The combined effect of high intensity intermittent training and vitamin D supplementation on insulin sensitivity in overweight and obese males and females

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Abstract

High intensity intermittent training (HIIT) is a time efficient mode of exercise, which has been shown to induce metabolic benefits, such as an improvement in insulin sensitivity (IS) and thus type 2 diabetes (T2D). Vitamin D_3 ($25(OH)D_3$) supplementation has also been shown to influence the pathogenesis of IS. The aim of this study was to investigate if there is a combined effect of HIIT and $25(OH)D_3$ supplementation on IS.

Twelve inactive and overweight adults (9 male, 3 female; age: 32 ± 18-45 y, BMI: 31.9 ± 2.8 $kg \cdot m^{-2}$) performed HIIT 3 times/week for 6 weeks, with oral glucose tolerance tests and peak oxygen consumption ($\dot{V}O_{2peak}$) tests done at baseline and post-intervention. The HIIT protocol consisted of 10 x 1 min intervals cycling at 97 ± 8 % $\dot{V}O_{2peak}$ separated by 1 min active recovery. Participants were randomised to ingest 4000 IU/day $25(OH)D_3$ (n=6) or a placebo (n=6). Plasma glucose, insulin, $25(OH)D_3$, adiponectin, leptin, and the lipid profile were analysed pre and post training.

Peak $\dot{V}O_2$ and power output was increased in all participants (P<0.01). Systolic blood pressure (BP) was reduced in all participants and the vitamin D group (P<0.05) but not the placebo group. Insulin area under the curve (AUC) was significantly reduced by 16.6% in the placebo group (P<0.05) but not overall for all participants or the vitamin D group. There was no change in fasting glucose or glucose AUC. Insulin sensitivity index (ISI) and homeostatic model assessment of insulin resistance (HOMA-IR) remained unchanged across all groups. All participants were $25(OH)D_3$ deficient (<20 $ng \cdot ml^{-1}$) at baseline, with an increase in $25(OH)D_3$ in the vitamin D group after 6 weeks (P<0.05). Adiponectin decreased in the placebo group but was unaltered in the vitamin D group. Leptin remained unaltered in all groups. Plasma triglycerides were reduced in all participants (P<0.05).

In conclusion, 6 weeks of HIIT increased physical capacity but had no effect on the ISI and HOMA-IR in overweight and obese males and females. The findings showed that 6 weeks of supplementation resulted in an increase in $25(OH)D_3$ and an improvement in systolic BP.

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ANOVA	analysis of variance
AUC	area under the curve
BMI	body mass index
bmp	beats per minute
DEXA	duel-energy X-ray absorptiometry
dL	deciliter
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme linked immune sorbent assay
g	gram
h	hour
Hb	haemoglobin
Hct	haematocrit
HDL	high density lipoprotein
HIIT	high intensity intermittent training
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
HR	heart rate
IL	interleukin
ISI	Insulin Sensitivity Index
IU	International Units
kDa	kilodaltons
kg	kilogram
L	litre
LDL	low density lipoprotein

m	metre
min	minute
ml	millilitre
mmol	millimole
ng	nanogram
nmol	nanomol
O_2	oxygen
OGTT	oral glucose tolerance test
PLT	platelet
PVC	plasma volume change
RBC	red blood cell
rpm	revolutions per minute
s	second
SD	standard deviation
T2D	type 2 diabetes
TNF-α	tumour necrosis factor – alpha
$\dot{V}O_{2max}$	maximal oxygen consumption
$\dot{V}O_{2peak}$	peak oxygen consumption
W	Watt
w/v	weight to volume
WBC	white blood cell
y	years
%	percent
µg	microgram
µl	microliter
25(OH)D	25-hydroxy vitamin D

1.0 Introduction

Obesity and an inactive lifestyle have been reported to be the leading causes of global mortality (WHO, 2010), with a staggering 27% of diabetes cases attributed to physical inactivity (Must *et al.*, 1999). The prevalence of type 2 diabetes (T2D) is rapidly increasing and is expected to increase from 171 million in the year 2000 to 366 million people in 2030 worldwide (Wild *et al*., 2004). The initial pathophysiological event in the development of T2D is usually insulin resistance and chronic hyperglycaemia (Kahn, 2003). Diabetes costs NHS Scotland £1 billion per annum and thus stresses the importance of investing in feasible strategies to improve physical activity levels in Scotland. Diabetes cases are also projected to rise by 17% in the next 25 years in Scotland alone (Hex *et al.*, 2012), and since the development of T2D is mainly attributed to lifestyle choices, the focus should be on preventing T2D through increased activity levels and diet. In some cases exercise has also been found to be a better form of prevention and treatment than medicine, presenting a cost-effective approach (Naci and Ioannidis, 2013).

The most commonly reported barrier to physical activity and exercise is “lack of time” (Stutts, 2002; Trost *et al.*, 2002). High intensity intermittent training (HIIT) involves repeated short bouts of exercise at an intensity above 85% of maximal capacity, and has been shown to provide similar, and in some cases greater, physiological and metabolic adaptations compared to work-matched moderate intensity endurance training (Burgomaster *et al.*, 2008; Babraj *et al.*, 2009; Racil *et al.*, 2013; Little *et al.*, 2014; Mitranun *et al.*, 2014). Currently, the UK Government Physical Activity Guidelines for Adults (2011) are based on moderate and vigorous intensities but consequently require a commitment time of 150 or 75 minutes/week of aerobic exercise, respectively.

There is also a graded increase in the prevalence of T2D with increasing severity of overweight and obesity, therefore individuals with respective body mass indices (BMI) of 25-30 $kg \cdot m^2$ and 30-35 $kg \cdot m^2$, should be a target group for exercise interventions (Must *et al.*, 1999).

Supplementation with cholecalciferol ($25(OH)D_3$), known commonly as vitamin D_3, has been reported to resemble the effects induced by exercise, specifically HIIT, on glucose metabolism and insulin sensitivity. The effects have been found to be dose and duration dependent, with vitamin D deficiency associated

with overweight and obesity (Pittas *et al.*, 2006; Pittas *et al.*, 2007; Gallagher *et al.*, 2012; Belenchia *et al.*, 2013; Gallagher *et al.*, 2013). The proposed mechanisms underpinning the effect of vitamin D on insulin resistance are the reduction of pro-inflammatory markers and enhancing circulating glucose homeostasis (Flores, 2005).

Specifically, adiponectin concentrations have been altered with a strong correlation presented. The adiponectin concentration in the circulating blood can be reported as a clinical indicator of insulin resistance as it induces insulin-sensitising properties (Fasshauer *et al.*, 2008). Elevated adiponectin levels can predict and indicate lower insulin resistance, therefore supplementing with vitamin D has the potential to improve the risk of developing T2D (Lin *et al.*, 2007).

There is very little research investigating a combined effect of vitamin D supplementation and exercise training, although recent studies have suggested that the effect on glucose control is independently regulated by increased vitamin D concentration and HIIT (Barker *et al.*, 2013; Kobza *et al.*, 2013).

2.0 Literature review

2.1 Pathophysiological events in Type 2 diabetes

Type 2 diabetes mellitus refers to a metabolic system collapse when there is reduced secretion or a loss of response to endogenous insulin resulting in a recurring state of hyperglycaemia (Alberti and Zimmet, 1998). The development of T2D has been primarily and extensively reported as relative contributions of insulin resistance and beta cell (β cell) dysfunction (Kahn, 2003). Through genome-wide association studies there is evidence that genetic predisposition plays a role in the development of T2D, although only a small proportion in comparison to a healthy diet and regular physical activity (Ardisson Korat *et al.*, 2014). Additionally, environmental and epidemiological factors such as geographical, cultural, and seasonal influences can predispose specific population groups to the onset of T2D (Wild *et al.*, 2004), with vitamin D deficiency playing a key role (Holick and Chen, 2008).

Beta cells are unique cells located in the islets of Langerhans in the pancreas that store and secrete the hormone insulin - responsible for regulating blood glucose concentrations. Beta cells quickly respond to changes and spikes in blood glucose concentration through the mediated secretion of insulin following activation of the insulin receptors located in the cell membrane (Kahn, 2003; Abdul-Ghani *et al.*, 2006). However, if β cell function is impaired or β cell mass is reduced the effectiveness of the insulin response to a lower glucose concentration and achieve homeostasis becomes diminished (Wolden-Kirk *et al.*, 2011). To compensate, the β cells up-regulate the production of insulin, which accelerates β cell turnover and thus reduces the life span of the cell. It can also result in permanent cell damage. Similarly, if hepatic cells become resistant to the insulin that is secreted then glucose control can become impaired (Saltiel and Kahn, 2001). It is primarily this dysfunctional utilisation of insulin, a reduction in the sensitivity to insulin, which can lead to chronic hyperglycaemia: the main physiological event in the development of T2D (Kahn, 2003). A breakdown in the cross-talk between the pancreatic β cell and peripheral tissue insulin sensitivity results in the progressive deterioration of glucose homeostasis (Gastaldelli, 2011).

Modifiable lifestyle risk factors such as obesity and low levels of physical activity are the main determinants of the metabolic disease in both males and females (Tuomilehto*, et al.*, 2001).

2.2 Obesity and insulin resistance

The critical role of weight and body composition has been identified through the consideration of the hyperbolic relationship between β cell insulin secretion and insulin sensitivity, illustrated in Figure 1 (Kahn *et al.*, 1993). Overweight/obesity and physical inactivity are regarded as the main contributors to insulin resistance, with many studies concluding that increased subcutaneous fat mass is associated with an increased risk of developing T2D (Gastaldelli *et al.*, 2000; Gastaldelli *et al.*, 2002; Snijder *et al.*, 2005; Guh *et al.*, 2009). Any defects in the insulin signalling cascade can cause the development of insulin resistance and impaired glucose tolerance (Saltiel and Kahn, 2001; Abdul-Ghani *et al.*, 2006). Subcutaneous and visceral fat accumulation can lead to chronic hyperglycaemia primarily due to an increased rate of gluconeogenesis resulting in elevated hepatic glucose secretion (Gastaldelli *et al.*, 2002; Gastaldelli *et al.*, 2007). Excess abdominal and subcutaneous fat is also associated with decreased muscle glucose uptake and increased release of free fatty acids (FFAs), which presents a hyperglycaemic and lipotoxic state (Gastaldelli, 2011). Systemic hypoinsulinemia also advances in the presence of fat accumulation as a result of the decreased expression of insulin receptors, which is mediated through the transcription process (Kahn *et al.*, 2006). Consequently, hepatic and peripheral insulin sensitivity are reduced following the development of impaired glucose tolerance through mechanisms associated with obesity. Straying from the hyperbolic curve (Figure 1) can result in impaired glucose tolerance and subsequently pre-diabetes if both abnormalities exist: insulin resistance and β-cell dysfunction (Kahn *et al.*, 1993).

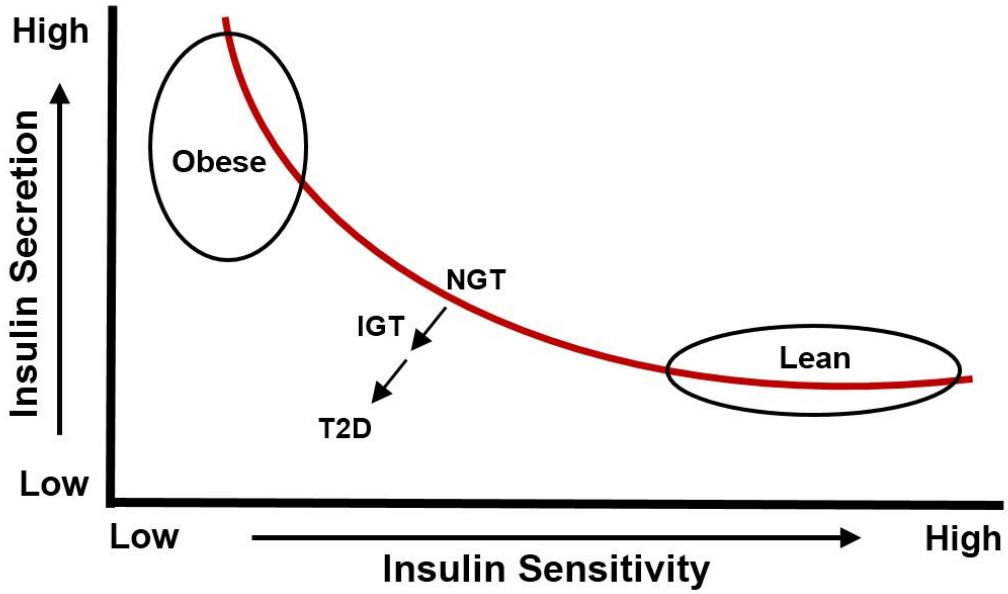

Figure 1: The hyperbolic relationship between insulin secretion and insulin sensitivity relative to weight/body composition. Adapted from Kahn et al. (1993). NGT, normal glucose tolerance; IGT, impaired glucose tolerance; T2D, type 2 diabetes mellitus.

Adipose tissue releases numerous hormones and anti- and pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumour necrosis factor – alpha (TNF-α), which are small proteins of 22 and 17 kilodaltons (kDa) respectively that influence insulin and glucose metabolism. However, an abnormal amount of fat mass and thus an excess of adipocytes can detrimentally affect insulin sensitivity (Kahn *et al.*, 2006). Therefore, the systemic circulation of pro- and anti-inflammatory cytokines could act as a predictor of the development or the presence of impaired glucose tolerance and thus T2D (Eder *et al.*, 2009). This overabundance of adipocytes has been associated with reduced concentrations of the adipose tissue-derived protein hormone adiponectin (Punthakee *et al.*, 2006), which expresses both anti- and pro-inflammatory properties (Ohashi *et al.*, 2012). One of the primary actions of adiponectin is to mediate numerous metabolic processes involving insulin regulation and action and glycaemic homeostasis (Lihn *et al.*, 2005).

