol/dl. A third classification of combined IGT/IFG is also available. Basal endogenous glucose output (EGO) is not increased in individuals with IGT and is typically considered a later abnormality that only occurs with the onset of type II diabetes.

The categorization of glucose homeostasis by both the American Diabetes Association and WHO are related directly to fasting and 2 hour glucose concentrations however insulin action and insulin secretion may be related differently to fasting and 2hour glucose concentrations (Weyer *et al*. 1999). Although having no direct measurement of insulin resistance in their study, subjects studied by Annuzzi *et al*. (1985) to examine the relationship between IGT and habitually physical activity had similar serum insulin levels in the IGT and control groups despite a substantial difference in blood glucose concentrations. This suggests a reduced insulin sensitivity may be present in IGT individuals.

Hsieh *et al*. (2005) sought to explore whether patients classified as IGT, IFG or IFG/IGT had differing and more severe defects in insulin resistance and decreased insulin secretion. They found that IGT, IFG and IFG/IGT groups had similar increased insulin resistance. A study involving Pima Indians, a population with a high prevalence of glucose intolerance and type II diabetes, showed that IFG and IGT had a comparable impairment in insulin release, but those with IFG had a greater impairment in early phase insulin secretion and, in addition, increased EGO in comparison with individuals with normal glucose homeostasis (Weyer *et al*. 1999). Those with IGT/IFG, even more profound abnormalities in insulin action, insulin secretion and EGO were found. In the groups experiencing IGT, IFG or IGT/IFG the decrease in insulin action was accounted for by a lower rate of nonoxidative glucose disposal.

It has been shown that lean subjects have lower insulin levels whether they are normal, IGT or diabetic (Matsumoto *et al*. 1997) and insulin resistance is a major characteristic of obesity and the development of type 2 diabetes (Stephens *et al*. 2005). Vega (2001) also reviewed that obesity was a risk factor associated with IGT, dyslipidemia, insulin resistance, hypertension, hyperurecemia and IFG. Physical training is effective at improving glucose tolerance (Lindgarde *et* al. 1983; Saltin *et al*. 1979), lifestyle interventions (Rosenberg *et al*. 2005), including weight loss (Chiasson and Rabasa-Lhoret 2004) and increased physical activity, are highly effective in preventing or delaying the onset of diabetes in people with IGT (Unwin, *et al*. 2002; Abuissa *et al*. 2005; Herman *et al*. 2005) and IGT is associated with low habitual physical activity during leisure time and reduced physical fitness (Annuzzi *et*

al. 1985). Also, exercise combined with weight loss rather than weight loss alone, enhances fasting skeletal muscle fat oxidation rates and improves insulin sensitivity in obese patients (Goodpaster *et al*. 1999). Indeed exercise may be the single most important lifestyle factor for both preventing and reversing insulin resistance (Kelly, 2000).

Improving insulin sensitivity through diet and exercise has been found to decrease the incidence of type II diabetes in randomised control trials (Abuissa *et al*. 2005) and targeting those at risk, especially those with IGT or IFG is imperative (Deedwania *et al*. 2005). Two-thirds of individuals with IGT do not go on to develop overt type II diabetes, but are almost at the same increased risk of cardiovascular events as diabetic individuals (Kelly, 2000). Again by targeting these individuals, the focus can be attributed to preventative mechanisms.

Nutritional interventions may also be important as a preventative mechanism. Amino acids, including L-carnitine, taurine, and L-arginine may play a role in the reversal of insulin resistance (Kelly, 2000). Anuradha *et al.* (1999) reported that adding taurine to the diet of fructose-fed rats moderated the fructosed induced exaggerated glucose levels and hyperinsulinemia and similarly Nakaya *et al.* (2000) noted a positive effect of taurine on insulin sensitivity in a rat model of type II diabetes. L-arginine (0.52 mg/kg/min) restored the impaired insulin mediated vasodilation observed in patients with obesity and type II diabetes and improved insulin sensitivity in healthy, obese and type II diabetic subjects when administered in low doses intravenously (Wascher *et al*., 1997).

Stephens and colleagues (2005) conclude that an increase of 2.7 mmol/(kg dm) total carnitine content observed could alleviate the decline in fat oxidation rates routinely observed at exercise intensities above 75% W_{max}. It is possible that such an increase could positively impact upon fat oxidation during exercise in metabolic diseases such as obesity and type II diabetes. Observing this rate of total carnitine content outside a lab environment however through oral supplementation has yet proved unachievable and Stephens himself acknowledges that maintaining plasma carnitine at a supraphysiological concentration (500 µmol/L) by infusing L-carnitine for five hours however did not measurably increase muscle total carnitine accumulation (Stephens *et al*. 2005). Also, any increase in skeletal muscle carnitine content increases fat oxidation at rest would have to be investigated, particularly because insulin-resistant conditions such as obesity and type 2 diabetes, are associated with an impaired ability of skeletal muscle to oxidize fatty acids both at rest and during exercise (Rosenberg *et al*. 2005; Goodpaster *et al*. 1999; Kelly 2000, Stephens *et al*. 2006 Stephens *et al*. 2007b). These reports should also be interpreted with caution as in trials where Stephens and colleagues have increased muscle carnitine content (Stephens *et al*. 2005; Stephens *et al*. 2006; Stephens *et al*. 2007a; Stephens *et al*. 2007b) healthy active volunteers have been used as subjects with relatively low subject numbers.

Rodgers *et al*. (2001) used isolated, perfused rat hearts to compare the effects of a physiologic levels of carnitine (50µM) and insulin (75 mU/l[0.5nM]) on glycolysis, oxidation of glucose and pyruvate and oxidation of palmitate. Their results showed for the first time that a physiologically relevant concentration of carnitine exerts insulin like metabolic effects on the intact heart. The actions of 50µM carnitine and 75 mU/L (0.5nM) insulin were very similar on all measurements, although those of

carnitine were approximately 25-30% lower in magnitude. Carnitine mimicked insulin's actions on glycolysis, glucose oxidation, pyruvate oxidation, and palmitate oxidation. Both the timing and the duration of the response were also similar. Also, the augmentation of glycolysis by each agent more than accounted for the attendant increase in glucose oxidation.

However this glycolysis driven glucose oxidation may explain the individual actions of insulin or carnitine, it may not account for their effects in combination (Rodgers *et al.*, 2001). Rodgers and colleagues observed further stimulation of glucose oxidation when combining carnitine and insulin however this was not accompanied by an additional enhancement of glycolysis. Although no solid mechanisms are proposed, the insulin like effects of a physiologic concentration of carnitine on cardiac metabolism holds potential therapeutic significance of carnitine supplementation in clinical diabetes. Also, by targeting obese and inactive populations, L-carnitine may help to reduce insulin resistance before clinical stages such as IGT have been reached.

2.5 β -Cell Function

The β-cell is metabolically distinct from almost all other mammalian cell types in several respects: it can utilize glucose in the physiologically relevant range (2-20 mM) as it expresses a combination of GLUT-2 and glucokinase; it possesses low lactate dehydrogenase and plasma membrane mono-carboxylate pyruvate / lactate transporter activity and correspondingly high activity in the mitochondrial malate / aspartate shuttle, so ensuring mitochondrial oxidation of NADH; it has high activity of both pyruvate carboxylase ensuring the anaplerotic and oxidative metabolism of glucose / pyruvate can co-exist. All of these adaptations are geared to enhancing mitochondrial

TCA cycle activity, oxidative phosphorylation and efficient ATP production (Newsholme *et al.*, 2005). An important aspect of β-cell function is the ability to increase insulin release in appropriate amount and time course to cope with acute changes in plasma glucose concentration (Ferrannini *et al.*, 2004).

Type II diabetes is also associated with β-cell dysfunction as well as insulin resistance (Cerf, 2006). The relative contribution in the alterations in insulin sensitivity versus β-cell function in the development of diabetes remains unclear, it is clear that reductions in both processes have already occurred by the time hyperglycemia develops (Chiasson and Rabasa-Lhoret 2004). Most of the pancreatic transcription factors that are important for β-cell maintenance are found in other islet cell types and may also reside in pancreatic ducts. In the mature pancreas, transcription factors play a role in achieving glucose homeostasis by regulating the expression of key genes involved in maintaining the β-cell phenotype, most notably the insulin gene. Improving our knowledge of these important transcription factors, establishing their hierarchy, finding novel transcription factors, and ultimately regulating their expression to ensure glucose homeostasis may be the key to prevent β-cell dysfunction (Cerf, 2006).

Defective function of the pancreatic β-cells is now accepted to be a hallmark of type I and type II diabetes although the importance of the β-cell in type I diabetes has long been accepted (Bergman *et al.*, 2002). Type II diabetes is often a "2-hit" phenomenon, in which insulin resistance is accompanied by a β-cell defect preventing compensatory upregulation of insulin secretion. It has been postulated (Bergman *et al.*, 2002) that increased insulin resistance should upregulate β-cell sensitivity,

whereas decreased insulin resistance would downregulate β-cell sensitivity. It is also reasonable to assume that β-cells would suffer from a reduced ability to upregulate insulin secretion in the face of insulin resistance when they are defective. It is only later in life when insulin resistance becomes profound that that the presence of any β-cell defect would become obvious. Careful assessment of β-cell response may however allow for early prediction of those who will eventually show type II diabetes. The compensatory ability of the β-cell is the single physiological process that protects well-fed individuals from type II diabetes.

It is possible that the insulin sensitivity of muscle, liver and adipose tissue may themselves play a regulatory role and are sensitized by insulin to ultimately reduce the hyperinsulinemia necessary to keep glycemia within normal ranges. The relative importance of β-cell upregulation versus insulin-mediated sensitization of target tissues to overall maintenance of glucose tolerance remains to be clarified. The most popular index of insulin secretion is perhaps the acute insulin response (AIR) (Cobelli *et al*., 2007).

The mechanisms by which amino acids enhance insulin secretion are varied. The cationically charged amino acid L-arginine does so by direct depolarization of the plasma membrane at neutral pH, but only in the presence of glucose. Other amino acids, which are co-transported with Na^+, can also depolarize the cell membrane as a consequence of Na^+ transport and thus induce insulin secretion by activating voltage dependant Ca^{2+} channels. In general, metabolism of key amino acids appears to be necessary for appropriate generation of regulatory signals which then impact upon insulin secretion (Newsholme, 2005).

The product of insulin response and insulin sensitivity provides a better measurement of β-cell function rather than the insulin or C-peptide response examined in isolation. When this relationship between insulin sensitivity and insulin secretion is taken into consideration, it becomes evident that the subjects who are at high risk of developing type II diabetes have demonstrated β-cell dysfunction at a time when they still have normal glucose tolerance (Chiasson and Rabasa-Lhoret 2004).

Insulin resistance seems to be explained mostly by the prevalence of obesity. The concept that β-cell failure is the primary defect leading to the development of diabetes is supported by the fact that β-cell dysfunction is present years before the glucose intolerance appears. Chiasson and Rabasa-Lhoret (2004) conclude from their review that a decrease in insulin resistance offers a protective effect on the β-cell, and therefore has a preservative effect on the β-cell function.

Amino acid metabolism under appropriate conditions, is known to enhance insulin secretion from primary islet cells and β-cell lines (Newsholme *et al*., 2005). L-Leucine stimulates insulin release in pancreatic β-cells; L-alanine can stimulate insulin secretion under specific conditions of nutrient ability; and L-arginine metabolism in the β-cell can give rise to glutamate production and this can influence insulin secretion (Newsholme *et al*., 2005). It has previously been shown (Broad *et al*., 2008) that L-Carnitine supplemented over a 14 day period, resulted in an increased resting plasma glutamate concentration. Essentially, the potential effects of L-Carnitine supplementation on insulin secretion through maintenance of glucose or fatty acid oxidation in the β-cell by buffering of acyl-groups, or through generation of

glutamate for maintenance of ATP/ADP ratio in the β-cell are attractive as these mechanisms of action have previously been demonstrated for other amino acids which have been shown to enhance insulin secretion (Kelly, 2000; Newsholme *et al.*, 2005).

The fact that elevations in circulating insulin are generally observed before impaired glucose tolerance led to the belief that impaired insulin secretion developed secondary to insulin resistance (Biden *et al.*, 2004). On the other hand, only a minority of obese insulin-resistant individuals progress to overt hyperglycemia (Chiasson and Rabasa-Lhoret 2004), and many do not even display impaired glucose tolerance (Biden *et al.*, 2004). This suggests that that insulin resistance is not sufficient in itself to impair β-cell function but that separate defects, operating at both the level of peripheral tissues and the pancreas were necessary for the development of the disease (Butler *et al.* 2003). Type II diabetes can thus now be viewed as failure of the β-cell to compensate for insulin resistance by enhancing insulin output (Biden *et al.*, 2004).

2. 6 Carnitine and Exercise

The most important claim in relation to L-Carnitine's role in improving exercise function relates to the role of carnitine in fat metabolism and the marketing claim of L-Carnitine promoting fat burning. L-Carnitine is often advertised to improve fat metabolism, reduce fat mass, and increase muscle mass (Maughan *et al.*, 2004) . This is based on the observation that as exercise intensity increases there is a subsequent decline in muscle free carnitine availability in support of the hypothesis that a decrease in free carnitine availability could possibly by directly responsible for the decreased fat oxidation (van Loon *et al.*, 2001). Endurance athletes may use carnitine to increase oxidation of fat during exercise and spare muscle glycogen and athletes in

sports such as boxing and horse riding where weight loss and maintenance are essential have been reported to use L-Carnitine supplementation (Brass, 2000).

General consumers must beware as it has been shown in a study of 12 over-the-counter carnitine formulations, the actual mean carnitine content was only 52% of that indicated on the label (Millington *et al*., 1993). Studies investigating the effect of carnitine on metabolism have used in excess of 3g/day, however supplements commonly available may contain a maximum of 300mg per serving.