In the pathogenesis of T2D there is increasing evidence that cytokines and associated hormones induce an acute-phase immune response and inflammatory reaction that contributes to chronic low-grade inflammation (Schmidt *et al.*, 1999; Spranger *et al.*, 2003). The pattern of circulating

inflammatory cytokines and other proteins that exhibit pro- and anti-inflammatory properties can modify the risk for the development of T2D (Spranger *et al.*, 2003; Smitka and Maresova, 2015). The association between obesity and insulin resistance is partly attributed to a decrease in adiponectin and thus an alteration in the pattern of circulating hormones and proteins (Hotamisligil *et al.*, 1994; Katergari *et al.*, 2015). Adiponectin has been reported to influence secretory intracellular-pathways that are mediated by signal transduction from the insulin receptor, with low levels associated with insulin resistance (Kadowaki *et al.*, 2006; Punthakee *et al.*, 2006). Reducing or preventing low adiponectin concentrations may directly or indirectly influence insulin action on cell membrane involved in hepatic and skeletal muscle glucose uptake. This has also been shown to be influenced by an increase in physical activity and exercise, with exercise intensity altering the capacity of muscle to oxidise fat and carbohydrates (Perry *et al.*, 2008; Racil *et al.*, 2013; Hansen *et al.*, 2015). Circulating adiponectin concentration may thus be a predictor of insulin resistance and inflammation-induced impaired glucose tolerance.

Leptin is a hormone that induces the feeling of satiety and thus can influence appetite and weight status (Klok *et al.*, 2007; Deighton *et al.*, 2013; Deighton *et al.*, 2014). Leptin is often associated with adiponectin, with an elevation in adiponectin-to-leptin ratio reported to correlate with a rise in fasting glucose. Although, this may not always be the case as adiponectin expression has been reported to have no direct association with plasma concentrations of leptin (Kern *et al.*, 2003). Indirect mechanisms may exist but are currently unknown.

A low concentration of adiponectin has also been negatively associated with visceral adiposity and other features of the MetS suggesting reduced systemic adiponectin is a predictor for the development of T2D (Hotta *et al.*, 2000). It has been demonstrated that an upregulation of adiponectin and leptin can induce glucose uptake and thus play a role in insulin regulation and action.

2.3 Physical activity and insulin sensitivity

A physically inactive lifestyle is associated with the development of impaired glucose metabolism and pre-diabetes (Sarvas *et al.*, 2015). Physical activity has been found to prevent T2D but is dependent on baseline risk of T2D (Gill and Cooper, 2008) and thus a range of activities and exercise at different intensities should be considered for preventing or delaying the onset of T2D. Exercise induces numerous physiological, metabolic, and cardiovascular adaptations that are directly linked to a reduction in the risk of developing chronic conditions, specifically impaired glucose tolerance (Trapp *et al.*, 2008; Babraj *et al.*, 2009; Wisloff *et al.*, 2009; Iellamo *et al.*, 2013). The post-prandial insulin response is influenced by physical activity status, with structured exercise demanding a relatively higher demand on hepatic and skeletal muscle metabolism, which alters the respiratory exchange ratio (RER) value and thus substrate availability and utilisation (Thompson *et al.*, 1998; Warren *et al.*, 2009; Thompson *et al.*, 2012). This efficient insulin control is required to regulate systemic glucose concentration in order to meet the increased fuel demand during exercise, the fuel source of which is dependent on exercise intensity (Marliss and Vranic, 2002). During exercise, the production, regulation and uptake of glucose is mediated by the glucose transporter type 4 (GLUT4) through insulin-controlled pathways (Park *et al.*, 2014), contributing to glycogen stores if not metabolically required. Insulin secretion is inhibited during exercise and thus relies on hepatic and skeletal muscle tissue cells being sufficiently sensitive to insulin to maintain glucose homeostasis. It is now well established that the expression and protein content of GLUT4 increases following endurance exercise (Kraniou *et al.*, 2004; Hansen *et al.*, 2015). As a result, GLUT4 is available for translocation to improve efficiency of skeletal muscle glucose uptake through insulin action, subsequently enhancing the insulin sensitive capacity of muscle cells (Goodyear and Kahn, 1998).

Following exercise, the up-regulation of metabolic demand stimulates the role of glycogen depletion in mediating improvements in insulin resistance (Jensen *et al.*, 2011; Metcalfe *et al.*, 2012). Exercise at higher intensities primarily rely on muscle glycogen as the main source of carbohydrate fuel (Romijn *et al.*, 1993; van Loon *et al.*, 2001). However, 6 weeks of low volume sprint interval

training (SIT) in healthy adults (Metcalfe *et al.*, 2012) has been found to induce a reduction in the rate of glycogen utilisation, indicating reduced glycogenolysis and consequently a reduction in systemic hyperglycaemia (Jensen *et al.*, 2011). To accommodate the reduction in glycogenolysis, there is a shift in substrate utilisation with an increase in the rate of lipolysis to meet and maintain the demand required (Romijn *et al.*, 1993). This induced skeletal muscle oxidative capacity remodelling can be caused by a number of exercise-induced metabolic adaptations, such as: mitochondrial capacity, mitochondrial biogenesis, skeletal muscle mass (Little *et al.*, 2011; Hansen *et al.*, 2015).

2.4 HIIT

2.4.1 HIIT as an alternative to moderate intensity continuous training

It is well established that exercise provides cardiovascular and metabolic adaptations that prevent adverse health outcomes such as the development of atherosclerosis (Roberts *et al.*, 2002), cardiovascular disease (Warburton *et al.*, 2005; Tjonna *et al.*, 2008; Tjonna *et al.*, 2009), and T2D (Knowler *et al.*, 2002; Bassuk and Manson, 2005; Stanford and Goodyear, 2014). Despite the general consensus that exercise induces responses and adaptations that improve insulin sensitivity, morphological and metabolic adaptations differentiate between different modes and intensities of exercise training (Wisloff *et al.*, 2007; Iellamo *et al.*, 2013).

Moderate intensity exercise involves a relatively large volume of continuous aerobic exercise (50-75 % $\dot{V}O_{2peak}$), high intensity intermittent training (HIIT) involves repeated short bouts, ranging from 5 s to 4 min long, at a constant load (75-100 % $\dot{V}O_{2peak}$) interspersed with active or passive recovery periods, and SIT involves ‘all-out’ sprints (≥100 % $\dot{V}O_{2peak}$) also separated with rest or active recovery periods.

High intensity intermittent training has garnered attention in recent years in a more general and clinical application as it has been found to promote adaptations that are often similar or superior to moderate intensity continuous training (Wisloff *et al.*, 2007; Tjonna *et al.*, 2008; Iellamo *et al.*, 2013; Racil *et al.*,

2013; Trombold *et al.*, 2013; Mitranun *et al.*, 2014; Jung *et al.*, 2015). Commonly, HIIT has been used in athletic populations for years by coaches to provide molecular adaptations to better performance outcomes. However, more recently HIIT has been established as a method for health-associated adaptations that are similar or in some cases greater than moderate intensity training, with scope for considerably shorter commitment time required from individuals. The most common reported barrier to physical activity and exercise is “lack of time” (Stutts, 2002; Trost *et al.*, 2002). Short sprints and minimal interspersed recovery periods comprising HIIT protocols can reduce the commitment time per session, to as little as 10 minutes (Gillen *et al.*, 2014), presenting a time efficient mode of exercise and overcome adherence issues (Jung *et al.*, 2015).

Accumulating evidence has shown that HIIT can improve insulin sensitivity in individuals at risk of developing T2D (Trapp *et al.*, 2008; Babraj *et al.*, 2009; Richards *et al.*, 2010; Metcalfe *et al.*, 2012; Iellamo *et al.*, 2013; Racil *et al.*, 2013; Whyte *et al.*, 2013; Mitranun *et al.*, 2014). Although, not all HIIT studies have demonstrated changes in all associated indices that contribute to insulin sensitivity. Despite an improvement in insulin sensitivity, some HIIT studies have observed no changes in fasting insulin (Babraj *et al.*, 2009; Shaban *et al.*, 2014), or no effect on fasting glucose or control (Talanian *et al.*, 2007; Whyte *et al.*, 2010; Racil *et al.*, 2013).

There is great variation in the protocols utilised in studies investigating the effects of HIIT, with sessions ranging from as low as 3 minutes (Gillen *et al.*, 2014) up to 1 hour in total (Talanian *et al.*, 2007). Recently, research by three research groups has focused on a 2 week intervention involving 8-10 sets of 60 s bouts on a cycle ergometer at ~90% $\dot{V}O_{2peak}$ or maximum heart rate (HR_{max}), interspersed with 60-120 s rest or cycling at a low intensity (Little *et al.*, 2010; Little *et al.*, 2011; Gillen *et al.*, 2012; Little *et al.*, 2014). The latter research involved overweight and obese participants, some with diagnosed T2D, and has reported improvements in insulin sensitivity, changes in glucose uptake through the action of GLUT4, decreases in glucose area under the curve (AUC), and alterations in metabolism, such as an increase in resting muscle glycogen.

A number of studies have utilised SIT interventions involving 'all-out' sprints ranging from 6 s (Adamson *et al.*, 2014), 10-20 s (Metcalfe *et al.*, 2012), and up to 30 s (Burgomaster *et al.*, 2005; Babraj *et al.*, 2009; Richards *et al.*, 2010; Racil *et al.*, 2013). Despite the improvements in insulin sensitivity gained from very intense protocols, the nature of the intervals, i.e. the intensity involved, may be discouraging to a population that is not accustomed to regular exercise. Therefore, more suitable protocols involving longer bouts but slightly lower intensities can be used to accommodate such population groups or provide an alternative to increase adherence. Adherence to exercise is low in individuals at risk of pre-diabetes (Jung *et al.*, 2014; Jung *et al.*, 2015), however there is preliminary evidence that individuals at risk of pre-diabetes can adhere to HIIT, 10 x 1 min at ~90 % of peak heart rate (HR_{peak}), as a time-efficient form of exercise more so than continuous moderate intensity exercise, 50 min at ~65 % HR_{peak} (Jung *et al.*, 2014; Jung *et al.*, 2015).

The interspersed recovery intervals should be determined based on the intensity and duration of the high intensity exercise bouts, allowing for full or partial recovery (Bompa, 1999). Also, depending on the length of the study intervention, periodisation in training should be adopted in order to increase the intensity/work load in line with correlated increases in peak physical capacity and skeletal muscle metabolic capacity. This allows for physiological and metabolic adaptations to occur before increasing the work load (Bompa, 1999).

2.4.2 Metabolic benefits of HIIT

Since HIIT is designed to implement a higher workload and power output compared to continuous moderate intensity exercise, the cardiovascular and metabolic systems respond accordingly to tolerate the demand (Romijn *et al.*, 1993; Laursen and Jenkins, 2002; Gibala *et al.*, 2012). The high stimulus effect of HIIT can further up-regulate the uptake of glucose to contracting muscles and the liver when compared to moderate or low intensity training. As a result, HIIT has been found to have the potential to augment the effective regulation and action of insulin through direct and indirect pathways (Kemmler *et al.*, 2015).

2.4.3 Acute effects of HIIT

During a single session of HIIT, HR has been reported to increase rapidly with time, and intermittently with each interval reference. The HR response to short (8 s bouts with 12 s recovery) and long (24 s bouts with 36 s recovery) 'all-out' intervals has been investigated in young women, reporting HR to increase to near maximal (calculated as 220 minus age) following 2 bouts (Trapp *et al.*, 2007). The increase in HR coincides with the number of intervals completed, with the long sprints generating a greater power output. In addition, Trapp *et al*. (2007) found epinephrine and norepinephrine to be significantly elevated post-exercise, suggesting that β-adrenergic receptor sensitivity in adipose tissue may be enhanced, therefore causing a drive in lipolysis. Essentially, HIIT can benefit the overweight and obese population due to an increase in fat oxidation and lipolysis, and fat loss (Talanian *et al.*, 2007; Trapp *et al.*, 2008; Mitranun *et al.*, 2014).

Acute HIIT studies have demonstrated changes and improvements in glycaemia and insulin sensitivity (Gillen *et al.*, 2012; Whyte *et al.*, 2013; Little *et al.*, 2014). In a 2 day trial, 4 x 30 s Wingate trials: standard all-out anaerobic sprint protocol (Bar-Or, 1987; Vandewalle *et al.*, 1987), were compared to a single 200 s (198 ± 10 s) sprint, matched for workload, in overweight/obese sedentary men. The extended interval improved insulin sensitivity index by 44.6 % and decreased insulin resistance (HOMA-IR) by 32 % compared to the control group (Whyte *et al.*, 2013). However, both protocols demonstrated no change in glycaemia, observed through glucose AUC following an oral glucose tolerance test (OGTT). Whyte and colleagues (2012) also investigated the effect of a 2 week intervention using the same protocol on the same population group, and found repeated Wingates to reduce insulin AUC and decrease fasting plasma insulin. Similarly, they reported no effect on glucose homeostasis.

Following a single session of HIIT involving a commonly utilised protocol of 10 x 60 s constant load bouts at 85 % HR_{max}, the duration spent in a hyperglycaemic state was reduced by 65 % in the 24 h post-training (Gillen *et al.*, 2012). Glucose AUC was also reduced, which concurs with other studies involving 60 s intervals, whereby a decrease in post-prandial glucose AUC was

reported (Little *et al.*, 2014). No effect was found following continuous moderate intensity exercise, highlighting the comparable high stimulus effect of HIIT on the regulation of insulin and glucose. The volume of high intensity exercise within each session may be accountable for the improvements in insulin sensitivity and glycaemic control as opposed to the intensity. Following a single session of HIIT there have been marked improvements in glucose tolerance compared to continuous moderate intensity exercise (Whyte *et al.*, 2010; Gillen *et al.*, 2012; Little *et al.*, 2014). Although, these may not be represented in the long term or induce physiological and metabolic adaptations that reduce the development of chronic hyperglycaemia.

2.4.4 Chronic effects and metabolic adaptations to HIIT

There has been an abundance of studies focusing on 2 week HIIT interventions that induce adaptations including increased $\dot{V}O_{2peak}$, skeletal muscle adaptations, and reduced insulin resistance particularly in overweight and obese individuals (Burgomaster *et al.*, 2005; Babraj *et al.*, 2009; Little *et al.*, 2010; Richards *et al.*, 2010; Whyte *et al.*, 2010; Little *et al.*, 2011). In a study involving healthy men with a normal BMI, 2 weeks of HIIT involving 4-6 Wingates observed no changes in fasting insulin or glucose concentrations (Babraj *et al.*, 2009). However, the AUC for both plasma glucose and insulin after a 75 g OGTT was reduced by 12 % and 37 %, respectively and insulin sensitivity was improved by 16.7 %. These results, along with a similar study involving 4-7 Wingates (Richards *et al.*, 2010) that has found an improvement in insulin sensitivity, suggest a training-induced adaptation in the insulin response even in those with normal glucose tolerance. It should be noted that these results were achieved with no change in body mass and an increase in calorie consumption. In a population with normal glucose tolerance and thus a normal response to a glucose tolerance test, i.e. an OGTT, observing no change in glucose homeostasis could indicate that a reduction in fasting insulin concentration can occur independently. Whyte *et al.* (2010) reported a protocol involving 4-6 Wingates reduced fasting plasma insulin by 25 % and insulin AUC by 15 % in overweight/obese males after just 2 weeks. Another study adopting a similar protocol but in obese males and females with T2D,

demonstrated a decrease in plasma glucose accompanied with a reduction in the HOMA-IR index (Shaban *et al.*, 2014).

Skeletal muscle is the primary tissue involved in the uptake of post-prandial glucose (Baron *et al.*, 1988), suggesting that an increase in muscle activity will elevate muscle glucose uptake during exercise. This is reflected in the pre- and post-HIIT response of glucose and insulin to an OGTT. The rate limiting step in glucose uptake and a contributor to insulin action is the bioavailability of GLUT4. HIIT has been shown to be a stimulus for increasing skeletal muscle mitochondrial activity through a reported increase in the protein content of GLUT4 by 119 % following 2 weeks of HIIT involving 8-12 x 60 s intervals in healthy males (Little *et al.*, 2010). Elevated GLUT4 protein content has also been accompanied by an improvement in insulin sensitivity in overweight adults by ~35 % following a similar protocol (Hood *et al.*, 2011). It may be concluded that the intra-cellular translocation of GLUT4 may be stimulated by insulin and be a determinant of insulin sensitivity (Park *et al.*, 2014). Muscle glucose uptake may also increase due to the higher skeletal muscle oxidative capacity and thus muscle fibre recruitment associated with the higher intensity workloads and physiological demand that is achieved through HIIT (Little *et al.*, 2011).

Glycogen stores deplete during exercise in order to meet substrate demand particularly when intensities are >70 % $\dot{V}O_{2peak}$ (Romijn *et al.*, 1993; van Loon *et al.*, 2001). The greater proportion of muscle fibres that need to replenish glycogen stores post-HIIT compared to post-moderate intensity training increases the demand for glucose uptake to skeletal muscle cells. In turn, this induces the requirement of GLUT4 translocation and availability. High intensity interval training has been shown to increase glycogen breakdown to a greater extent than moderate intensity continuous training and thus allows for effective post-prandial storage of glucose, reducing hyperglycaemia, preventing a reduction in insulin resistance (Jensen *et al.*, 2011). Resting muscle glycogen stores have been shown to increase by as much as 17 % and 26 % in healthy adults following 2 weeks of HIIT (Burgomaster *et al.*, 2005; Little *et al.*, 2010). Burgomaster *et al.* (2005) demonstrated that only 2 weeks of HIIT involving 4-7 x 30 s sprints can increase muscle oxidative capacity. This has been

demonstrated through an increase in the maximal activity and protein content of citrate synthase: the ‘pace-making enzyme’ involved in glycolysis, which stimulates the rate of glucose production and subsequently availability. Furthermore, Little *et al*. (2010) utilised 8-12 longer bouts of 60s and also found an increase in muscle glycogen. Although this increase was slightly less than that reported by Burgomaster and colleagues (2005), they also reported an increase in the protein content of GLUT4 after the same 2 week training period. It could be concluded that the intensity and not volume of interval training is the main determinant to augment metabolic adaptations.

When the common 10 x 60 s HIIT protocol was applied to an overweight/obese and T2D population, glycaemia was reduced over a 24 h period with an accompanying decrease in blood glucose AUC (Little *et al.*, 2011). This improvement in the ability to tolerate glucose concentrations assumes an effect on insulin sensitivity is possible, although this was not observed. Such metabolic adaptations to short-term intervention studies can reduce the risk of developing inactivity-related disorders, such as T2D. However, the latter protocol has not yet been investigated in overweight and obese adults free of diabetes, or in training interventions exceeding 2 weeks.

Another metabolic adaptation following HIIT is an increase in lipid oxidation. Whole body and skeletal muscle capacity for fatty acid oxidation has been shown to increase following HIIT. Longer intervals, 10 x 4 min at 90 % of $\dot{V}O_{2peak}$, have demonstrated increased fatty acid oxidation but unchanged whole body glucose, despite an increase in maximal muscle citrate synthase activity (Talanian *et al.*, 2007). This may predict enhanced insulin utilisation due to the more effective control of insulin-mediated glucose regulation to achieve homeostasis. It should be taken into consideration that this study involved healthy, recreationally active females with normal glucose tolerance. In overweight and obese males, 2 weeks of HIIT utilising a similar protocol of 10 x 4 min at 85 % $\dot{V}O_{2peak}$ observed a beneficial change in the resting inflammatory profile but no improvement in insulin sensitivity or glycaemic control (Leggate *et al.*, 2012). A reduction in the enzyme fatty acid synthase (FAS) was reported following the 2 weeks of HIIT providing further evidence for increased fatty acid oxidation after exposure to regular exercise (Tjonna *et*

al., 2008). Despite no reduction in body mass, which has been found as a result of HIIT training in other studies (Tjonna *et al.*, 2008), the study by Leggate *et al.* (2012) observed a decrease in waist circumference. Longer interventions have been recommended in order to investigate if greater or differential benefits in insulin sensitivity, fatty acid oxidation, or changes in body composition can be gained through HIIT.

Longer HIIT intervention studies involving 6-12 weeks of training have reported beneficial alterations in glycaemic control in overweight/obese and healthy adults (Metcalfe *et al.*, 2012; Adamson *et al.*, 2014). An intervention consisting of extremely short cycling sprints, 10 x 6 s, reduced glucose AUC in overweight males and females by 6 % (Adamson *et al.*, 2014). Contrastingly, Metcalfe *et al.* (2012) found an increase in glucose AUC following 6 weeks of low-volume HIIT: 2 x 10-20 s sprints, though this was only found in healthy but sedentary females. Interestingly, the authors also reported a 28 % improvement in insulin sensitivity in males but no change in females. This demonstrates that beneficial adaptations can be observed despite the relatively low volume of training per session, and indicates gender difference may exist in response to HIIT. In light of the results, it can be concluded that 6-12 weeks of HIIT may be sufficient to induce alterations in the glycaemic profile and thus have the potential to improve insulin sensitivity.

A reduction in insulin resistance also reflects insulin sensitivity and has been reported following 12 weeks of HIIT in overweight/obese populations both with and without the presence of T2D (Racil *et al.*, 2013; Mitranun *et al.*, 2014). In overweight and obese males and females with T2D, a HIIT protocol involving 4-6 x 60 s at 80-85 % $\dot{V}O_{2peak}$ elicited a reduction in insulin resistance (HOMA-IR) that was accompanied with a reduction in fasting plasma glucose by 14 % (Mitranun *et al.*, 2014). This demonstrates the benefits of HIIT in a population already exhibiting the pathophysiological events of T2D. A reduction in insulin resistance (HOMA-IR) in obese females without diabetes has also been reported following 12 weeks of 6 Wingates per session, however despite the reduction in fasting plasma insulin, there was no change in glucose regulation (Racil *et al.*, 2013). It appears that the frequent HIIT induces chronic

physiological and cardiovascular adaptations that can ultimately improve insulin sensitivity and related glycaemic indices.

2.4.5 Effect of HIIT on adiponectin and leptin

Exercise, specifically HIIT, has been demonstrated to elevate adiponectin concentration. Following 12 weeks of HIIT involving 2 series of 6 Wingates, adiponectin was increased by 25 % in overweight and obese young females (Racil *et al.*, 2013). This was accompanied by a 30 % improvement in insulin resistance and a reduction in blood insulin concentration, however no change in systemic glucose was observed. Interestingly, HIIT was more effective in elevating adiponectin concentration (7.4 ± 1.5 $\mu g \cdot ml^{-1}$ to 9.9 ± 1.5 $\mu g \cdot ml^{-1}$; n=11) than continuous moderate intensity exercise (6.7 $\mu g \cdot ml^{-1}$ ± 7.7 $\mu g \cdot ml^{-1}$; n=11). It should be noted that the change in adiponectin was in parallel to a reduction in body mass. Contrastingly, lower adiponectin concentration has been reported following 2 weeks of HIIT involving longer 4 min high intensity intervals in overweight and obese males (Leggate *et al.*, 2012). However, different isoforms of adiponectin exist and since the hormone displays both pro- and anti-inflammatory properties through the up and down regulation of specific proteins and hormones, this may explain the inconsistency in the literature. In addition, the length of HIIT interventions utilised in published literature varies substantially, and as a result, the influence on adiponectin and leptin concentrations will differentiate between short and long term responses reported.

2.5 Vitamin D

2.5.1 Vitamin D metabolism and status

Vitamin D deficiency ($25(OH)D_3$ <20 $ng \cdot ml^{-1}$) has been associated with reduced insulin sensitivity (Pittas *et al.*, 2006; Pittas *et al.*, 2007; Wolden-Kirk *et al.*, 2011). Vitamin D is classified as an active pre-hormone in the body and not technically a vitamin as it must be converted into the active form ($25(OH)D_3$) from 7-dehydrocholesterol via two consecutive hydroxylation

reactions before being biologically and metabolically available to bind to target tissues to subsequently elicit intracellular effects (Kumar, 1984). The main source of 25(OH)D_3 is through skin exposure to Ultra Violet (UV) B rays of a specific wavelength (290-315 nm) amounting to approximately 80 % of total vitamin D status (Holick, 2002). However, despite being primarily dependant on sun exposure, vitamin D_3 can also be ingested through the diet from sources such as lean meats, poultry, beans, eggs and fatty fish, amounting to the remaining 20 % (Holick, 2002, 2009; Zhang and Naughton, 2010). Since the formation of 25(OH)D_3 only occurs when sun rays of a specific intensity directly project onto the skin; this gives rise to seasonal and geographical variances: “sun effect” and the “latitude effect” (MacLaughlin *et al.*, 1982; Chapuy *et al.*, 1997).

It has been estimated through studies investigating the prevalence of vitamin D deficiency and vitamin D status that 30 – 50 % of all children and adults worldwide have established vitamin D deficiency or are at risk (Holick and Chen, 2008). The prevalence of vitamin D deficiency has been reported to be high, particularly in the winter and spring months, in England and Britain as a whole (Hypponen and Power, 2007; Hirani, 2013). The study by Hypponen and Power (2007) included men and women from the 1958 British birth cohort, all aged 45 years old and were born in England, Scotland or Wales. In the latter study almost all of the participants were vitamin D deficient despite the seasonal effect: serum 25(OH)D concentrations peaked in September and were at their lowest in February. Furthermore, Scotland had significantly lower concentrations compared to the other areas of Britain in the summer and autumn months (35.4 $nmol \cdot L^{-1}$ compared to 40.6-42.6 $nmol \cdot L^{-1}$) and the winter and spring months (50.9 $nmol \cdot L^{-1}$ compared to 60.4-62.4 $nmol \cdot L^{-1}$). Hirani *et al*. (2013) reported the prevalence of vitamin D insufficiency and deficiency in men and women aged 65 years and older in England to be 33.8 % and 51.7 %, indicating only 14.5 % exhibit a sufficient status. A study evaluating the vitamin D status of men and women in Scotland reported a high prevalence of deficiency, with a mean 25(OH)D concentration of 35.9 ± 22.5 $nmol \cdot L^{-1}$ (Zgaga *et al.*, 2011). This highlights the importance of interventions, primarily

supplementation and/or dietary, aimed at increasing the vitamin D status of many populations.

Epidemiological studies have shown that vitamin D deficiency is associated with a higher prevalence of T2D (Pittas *et al.*, 2006) and the development of MetS (Barchetta *et al.*, 2013). Therefore, it is crucial to maintain an adequate vitamin D status, however supplementation is presented as a safe alternative to UV light exposure, which is deemed as unsafe by the Institute of Medicine (IOM, 2010). In overweight and obese individuals, lower 25(OH)D_3 concentrations have been reported in individuals with the presence of MetS compared to those not affected by MetS, this was independent from the presence of T2D (Barchetta *et al.*, 2013). The condition MetS involves a combination of at least three of five or six medical conditions that are associated with one another: abdominal obesity, physical inactivity/sedentary lifestyle, hypertension, hyperglycemia, elevated plasma or serum triglycerides, and low high-density lipoprotein (HDL) concentrations.

2.5.2 Vitamin D and insulin sensitivity

Vitamin D has also been demonstrated to have a functional role in the improvement of glucose tolerance through decreases in mean fasting glucose in those with and without T2D (Salehpour *et al.*, 2013; Talaei *et al.*, 2013). Many studies have shown that increased vitamin D concentration, induced by supplementation, can improve fasting plasma insulin and insulin resistance in overweight/obese males and females, with or without T2D and vitamin D deficiency (von Hurst *et al.*, 2010; Nazarian *et al.*, 2011; Harris *et al.*, 2012; Belenchia *et al.*, 2013; Talaei *et al.*, 2013).

There are several mechanisms underpinning the insulin-sensitising action of vitamin D. Hypovitaminosis D has been associated with impaired β-cell function (Pittas *et al.*, 2007), chronic low-grade inflammation, and a decrease in parathyroid hormone (PTH) concentration (Harris *et al.*, 2012). Even after adjusting for weight loss, dietary changes and increased physical activity, a higher 25(OH)D_3 concentration may reduce the risk of developing T2D (Pittas *et al.*, 2012). A direct effect of vitamin D may be through the binding of

25(OH)D_3 to the vitamin D receptors (VDR's) found on β-cells and activating intra-cellular signalling pathways (Guo *et al.*, 2013), involved in transcriptional activation of the human insulin gene and thus up-regulation of insulin production and secretion (Maestro *et al.*, 2002).