Due to the hypothesis that muscle free carnitine availability can limit fat oxidation during conditions of high PDC flux (Stephens *et al*. 2005; Stephens *et al*. 2007b; Stephens *et al*. 2007c; Roepstorff *et al*. 2005; Karlic and Lohninger 2004; van Loon *et al*., 2001), increasing muscle total carnitine content could potentially alleviate the decline in fat oxidation rates routinely observed during high intensity exercise (van Loon *et* al., 2001; Brass, 2000; Stephens *et al*. 2005; Stephens *et al*. 2006; Stephens *et al*. 2007b). During intense exercise, carnitine buffers excess acetyl-CoA production, forming of acetylcarnitine, when its rate of condensation with oxaloacetate is less than its rate of formation by pyruvate oxidation (Constantine-Teodosiu *et al*. 1991). However, this acetylation depletes the free carnitine pool, and it has been suggested that the resulting reduction in the availability of free carnitine in the reaction catalyzed by CPT I, the rate-limiting step in LCA-CoA entry into the mitochondria (McGarry and Brown, 1997), might be limiting to fat oxidation under these conditions (Stephens *et al*. 2005). If skeletal muscle free carnitine availability were to be increased, then the inhibition of fat oxidation observed during exercise could possibly be alleviated.

2.7 Uptake of Carnitine during Supplementation

A higher muscle carnitine content has been observed in trained humans and equine subjects compared to untrained controls (Lennon *et al*. 1983; Foster and Harris, 1992) and the trained subjects have a higher capacity to utilize fatty acids during exercise thus promoting a glycogen sparing response (Hawley *et al*. 1998) . Since the storage in the form of glycogen is limited, the ability to perform high intensity exercise will be decreased with progressive glycogen depletion (Brouns, 1997). An adaptation leading to an increased capacity to utilise fatty acids for ATP resynthesis will lead to a sparing of endogenous CHO with the consequence that endurance capacity may be increased (Bacurau *et al*. 2003; Karlic and Lohninger 2004). In order to view this sort of response during carnitine supplementation alone, an increase in muscle carnitine concentration would have to be observed.

On occasion, muscle carnitine concentration has been shown to increase with long term supplementation in patients with chronic renal failure (Ahmad *et al*. 1990), chronic hemodialysis (Siami *et al*. 1991) and peripheral vascular disease (Brevetti *et al* 1988) but the authors offer no explanation for any effects in these disorders. One potential explanation is that in renal failure patients, dialysis can lead to the filtration of carnitine resulting in low carnitine muscle stores (Reda *et al*., 2003; Poorabbas *et al*., 2007), so a period of supplementation may lead to an uptake of carnitine to that higher than the base level. The duration of carnitine treatment however may be a particularly critical variable (Brass, 2000). It must also be acknowledged that in patients with carnitine deficiency, who can have extremely low carnitine tissue levels,

diffusion may be an important mechanism by which carnitine is transported to the tissues such as skeletal muscle and heart (Wachter *et al*. 2002) rather than usual active transport using OCTN2 transporters.

By supplementing 28mg/kg, intragastrically for the last 4 weeks of a 6 week moderate intensity training programme in male Wistar rats, Bacurau *et al*. (2003) increased fatty acid oxidation in skeletal muscle by a mechanism that includes increasing total carnitine content within the soleus muscle and increasing the total content of acyl-carnitine. This increase in acyl-carnitine concentration was matched by an increase in free CoA that enabled Krebs cycle flux enhancement by acting on pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase. These results were increased in trained rats.

Stephens *et al*. (2005) was the first study to demonstrate that human skeletal muscle total carnitine content can be acutely increased after L-carnitine administration via intravenous infusion and that this occurred as a result of an insulin-mediated augmentation of muscle carnitine accumulation. Maintaining plasma carnitine at a supraphysiological concentration (500 µmol/L) by infusing L-carnitine for five hours however did not measurably increase muscle total carnitine accumulation. This supports the argument that muscle carnitine content cannot be increased by L-carnitine supplementation alone, and subsequent work on oral L-carnitine supplementation has not examined muscle carnitine content but it is estimated it would require in excess of 100 days to effect a change in muscle carnitine stores (Stephens *et al*. 2007c)

Indeed, further study (Stephens *et al*. 2007b) has shown that only high circulating serum insulin concentrations ($\geq$90 mU/L) are capable of stimulating skeletal muscle carnitine accumulation close to the upper physiological limit difficult to achieve by dietary means alone. This finding is in concordance with the hypothesis that insulin would augment Na^+ -dependent skeletal muscle carnitine transport via OCTN2, secondary to its action of increasing sarcolemmal $Na^{+-}K^+$ ATPase pump activity and therefore intracellular Na^+ flux (Stephens *et al*. 2005; Stephens *et al*. 2006; Stephens *et al*. 2007b; Stephens *et al*. 2007c) but also show that it will require a relatively high serum insulin concentration to achieve this effect (Stephens *et al*. 2007b).

To try to alleviate this issue, Stephens has aimed to stimulate whether insulin, released as a consequence of carbohydrate ingestion could increase the retention of carnitine in humans over the course of 1 day or 2 week of L-carnitine supplementation (Stephens *et al*. 2007a). Plasma total carnitine concentration and urinary total carnitine excretion were significantly reduced when L-carnitine ingestion was accompanied by carbohydrate feeding. When analysed along with their previous work in the Nottingham lab, they concluded that carbohydrate feeding augments whole body carnitine retention in humans. There is however no muscle data to contribute to these findings. These findings could have a significant effect on the integration of fat and carbohydrate oxidation in contracting skeletal muscle as the retention is likely to reside in skeletal muscle because insulin is known to stimulate muscle total carnitine accumulation (Stephens *et al*. 2005; Stephens *et al*. 2006; Stephens *et al*. 2007b; Stephens *et al*. 2007c). The elevated muscle glycogen levels found also indicate a potential direct insulin action of carnitine (Rodgers *et al*., 2001).

In this final study however (Stephens *et al*. 2007a) carbohydrate based beverages were used and the author acknowledges that any increase in muscle carnitine content following a single dose or 2 week of L-carnitine feeding in the presence of elevated circulating insulin is likely to be small due to the poor bioavailability of orally administered L-carnitine (Harper *et al*. 1998; Serge *et al*. 1988; Brass 2000; Stephens *et al*. 2007a). Thus muscle carnitine accumulation was not measured but estimated indirectly using measurements of plasma or urinary carnitine concentrations. Carnitine metabolites (TMA etc.) were however not measured which would have given a more accurate indication of urinary excretion (Galloway; personal correspondence).

The majority of studies have however failed to increase muscle carnitine concentration whether it be via oral or intravenous supplementation. In *vivo* oral L-carnitine has been observed to increase plasma L-Carnitine concentrations, but uptake in muscle remained unchanged (Fritz, 1967) and this fits well with the observations (Engel *at al*. 1984; Stephens *et al*. 2007c; Wachter *et al*. 2002) that L-carnitine is taken up by muscle against a concentration gradient – plasma 40 to 60 µmol/L to muscle 3 to 4 mmol/L. The gradient is so large that even a substantial oral intake would not result in a measurable change. Intravenous administration of a single dose of L-Carnitine for up to 2 weeks failed to show an increase in the working capacity or endurance performance in humans (Brass *et al*. 2004). Brass *et al*. (1994) showed that muscle carnitine content glycogen utilisation was not modified by carnitine administration in any protocols used and hypothesise that any efficacy of carnitine modifying exercise performance must therefore reflect either a nonmuscle site of action and although muscle carnitine concentration was increased by 17% after 2 x

2g/day supplementation for 3 months in moderately trained subjects (Wachter *et al.* 2002), the authors acknowledge that this was not significant and the study had low sample numbers and no effect on P_{max}, $VO2_{max}$ and RER_{max}.

There is poor bioavailability of L-Carnitine administered orally (<20% for a 2-6g dose; Harper *et al.* 1998; Serge *et al.* 1988; Brass 2000; Stephens *et al.* 2007a) and this is potentially the main reason for oral L-Carnitine administration failing to increase muscle carnitine concentration and thus alter energy metabolism (Brass, 2000; Stephens *et al.* 2007a; Hultman *et al.* 1991)). In humans, the finding that intravenous L-Carnitine supplementation also has no impact on muscle carnitine content suggests that muscle carnitine transport is most likely the limiting factor to muscle carnitine accumulation in humans showing no signs of metabolic disease or carnitine deficiency (Stephens *et al.* 2007c; Wachter *et al.* 2002). These observations show that beneficial effects of the long-term administration with L-Carnitine on the physical performance of healthy subjects cannot be explained by an increase in their carnitine skeletal muscle stores. Altered muscle carnitine handling in pathophysiologic states (Brass *et al* 2004) may be the reason for the increased muscle carnitine concentrations in some clinical situations discussed.

2.8 Carnitine in Clinical Conditions

Due to the findings that Carnitine supplementation may not in fact promote fatty acid oxidation and may promote carbohydrate metabolism (Galloway and Broad, 2005), Carnitine supplementation may be beneficial to those at risk of developing metabolic disorders such as impaired glucose tolerance and type 2 diabetes.

Indeed, De Gaetano *et al*. (1999) observed that intravenous glucose plus carnitine administration stimulated glucose disposal calculated through indirect calorimetry compared with placebo (glucose plus saline infusion) in healthy subjects. This supported the existence of an insulin-independent action of carnitine, whilst not excluding the possibility of an associated insulin-dependant reaction as well. This is consistent with the hypothesis that carnitine induced glucose disposal increased glucose oxidation. They also postulate that carnitine could have a role in the therapy of diabetes.

Due to the calorimetric basis of the results obtained (De Gaetano *et al*., 1999), these results should be interpreted with caution. Indirect calorimetry is the mothod by which the type and rate of substrate utilization, and energy metabolism are estimated in vivo starting from gas exchange measurements (Ferrannini, 1988). Indirect calorimetry assumes generally that the composition of fats, proteins and carbohydrates are sufficiently similar from one food source to another so that the corresponding heat equivalent of oxygen and respiratory quotients can be regarded as constant (Livesey and Elia, 1988), thus the asumptions and the estimations should be interpreted with caution.

Mingrone et al (1999) created at least a short term improvement in insulin sensitivity by enhancing whole-body glucose uptake (8%) and increasing glucose storage after a two hour infusion of L-carnitine administered to patients with type II diabetes. These results were mirrored in healthy active controls. The difference in total glucose uptake between carnitine and saline infusion was accounted for by a difference in the non oxidative disposal of glucose and glucose storage was significantly higher in both

controls and diabetic patients under carnitine infusion and the authors raise the question as to whether oral carnitine supplementation could possibly produce similar effects.

A previous study had shown in a well controlled trial that whole body glucose metabolism was significant higher after carnitine infusion in subjects with type II diabetes (Capaldo *et al*. 1991). They mirrored the results of Ferrannini *et al* (1988) and the results were independent of body weight and the degree of insulin resistance experienced by the subjects. Capaldo (*et al*. 1991) hypothesise that the majority of glucose uptake taken up by the peripheral tissues is directed towards glycogen synthesis or complete oxidation.

Again after infusion of ALC over a 3 hour period Giancaterini *et al*. (2000) increased glucose disposal in type II diabetics. It has been hypothesised that there is a shift in substrate utilization from carbohydrates to lipids in type II diabetes, with a large proportion of the increase in lipid oxidation accounted for by an increase in intramuscular triglyceride mobilization (Kiens *et al*. 1987). Giancaterini *et al*. (2000) propose an action for these results: as impaired PDH activity and increased β-oxidation have been described in type II diabetic patients low dose ALC administration could represent a feasible way to physiologically correct this enzymatic impairment of substrate utilization.

It has also been shown that administration of L-carnitine holds potential to improve insulin sensitivity (Kelly, 2000) in other clinical settings. Kelly (2000) reviewed that: in a study evaluating the effect of parental administration of L-carnitine on metabolic

parameters subsequent to post surgical stress, Heller et al (1986) concluded that carnitine administration was capable of reducing the associate trend toward insulin resistance; and that Gunal (1999) showed a single intravenous dose of L-carnitine had a positive effect on insulin sensitivity in patients with renal failure. Also patients with chronic renal failure (Ahmad *et al*. 1990) and peripheral vascular disease (Brevetti *et al* 1988) have been reported to increase their exercise capacity after treatment with carnitine, and carnitine supplementation has also been suggested to be beneficial in treating chronic fatigue syndrome (Plyioplys and Plyioplys, 1997).

The work that has been carried out has however been achieved mainly through intravenous L-carnitine infusion and little work has researched the use of oral L-carnitine supplementation, or in those who are not yet at the clinical stage but may still be at risk i.e. the obese population.

2.9 Non-Clinical Carnitine Supplementation

In contrast with the idea of accelerating fatty acid oxidation by carnitine supplementation, data from animal heart models suggests that exogenous carnitine can induce an increase in glucose oxidation at the expense of fatty acid oxidation (Broderick *et al*. 1992). The mechanism of carnitine-induced enhanced glucose oxidation may involve activation of pyruvate dehydrogenase secondary to reductions in acetyl-CoA content as acetylcarnitine is generated (Uziel *et al*. 1988).

The effect that an increase in skeletal muscle carnitine content had on the integration of muscle fat and carbohydrate oxidation during and after hyperinsulinemic clamp

conditions was investigated by Stephens and colleagues (Stephens *et al*. 2006). Their most significant finding was that a 15% increase in skeletal muscle carnitine content, achieved via intravenous L-carnitine infusion during a 6-h euglycemic hyperinsulinemic clamp conditions resulted in a 30% decrease in muscle PDC activity and a 40% decrease in muscle lactate content. An overnight increase in muscle glycogen content was also observed in their carnitine trial again suggesting a direct insulin effect of carnitine.

They concluded that the increase in muscle carnitine content observed inhibited glycolytic flux (decrease in lactate) and carbohydrate oxidation at the level of the PDC, thereby diverting muscle glucose uptake toward glycogen storage (nonoxidative glucose disposal). This would hold potential benefits of carnitine in the use of recovery products aimed at glycogen replenishment after exercise. They propose a mechanism whereby the reduction in PDC activation could have been caused by a carnitine-mediated increase in skeletal muscle long-chain fatty acid oxidation via CPT I. It could also suggest however, a direct effect of intracellular carnitine on glycogen synthase activity and therefore rates of glycogen synthesis.