2.5.3 Vitamin D deficiency and obesity

The effect of excess fat accumulation on 25(OH)D_3 is often overlooked, however has been found to be a major biological determinant of vitamin D deficiency (Bolland *et al.*, 2007). Vitamin D deficiency is categorised as a concentration of <20 $ng \cdot ml^{-1}$ of 25(OH)D, insufficient concentrations are between 21-29 $ng \cdot ml^{-1}$ and sufficient concentrations are above 30 $ng \cdot ml^{-1}$ (Holick *et al.*, 2011). Vitamin D sufficiency is associated with beneficial alterations in the treatment and pathogenesis of several chronic health conditions, such as hypertension, multiple sclerosis, osteoporosis, and diabetes (Holick, 2009; Zhang and Naughton, 2010). The association between vitamin D concentration and fat mass is due to the sequestering of fat-soluble vitamin D, thereby reducing the bioavailability of systemic vitamin D (Wortsman *et al.*, 2000). This uptake of vitamin D by adipocytes renders the body as a whole deprived of vitamin D if there is an imbalance between the rate of sequestering vitamin D and the rate of 25(OH)D_3 synthesis. A strong causal relationship occurs in the effect of fat mass on 25(OH)D_3 concentrations, with each 1 kg difference in body mass inducing a deficit change in 25(OH)D_3 concentration (Bolland *et al.*, 2007).

2.5.4 Vitamin D supplementation

It is suggested that maintaining an adequate vitamin D status can be achieved through daily supplementation (Holick *et al.*, 2011). The IOM recommends a daily dietary intake of 600-800 International Units (IU) per day (IOM 2010). Note that 100 IU of vitamin D is equivalent to 100 µg. Similarly, the US Endocrine Society Guidelines recommend a daily intake of at least 600-800 IU/day for adults aged 19-50 years (Holick *et al.*, 2011). However, greater intake is necessary to replete vitamin D concentrations and counteract

insufficiency particularly in a population group that may be at risk of inefficient utilisation of ingested vitamin D, i.e. the obese (Ekwaru *et al.*, 2014). Excess adipose tissue in overweight/obese individuals exhibits unfavourable sequestering of 25(OH)D, therefore posing a need for increased consumption via supplementation. Intervention studies have supplemented with a vast variation in dosage from 400-10,000 IU/day. Ideally supplementation should be population-specific and prescribed based on the current vitamin D status and characteristics of the individual: i.e. geographical location, presence of T2D, or overweight/obese. However, generally baseline vitamin D concentrations are not known prior to supplementation. It is primarily central subcutaneous and visceral fat accumulation that affects the bioavailability of systemic 25(OH)D (Wamberg *et al.*, 2013).

Vitamin D can be supplemented in two forms: vitamin D_2 known as ergocalciferol, and vitamin D_3 known as cholecalciferol. The two forms should not be used interchangeably as they do not have equal nutritional values. It has been reported that vitamin D_3 supplementation increases serum 25(OH)D concentration more effectively than vitamin D_2, by as much as 70 % more (Trang *et al.*, 1998). Three months of vitamin D_3 supplementation has been shown to raise serum 25(OH)D more than vitamin D_2, indicating an effective response to vitamin D_3 (Nimitphong *et al.*, 2013). However, it should be noted that the dosage was only 400 IU/day and fasting 25(OH)D concentration did not exceed 30 $ng \cdot ml^{-1}$ (sufficiency) indicating dose of supplementation is important.

The IOM have recommended an upper level intake of 4000 IU/day to prevent any associated harm or risk from potentially excessive or high vitamin D consumption (Jones, 2008). The Endocrine Society suggest that a daily dose of 1500-2000 IU/day or more may be required to elevate circulating 25(OH)D concentration above 30 $ng \cdot ml^{-1}$. They also suggest a dose of 10,000 IU/day may be required to correct vitamin D deficiency; however this dose is substantially higher than the upper intake limit issued by the IOM (2010). The differences between set guidelines and recommendations should be noted and taken into consideration when establishing a supplementation dose. Doses should also be prescribed on a population-specific basis.

2.5.5 Vitamin D supplementation and insulin sensitivity

There are numerous methodological variations in studies investigating the effects of vitamin D supplementation on glucose tolerance, insulin sensitivity and hormones involved in metabolism such as adiponectin. For example, the length of supplementation and the dose varies between interventions with the majority of studies involving overweight and obese male and females, some with diagnosed T2D. Doses of 1000 IU/day for 12 weeks in overweight/obese females free of diabetes increased 25(OH)D_3 concentration by 51 % (Salehpour *et al.*, 2013). Similarly, 12 months of this dose elevated 25(OH)D_3 concentration by 31 % in overweight adults with T2D (Breslavsky *et al.*, 2013). However, the latter study reported vitamin D status to remain deficient after the 12 months, suggesting established insulin resistance may be a determinant of low 25(OH)D_3 status.

There are few studies that demonstrate changes in glycaemic control post-supplementation but a reduction in fasting plasma glucose has been reported in two studies by 6.4 % and 5 %, respectively (Salehpour *et al.*, 2013; Talaei *et al.*, 2013). Talaei *et al*. (2013) supplemented males and females with T2D with relatively high supplementation dose of 50,000 IU/week, equivalent to 7142 IU/day over the course of 8 weeks. Whereas, Salehpour *et al*. (2013) provided a much lower dose of 1000 IU/day to overweight and obese females over 12 weeks and found a similar result in fasting glucose. The most common supplement doses are 2000 IU or 4000 IU/day, with studies supplementing with these doses for periods of 12-24 weeks. The lower 2000 IU/day dose has been found to increase vitamin D concentration by 19 % in obese adolescents after 12 weeks (Nader *et al.*, 2014), 25 % in overweight/obese adults with pre-diabetes after 16 weeks (Mitri *et al.*, 2011), and 64 % in adults with T2D after 24 weeks (Ryu *et al.*, 2014). These studies observed no change in fasting insulin, insulin sensitivity, or glycaemia but reported sufficient 25(OH)D3 status (>30 $ng \cdot ml^{-1}$) following supplementation. Vitamin D_3 was also increased in obese adolescents after 12 weeks with no change in insulin sensitivity in the 2000 IU/day group but not the 400 IU/day group (Javed *et al.*, 2015). Interestingly, a higher dose of 4000 IU/day for 12 weeks in African Americans with pre- or early–diabetes has been demonstrated to increase insulin

secretion, and although insulin sensitivity was reduced following supplementation, the decline was only 4 % compared to 12 % observed in the control group (Harris *et al.*, 2012). However, the vitamin D concentration was sufficient at baseline (39 $ng \cdot ml^{-1}$) in both groups and thus the comparably lower reduction in insulin resistance observed after 12 weeks supplementation may be attributed to the substantial rise in concentration to 81.1 $ng \cdot ml^{-1}$. The vitamin D status of the placebo group remained unchanged. Additionally, the difference between week 6 and 12 was minimal in comparison to the overall increase in concentration, suggesting there may be a plateau in the dose-response relationship depending on current blood vitamin D concentrations.

Six month interventions involving 4000 IU/day have also demonstrated increases in $25(OH)D_3$ concentrations by 50 % and 72 %, accompanied with improvements in insulin resistance (von Hurst *et al.*, 2010; Belenchia *et al.*, 2013). Belenchia and colleagues (2013) investigated obese adolescents and observed a greater reduction in fasting plasma insulin than the overweight middle-aged female population included in von Hurst *et al.* (2010) study: 28.1 % compared to 15 %. The baseline $25(OH)D_3$ concentrations were considerably lower in the adults since they had declared vitamin D deficiency and it was claimed that insulin resistance improved when $25(OH)D_3$ concentrations reached 32 $ng \cdot ml^{-1}$. This falls in line with the hypothesis that insulin sensitivity is improved when the body enters and remains in a vitamin D sufficient status.

Higher doses designed to achieve serum $25(OH)D_3$ concentrations of ≥30 $ng \cdot ml^{-1}$ have controversially observed no changes in glucose or insulin concentrations, despite the long intervention lengths of 12 months and 26 weeks (Davidson *et al.*, 2013; Wamberg *et al.*, 2013). Supplementing with a weekly dose of ≥64,731 IU rapidly increased $25(OH)D_3$ concentrations but did not have an effect on insulin secretion or insulin sensitivity in individuals with pre-diabetes (Davidson *et al.*, 2013). Following 26 weeks of supplementation, elevated vitamin D concentrations had no effect on glucose or insulin concentrations in deficient obese adults, again this is despite a high supplementation dose of 7000 IU/day (Wamberg *et al.*, 2013). In contrast, a 4

week intervention involving a high dose of 10,000 IU/day increased insulin sensitivity by 37 % and reduced the acute insulin response to glucose (Nazarian *et al.*, 2011). Supplementation with 5000 IU/day for 12 weeks has also been found to effectively increase vitamin D concentrations from deficient to sufficient and was associated with a significant improvement in β-cell activity and a tendency for improvement in insulin resistance, although not statistically significant (Al-Sofiani *et al.*, 2015). Improved β-cell function and activity can potentially reduce the risk of developing T2D, however a change in insulin sensitivity is usually parallel to reduce or prevent hyperglycaemia. Furthermore, this may only apply to those with T2D, whereas in those free of diabetes, elevating vitamin D concentrations and improving β-cell function and insulin sensitivity may prevent the development of pre-T2D.

Studies focussing on the dose-response relationship of $25(OH)D_3$ supplementation found a significant interaction between dose and time with a linear relationship observed (Gallagher *et al.*, 2012; Gallagher *et al.*, 2013). The elevated concentration in the obese older adults after 6 months was not significantly different to that found after 12 months for all doses (400-4800 IU/day) (Gallagher *et al.*, 2012). This assumes that once $25(OH)D_3$ concentrations reach a certain threshold, supplementation can be reduced to a dose that will maintain vitamin D sufficiency and ameliorate unnecessary over-consumption of the pre-hormone.

It is hypothesised that obesity is a determinant of vitamin D deficiency and supplementation has been shown to overcome this (Belenchia *et al.*, 2013). It has been found that the dose-response relationship between vitamin D supplementation and 25(OH)D concentrations follows an exponential curve (Figure 2), and the relationship between vitamin D supplementation and body mass has a curvi-linear response (Figure 3) (Ekwaru *et al.*, 2014). Weight status induces a substantial influence on $25(OH)D_3$ concentrations and thus justifies the modification of supplementation dose for overweight and obese individuals. Recommendations for vitamin D intake by the IOM do not take into consideration the dose-response with regards to weight/BMI, however the Endocrine Society acknowledge body mass differentials and recommend higher doses for overweight and obesity to achieve sufficiency, concurring with

many studies (Gallagher *et al.*, 2012; Choi *et al.*, 2013; Gallagher *et al.*, 2013; Zittermann *et al.*, 2014).

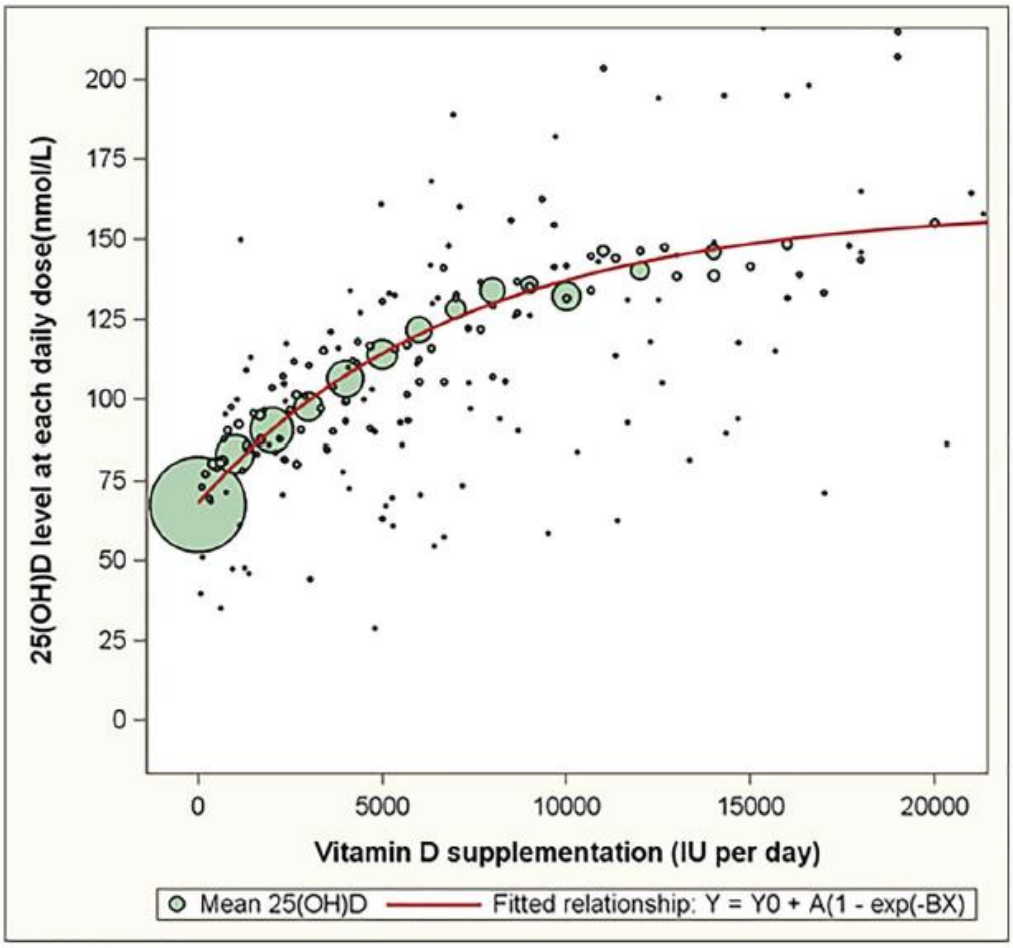

Figure 2: The dose-response relationship between vitamin D supplementation and 25(OH)D concentration. Taken from Ekwaru et al. (2014): page 4.

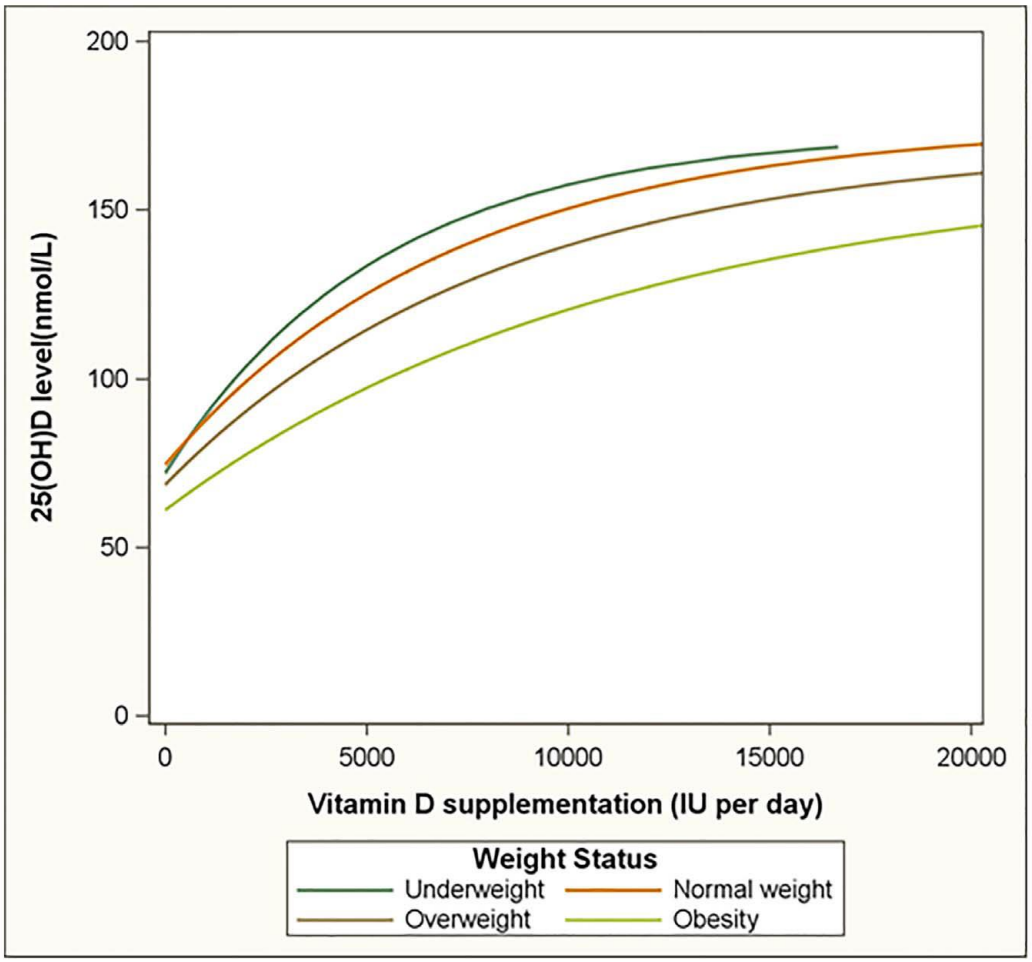

Figure 3: The dose-response relationship between vitamin D supplementation and 25(OH)D concentration by weight status based on BMI category. Taken from Ekwaru et al. (2014): page 7.

2.5.6 Effect of vitamin D supplementation on adiponectin and leptin

Elevated vitamin D concentration has been associated with increases in adiponectin. In adults with features of MetS, systemic adiponectin was increased by 21 % after 12 months of supplementation with 1000 IU/day, however no change in insulin resistance was observed (Breslavsky *et al.*, 2013). An inverse correlation has been reported between insulin sensitivity and adiponectin at baseline in adults with T2D (Al-Sofiani *et al.*, 2015) suggesting adiponectin may play a role in metabolic pathways that control glycaemia. Adiponectin favours the uptake of glucose into skeletal muscle and thus is regarded as a predictor for reducing hyperglycaemia (Punthakee *et al.*, 2006). This is assumed to be through insulin-mediated pathways, suggesting changes in the blood insulin profile. Changes in free-fatty acid concentrations

and metabolism that are induced by exercise can also determine improvements in glycaemia, which may be linked to changes in circulating adiponectin (Johannsen *et al.*, 2013).

2.6 Summary

Independently, both exercise and vitamin D_3 supplementation indirectly and directly induce beneficial responses and adaptations on insulin sensitivity and glucose regulation.

Vitamin D supplementation with the aim to increase concentrations, ideally to a sufficient concentration (>30 $ng \cdot ml^{-1}$), has been reported to induce a reduction in fasting glucose (Salehpour *et al.*, 2013; Talaei *et al.*, 2013), and improve insulin sensitivity (von Hurst *et al.*, 2010; Nazarian *et al.*, 2011; Belenchia *et al.*, 2013; Talaei *et al.*, 2013). Independent of vitamin D supplementation studies, the benefit of HIIT have been demonstrated through reported reductions in fasting insulin (Trapp *et al.*, 2008; Whyte *et al.*, 2010; Iellamo *et al.*, 2013; Racil *et al.*, 2013), reductions in fasting glucose (Mitranun *et al.*, 2014; Shaban *et al.*, 2014), and improvements in glucose tolerance shown through reductions in glucose and insulin AUC following an OGTT (Babraj *et al.*, 2009; Little *et al.*, 2011; Adamson *et al.*, 2014).

Both HIIT and vitamin D_3 supplementation can independently improve glucose regulation and the body's response to insulin, and influence concentrations of leptin (Trapp *et al.*, 2008; Belenchia *et al.*, 2013; Ghavamzadeh *et al.*, 2014) and adiponectin (Leggate *et al.*, 2012; Breslavsky *et al.*, 2013; Racil *et al.*, 2013). However, there is very little research investigating a combined effect of vitamin D supplementation and exercise training on these markers. Recent studies have suggested that the effect on glucose control is independently regulated by vitamin D status/supplementation and exercise training (Barker *et al.*, 2013; Kobza *et al.*, 2013), although HIIT has not been used as the exercise model in parallel to supplementation.

2.7 Aims and hypothesis

This project will aim to investigate whether there is a combined effect of HIIT and vitamin D supplementation on insulin sensitivity, and determine the magnitude of effect HIIT has on markers of physical capacity and metabolism.

It is hypothesised that the additive effect of vitamin D will augment the improvement in insulin sensitivity observed through 6 weeks of HIIT. Furthermore, HIIT alone will induce cardiovascular and metabolic adaptations that benefit the insulin and glycaemic profile.

3.0 Materials and methods

3.1 Overview of study

The study is a placebo-controlled randomised trial investigating the combined effect of HIIT and 25(OH)D_3 supplementation on insulin sensitivity: assessed via a 75 g OGTT. Overweight/obese and inactive adults were recruited to complete 6 weeks of HIIT and receive either a 25(OH)D_3 supplement or a placebo, 1 per day (42 in total), for the duration of training. In order to introduce periodisation into the training programme, the workload of the high intensity intervals was increased by 10 % of peak oxygen uptake ($\dot{V}O_{2peak}$) every 2 weeks/6 sessions. This ensured participants were working in a specific HR zone throughout the entire intervention. Pre- and post-training measurements were taken to determine the metabolic adaptations following 6 weeks of HIIT with or without 25(OH)D_3 supplementation, with physiological data taken every 2 weeks to monitor progress.

3.2 Participants and ethical approval

Participants (n=12, 9 males and 3 females) were recruited from the Edinburgh area via: posters, social media (Facebook, Twitter and Gumtree), letters sent to Edinburgh based companies and organisations, and presentations to university students. Participants' baseline characteristics are provided in Table 1.

The inclusion criteria requested Caucasian overweight and obese males and females between the ages of 18 to 45 y with a BMI of 27-35 $kg \cdot m^{-2}$ who undertake 2 or less bouts of light to moderate intensity exercise per week. Only Caucasian individuals were included as skin colour/pigmentation is associated with T2D and hyperglycemia (Parra *et al.*, 2004). The exclusion criteria excluded persons who have diagnosed diabetes, current smokers (or a smoker who has quit less than 15 months ago), use of tanning beds or undergo UV light therapy, take vitamin D tablets or any multivitamins containing vitamin D, take part in more than 2 bouts of light to moderate intensity exercise per week. Females were included only if they were on hormonal control as the menstrual cycle alters glucose metabolism (Widom *et al.*, 1992).

All participants were asked to complete a health and physiology screening questionnaire (Appendix 1 A) and provide written and verbal consent (Appendix 1 B) to verify eligibility for the study.

Ethical approval for the study was granted by the Faculty of Health, Life, and Social Sciences Research Integrity Approvals Group at Edinburgh Napier University.

Table 1: Baseline participant characteristics

	All	**Placebo**	**Vitamin D**
N (males:females)	12 (9:3)	6 (5:1)	6 (4:2)
Age, y	32 ± 8	32 ± 9	33 ± 7
Height, m	1.73 ± 0.10	1.77 ± 0.09	1.69 ± 0.10
Body Mass, kg	96.0 ± 15.6	90.6 ± 11.9	101.5 ± 18.0 *
BMI, $kg \cdot m^{-2}$	31.9 ± 2.8	29.7 ± 2.0	34.1 ± 1.5
Waist circumference, cm	100 ± 10	96 ± 9	103 ± 11
Hip circumference, cm	111 ± 6	107 ± 4	116 ± 5
Waist-to-hip ratio	0.90 ± 0.08	0.90 ± 0.09	0.89 ± 0.08
Resting HR, bmp	73 ± 10	71 ± 10	75 ± 10
Systolic blood pressure, mmHg	132 ± 10	130 ± 13	133 ± 6
Diastolic blood pressure, mmHg	81 ± 8	79 ± 1	84 ± 6
Fasting glucose, $mmol \cdot L^{-1}$	4.8 ± 0.5	4.7 ± 0.5	4.9 ± 0.6
Fasting insulin, $mU \cdot L^{-1}$	7.0 ± 3.8	8.1 ± 3.5	8.4 ± 5.3

*Significantly different compared with placebo (P<0.05). All data is presented as mean ± standard deviation (SD).

3.3 Control measures

Participants were asked to abstain from consuming caffeine and alcohol and engaging in strenuous exercise in the 24 h prior to the $\dot{V}O_{2peak}$ test and the OGTT. Participants were asked to maintain their normal diet and habitual activity for the duration of the intervention.

Environmental conditions were not controlled but were recorded at the beginning of each visit: temperature, barometric pressure, and humidity.

3.4 Visit 1

3.4.1 Anthropometric measurements and blood pressure

On arrival to the Sport and Exercise Science laboratory participant's height and body mass were measured via a Stadiometer (Harpenden Portable, Holtain Limited, UK) and scales (Seca, 808, Germany), respectively. Waist and hip circumference were also measured using a standard measuring tape. Waist circumference was measured half way between the iliac crest and the lowest rib. Hip circumference was measured at the widest part of the hips (WHO, 2008). These measurements were then used to calculate the waist-to-hip ratio.

Body mass was measured at the beginning of every visit to the laboratory. Waist and hip circumferences were measured at the start of each 2 week stage (sessions 1, 7 and 13) and in the post-intervention session (visit 21).

Arterial blood pressure was measured on the participant's non-dominant arm using a digital automatic blood pressure monitor (Omron, R5-1, Japan). Participants remained in a seated position for 10 min prior to the first measurement. Blood pressure was taken 3 times and an average of the second and third readings was reported. If blood pressure was >150/100 mmHg, participants were excluded from the study and informed to contact their GP.

3.4.2 Peak oxygen uptake test

Participants $\dot{V}O_{2peak}$ was determined using a continuous incremental exercise test on an electromagnetically-braked cycle ergometer (Velotron Pro, Racer Mate, USA), performed to volitional exhaustion (Yoon *et al.*, 2007; Poole *et al.*, 2008). An on-line breath-by-breath gas analysis system (Cortex, MetaLyzer 3B, Germany) was used to measure expired air continuously throughout the test. Calibration of the flow sensor and the gas analysis system was performed 30 min prior to each use. Heart rate was also continuously monitored throughout the test by a HR monitor (Polar, RS400, Finland) and the HR sensor linked to the gas analysis system. After a 5 min warm up at 50 W, the

intensity increased by 25 W every 2 min until volitional exhaustion was achieved. Participants were instructed to maintain a pedalling rate of 60 revolutions per min (rpm). Participants were verbally encouraged to perform to volitional exhaustion and the test was terminated when the pedalling rate fell below 55 rpm. A 5 min cool down at 50 W was then immediately completed. Peak oxygen uptake was identified as the highest $\dot{V}O_2$ over a 30 s period during the test.

3.4.3 Familiarisation trial

In the same session, following 30 min rest in a seated position after completion of the $\dot{V}O_{2peak}$ test, a familiarisation trial for the HIIT protocol was performed. This involved a shortened version of the HIIT protocol consisting of 5 x 1 min intervals at 100 % $\dot{V}O_{2peak}$ interspersed with 1 min at 30 W. Breath-by-breath data and HR data was gathered for the duration of the familiarisation session. This session was to familiarise the participant with the structure of the HIIT protocol and to ensure that HR_{peak} was within the desired HR zone of 85-95 % HR_{peak} (determined in the $\dot{V}O_{2peak}$ test) prior to the training sessions.

3.5 Visit 2

3.5.1 Oral glucose tolerance test

This test occurred 4-5 days after the $\dot{V}O_{2peak}$ test session to prevent any residual effects influencing the metabolic/biological markers measured during the glucose test. Participants attended the laboratory between 7.30-8.30am following an overnight fast (≥10 h) to complete a 75 g OGTT. Resting blood samples (3 x 4 ml) were taken via an indwelling cannula, then participants consumed 82.5 g of dextrose monohydrate dissolved in 290 ml of water and 10 ml of lemon juice over a 5 min period. Further blood samples (2 x 4ml) were subsequently taken every 30 min for 2 h. Participants were required to remain in a seated position for the 2 h period.