It has also be shown that steady-state plasma total carnitine concentration was significantly lower when L-carnitine was administered intravenously in the presence of high (~90 mU/L) and very high (~170 mU/L) serum insulin concentrations compared with when serum insulin concentration was maintained at ~10 mU/L (fasted) or ~50 mU/L during insulin clamp conditions (Stephens *et al*. 2007b). Collectively, this indicated that insulin will not stimulate muscle carnitine retention unless a serum insulin ≥90 mU/L is achieved during hypercarnitinemia.

The aim of the present study was to investigate insulin like actions of carnitine on healthy active males and sedentary obese subjects. Due to the findings that Carnitine supplementation may not in fact promote fatty acid oxidation and may promote carbohydrate metabolism (Hultman *et al*., 1991; Broderick *et al*. 1992; Millington *et al*., 1993; Brass *et al*., 1994; Hawley *et al*., 1998; Karlic and Lohninger, 2004; Maughan *et al*., 2004, Abramowicz and Galloway, 2005), Carnitine supplementation may be beneficial to those at risk of developing metabolic disorders such as impaired glucose tolerance and type 2 diabetes. Obesity is a risk factor associated with IGT, dyslipidemia, insulin resistanc, hypertension, hyperurecemia and Impaired Fasting Glucose (IFG) (Vega, 2001) and nutritional interventions including L-Carnitine may play a role in the reversal of insulin resistance (Kelly, 2000). This provides the basis of the study supplementing to adult males with a BMI which would class them as being at risk from developing such metabolic conditions.

Furthermore, if carnitine has an impact upon insulin sensitivity or insulin release in response to an oral glucose load it could result in suppressed mobilization of non-esterified fatty acids (NEFA; Stumvoll *et al*., 2000) and thus impact upon substrate use during low intensity exercise during which plasma derived NEFA's are a main contributor to skeletal muscle oxidation. This provides the basis for measuring substrate use at low intensity exercise in subjects.

Chapter 3 - Methods

3.1 Subjects

Sixteen non-vegetarian males of ranging BMI aged 18-50 years were recruited for this study. Each participant attended the laboratory on 4 occasions over a 5-6 weeks period. Two weeks placebo supplementation followed by two weeks L-carnitine supplementation was undertaken together with the completion of food intake diaries and exercise logs during this period. Subject characteristics can be viewed on table 4.1.1. The University of Stirling's Ethics Committee in accordance with the Declaration of Helsinki approved the study.

Before taking part in the study, all subjects underwent a routine medical screening (height, weight, blood pressure) and completed a general health questionnaire. Subjects with any type of metabolic conditions (e.g. type 2 diabetes) were excluded from the study. All subjects gave their consent to take part in the study and were aware that they were free to withdraw from the experiment at any point.

3.2 Experimental Protocol

Each subject was required to visit the lab on 4 different occasions. During the first visit, subjects completed a three stage incremental predicted maximum aerobic capacity test. During this test subjects completed the incremental predicted VO_2max test on an electronically braked cycle ergometer (Lode Excaliber Sport) in three different 3-minute stages, with the workload being increased in relation to heart rate. Using the predicted maximum heart rate (220 – age) subject's maximum aerobic

capacity was estimated and from this, the workload required to work at 40% of this Predicted VO$_2$max was estimated.

Participants were then assigned to either the lean or overweight / obese groups based on BMI and estimated % body fat measurements. For visits 2, 3 and 4 subjects reported to the lab at the same time of day on the same weekday in a fasted state in order to participate in an oral glucose tolerance test (OGTT). These visits were separated by two weeks. The purpose of visit 2 was to perform a preliminary familiarisation trial. This preliminary trial was included to ensure that all participants were familiarized with the OGTT protocol and to ensure that the exercise intensity was appropriate to achieve a steady state oxygen cost of 40% of predicted VO$_2$max.

On arrival, subjects height, weight and blood pressure was measured. Subjects were then asked to rest in a supine position on a bed while an intravenous cannula was inserted into an antecubital vein. A resting sample (7.5ml) was collected before subjects ingested a pre mixed 75g glucose (330ml) drink within 2 minutes (end of drink, t= 0 minutes). Further 7.5ml blood sample was collected at t= 15, 30, 45, 60, 90 and 120 minutes. After drawing of samples, a small volume of sterile saline (1-2ml) was used to flush the cannula to ensure patency.

Five minutes prior to the last sample collection, subjects sat on the electronically braked cycle ergometer (Lode Excaliber Sport). After the final resting blood sample was collected, subjects started exercising at 40% of their predicted VO$_2$max for 20 minutes. Subjects were asked to cycle at a cadence of between 60-80 RPM that they found comfortable, which was kept constant for the 20 minutes and repeated during

visits 3 and 4. Further 7.5ml blood samples were collected at 10 and 20 minutes of exercise.

During the exercise time, expired gas was continuously collected and analysed using the Sensormedics Vmax29 system in order to measure VO_2, RER and the data used to estimate rate of carbohydrate and fat oxidation. Heart rate was also recorded using a polar HR monitor at 1 minute intervals to assess cardiorespiratory response to exercise. At the end of the 20 minutes, the workload was decreased and subjects pedalled until they reached a heart rate below 120 beats per minute before the intravenous cannula was removed.

Previous work in the lab has shown that it can take up to 3 weeks after L-Carnitine supplementation for plasma carnitine to undergo a successful wash out period from subjects (Broad, unpublished observations). Hence, we used a single blind ordered design with placebo supplementation administered first. During visits 2 and 3, subjects were asked to record food and fluid intake and exercise completed on 3 days prior to the trial and then replicate this on each visit. There was no heavy exercise performed on the day before or the morning of the trial. This allowed them to repeat the exact food intake and exercise as they had completed for three days before visit 3 and 4. They were also given supplement capsules each containing 0.5g placebo to be taken orally two capsules, three times daily for 14 days until the next visit (glucose polymer – 3g/day).

Between visits 3 and 4, subjects were again given food intake diaries and exercise logs to complete in order to replicate their previous food an exercise intake. They

were also given capsules containing 0.5g L-Carnitine L-Tartrate capsules to be taken orally two capsules, three times daily for 14 days until visit 4 (3g/day). On each visit, the final dose was taken the evening before with the subject's evening meal. Subjects were not aware which supplementation they were taking during the visits and believed this to be randomised.

3.3 Blood Collection and Analysis

Blood samples were drawn into a dry syringe during visits 2, 3 and 4. They were dispersed into one 2.5ml (KEDTA) and one 5ml (Lithium Heparin) blood collection tubes. All samples were immediately mixed and centrifuged at 4000 rpm for 10 minutes at $4^{o}C$. Plasma was transferred to microcentrifuge tubes frozen at $-80^{o}C$ until analysis.

Plasma glucose concentration was analysed using mass spectrometry by the glucose oxidase (ABX diagnostics) method at a later date. Plasma insulin (ELISA method, DRG human insulin kit) and plasma NEFA (enzymatic analysis, Wako NEFA-C kit) were also analysed. Glucose and insulin data were used to calculate the usual sensitivity indices (HOMA-IR, AUC glucose, AUC insulin) with the additional parameters of insulin sensitivity index, β cell function (1st phase and 2nd phase), and estimated metabolic clearance rate calculated using the methods of Stumvoll *et al*., 2000 (Appendix F).

3.4 Statistical Analysis

Changes were compared by repeated measures analysis of variance. Data from the OGTT trials were analysed using a repeated measures analysis of variance (RMANOVA) with trial (placebo or carnitine) and time (0 min to 120 min timepoints) as within subjects factors and group (lean or overweight / obese) as a between subjects factor. Following observations of a main effect of trial, time, or trial x time interaction these were followed up with post-hoc analysis using one-way ANOVA and paired samples T-test where appropriate to determine at which location the differences occurred. If a group effect was observed then data was separated into the groups and the analysis re-run on each group alone without a between subjects factor. Pearson correlation analysis was used to determine whether any relationships existed between BMI, estimated % body fat, and predicted VO_2max and the magnitude of the change in response of the insulin sensitivity indices between placebo and carnitine trials. Data from the exercise task were analysed using RMANOVA with the 120 min OGTT values as the exercise period baseline, again using trial and time within subjects effects and group as a between subjects effect. Significance was taken as $p < 0.05$.

Chapter 4 - Results

4.1 Study Adherence

Subjects were allocated to either the lean (n=8) or overweight/obese (n=8) groups

after their initial OGTT familiarisation and physical characteristics had been

determined. Tables 4.1.1 shows the mean (Standard Deviation) subjects

characteristics for the whole group and mean (95% CI) for the lean and overweight /

obese groups. All subjects completed the study and reported no side effects to the

supplementation.

Table 4.1.1 Subject Characteristics. Values are mean (SEM) for whole group, and

mean (95% CI) for lean and overweight / obese groups.

Variable	Whole Group	Lean	Overweight / Obese
N	16	8	8
Age (years)	26.5 ± 2.2	24.0 (18.9-29.1)	29.0 (22.2-35.8)
Height (cm)	179.3 ± 1.5	180.0 (176.0-184.0)	178.7 (174.1-183.3)
Body mass (kg)	89.7 ± 4.3	76.1 (72.2-80.0)*	103.3 (94.3-112.3)*
BMI (kg/m^2)	28.0 ± 1.4	23.5 (22.8-24.2)*	32.5 (29.1-35.9)*
Estimated body fat (%)	19.1 ± 2.1	12.3 (9.3-14.3)*	25.9 (21.5-30.3)*
predicted VO$_2$max (L.min^{-1})	4.06 ± 0.19	4.12 (3.67-4.57)	4.01 (3.38-4.64)
Predicted VO$_2$max (ml.kg^{-1}.min^{-1})	46.7 ± 2.8	53.9 (49.3-58.5)*	39.5 (32.2-46.9)*
Fasting glucose (mmol/L)	5.4 ± 0.2	5.6 (5.1-6.1)	5.1 (4.6-5.6)
Fasting Insulin	13.7 ± 3.5	6.9 (5.6 – 8.2)	15.4 (5.8 – 23.8)

* indicates a significant difference between lean and overweight / obese groups

4.2 Glucose and Insulin Response to OGTT

Figure 4.2.1 shows the plasma glucose response to the OGTT for all 16 subjects who completed the trials. Repeated measures analysis of variance revealed no significant main effect of carnitine supplementation but there was a significant time effect ($p < 0.01$) and trial x time interaction ($p < 0.01$) for plasma glucose response to supplementation with a lower peak plasma glucose during the OGTT at 30 minutes ($p < 0.05$), in the carnitine trial compared with the placebo trial, and a higher plasma glucose concentration ($p < 0.05$) at 90 minutes on the carnitine trial compared with the placebo trial. However, Δ total AUC glucose was not significantly different between trials (Figure 4.2.3).
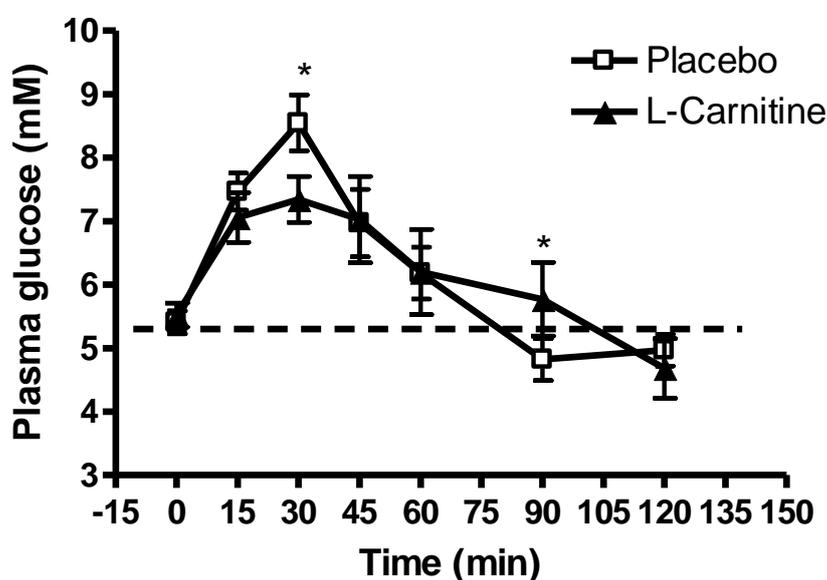
Figure 4.2.1 Plasma Glucose Response to OGTT after 14 days of 3g L-Carnitine L-Tartrate per day. Data is for n=16. Values are mean (SEM). * indicates significant difference ($p < 0.05$) from placebo trial.
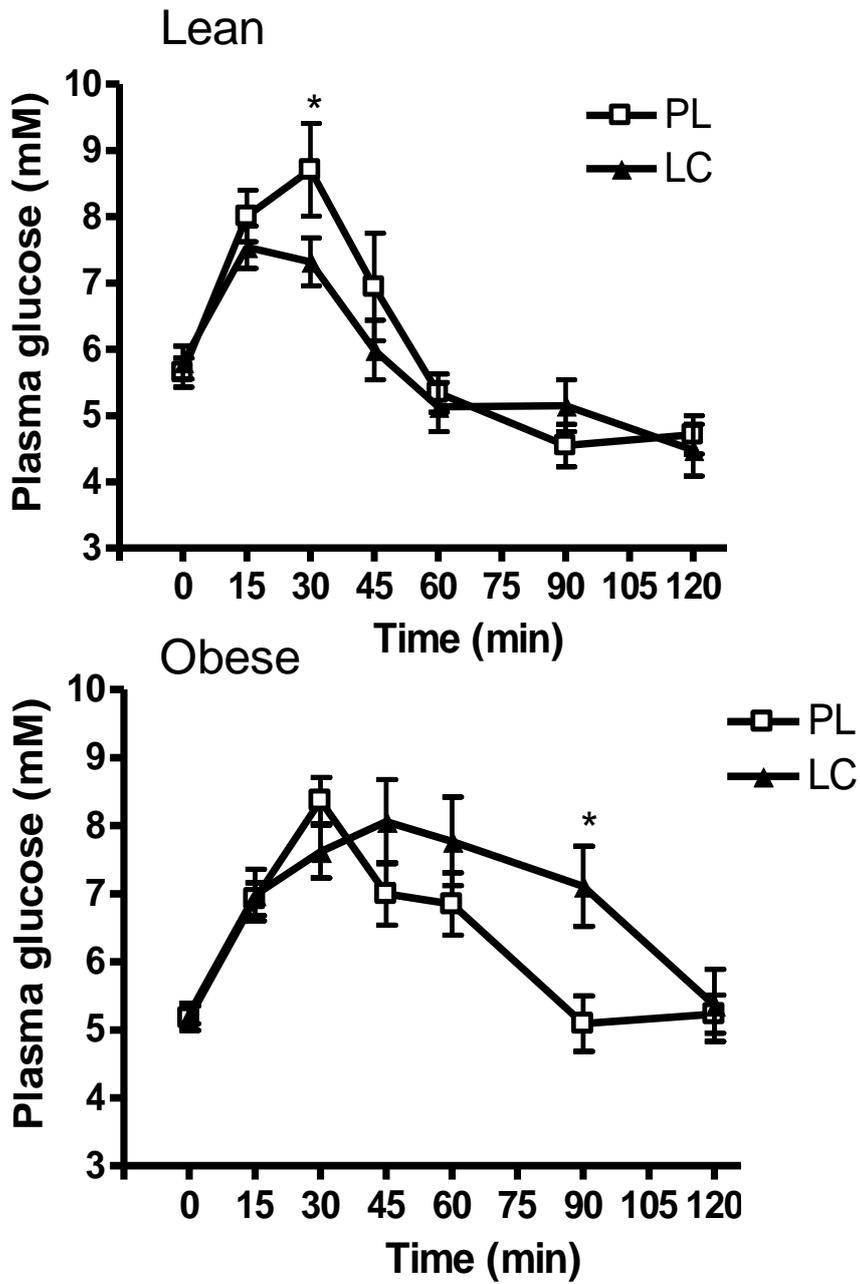
Figure 4.2.2 - Plasma Glucose Response to OGTT for Lean (n=8) and Obese (n=8) between L-Carnitnie (LC) and Placebo trials (PL) after 14 days of 3g L-Carnitine L-Tartrate per day. Values are mean (SEM). * indicates significant difference (p<0.05) from placebo trial.
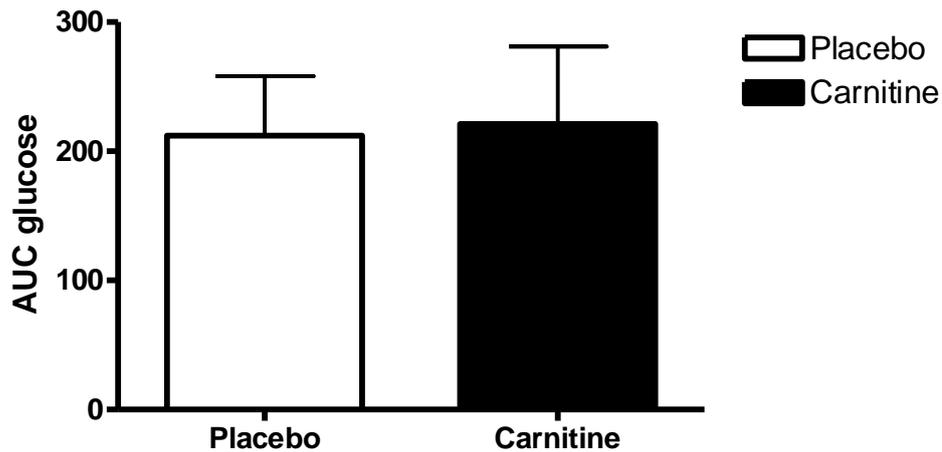
Figure 4.2.3 Area Plasma Glucose Curve after 14 days of 3g L-Carnitine L-Tartrate per day. Values are mean (SEM). Data is for n=16 as no main group effect was noted.