3.6 Visits 3-20

3.6.1 High intensity intermittent training

Training was initiated within 1-3 days following the OGTT, depending on participants' availability, and consisted of 6 weeks training and 3 sessions/week of HIIT: separated by 1-2 days to allow muscle recovery and rest. The HIIT protocol is based on that utilised in recent studies (Little *et al.*, 2010; Little *et al.*, 2011; Little *et al.*, 2014; Mancilla *et al.*, 2014). Participants completed a warm up and cool down consisting of 5 min at a low power output of 50 W. The protocol consisted of 10 x 1 min intervals at a power output corresponding to: 100 % $\dot{V}O_{2peak}$ (determined in visit 1) in sessions 1-6; 110 % of $\dot{V}O_{2peak}$ in sessions 7-12; 120 % of $\dot{V}O_{2peak}$ in sessions 13-18. The percentage of $\dot{V}O_{2peak}$ (100, 110 and 120 %) and corresponding workload/power output for the high intensity intervals was determined using the equation of the trend-line from a plotted graph of $\dot{V}O_2$ (x axis) against power output (y axis), and adjusted accordingly following the familiarisation trial. The intervals were interspersed with 1 min active recovery periods set at 30 W. Figure 4 illustrates a schematic timeline of a single session of the HIIT. Heart rate was also continuously monitored throughout the test by a HR monitor (Polar, RS400, Finland)

Figure 4: Schematic diagram of a HIIT session

3.6.2 25(OH)D_3 Supplementation

Participants were randomised (1:1) to receive a 6 week course of 25(OH)D_3 capsules or placebo tablets- 1 per day. Randomisation of the supplements and placebo was blind to both participant and researcher and controlled by the laboratory technicians. Supplements and placebo were distributed in brown

envelopes to participants, which included instructions for consumption. Each of the 25(OH)D_3 capsules (Solgar, New Jersey, USA) contained a dose of 4000 IU/day (100 mg), which corresponds to the upper intake level set by IOM (2010). The placebo was a commercially available lactose tablet (Placebo-World, Powys, UK), with one consumed daily. Participants were asked to consume one supplement or placebo per day at a similar time in the morning, and were reminded at each training session to continue taking the tablets daily. Adherence to supplement and placebo consumption was self-reported by participants and is reported as 100 % compliance.

3.7 Visit 21

3.7.1 Post-training OGTT and $\dot{V}O_{2peak}$ test

The OGTT and $\dot{V}O_{2peak}$ were repeated approximately 72 h post-training. The same procedures were followed for both trials and completed at the same time of day. Upon completion of the OGTT, participants rested for 30 min in a seated position before performing the $\dot{V}O_{2peak}$ test.

3.8 Blood sampling

All venous blood samples (44 ml in total) were taken from the antecubital vein in the forearm via cannulation using a 20 G BD Venflon Pro Safety I.V. cannula (Sweden). Connecta tubing was used to prevent movement of the positioning of the needle. The cannula was kept patent via syringe flushing between samples with 5 ml of 0.9 (w/v) saline solution. A Terumo syringe was used to extract the first 2.5 ml of blood and was discarded prior to sample collection. Blood samples were collected in 4 ml capacity BD vacutainers: grey-topped vacutainers containing sodium fluoride/potassium oxalate for glucose concentration analysis and lavender-topped vacutainers containing K_3 ethylenediaminetetraacetic acid (EDTA) (approximately 1.0 mg) for insulin, vitamin D, and adiponectin concentration analysis. The blood samples were inverted 8 times then placed on ice.

For plasma analysis, the whole blood was centrifuged (Satorius Universal, 320R, Germany) at 1500 rpm for 15 min at 4 °C within 30 min of collection, and the resulting plasma aliquoted into eppendorfs and stored at -80 °C for subsequent analysis.

Participants were in a seated position on a laboratory bed for all blood sampling.

3.9 Blood sample analysis

All assays and analysis methods were performed in duplicate. All plasma samples were thawed prior to analysis.

3.9.1 Enzyme-linked immunosorbent assays (ELISA)

Plasma insulin concentrations were determined using a commercially available ELISA kit, with human low and high controls (Mercodia, Uppsala, Sweden). Fasting plasma 25(OH)D_3 (IDS PLC, Tyne and Wear, UK), adiponectin, and leptin concentrations were analysed via commercially available ELISA kits before and after the 6 week intervention (R&D Systems, Minneapolis, USA).

3.9.2 Biochemical analysis

Plasma glucose, total cholesterol, triglyceride, and HDL cholesterol concentrations were analysed using a bench top clinical chemistry analyser (Randox, RX Monza, UK). Low density lipid (LDL) cholesterol concentration was calculated using the following equation:

$$\text{LDL cholesterol} = Total\ cholesterol - \frac{Triglycerides}{2.2} - HDL\ cholesterol$$

3.9.3 Haematological analysis

Whole blood was analysed for full blood cell count in vacutainers containing K_3 EDTA using a haematology analyser (Sysmex, XS 1000i, USA). Haemoglobin (Hb) concentration, haematocrit (Hct) content, red blood cell count, and white blood cell count and components were measured.

3.9.4 Coefficient of variation (CV)

All CV values for all assays and analysis methods are displayed in Table 2.

Table 2 CV values for all assays and analysis methods

	Analysis method	Intra-CV (%)	Inter-CV (%)	
			Control 1	**Control 2**
Insulin	ELISA	4.76	0.22	4.23
Vitamin D	ELISA	3.43		
Adiponectin	ELISA	3.72		
Leptin	ELISA	5.15		
Glucose	Clinical chemistry analyser	0.17		
Cholesterol	Clinical chemistry analyser	0.1		
HDL cholesterol	Clinical chemistry analyser	0.72		
Triglyceride	Clinical chemistry analyser	0.24		
Haematology	Clinical chemistry analyser	3.64		

3.9.5 Plasma volume changes (PVC)

Changes in plasma volume from pre-to post-training were calculated using a previously outlined method (Dill and Costill, 1974) to determine if PVC would affect the outcomes.

3.9.6 Insulin Sensitivity Index (ISI)

Insulin sensitivity was assessed as the insulin sensitivity index (ISI) calculated using the OGTT results and formula proposed by Matsuda and DeFronzo (1999).

$$\text{ISI} = \frac{10000}{\sqrt{(FPG * FPI) * (G * I)}}$$

Homeostatic model assessment-insulin resistance (HOMA-IR) was also calculated.

$$\text{HOMA-IR} = \frac{FPG * FPI}{22.5}$$

Where:

FPG is the fasting plasma glucose

FPI is the fasting plasma insulin

G is the mean plasma glucose during the OGTT

I is the mean plasma insulin during the OGTT

3.10 Statistical analysis

Data was checked for skewness. All data except the insulin data from the OGTT was normally distributed and parametric tests were used. The insulin data was log transformed prior to statistical analysis. Differences in participant characteristics, $\dot{V}O_{2peak}$ test outcomes, fasted plasma glucose, insulin, glucose AUC, insulin AUC, $25(OH)D_3$, adiponectin, leptin, and lipids, pre- and post-intervention, were analysed using a two-way repeated measures analysis of variance (ANOVA) for overall effect. A repeated measures ANOVA was also used to analyse the insulin and glucose response to an OGTT. If a main effect of time and an interaction effect between groups was observed, Bonferroni post-hoc analysis were performed using paired samples t-tests to compare pre-training to post-training. All statistical analyses were performed using SPSS software (20.0) and data presented as means ± SD. Significance was accepted at $P<0.05$.

4.0 Results

4.1 25-hydroxyvitamin D_3

There was a main effect of time for 25(OH)D_3 (F=7.847, *P*=0.019, Figure 5 **A**), however no interaction effect between groups (F=0.344, *P*=0.570). All participants were deficient at baseline (<20 ng·ml^{-1}) as shown by the individual responses in Figure 5 **B**. Following 6 weeks of supplementation, the vitamin D group resulted in 50 % of participants no longer deficient (>20 ng·ml^{-1}) and 16.6 % reporting sufficiency (>30 ng·ml^{-1}), and participants as a whole resulted in 41.6 % no longer deficient and 8.4 % sufficient post-training.

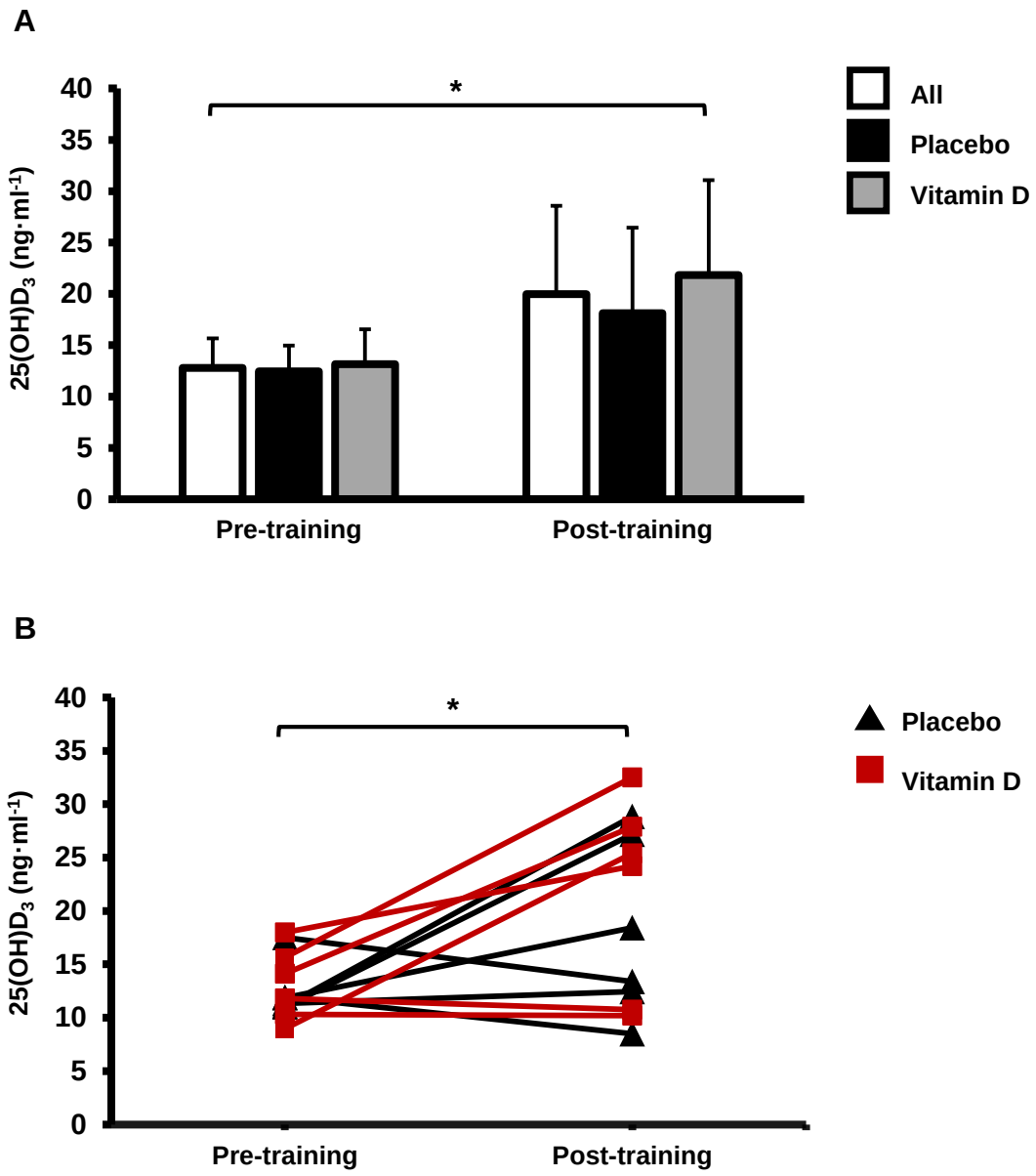

*Figure 5: (**A**) Pre- and post-training 25(OH)D3 concentration for all participants (n=12), the placebo group (n=6), and the vitamin D group (n=6); (**B**) individual changes in 25(OH)D_3 pre- to post-training. *Denotes a main effect of time (P<0.05).*

4.2 Exercise outcomes

Exercise intensity across all intervals during HIIT averaged 89 ± 5 % of HR_{peak} and 97.2 ± 8.4 % of $\dot{V}O_{2peak}$. Table 3 shows the mean power output that participants cycled for all 10 intervals during sessions 1, 7 and 13 when the intensity was increased (F=359.097, *P*=0.000) by the corresponding workload to 10 % of $\dot{V}O_{2peak}$ to allow periodisation of training. Table 3 also presents HR and $\dot{V}O_2$ averaged across all 10 intervals in these sessions. There was no change in HR between the three sessions (F=0.053, *P*=0.949) but $\dot{V}O_2$ observed a time effect (F=29.651, *P*=0.000) with an increase every 2 weeks (*P*≤0.001).

Table 3: Power output, HR and V̇O2 averaged across all 10 intervals in HIIT session 1, 7, and 13.

	Session 1	**Session 7**	**Session 13**	***P* value**
Power output (W)	227 ± 42	250 ± 46	273 ± 50	<0.001
HR (bpm)	166 ± 13	166 ± 14	166 ± 15	0.949
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	27.2 ± 2.7	28.7 ± 2.3	30.3 ± 3.2	<0.001

All data is presented as mean ± SD.

Figure 6 illustrates the percentage of baseline peak HR (**A**) and peak $\dot{V}O_2$ (**B**) that participants were cycling at for each of the intervals in session 1, 7, and 13. In each of these sessions $\dot{V}O_{2peak}$ averaged 91.7 ± 7.7 %, 97.0 ± 7.8 %, and 101.5 ± 8.4 % of baseline $\dot{V}O_{2peak}$. Participants performed the HIIT training in the same HR zone for each session as shown in Figure 6 **A**, with an observed increase in $\dot{V}O_{2peak}$. As a result of every 2 weeks of training, $\dot{V}O_2$ was increased (F=29.651, *P*<0.001).