Plasma insulin peaked at 30 minutes for both the placebo and carnitine trials when observing all subjects (figure 4.2.4). There were however no significant differences in plasma insulin levels in response to OGTT after supplementation and no trial, or time x trial interaction but there was a significant time effect as expected ($p<0.01$). There was no between-subjects group effect ($p=0.16$) indicating that the insulin response was similar in both the lean and overweight / obese participants (figure 4.2.3). Total AUC insulin (figure 4.2.5) was also not different between trials.
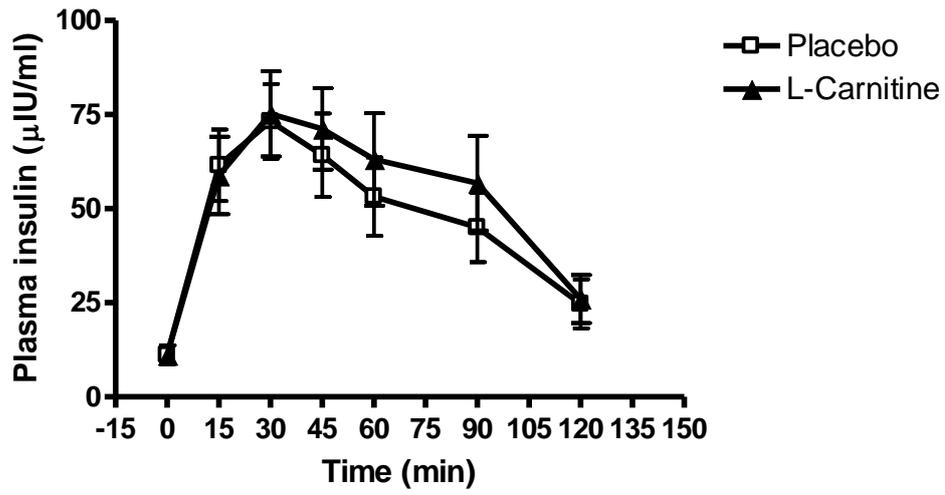
Figure 4.2.4 Plasma Insulin response to OGTT after 14 days of 3g L-Carnitine L-Tartrate per day. Values are mean (SEM). Data is for n=16.
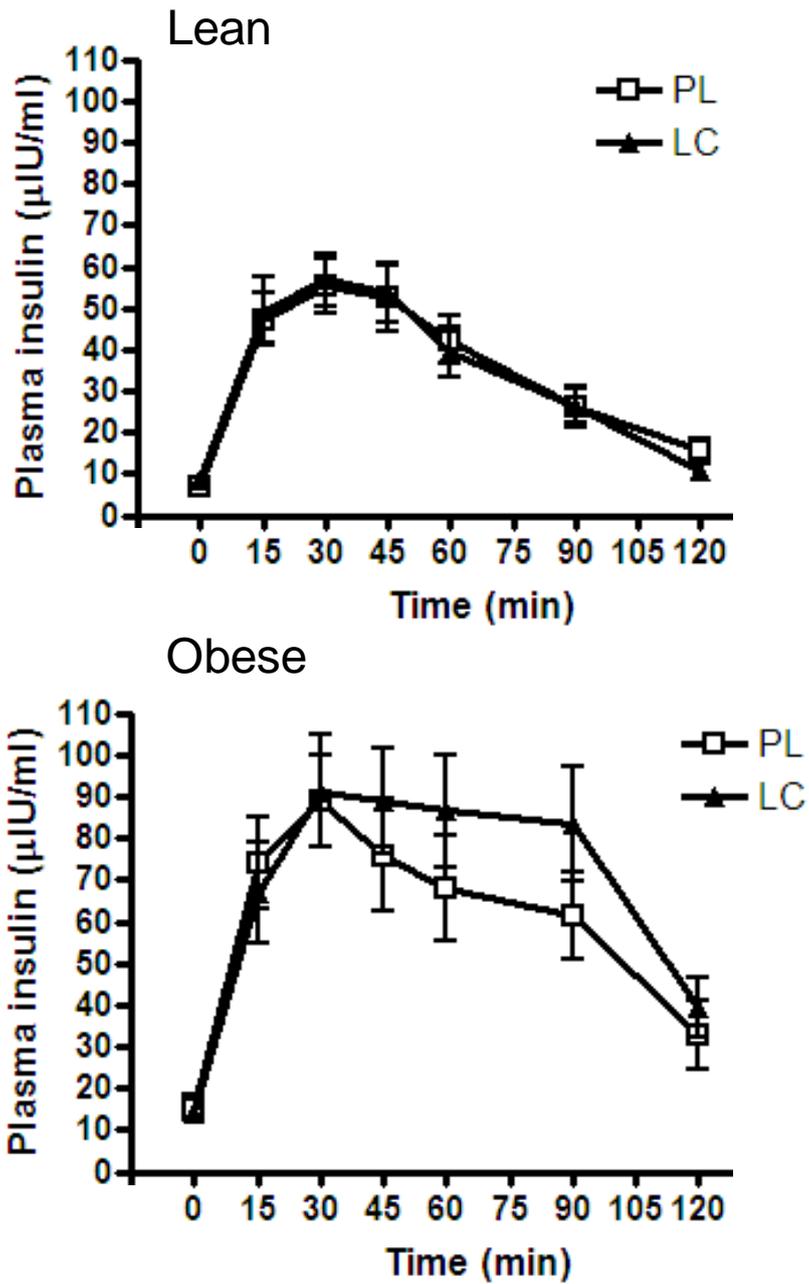
Figure 4.2.5 Plasma Insulin response to OGTT for Lean (n=8) and Obese (n=8)

subjects after 14 days of 3g L-Carnitine L-Tartrate per day. Values are mean (SEM).

Trials are placebo (PL) and L-carnitine (LC).
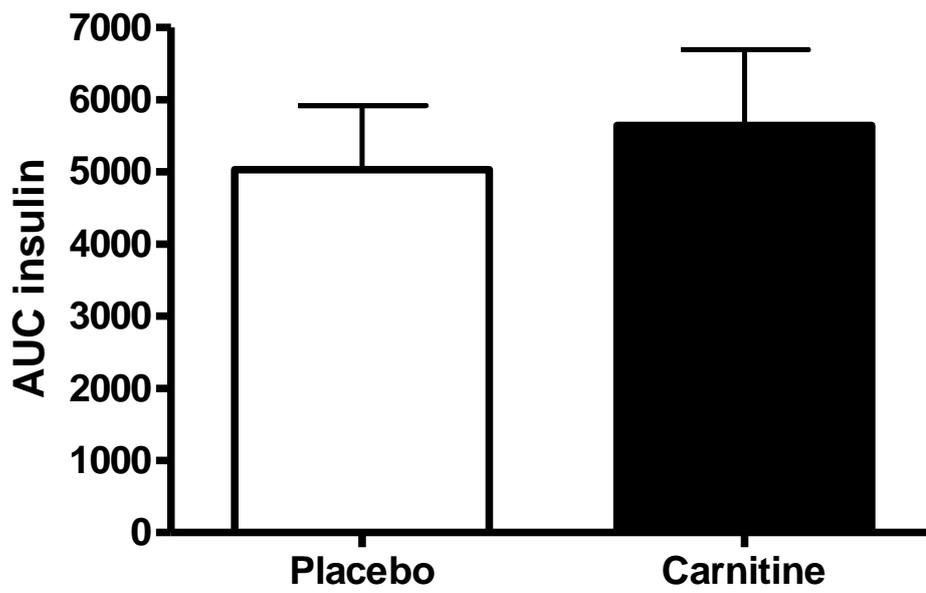
Figure 4.2.6 Area under Plasma Insulin Curve. Values are mean (SEM). Data is for

n=16 as no main group effect was noted.

4.3 Other Insulin Sensitivity Responses to OGTT

The estimated first phase and second phase β–cell function was elevated when comparing all subjects (P<0.05) as shown in figure 4.3.1.

A
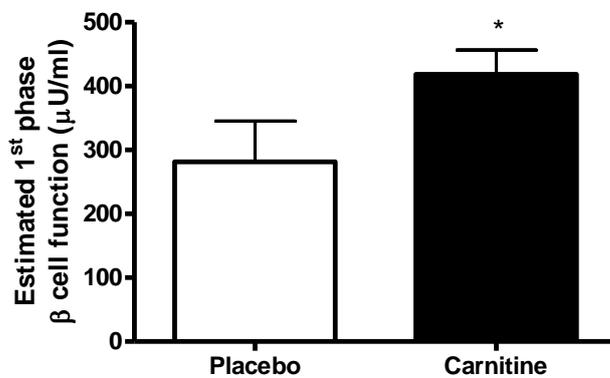
B
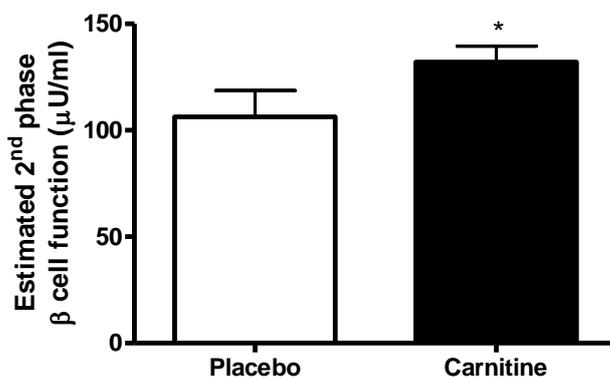
Figure 4.3.1 Estimated first (A) and second (B) phase β–cell function during the OGTT on Placebo and L-Carnitine trials after 14 days of 3g L-Carnitine L-Tartrate per day. Values are mean (SEM). * indicates significant difference from placebo. Data is for n=16 as no main group effect was noted. Calculated using the methods of Stumvoll *et al*., 2000.

There was no change in HOMA-IR (2.6 ± 0.6, PL vs. 2.7 ± 0.4, LC), estimated insulin sensitivity index (0.11 ± 0.01, PL vs. 0.11 ± 0.01, LC) or estimated metabolic clearance rate (9.4 ± 0.5, PL vs. 9.4 ± 0.5, LC) between trials and no differences in response were noted between lean and overweight / obese groups.

4.4 NEFA Responses to OGTT with Carnitine Supplementation

There was no significant main effect of trial and no trial x time interaction in the plasma NEFA response during the OGTT. There was a significant time effect ($p<0.01$) with a decline in plasma NEFA occurring throughout the OGTT (figure 4.4.1).
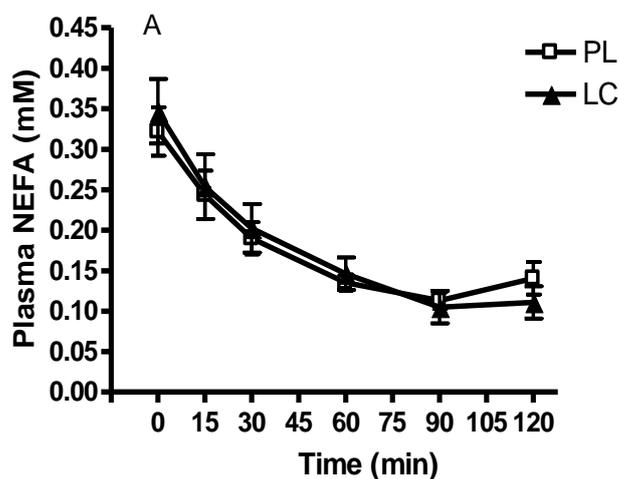
Figure 4.4.1 – Plasma NEFA response to OGTT on the L-Carnitine after 14 days of 3g L-Carnitine L-Tartrate per day and placebo trials. Values are mean (SEM). Data is for n=16 as no main group effect was noted. No significant differences between trials.
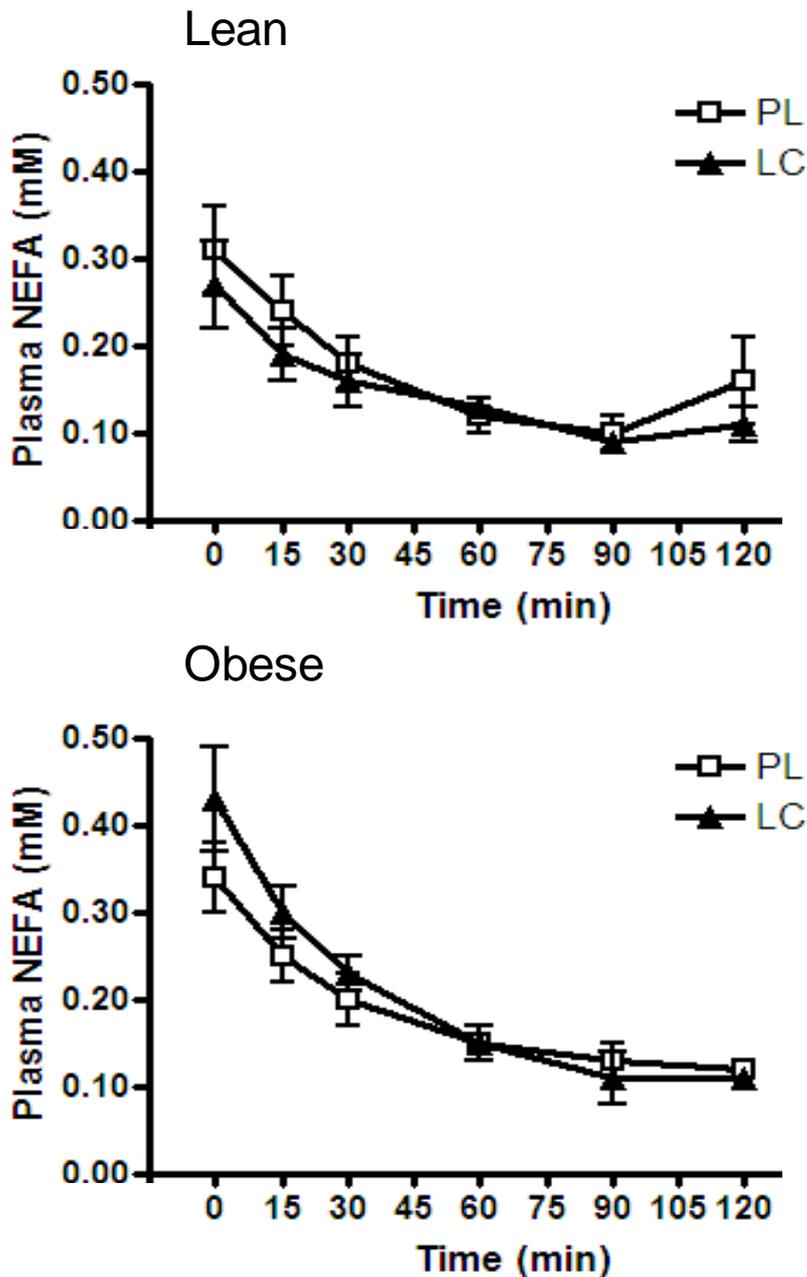
Figure 4.4.2 – Plasma NEFA response to OGTT on the L-Carnitine (LC) after 14 days of 3g L-Carnitine L-Tartrate per day and placebo (PL) trials between Lean (n=8) and Obese (n=8) subjects. Values are mean (SEM). No significant differences between trials.

4.5 Response to Exercise

End of OGTT (pre-exercise) plasma glucose concentration was not different between

trials between trials (4.97 (0.25) mM on placebo and 4.68 (0.47) mM on L-Carnitine)

and there was no significant difference between trials during exercise. However, a

trial by group interaction ($p < 0.05$) revealed that plasma glucose was significantly

lower ($p < 0.05$) in the lean group at 10 minutes of exercise on the carnitine trial

compared with the placebo trial (4.94 (0.28) mmol/L on placebo vs. 4.14 (0.36)

mmol/L on carnitine), but this effect was not observed in the overweight / obese

group (4.64 (0.15) mM on placebo vs. 4.66 (0.31) mM on carnitine). There was also a

trial by group interaction for insulin response during exercise ($p < 0.05$) which revealed

that plasma insulin was significantly higher ($p < 0.05$) in the overweight / obese group

at the end of exercise on the carnitine trial compared with the placebo trial (9.8 (0.8)

µlU/mL on placebo vs. 13.7 (1.8) µlU/mL on carnitine), but this effect was not

observed in the lean group (7.2 (0.7) µlU/mL on placebo vs. 7.2 (0.9) µlU/mL on

carnitine).

Statistical analysis revealed that there was no significant change in Heart Rate or VO_2

respectively, during 20 minutes exercise at 40% of predicted VO_2max following a 2

hour OGTT between trials. Mean (SEM) heart rate was 114 (3) b.p.m. on the placebo

trial and 111 (4) on the carnitine trial, mean (SEM) VO_2 was 1.69 (0.11) L.min^{-1} on

the placebo trial and 1.70 (0.11) L.min^{-1} on the carnitine trial but neither of these were

significant differences. Mean (SEM) RER during the steady state (40% of predicted

VO_2max) exercise period was 0.77 (0.02) on the placebo trial and 0.77 (0.02) on the

carnitine trial and as a result total carbohydrate oxidation and total fat oxidation rates were not different.
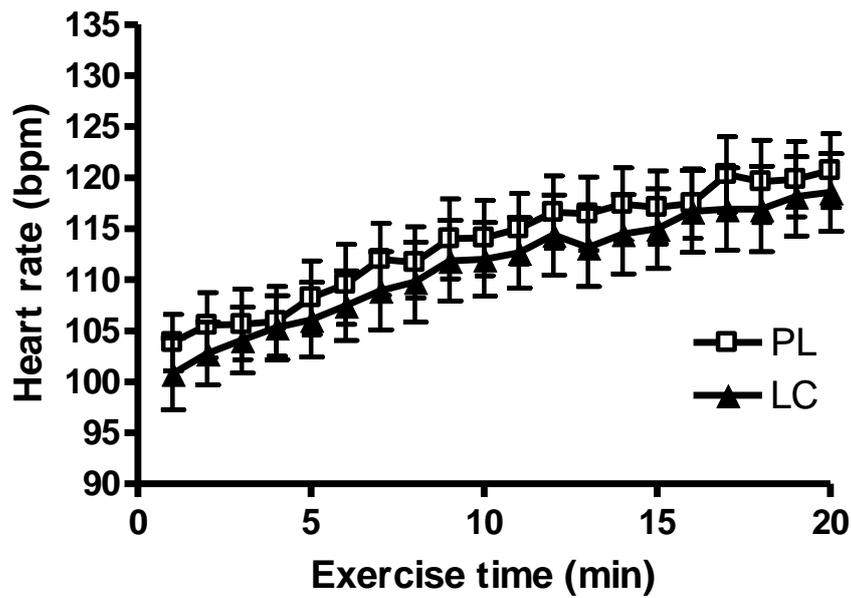
Figure 4.5.1 Heart Rate at 40% of predicted VO$_2$max following a 2 hour OGTT after 14 days of 3g L-Carnitine L-Tartrate per day. Values are mean (SEM). Data are for n=16 as no main group effect was noted.
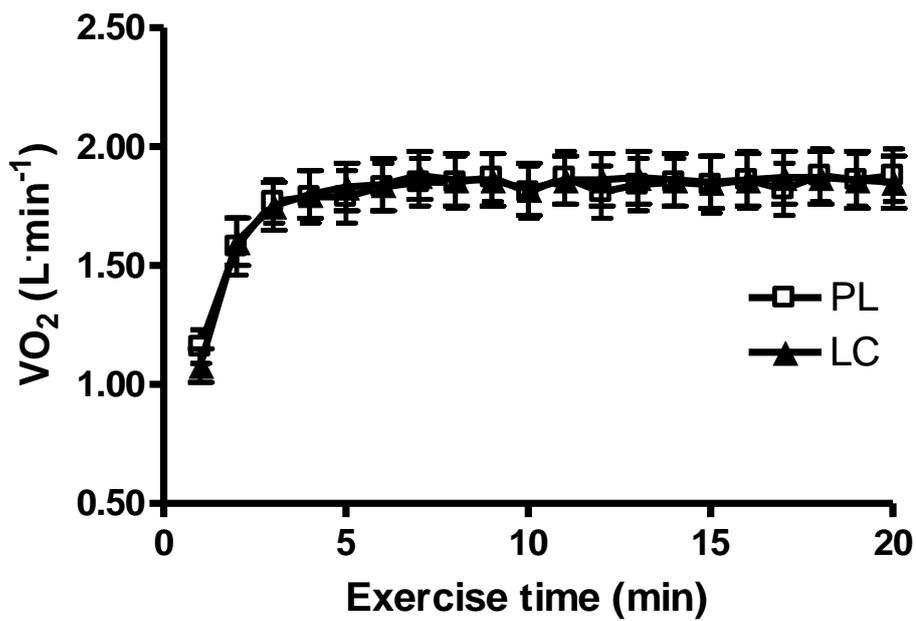
Figure 4.5.2 VO_2 at 40% of predicted VO_2max following a 2 hour OGTT after 14 days of 3g L-Carnitine L-Tartrate per day. Values are mean (SEM). Data are for n=16 as no main group effect was noted.

There was no difference at the end of OGTT (pre-exercise) plasma NEFA concentration between trials and no difference in the end of exercise concentrations (exercise t=20) as shown in figure 4.5.3.
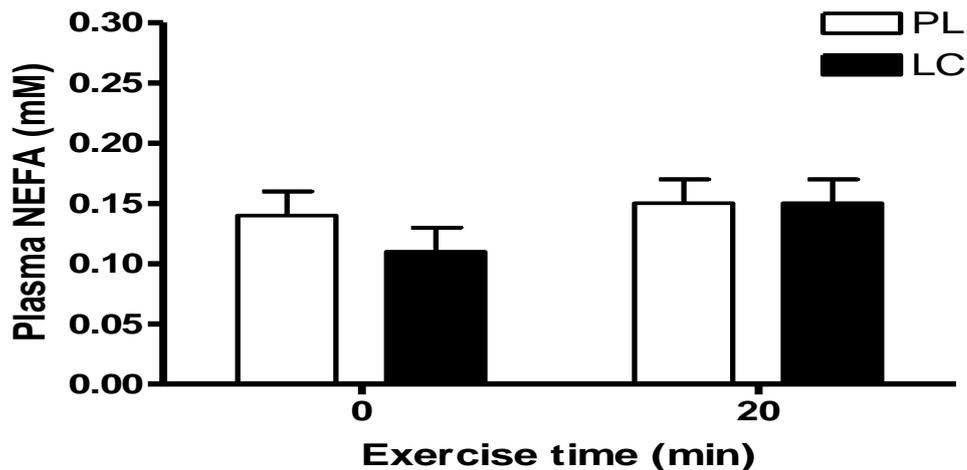
Figure 4.5.3 – Plasma NEFA response to submaximal exercise on the placebo and carnitine trials after 14 days of 3g L-Carnitine L-Tartrate per day. Date is for n=16 as no main group effect was noted. Values are mean (SEM). No difference between trials.

4.6 Correlations Between Change in Variables in Response to Carnitine Supplementation with BMI, Body Fat and Predicted VO$_2$max

There were significant correlations between the change in AUC glucose with BMI, estimated % body fat and predicted VO$_2$max (Table 4.6.1). A positive correlation was noted for change in AUC glucose with BMI and % body fat with a lower BMI and a lower % body fat being associated with a lower (more negative) change in AUC glucose. A negative change in AUC glucose means a lower total AUC glucose on the carnitine trial compared with placebo. However, a negative correlation was observed for change in AUC glucose to predicted VO$_2$max with those having a higher predicted VO$_2$max also having a lower (more negative) change in AUC glucose. In addition, change in AUC glucose was significant positively correlated with change in AUC insulin as expected (Pearson correlation 0.55, p<0.05) showing that a lower AUC

glucose response was associated with a lower total AUC insulin in the OGTT on

carnitine.