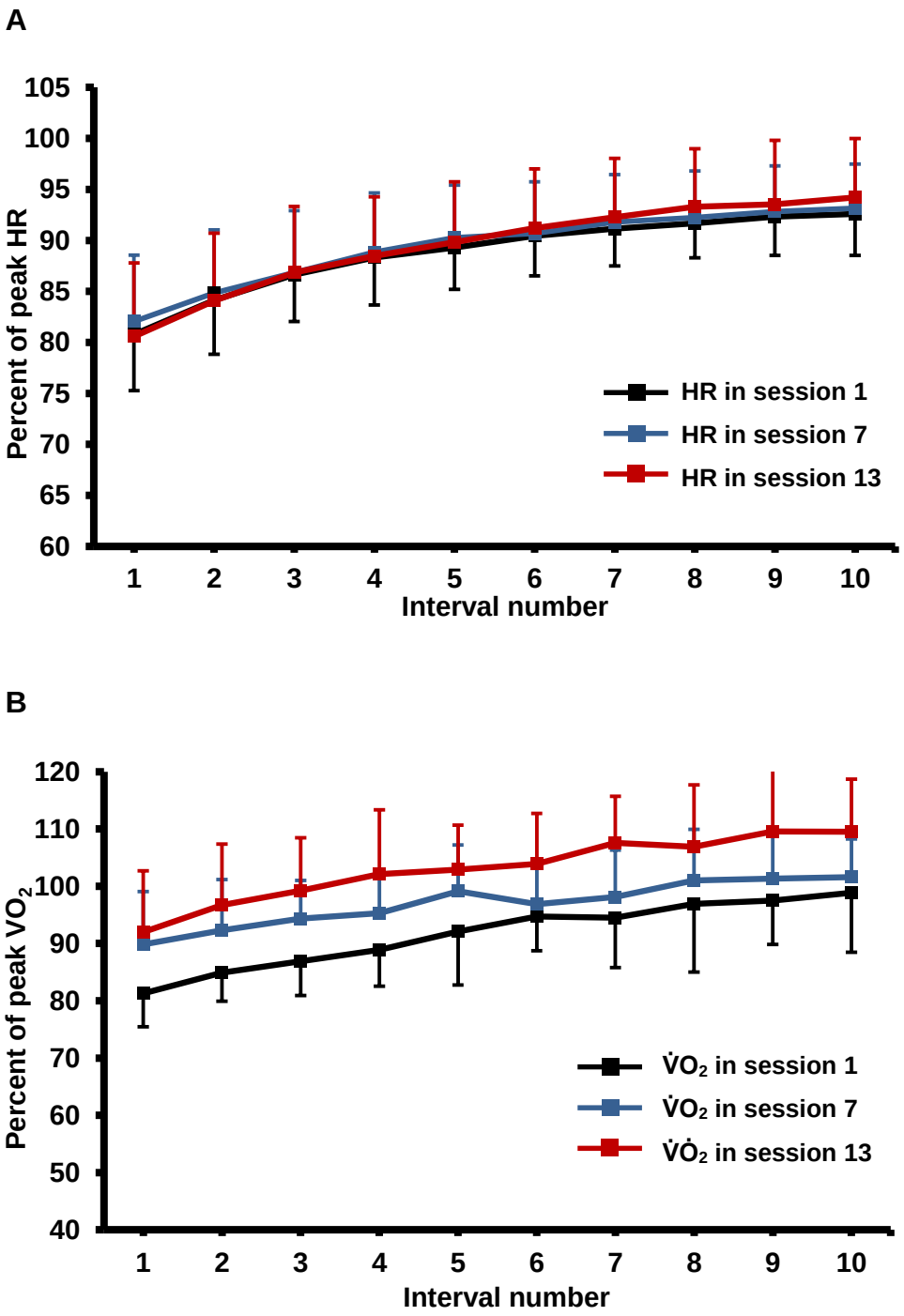

*Figure 6: The percentage of baseline (**A**) peak HR and (**B**) peak $\dot{V}O_2$ that participants were cycling at during the individual intervals in the first, seventh and thirteenth HIIT sessions (n=12).*

As displayed in Figure 7, the absolute and relative $\dot{V}O_{2peak}$ at baseline for all participants was 2.86 ± 0.56 L·min^{-1} and 29.7 ± 2.7 ml·min^{-1}·kg^{-1}, respectively, and occurred at a mean power output of 227 ± 42 W. The absolute and relative $\dot{V}O_{2peak}$ at baseline for the placebo group was 3.05 ± 0.65 L·min^{-1} and 28.8 ± 3.1 ml·min^{-1}·kg^{-1}, respectively, and occurred at a mean power output of 237 ± 51 W; and the vitamin D group was 2.67 ± 0.41 L·min^{-1} and 30.7 ± 2.2 ml·min^{-1}·kg^{-1}, respectively, and occurred at a mean power output of 217 ± 31 W.

As a result of the 6 weeks of HIIT training there was a main effect of time in absolute $\dot{V}O_{2peak}$ (F=14.797, *P*=0.004, Figure 7 **A**). Relative $\dot{V}O_{2peak}$ observed a main effect of time (F=16.631, *P*=0.003, Figure 7 **B**). There was no interaction effect between groups for absolute or relative $\dot{V}O_{2peak}$ at 6 weeks.

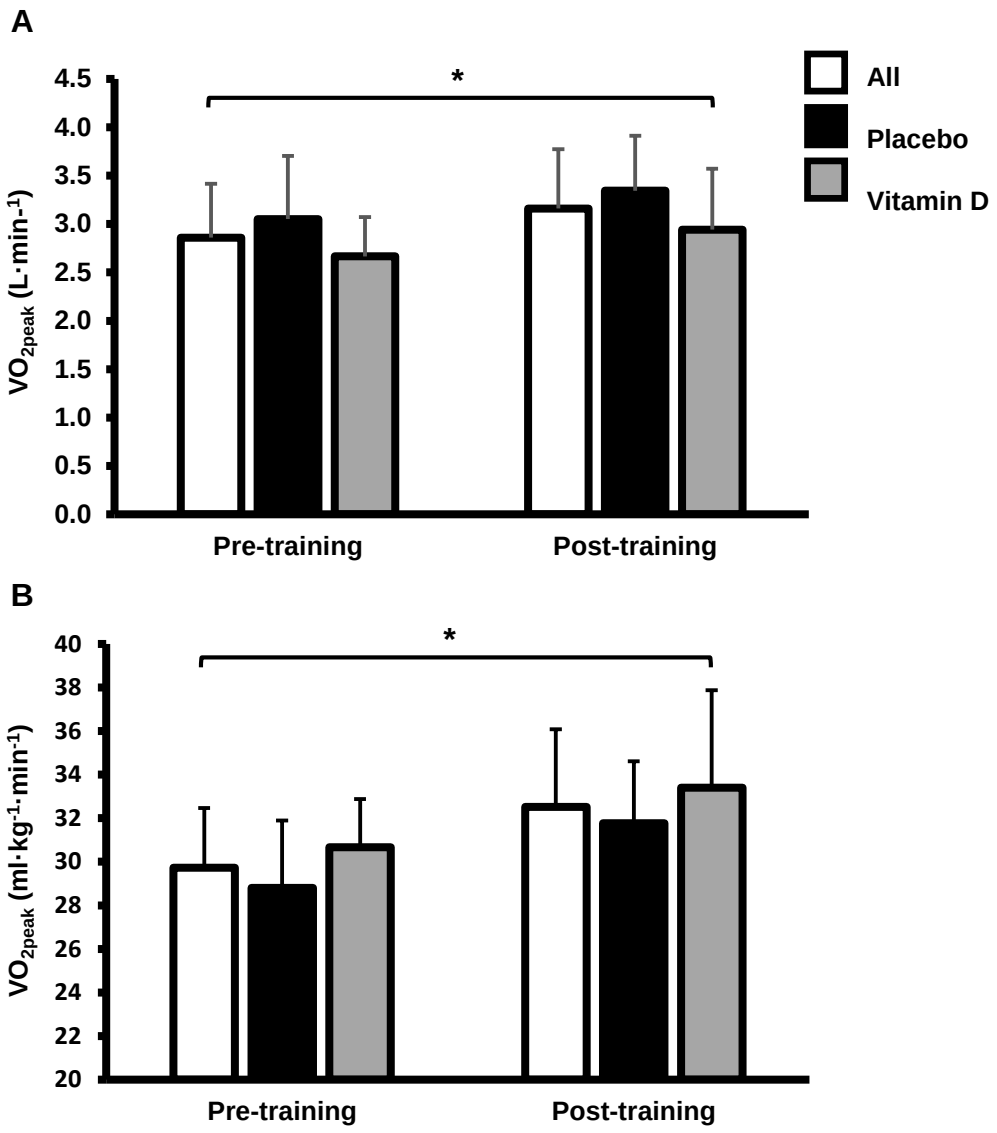

*Figure 7: Absolute $\dot{V}O_{2peak}$ (**A**) and relative $\dot{V}O_{2peak}$ (**B**) pre- and post-training for all participants (n=12), the placebo group (n=6), and the vitamin D group (n=6). *Denotes a main effect of time (P<0.05).*

There was a main effect of time on absolute peak power output (F=23.233, *P*=0.001, Figure 8 **A**). This was consistent in peak power relative to body mass, with a main effect of time (F=21.584, *P*=0.001, Figure 8 **B**). There was no interaction effect between groups for absolute or relative peak power output at 6 weeks.

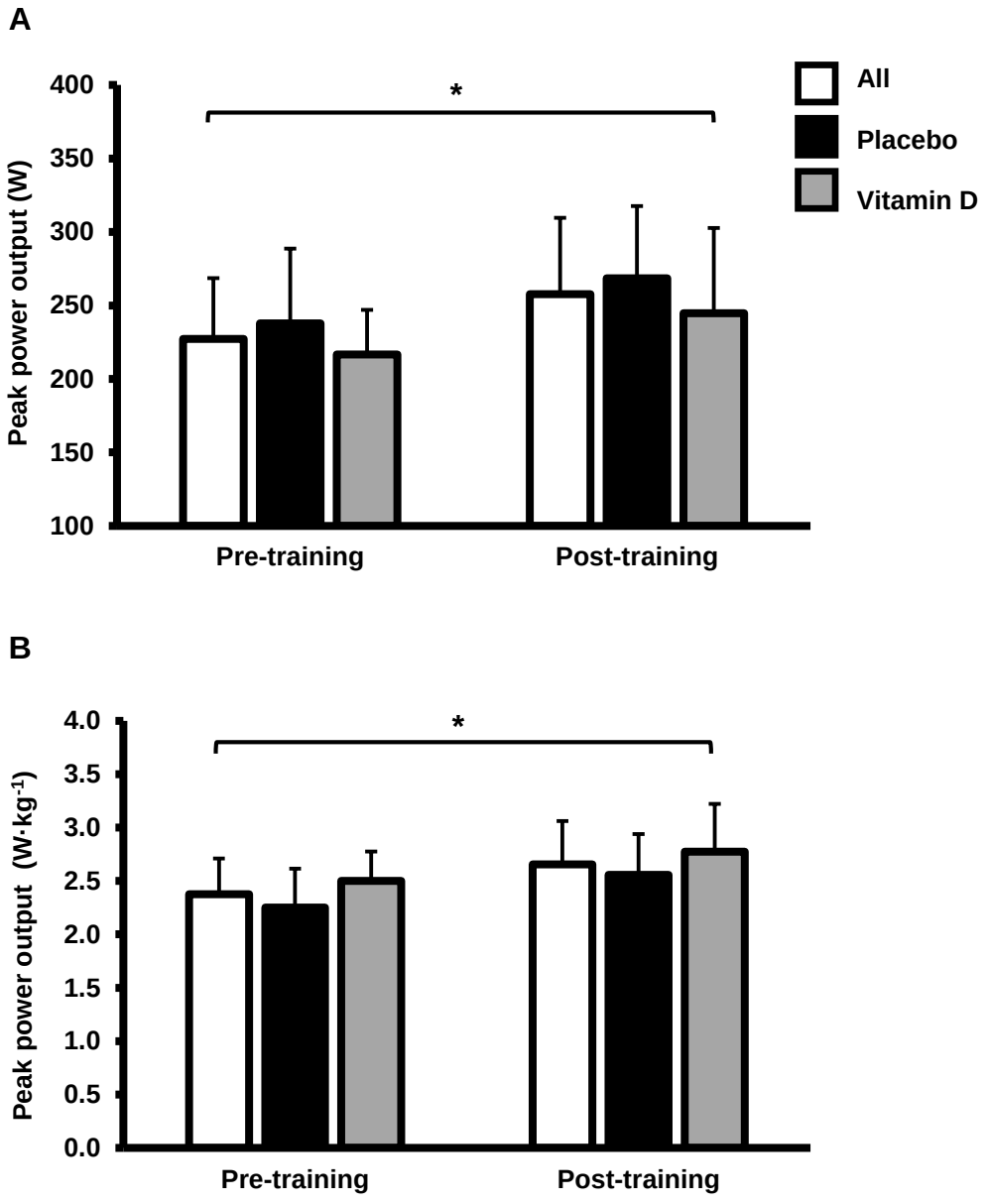

*Figure 8: Absolute W_{peak} (**A**) and relative W_{peak} (**B**) pre- and post-training for all participants (n=12), the placebo group (n=6), and vitamin D group (n=6). *Denotes a main effect of time (P<0.05).*

4.3 Participant characteristics

4.3.1 Physical and metabolic characteristics

Physical and metabolic characteristics of all participants are shown in Table 4. All participants completed all supervised sessions and intervals within the sessions with no complications or interruptions: 100 % adherence and compliance.

No significant differences existed between the placebo group and vitamin D group at baseline except for body mass (P=0.036) and hip circumference (P=0.020).

Hip circumference (F=4.216, P=0.067) and waist circumference (F=0.473, P=0.507) observed no main effect of time. Additionally, body mass did not differ from pre- to post-training (F=0.781, P=0.398). There was a main effect of time on systolic blood pressure (F=8.448, P=0.016). Diastolic blood pressure did not observe an effect of time in any group (F=1.337, P=0.274). Resting HR also did not differ after 6 weeks of HIIT (F=3.301, P=0.099). There were no interaction effects between groups for any of the physical and metabolic characteristics.

Table 4: Pre- and post-training values for all participants, the placebo group, and the vitamin D group

	All		**Placebo**		**Vitamin D**		**Main Effect of Time**	**Interaction Effect Between Groups**
	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training	*P* Value	*P* Value
N (males:females)	12 (9:3)	12 (9:3)	6 (5:1)	6 (5:1)	6 (4:2)	6 (4:2)		
Body Mass, kg	96.0 ± 15.6	95.4 ± 14.6	101.5 ± 18.0	100.0 ± 16.5	90.6 ± 11.9	90.8 ± 12.0	0.398	0.664
BMI, $kg \cdot m^{-2}$	31.9 ± 2.8	31.7 ± 2.6	34.1 ± 1.5	33.6 ± 0.7	29.7 ± 2.0	29.7 ± 2.2	0.445	0.643
Waist circumference, cm	100 ± 10	99 ± 10	103 ± 11	103 ± 8	96 ± 9	95 ± 10	0.507	0.148
Hip circumference, cm	111 ± 6	110 ± 8	116 ± 4	116 ± 6	107 ± 4	105 ± 5	0.067	0.054
Waist-to-hip ratio	0.90 ± 0.08	0.90 ± 0.07	0.89 ± 0.08	0.89 ±0.09	0.90 ± 0.09	0.91 ±0.06	0.935	0.548
Waist-to-height ratio	0.57 ± 0.04	0.57 ± 0.05	0.58 ± 0.03	0.59 ± 0.02	0.57 ± 0.06	0.55 ± 0.05	0.551	0.131
Resting HR rate, bmp	73 ± 10	68 ± 9	75 ± 10	72 ± 8	71 ± 10	65 ± 8	0.099	0.951
Systolic blood pressure, mmHg	132 ± 10	124 ± 13	133 ± 6	125 ± 16	130 ± 13	123 ± 11	0.016	0.345
Diastolic blood pressure, mmHg	81 ± 8	79 ± 8	84 ± 6	78 ± 12	79 ± 10	79 ± 4	0.274	0.911

All data is presented as mean ± SD.