Table 4.6.1 – Correlates of change (Δ) in insulin sensitivity indices and calculated
parameters following carnitine supplementation to BMI, % body fat and predicted
VO$_2$max. Values are Pearson correlation coefficients (p-value). N = 16

Variable	BMI	% Body Fat	predicted VO$_2$max
ΔAUC glucose	0.58 (0.02)*	0.65 (0.01)*	-0.65 (0.01)*
ΔAUC insulin	0.34 (0.22)	0.42 (0.12)	-0.30 (0.28)
Δ1st phase β-cell function	0.00 (0.99)	-0.10 (0.72)	-0.05 (0.86)
Δ2nd phase β-cell function	0.00 (0.99)	-0.10 (0.72)	-0.07 (0.81)
ΔInsulin Sensitivity Index	-0.37 (0.20)	-0.46 (0.10)	0.46 (0.10)
ΔMetabolic Clearance Rate	-0.39 (0.17)	-0.48 (0.09)┼	0.46 (0.10)
ΔHOMA-IR	0.07 (0.83)	0.14 (0.65)	-0.05 (0.88)

*significant correlation between variables. ┼ tendency to be significant (p<0.10)

Chapter 5 – Discussion

5.1 Main Findings

The main finding of the present study was that two weeks supplementation of L-Carnitine L-Tartrate (3g/day) significantly reduced peak glucose when assessed using an oral glucose tolerance test. This was more apparent in lean, active individuals than obese / sedentary subjects. Other findings included that β cell function (1st phase and 2nd phase) was increased after LC supplementation, however this was calculated using the methods of Stumvoll *et al*., 2000 of which the peak glucose concentration plays a direct part.

5.2 Carnitine and glucose disposal / oxidation

One proposed mode of action for the reduced peak glucose shown after LC supplementation could be an increased uptake and storage of glucose in skeletal muscle. Stephens *et al*. (2006), increased skeletal muscle carnitine content and observed a decrease in muscle lactate content and PDC activity and increased glycogen storage in conditions of high carbohydrate availability. The authors attributed the response to a possible carnitine-mediated increase in muscle fat oxidation, as muscle LCA-CoA was increased. Although muscle carnitine was not measured in the present study, it would have been unlikely in the current study that muscle carnitine was altered as oral supplementation in excess of 100 days may be required to alter muscle carnitine concentrations (Stephens *et al*., 2007c). Therefore an alternative mechanism for increased glucose disposal could be proposed that would fit the current observations and those of Stephens *et al*. (2007c) of carnitine mediated glucose uptake.

The blunting of peak glucose concentration to a glucose challenge following LC supplementation despite similar insulin release in the current study indicates that plasma carnitine levels may have direct insulin like effect. This direct insulin like effect may be on tissues such as skeletal and cardiac muscle, or could play a metabolic role in buffering acyl groups in β-cell mitochondrial metabolism which would act to maintain glucose oxidation and thus potentially enhance insulin release for a given glucose concentration (Gonzalez-Ortiz *et al*., 2008). Newsholme *et al*., (2005) suggests that amino acids which are co-transported with Na^+, can also depolarize the cell membrane as a consequence of Na^+ transport and thus induce insulin secretion by activating voltage-dependant Ca^{2+} channels. The work of Stephens and colleagues ((Stephens *et al*., 2005; Stephens *et al*., 2006; Stephens *et al*., 2007b; Stephens *et al*., 2007c) has shown the skeletal muscle carnitine accumulation can be manipulated close to the upper physiological limit difficult (Stephens *et al*., 2007b) to achieve by creating a serum insulin concentration greater than 90 mU/L. This is in concordance with the hypothesis that insulin would augment Na^+-dependant skeletal muscle carnitine transport via OCTN2 (Newsholme *et al* 2005), secondary to it's action of increasing sarcolemmal Na^{+-} K^+ ATPase pump activity and therefore intracellular Na^+ flux. There theory is enhanced given the change in glucose disposal in combination with no significant differences in insulin levels (Figures 4.2.2 and 4.2.5).

The potential for LC to reduce peak plasma glucose through a direct insulin like action is supported by previous observations of insulin like actions of physiological concentrations of carnitine on rat cardiac muscle (Rodgers *et al*., 2001) and the

observed increased skeletal muscle glycogen storage following carnitine infusion in humans (Stephens *et al*., 2006). The insulin like action of L-Carnitine could explain lower peak glucose observed during the OGTT in the present study if there was no increased non-oxidative glucose disposal but the absence of skeletal muscle biopsy data does not allow us to confirm whether our likely modest increase in fasting plasma carnitine concentration of between 50-100% of normal fasting concentration (Galloway and Broad, 2005) is sufficient to induce this action.

Stephens *et al*., (2006) estimated that the difference in skeletal muscle glycogen content (glucosyl units) between control and carnitine at 24h was approximately 170 Mmol/kg dry muscle or 40mmol/kg wet muscle. Because of carnitine's role in long chain fatty acid translocation (Reda *et al*., 2003; Karlic and Lohninger 2004), it is entirely plausible that a reduction in PDC activation could have been caused by a carnitine mediated increase in skeletal muscle activation via CPT I (Stephens *et al*. 2006). The decrease of PDC activity after the insulin clamp in their trial was paralleled by a reduction in muscle lactate content and resulted in an accumulation of muscle glycogen overnight, conditions that are consistent with the idea that carbohydrate oxidation was inhibited. In support of this theory, muscle Long chain acetyl-CoA content returned to basal overnight during the carnitine visit (whereas is remained suppressed during the control visit), which would also suggest an increase in fat oxidation.

In contrast to this suggestion, the results of the Stephens *et al*. (2006) study could also suggest a direct effect of intracellular carnitine on glycogen synthase activity and therefore rates of glycogen synthesis. An increase in glycogen synthesis would

reduce the amount of glucose entering glycolysis under insulin clamp conditions and therefore pyruvate and lactate accumulation and PDC activity. The decrease in muscle lactate content and PDC activity was evident however, before the increase in muscle glycogen content. Thus this mechanism is unlikely.

Similarly to the present study, De Gaetano *et al*. (1999) observed that carnitine increased the rate of glucose disposal compared with placebo. De Gaetano *et al*. (1999) used intravenous carnitine infusion to increase circulating carnitine levels and whole body-glucose uptake was measured using the Frequently Sampled I.V. Glucose Tolerance Test. Their results support the existence of an insulin-independent action of carnitine while not excluding the possibility of an associated insulin-independent action as well. Glucose oxidation rate, calculated through indirect calorimetry, was significantly increased after carnitine intravenous bolus. The authors acknowledge that results obtained from the calorimetric analysis have to be interpreted with caution. They also proposed no modes of actions for the results that they observed.

De Gaetano *et al*. (1999) postulate that carnitine might play a role in the therapy of diabetes mellitus by improving insulin sensitivity and work has been completed using clinical populations (Mingrone *et al*. 1999; Ferrannini *et al.* 1988; Capaldo *et al*. 1991; Giancaterini *et al*. 2000; Kelly, 2000; Heller et al 1986; Gunal 1999; Ahmad *et al*. 1990; Brevetti *et al* 1988; Plyioplys and Plyioplys, 1997). Whilst studying patients with type II diabetes Giancaterini *et al*. (2000) intravenously increased acetyl L Carnitine plasma concentrations and observed a higher glucose uptake. No significant effect was demonstrated on glucose oxidation or lipid oxidation even with high doses of Carnitine infusion.

In addition, Rahbar *et al*., (2005) have observed lowering of fasting glucose following 12 weeks of carnitine supplementation in patients with type II diabetes, and Power *et al*., (2007) have subsequently also confirmed these observations in a mouse model and suggest that carnitine could be used as an adjunctive therapy in diabetes. Furthermore, Poorabbas *et al*., (2007) have observed that plasma carnitine concentration is significantly lower in patients with complications associated with their type II diabetes (retinopathy, neuropathy and hyperlipidaemia). However, some confusion exists in the literature due to other poorly powered studies (n=6) that have not observed these beneficial actions of carnitine in type II diabetes patients. Taken together, the results of the previous studies discussed here points towards an extracellular effect of carnitine most likely influencing glucose uptake by tissues such as cardiac and skeletal muscle or liver.

One point the authors consider is the availability of ALC at the intracellular effector level. Two pools of acetyl-coa molecules coexist in the cell: the larger one is located in the mitochondria and the smaller one in the cytosol. The mitochondrial acetyl-CoA pool derives directly from the β-oxidation of long chain acyl-CoA and indirectly from pyruvate decarboxylation catalyzed by the PDC, ALC infusion very likely produces an increase in the acetyl-CoA cytosolic pool and simultaneously increases the ALC cellular concentration, forcing the passage of the acetyl moieties across the mitochondrial membrane. Therefore, an inhibition of intramitochondrial acyl-CoA β-oxidation would occur with a subsequent reduction of acetyl-CoA formation. As observed in animals, the natural consequence of this process should be an inhibition

of FFA oxidation and a simultaneous increase of glucose storage (Mingrone *et al*., 1999).

Mingrone *et al*. (1999) observed that glucose oxidation, evaluated by indirect calorimetry measurements, significantly increased in type II diabetic patients after carnitine infusion, while in normal controls glucose oxidation under carnitine was not significantly modified from the saline infusion value. Carnitine was administered by intravenous infusion. L-carnitine infusion also stimulated glucose uptake in both healthy and type II diabetic patients. In type II diabetic patients, in whom a defect of the PDH activity is present, L-carnitine would normalize PDH activity, thus stimulating oxidative utilization of glucose. In other words, when PDH is normally active, as in the case of the healthy subjects, the excess glucose uptake induced by the carnitine could be accumulated in the cells as glycogen. When it overcomes the capacity of the cell (already highly stimulated by insulin) to oxidize glucose: on the contrary, when PDH activity is impaired, such as in diabetic patients (Mingrone *et al*. 1999), L-carnitine would enhance it.

Mingrone *et al*. (1999) support this hypothesis by analysing evidence that derives from the observed fall in plasma concentration of lactate in L-carnitine-infused diabetic patients. An important enzyme in catalyzing the rate-limiting step in lactate utilization is PDH, and a decrease in PDH activity would result in a reduction of the oxidation of pyruvate to acetyl-CoA and could contribute to an increased conversion of pyruvate to lactate via lactate dehydrogenase (Mingrone *et al*., 1999). Since there is evidence that L-carnitine stimulates the activity of PDH (Broderick *et al*., 1991), the observation of an increase in oxidative glucose utilization during L-Carnitine

infusion in diabetic patients (Mingrone *et al*., 1999) could be explained by carntines ability to overcome the defect of PDH activity in type II diabetic patients.

The mechanism proposed underlying the improvement in glucose disposal induced by intravenous carnitine infusion in type II diabetic patients by Capaldo *et al*. (1991) again focuses around the majority of glucose taken up by peripheral tissues being directed towards glycogen synthesis or complete oxidation. They also showed a pronounced decline in lactate concentration during carnitine infusion mirroring that shown by Mingrone *et al*. (1999).

5.3 Combined Carnitine and Carbohydrate Supplementation

It has been shown (Stephens *et al*. 2007a) that plasma total carnitine concentration and urinary total carnitine excretion were significantly reduced when L-carnitine ingestion was accompanied by carbohydrate feeding. The conclusion from this particular work was that carbohydrate feeding augments whole body carnitine retention in humans. Given that skeletal muscle is the major site of carnitine storage within the body (Brass, 1995), and that maintaining hypercarnitinemia for 5 hours in the presence of hyperinsulinemia increases skeletal muscle total carnitine accumulation (Stephens *et al*. 2005; Stephens *et al*. 2007c) as was achieved in this study (Stephens *et al*. 2007a), it is not unreasonable to suggest that the greater retention occurred mainly in the muscle. This finding is in concordance with the hypothesis that insulin, released as a consequence of carbohydrate ingestion, would augment Na^+-dependent skeletal muscle carnitine uptake by increasing $Na^{+-}K^+$ ATPase pump activity in humans (Stephens *et al*. 2005; Stephens *et al*. 2006;

Stephens *et al*. 2007b; Stephens *et al*. 2007c). Essentially, elevated plasma carnitine

concentration together with carbohydrate ingestion would promote an increase in

glucose uptake.

Although subjects in the present study were advised to take 2 x 0.5g L-Carnitine L-

Tartrate three times a day (3g/day) with a meal, this was not tightly controlled. Also,

total carnitine concentration and urinary total carnitine excretion were not measured.

Thus, to credit the decrease in peak glucose concentration to an increase in muscle

glycogen synthesis (Stephens *et al*., 2006) stimulated by increased muscle carnitine

concentration would be conjecture.

5.4 Exercise Response

All subjects completed 20 minute exercise at 40% of predicted VO_2max after the 2

hour OGTT. There was no difference in the end of OGTT (pre-exercise) plasma

glucose concentration and plasma NEFA concentration between trials and no

difference at the end of exercise. There was also no significant difference between

heart rate, RER and VO_2 during 20 minutes of steady state exercise at 40% predicted

VO_2max. Thus, although increased glucose disposal was observed at rest, LC had no

effects on exercise metabolism.

Although nor significant, heart rate did appear to be lower at each time point during

the exercise on the L-Carnitine trial (figure 4.5.1). A trial by group interaction

revealed that blood glucose was significantly lower in the lean group after 10 minutes

of exercise after carnitine supplementation compared with the placebo trial and a trial

by group interaction also revealed that plasma insulin was significantly higher in the overweight / obese group after carnitine supplementation compared with placebo.

Rodgers *et al*., (2001) showed for the first time that a physiologically relevant concentration of carnitine exerts insulin-like metabolic effects on the intact heart. The actions of 50μM carnitine and 75 mU/L insulin were very similar on all measurements and specifically carnitine mimicked insulin's actions on glycolysis, glucose oxidation, pyruvate oxidation and palmitate oxidation with both timing and duration of responses similar. The heart rate results observed in the present study may be attributed to the increase rate of glycolysis as observed by Rodgers *et al*., (2001).

No change in RER, VO_2 muscle lactate, plasma lactate and muscle glycogen was observed at low intensity and high intensity exercise in a previous study (Brass *et al*., 1994) which used varying doses of 2 hours intravenous carnitine infusion but without glucose ingestion. The authors argue that carnitine does not modify any important parameters of fuel metabolism during exercise in healthy subjects, attributed to the fact that short term administration of carnitine would have little impact on muscle carnitine metabolism (Fritz, 1967; Brass *et al*., 1994; Stephens *et al*,. 2005; Stephens *et al*. 2007a; Stephens *et al*. 2007b; Stephens *et al*. 2007c; Engel *et al*. 1984; Wachter *et al*. 2002; Hultman *et al*. 1991; Brass *et al*., 2000). Brass *et al*., (2004) conclude that short term administration of carnitine has no effect on exercise physiology in normal subjects and that the efficacy of carnitine in modifying exercise performance must therefore reflect either a nonmuscle site of action, altered muscle carnitine handling in

pathophysiologic states or altered muscle carnitine content caused by long term carnitine administration (as supported by Stephens *et al.* 2007c).

It has previously been shown that a combination of hypercarnitinemia (~500 μmol/l) and hyperinsulinemia increased muscle total carnitine content (Stephens *et al.*, 2005). This particular Stephens study (Stephens *et al.*, 2005) demonstrated that insulin could acutely increase muscle total carnitine content in humans during hypercarnitinemia, which is associated with an increase in OCTN2 transcription.

Stephens *et al.*, (2006) later established that a threshold exists for the stimulatory effect of insulin on plasma L-Carnitine clearance in humans. They concluded that insulin can stimulate plasma carnitine clearance, most likely into skeletal muscle, but only when insulin is present at a physiologically high concentration (≥ 90 mU/l). It must be noted however, that muscle carnitine was not directly measured by Stephens *et al.* (2006).

In the present study, subjects were advised to supplement 3g/day L-Carnitine L-Tartrate (3 x 1g with meals). Although these meals were not controlled (except for asking of a repetition between trials) subjects would be experiencing increased insulin concentrations at the point of carnitine administration. During the OGTT trials, plasma insulin reached 76.5 mU/l and 79.6 mU/l for the placebo and carnitine trials respectively after oral administration of 75g glucose. Although, not at the level Stephens *et al.*, (2006 insulin) found to stimulate plasma carnitine clearance (≥ 90 mU/l), perhaps the repeated administration of carnitine with meals and the resulting increase in insulin may promote a similar effect. It must however be acknowledged

that for oral administration of L-Carnitine in the present study, it has been concluded that over 100 days of supplementation may be required to increase muscle carnitine concentration (Stephens *et al*., 2007c). For this reason, we can conclude that any effects we have observed will be due to an action of carnitine outside the muscle.

Work examining 2 weeks L-Carnitine supplementation saw enhanced carbohydrate metabolism in males working at 64% VO_2max for 30 minutes (Abramowicz and Galloway, 2005). The relevance to the current study is that carnitine was administered orally (3g/day) with a carbohydrate based meal. It does thus seem as if supplemental carnitine ingested with carbohydrate over a period of two weeks can increase carbohydrate oxidation at rest (Ferrannini *et al*., 1988) and exercise (Abramowicz and Galloway, 2005). This has also been demonstrated in working heart models where increases in glucose oxidation have been observed when carnitine is added to the perfusate (Broderick *et al*., 1992; Lopaschuk, 2000). Thus an increase in carbohydrate oxidation could be observed if greater glucose disposal was leading to greater glycogen storage (Stephens *et al*., 2007a).

Wachter *et al*., (2002) increased skeletal muscle carnitine content by 17% in healthy moderately trained males after 3 months supplementation, but this was not a significant result. Again, like the previous study there was no effect during exercise at 40% VO_2max on VO_2, RER or Heart Rate. There was also no change at exercise intensities of 20% and 60% VO_2max and no effect on blood lactate.

Short term carnitine administration (2 hours intravenous administration of 185 µmol/kg carnitine) has shown no effect on exercise physiology in normal subjects

(Brass *et al*., 1994). Perhaps for short term administration as was administered in the

Stephens *et al*., (2007b) and the Brass *et al*., (1994) studies, the plasma insulin levels

may be required to be manipulated to a physiologically high concentration ($\geq$90

mU/l). It has also been demonstrated that as exercise intensity increase to > 70%

VO_2max, muscle free carnitine falls to ~ 30% (5.6 mmol/kg dm) of the total carnitine

store and is paralleled by a 35% decrease in the rate of fat oxidation (van Loon *et al*.

2001). Exercise intensity and training status may also play an important element in

the response to carnitine supplementation as these are key determinants of substrate

selection (Wachter *et al*., 2002; Karlic and Lohninger, 2004; Bacarau *et al*., 2003).

Carnitine administration did not increase muscle carnitine content or physical

performance when administered long term (2 x 2g/day for three months) in

moderately trained humans (Wachter *et al*., 2002) however high intensity exercise

resulted in a significant increase in plasma short-chain acylcarnitine concentration

compared with low intensity exercise. Trained rats also showed an increase in

mitochondrial carnitine after supplementation when compared with sedentary rats

(Bacarau *et al*., 2003).

In summary, the observations in the present study that may be as a result of the

hypothesis that carnitine is not modifying any important parameters of fuel

metabolism during exercise in healthy subjects, attributed to the fact that short term

administration of carnitine would have little impact on muscle carnitine metabolism

(Fritz, 1967; Brass *et al*., 1994; Stephens *et al,*. 2005; Stephens *et al*. 2007a; Stephens

et al. 2007b; Stephens *et al*. 2007c; Engel *et al*. 1984; Wachter *et al*. 2002; Brass *et al*.

2004; Hultman *et al.* 1991; Brass *et al*., 2000). The present study was a two week

dose of oral L-Carnitine L-Tartrate, but as we have previously discussed, this protocol would have been unlikely to impact upon muscle carnitine metabolism.

At the onset of exercise, signals from inside and outside the muscle cell increase the availability of carbohydrate and fat to provide the fuel required for ATP production. There is room for shifts between the proportion of energy that is provided from CHO and fat. It has long been known that increasing the availability of endogenous or exogenous CHO can increase the oxidation of CHO and decrease the oxidation of fat (Spriet and Watt, 2003).

In the current study, the lean population were also active and this may explain the greatest glucose lowering effect of supplementation. Glucose ingestion before exercise results in increased plasma glucose and insulin, PDH activity and reduced plasma Free fatty acids (Spriet and Watt, 2003). Coyle *et al*. (1997) demonstrated reduced entry of long chain fatty acids into the muscle and the mitochondria following CHO ingestion in endurance trained men.

5.5 Possible Mechanisms of Action

It would not be unreasonable to propose that an increase in the muscle carnitine pool following prolonged oral supplementation may in part be related to both exercise training status and combined carnitine and carbohydrate meal ingestion (Abramowicz and Galloway, 2005). Carnitine supplementation increased the total amount of carnitine in the mitochondria by 49.4% in sedentary rats and by 79.5% in trained ones

indicating that the association between supplementation and moderately-intensity training seems to be much more efficient in promoting carnitine incorporation into soleus muscle cells (Bacarau *et al*., 2003).

5.6 Fat Oxidation Response

In the present study, there was no significant main effect of trial and no trial x time interaction in the plasma NEFA response during the OGTT. There was also no difference at the end of OGTT (pre-exercise) plasma NEFA concentration between trials and no difference in the end of exercise concentrations as shown in figure 4.5.3. Exercise RER was not different further indicating no effect on fat metabolism at an exercise intensity 40% VO_{2max} after L-Carnitine supplementation. This supports the majority of the literature in that oral carnitine supplementation does not influence fatty acid mobilisation (Hultman *et al*., 1991; Broderick *et al*. 1992; Millington *et al*., 1993; Brass *et al*., 1994; Hawley *et al*., 1998; Karlic and Lohninger, 2004; Maughan *et al*., 2004, Abramowicz and Galloway, 2005).

Muller *et al*., (2002) however did observe that L-Carnitine significantly increased fatty acid oxidation. Muller *et al*., (2002) assessed fatty acid oxidation by the cumulative $^{13}CO_2$ exhalation method. Following the same protocol as Muller *et al*., (2002), Wutzke and Lorenz (2004) also observed increased fatty acid oxidation by the cumulative $^{13}CO_2$ exhalation method in obese subjects. Both studies however measured their subjects at rest whereas fat oxidation rates in the present study were measured during exercise using indirect calorimetry. Supplementation was similar (3g/day) in all studies (for 14 days in the present study and 10 days in the Muller *et al*., (2002), Wutzke and Lorenz (2004) studies) but the present study used male

subjects only. Both methods of measurement are equally reliable, thus more work is required in the future using the protocol of the present study but measuring whole body indirect calorimetry at rest as well as exercise. Wutzke and Lorenz (2004) acknowledge that the measured $^{13}CO_2$ exhalation over 14 hours reflects mainly the dietary fat oxidation and that their observation of an increased fat oxidation during LC supplementation does not correspond to the findings of unchanged body fat mass, total body water, lean body mass and body weight within their supplementation period. Muller *et al*. (2002) attribute their finding to increased serum carnitine concentrations after supplementation but proposed no mechanisms for their observed increase in fatty acid oxidation.

5.7 Potential Role of Incretins in Response to Carnitine

The gastrointestinal tract is increasingly viewed as a critical organ in glucose metabolism because of its role in delivering glucose to the circulation and in secreting multiple glucoregulatory hormones that, in concert with insulin and glucagon, regulate glucose homeostasis (Maggs *et al*., 2007). A change in gastrointestinal tract function, or a change in gastric emptying may also explain the plasma glucose concentration results observed. Indeed, a slowing in the rate of gastric emptying would also appear to explain what seems to be a delayed insulin response and / or slower appearance of glucose into the circulation. Gastric emptying is a complex process, dependent on many factors including the physiochemical composition of food, the integration of motor activity in the stomach and duodenum, the nutrient- and mechanoreceptor-mediated feedback from receptors in the lumen of the small intestine and the secretion of gastrointestinal hormones (Hunt *et al*. 1968; Horowitz *et*

al., 1994). Prior nutrient intake may influence subsequent gastric emptying function (Maggs *et al*., 2007).

Glucose-dependent inhibitory peptide (GIP) and glucagon- like peptide-1 (GLP-1) are incretin hormones that are released in response to carbohydrate- and lipid containing meals (Herrmann-Rinke, *et al*., 1995; D'Alessio, *et a*., 2004). GIP has lost most of its insulinotropic activity in patients with type 2 diabetes: GLP-1 in contrast, shows promise as a potential diabetes treatment primarily because of its ability to enhance glucose-stimulated insulin secretion, slow gastric emptying and inhibit glucagon secretion, all of which act to regulate plasma glucose concentrations in patients with type 2 diabetes (Maggs *et al*., 2007). However, little is known about incretin responses in healthy and overweight / obese populations of the potential role of carnitine on secretion of incretin hormones. In relation to the current study, the incretin principles are of interest but the question of what potential effects may be occurring in healthy active, and overweight sedentary populations must be raised.

As previously discussed, the blunting of peak glucose response to a glucose challenge following LC supplementation despite similar insulin release in the current study indicates that plasma carnitine levels may have a direct insulin like effect. The potential for LC to reduce peak plasma glucose through a direct insulin like action is supported by previous observations of insulin like actions on rat cardiac muscle (Rodgers *et al*., 2001) and the observed increased skeletal muscle glycogen storage following carnitine infusion in humans (Stephens *et al*., 2006). With GLP-1's ability to enhance glucose-stimulated insulin secretion, slow gastric emptying and inhibit glucagon secretion, there is potential that carnitine may influence GLP-1. Potentially,

an increased incretin response (GLP-1) could promote an increase insulin response for a given glucose concentration and explain the observations in the present study of a lower peak glucose concentration after carnitine supplementation whilst insulin levels remained unchanged.

5.8 Limitations of Study

It must be acknowledged that the plasma glucose and insulin samples were frozen immediately after separation. Giampeitro *et al*., (1980) showed a significant positive relation, although minimal, between storage interval and percentage decrease in glucose concentration when frozen at $-20^{o}C$. In the present study, prompt separation after blood withdrawal would have avoided glucose degradation, and storage at $-80^{o}C$ would also have alleviated this issue.

It must also be acknowledged that no muscle tissue was collected and plasma carnitine concentrations were not measured.

Chapter 6 - Conclusion

6.1 Conclusion

Although the present data does not allow concrete conclusions to be drawn as to the mechanisms responsible for the results, we have shown that 2 weeks L-Carnitine L-Tartrate supplementation can decrease peak glucose levels during an OGTT despite similar insulin responses between control and L-Carntine trials. This may be attributed to either a decreased rate of gastric emptying, an increased incretin response, with GLP-1 being of particular interest, or increased oxidative or non-oxidative glucose disposal into skeletal muscle. These results may also be attributed to a direct insulin like carnitine response, although with the supplementation timescales, it is unlikely that muscle carnitine concentration would increase, but the dose administered should be sufficient to raise resting plasma carnitine levels by around 50% - 100%.

6.2 Recommendations for Future Study

It is recommended that oral L-Carnitine supplementation warrants further investigation following the protocol used in the present study. Of particular interest, would be plasma carnitine levels, muscle carnitine concentrations and muscle glycogen levels. By also measuring incretin response and carnitine effects on β-cell function in vitro, this would help to either confirm or deny that incretins, especially GLP-1, or β-cells were contributing to an insulin like effect due to carnitine supplementation. Rate of gastric emptying data would also provide interesting reading.

Should carnitine be shown to have a direct insulin like effect, then further research should be conducted looking at the practical implications of such findings for clinical situations such as subjects with impaired glucose tolerance, and type II diabetes.

It is also recommended that further study is warranted focussing on an elite athlete population. The hypothesis that carnitine supplementation may increase muscle glycogen holds potential to improve endurance performance or recovery from exercise especially at the elite level where small differences could be the difference between winning and losing.

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The effects of L-carnitine supplementation on response to glucose ingestion and on fat metabolism at rest and during exercise.

Principle Investigator: Thomas Craig

Other Investigators: Dr Stuart Galloway

You are being invited to take part in a research study. Before you decide to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

There has been an increasing interest in the role that L-carnitine supplementation may play in the fuels utilised by the body. Some reports suggest that it may enhance fat metabolism whereas others suggest that it may be the opposite and enhance glucose (carbohydrate metabolism) The purpose of this study is thus to test the theory that the use of L-carnitine supplementation may improve insulin sensitivity, enhance whole body glucose uptake, increase glucose storage and possibly improve fat metabolism at rest or during exercise.

Why have I been chosen?

You have been chosen as you may have a BMI (Body Mass Index) that suggests you are overweight and sedentary or that you are lean and active.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What do I have to do?

This study will require you to attend the Gannochy Sports Centre at the University of Stirling on 4 separate occasions over a five week period. The first visit will involve a predicted maximal aerobic test using a cycle ergometer (gym cycle) to measure your predicted maximum exercise capacity. This will necessitate you to cycle at increasing workloads while we monitor your heart rate and breathing. We have chosen to use a predicted maximal aerobic test in order to decrease any stress or discomfort you may feel. The visit will last approximately 40 minutes.

The next three visits will each last approximately two and a half hours. On each of these visits you will be asked to drink a carbohydrate drink. Over the next three hours, small samples of blood will be taken via an indwelling venous cannula (i.e. from a small plastic tube positioned in an arm vein). This is similar to a hospital visit but smaller samples of blood (6ml) will be taken. After this, we will ask you to undertake 20 mins of exercise on the cycle ergometer at up to 40% of your predicted maximal oxygen capability. During this time, we will monitor whether your body is metabolising fat or carbohydrate by measuring your breathing.

Between visits two and three, you will be given either L-carnitine L-tartrate or a placebo (a placebo is almost like a fake tablet but with no L-carnitine in it). You will not know which you are taking, as they will be randomly selected. Between visits three and four you will be given either L-carnitine L-tartrate or a placebo. Again you will not be made aware of what you are taking until after you have completed the study. You will be given instructions on how and when to take the supplements and we will follow this up with phone calls during this time to check that everything is fine.

To standardise the trials you will be required to report to the sports centre at the same time of day for each visit. You will be asked to produce a food diary and exercise diary during the time of the study, and we wish you to adhere to the same food intake and exercise for at least the three days before each visit. You must not eat in the morning of each visit. This is known as an overnight fast and helps us standardise tests. You should try to lead the same lifestyle as you did before the study, but avoid alcohol for at least two days before each visit.

Will I experience any problems?

It is very unlikely you will experience any problems. During the predicted maximal exercise capacity test you may experience some discomfort if you are not used to exercising. During the final three visits you may experience slight discomfort when we take the blood samples. We will however, do our up most to keep discomfort to a minimum.

If taking part in this research project harms you, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the University Ethics Committee panel will deal with these.

What will I get out of the study?

You may not gain any personal benefit from this study. It is hoped though that the results of this study will lead to future research within the University of Stirling to possibly test new ways of tackling and preventing obesity / type 2 diabetes.

You will gain some insight into current research in this area and the experimental methods used. Your results will be strictly confidential but at the end of the study we will be very pleased to discuss the findings with you.

It is unlikely you will lose weight in the 5 weeks you participate in this study as a direct result of particiapting. However we will give you advice on nutrition, exercise, physical activity and weight loss if this is something you would like.

Who has reviewed the study?

The ethics committee in the Sport Studies department at Stirling University has approved the study.

If you need more information, or have any problems at any time during the study, please do not hesitate to contact one of the investigators.

Thank you for taking part in this study.

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CONSENT FORM

CONSENT BY PATIENT/VOLUNTEER TO PARTICIPATE IN:
The effects of L-carnitine supplementation on response to glucose ingestion
and on fat metabolism at rest and during exercise

....
....

Name of Patient/Volunteer:

....

Name of Study:

....

Principal Investigator:

....

I have read the patient/volunteer information sheet on the above study and have had the opportunity to discuss the details with ....Thomas Craig / Dr Stuart Galloway...
and ask questions. The principal investigator has explained to me the nature and purpose of the tests to be undertaken. I understand fully what is proposed to be done.

I have agreed to take part in the study as it has been outlined to me, but I understand that I am completely free to withdraw from the study or any part of the study at any time I wish. I understand and agree that my participation in the study is entirely at my own risk.

I understand that these trials are part of a research project designed to promote medical or scientific knowledge, which has been approved by the Sports Studies Ethics Committee, and may be of no benefit to me personally. The Sports Studies Ethics Committee may wish to inspect the data collected at any time as part of its monitoring activities.

I also understand that my General Practitioner may be informed that I have taken part in this study if any unusual or surprising observations are made.

I hereby fully and freely consent to participate in the study which has been fully explained to me.

Signature   of   Patient/Volunteer:
....
....

Date:
....
....

I confirm that I have explained to the patient/volunteer named above, the nature and purpose of the tests to be undertaken.

Signature of Investigator:
....
....

Date :
....
......

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DEPARTMENT OF
SPORTS STUDIES

Date	Physiologist	Signature

Pre-Participation Health Screen Questionnaire (PPHS-Q)

PPHS-Q is an exercise – specific checklist for classification of training categories.
Accuracy in completion of the PPHS-Q is of the utmost importance

The purpose of the Fitness Centre (FC) pre-participation health screen is:
• To optimise safety during exercise testing and programme description.
• To identify medical risk factors which may contra-indicate exercise.
• To identify those with special needs.

Name:_____Age:_____ FC no_____

Address_____ Gender:_____

_____Tel:_____(H) _____(W)

Doctor's name:_____Tel:_____(H)_____(W)

Section A: Medical History Summary and Recommendations	
Date:	
Date:	
Date:	

Section B: Coronary Heart Disease Risk Index	Date	Date	Date
1. 1-10	No supervision required – exercise at will			
2. 10-17	No supervision required – use general exercise guidelines			
3. 18-27	No supervision required – use prescribed programme only			
4. 28-40	Use prescribed programme – Personal Training recommended			
5. 41+	Use prescribed programme – Personal Training and re-test within 8 weeks recommended			

Section C: Physical Activity Index

Activity Level	Times per week	Date: Risk level			
Inactive	0 occasional	Very High			
Semi-active	1	High			
Active	2-3	Moderate			
Very active	4 or more	Low			

SECTION A   MEDICAL
HISTORY

Have you ever been told that you have had or have any of the following conditions? If yes, please mark with an X in the appropriate box:

CARDIAC (Heart Related Diseases)

o	Heart Attack	o	High blood pressure
o	Coronary thrombosis (blood clot)	o	Rheumatic fever
o	Narrowing of arteries	o	Angina / Chest Pain
o	High cholesterol	o	Congenital Heart Disease
o	Further / comments		

PULMONARY (Lung Diseases)

o	Asthma	o	Exercise-induced asthma
o	Chronic Bronchitis	o	Emphysema
o	T.B.		
o			
o	Other / comments		

OTHER

o	Type I Diabetes (insulin dependent)	o	Type II Diabetes (non-insulin dependent)
o	Anaemia (ion deficiency)	o	Rheumatic fever
o	Kidney disease	o	Angina / Chest Pain
o	Rheumatoid Arthritis	o	Congenital Heart Disease
		o	Pregnant
o	Other / comments		

ORTHOPAEDIC SURGERY (Musculo Skeletal)
Surgery

o	Neck	o	Hip
o	Back	o	Knee
o	Shoulder	o	Ankle
o	Arm	o	Foot
o	Other / comments		

INJURY
Have you suffered any of the following injuries? If so, how long ago?

o	Neck vertebrae	o	Back vertebrae

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o	Rotator cuff	o	Impingement Syndrome (shoulder)
o	Tennis elbow	o	Runner's knee
o	ITB	o	Lower leg
o	Achillies Tendonitis	o	Plantar Fascitis
o	Other / comments		

MEDICATION

Do you use medication at present for any of the following? (If yes, please state the drug)

o	Heart rhythm	o	Blood pressure
o	Blood clotting.	o	Blood circulation
o	Asthma	o	Bronchitis
o	Emphysema	o	Flu
o	Diabetes	o	Thyoid dysfunction
o	Cholesterol	o	Anaemia
o	Kidney	o	Liver
o	Arthritis	o	Muscle injury
o	Other / comments		

SECTION B   CARDIOVASCULAR DISEASE RISK INDEX

Please read the following questions carefully and answer each accurately. Mark your choice with an X.

History of heart attack or bypass surgery / angioplasty

0	o	None	5	o	1 – 2 years ago
2	o	Over 5 years ago	8	o	< 1 year ago
4	o	3 – 5 years ago			

Family history of heart disease

1	o	No known history
2	o	1 relative with cardiovascular disease over the age of 60
3	o	2 relatives with cardiovascular disease over the age of 60
4	o	1 relative with cardiovascular disease under the age of 60
6	o	2 relatives with cardiovascular under the age of 60
8	o	Heart – related sudden death:
	o	Male, first degree relative before the age of 55
	o	Female, first degree relative before the age of 65

Age / Gender Index   Smoking status

0	o	Male / female under 30 years of age	0	o	None
1	o	30 – 40 years of age	1	o	Pipe

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2	o	Female 40 - 50 years of age	2	o	1 – 10 cigarettes daily
3	o	Male 40 – 50 years of age	3	o	11 – 20 cigarettes daily
3	o	Female 50 – 60 years of age	4	o	21 – 30 cigarettes daily
4	o	Male 50 – 60 years of age	5	o	31 – 40 cigarettes daily
4	o	Male / female 60+ years of age	6	o	41 – 60 cigarettes daily
			8	o	+ 60 cigarettes daily

State how long you have smoked for:
Years_____months_____

How would you describe your bodyweight?

0	o	Ideal weight
2	o	0 – 5kg overweight
4	o	6 – 10kg overweight
6	o	11 – 15kg overweight
8	o	+ 15kg overweight
10	o	Underweight

Total Cholesterol

0	o	< 5 mmol / L
1	o	5.0 – 5.2 mmol / L
3	o	5.3 – 5.9 mmol / L
5	o	6.0 – 6.2 mmol / L
6	o	6.3 – 6.9 mmol / L
7	o	7.0 – 7.5 mmol / L
8	o	> 7.5 mmol / L
	o	Not sure

Systolic Blood Pressure

0	o	< 130 mmHg
1	o	130 – 140 mmHg
2	o	141 – 150 mmHg
3	o	151- 160 mmHg
4	o	> 160 mmHg
	o	Not sure

Diastolic Blood Pressure

0	o	< 80 mmHg
1	o	81-90 mmHg
2	o	91 – 100 mmHg
3	o	101 – 110 mmHg
4	o	> 110 mmHg
	o	Not sure

Diabetes

0	o	None
1	o	Type 11 (non-insulin dependent)
2	o	Type 1 (insulin dependent)

Occupational activity level

1	o	Intense physical labour
2	o	Moderate (walk often etc.)
3	o	Sedentary

Work Stress Tension

0	o	No stress, very relaxed
1	o	Moderate work stress and relaxed personality
2	o	High work stress but cope well
3	o	Very high work stress and tense personality
4	o	Very high work stress, highly strung personality

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Physical Activity Status (for a minimum of 30 minutes a session)

1 o Exercise 4 or more times per week

2 o Exercise 2 – 3 times per week

3 o Recreational sport once a week

4 o Recreational sport occasionally or complete lack of exercise

SECTION C   EXERCISE PARTICIPATION

Do you participate in any of the activities more than twice weekly?
(Please tick all relevant activities)

o Jogging more than 5 km	o Aerobic classes 45 min
o Cycling more than 45 min.	o Tennis 90 min
o Swimming more than 600 m	o Squash 45 min.
o Gym (Combined strength / aerobic)	o Team sport (outdoor) – rugby hockey, soccer
o Gym (weights only)	o Team sport (indoor) – basketball, netball, etc
o Gym (aerobic only)	o Canoeing / Rowing 45 min

SECTION D

I have read, understood and completed this questionnaire to the best of my knowledge.
I am aware of the risk involved in fitness testing and understand the test procedures that I will perform. I give consent to participate in this assessment.

TEST 1

Date:_____

SIGNATURE:_____ WITNESS:_____
(for a minor)

SIGNATURE OF PARENT:_____

TEST 2

Date:_____

SIGNATURE:_____

WITNESS:_____

SIGNATURE OF PARENT:_____ (for a minor)

TEST 3

SIGNATURE:_____

Date:_____

SIGNATURE OF PARENT:_____

WITNESS:_____
(for a minor)

TEST 4

SIGNATURE:_____

Date:_____

SIGNATURE OF PARENT:_____

WITNESS:_____
(for a minor)

L-Carnitine Study – Example of Exercise and Food Diary

Please fill out all food intake a exercise and physical activity. Example is given in the first box.

Date: Example

Exercise and Physical Activity 8am: walk from bus stop to office, 12pm:walk from office to canteen and back 4.30pm: gym session, 15 mins cardio, 30 mins weights, 5.30pm: 15 minutes in pool, walk to and from car 8.00pm: general housework, playing with kids in front room
Food Intake 7am: 2 weetabix with sugar, tea with 2 sugar, banana 10am; mars bar, can diet coke 12pm: lunch, cheese baguette with mayo, walkers sensations, bottle diet fanta 3.oopm: banana, tea 2 sugar digestive biscuit 6.30pm: dinner, glass semi skimmed milk, mince and tatties, tea 2 sugar and chocholate buscuit. 9pm: slice of toast and tea with 2 sugars

Date:

| Exercise and Physical Activity |
| |
| Food Intake |
| |

Date:

| Exercise and Physical Activity |
| |
| Food Intake |
| |

Date:

| Exercise and Physical Activity |
| |
| Food Intake |
| |

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Predicted VO$_{2max}$ Test Sheet

Subject Name…………………………..   Subject Id………………………
Test Number……… DOB…………..   Height………
Weight………..   Body Fat………   Precict HR$_{max}$………………….

Stage	TIME	Workload	Sample	Result
	1 min 50s		HR	
	2 min 5s		BP	
	2 min 35s		RPE	
1	2 min 50s		HR	
2	4 min 50s		HR	
	5 min 5s		BP	
	5 min 35s		RPE	
	5 min 50s		HR	
3	7 min 50s		HR	
	8 min 5s		BP	
	8 min 35s		RPE	
	8 min 50s		HR	
4	10 min 50s		HR	
	11min 5s		BP	
	11 min 35s		RPE	
	11 min 50s		HR	

Predicted VO$_{2max}$…………………   40% VO$_{2max}$…………………………...

Test Carried out by………………………………

Date…………………………………………...

APPENDIX F

Estimate 1st Phase Insulin Release = 1.283 + 1.829 x INS30 – 138.7 x GLUC30 + 3.772 X INS0

Estimated 2nd Phase Insulin Release= 287 + 0.4164 x INS30 – 26.07 x GLUC30 + 0.9226 x INS30

Stumvoll *et al.*, 2000