4.3.2 Haematological values

All haematological values are displayed in Table 5. There were no main effects of time or interaction effects between groups for any of the haematological markers.

4.3.3 PVC

There were no significant differences in plasma volume from pre- to post-training (F=0.310, *P*=0.585), therefore the unadjusted data is reported and no changes were made to any outcomes.

Table 5: Haematological values for all participants, the placebo group, and the vitamin D group pre- and post-training.

	All		Placebo		Vitamin D		Main Effect of Time	Interaction Effect Between Groups
	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training	*P* Value	*P* Value
WBC (10^3·µL)	6.2 ± 1.6	5.8 ± 1.4	5.5 ± 1.2	5.7 ± 1.4	6.8 ± 1.8	5.8 ± 1.5	0.346	0.159
RBC (10^3·µL)	4.9 ± 0.3	5.0 ± 0.6	5.0 ± 0.3	5.1 ± 0.8	4.9 ± 0.3	4.8 ± 0.5	0.596	0.249
Hb (g·dL)	14.7 ± 1.0	14.8 ± 1.8	14.9 ± 0.8	15.1 ± 1.9	14.6 ± 1.2	14.4 ± 1.7	0.633	0.225
Hct (%)	43.2 ± 2.5	43.7 ± 5.1	43.7 ± 2.5	44.9 ± 6.1	42.7 ± 2.7	42.6 ± 4.1	0.485	0.244
PLT (10^3·µL)	265 ± 73	267 ± 118	252 ± 64	237 ± 98	279 ± 84	297 ± 138	0.646	0.798
Neutrophils (10^9·L)	3.6 ± 1.5	3.2 ± 1.0	3.1 ± 0.9	3.3 ± 0.9	4.1 ± 1.8	3.1 ± 1.0	0.315	0.113
Lymphocytes (10^9·L)	1.83 ± 0.41	1.86 ± 0.45	1.69 ± 0.39	1.73 ± 0.55	1.97 ± 0.41	1.99 ± 0.31	0.928	0.939
Monocytes (10^9·L)	0.48 ± 0.09	0.50 ± 0.18	0.46 ± 0.09	0.47 ± 0.19	0.50 ± 0.09	0.52 ± 0.20	0.897	0.982
Eosinophils (10^9·L)	0.19 ± 0.08	0.16 ± 0.07	0.22 ± 0.11	0.14 ± 0.07	0.16 ± 0.04	0.17 ± 0.08	0.170	0.192
Basophiles (10^9·L)	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.498	0.692

WBC, white blood cell count; RBC, red blood cell count; Hb, haemoglobin; Hct, haematocrit, PLT, platelets. All data is presented as mean ± SD.

4.4 OGTT outcomes and insulin sensitivity

There was a main effect of time in the insulin response to a 75 g OGTT in all participants (F=5.129, *P*=0.009, Figure 9 **A**), with further analysis showing significant differences between pre- and post-training at 30 min (*P*=0.025) and 120 min post-prandial (*P*=0.004). No main effect of time was observed for the glucose response to an OGTT (F=1.026, *P*=0.511, Figure 9 **B**).

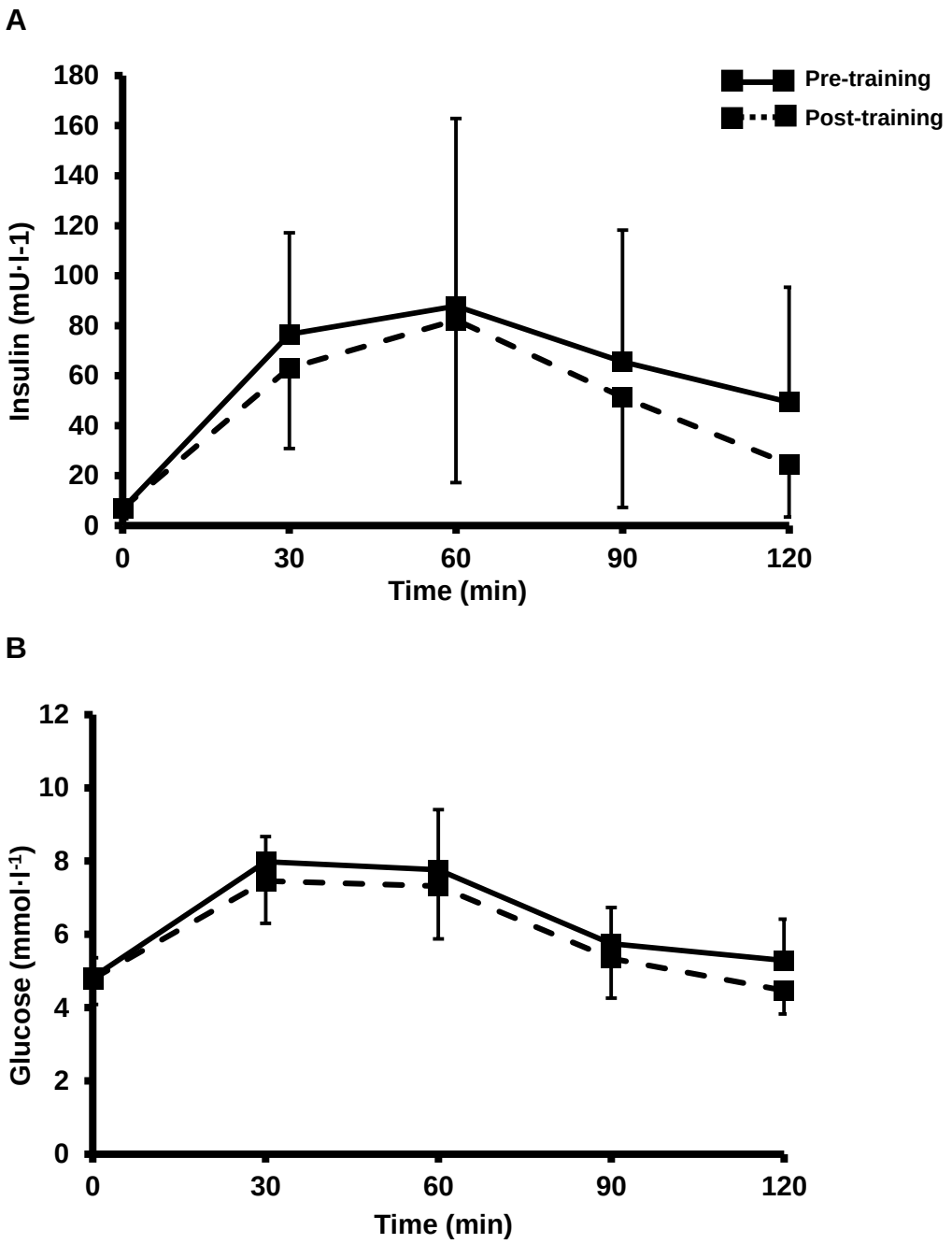

*Figure 9: Plasma glucose (**A**) and insulin (**B**) responses to a 75 g OGTT pre- and post-training for all participants (n=12).*

The glucose and insulin responses to the 2 h OGTT pre- and post-training for both groups are shown in Figure 10 **A** and **B**. There was no interaction effect between the two groups for insulin (F=0.949, *P*=0.353) or glucose (F=1.091, *P*=0.321) responses.

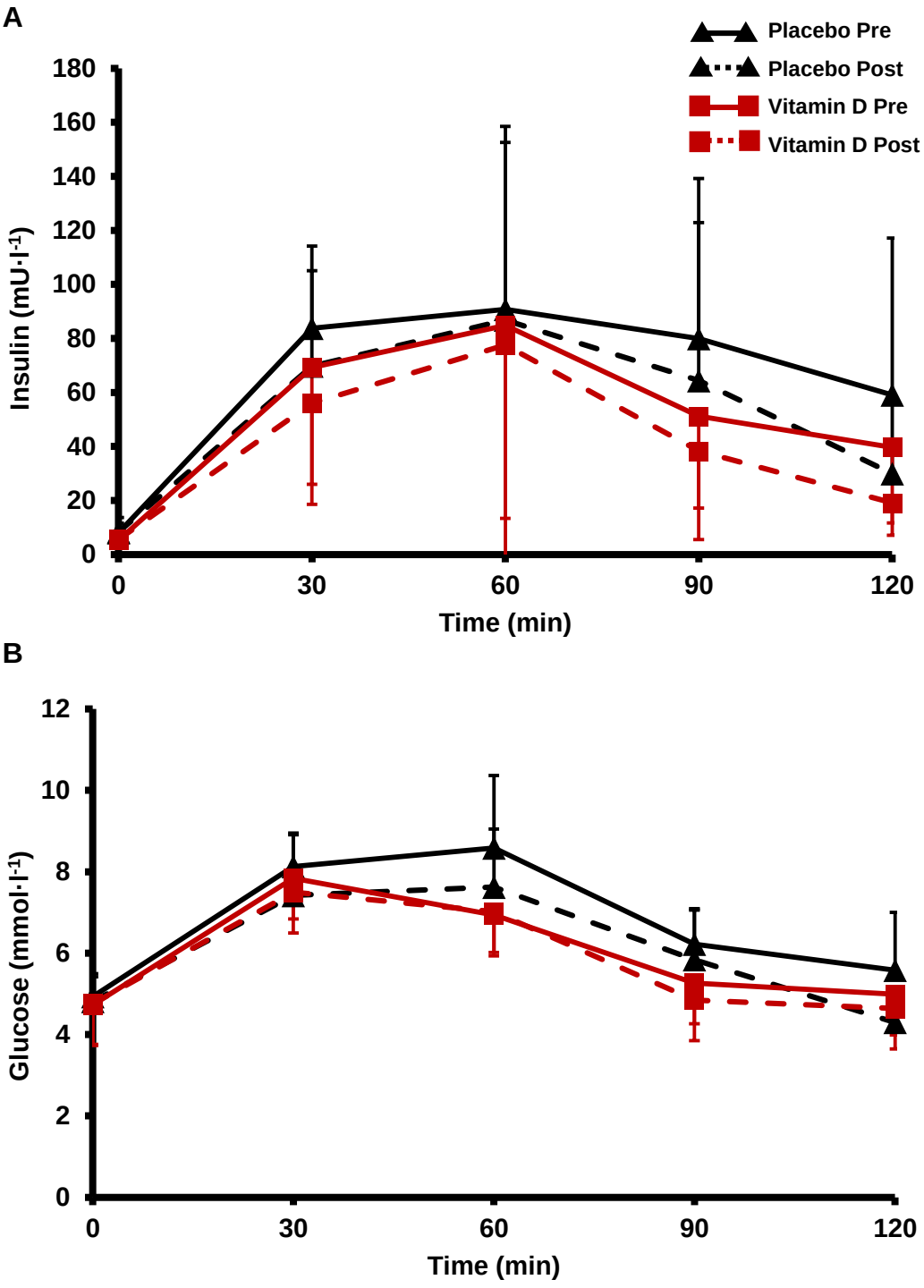

*Figure 10: Plasma glucose (**A**) and insulin (**B**) responses to a 75 g OGTT pre- and post-training for the placebo group (n=6) and the vitamin D group (n=6).*

The glycaemic indices including AUC and insulin sensitivity are shown in Table 6. There were no significant changes in fasting plasma insulin (F=0.100, *P*=0.758) and glucose (F=1.487, *P*=0.251) concentration after 6 weeks of HIIT. No main effect of time was observed for glucose AUC (F=2.997, *P*=0.114). Insulin sensitivity index (ISI) (Matsuda and DeFronzo, 1999) and HOMA-IR (homeostatic model assessment-insulin resistance) remained unchanged pre- to post-training (F=2.131, *P*=0.175; F=0.237, *P*=0.637).

Table 6: Pre- and post-training glycaemic indices

	All (n=12)		Placebo (n=6)		Vitamin D (n=6)		Main Effect of Time	Interaction Effect Between Groups
	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training	*P* Value	*P* Value
Fasting glucose, $mmol \cdot L^{-1}$	4.8 ± 0.5	4.9 ± 0.7	5.0 ± 0.6	4.8 ± 0.6	4.7 ± 0.5	4.8 ± 0.8	0.251	0.118
Fasting insulin, $mU \cdot L^{-1}$	6.7 ± 3.3	7.0 ± 4.3	8.1 ± 3.5	8.4 ± 5.3	5.3 ± 2.6	5.5 ± 3.0	0.758	0.995
Glucose AUC, $mmol \cdot h^{-1} \cdot L^{-1}$	796 ± 98	742 ± 97	846 ± 114	763 ± 114	747 ± 48	722 ± 83	0.114	0.379
Insulin AUC, $mU \cdot h^{-1} \cdot L^{-1}$	7734 ± 5327	6366 ± 4094	8638 ± 5087	7204 ± 5079	6836 ± 5882	5527 ± 3064	0.123	0.940
Insulin sensitivity index†	10.12 ± 8.75	15.71 ± 19.62	7.48 ± 8.99	18.15 ± 26.84	12.77 ± 8.42	13.28 ±10.61	0.175	0.214
HOMA-IR	1.47 ± 0.78	1.57 ± 1.06	1.80 ± 0.82	1.89 ± 1.26	1.14 ± 0.64	1.24 ± 0.8	0.637	0.965

HOMA-IR, homeostatic model assessment-insulin resistance. † Insulin sensitivity index calculation derived by Matsuda & DeFronzo (1999). All data is presented as mean ± SD.

4.5 Adiponectin and leptin

A main effect of time was observed for plasma adiponectin after the 6 week training period (F=13.604, *P*=0.004, Figure 11 **A**), however there was no interaction effect between groups (F=3.353, *P*=0.097, Figure 11 **A**). The individual responses for adiponectin are shown in Figure 11 **B**.

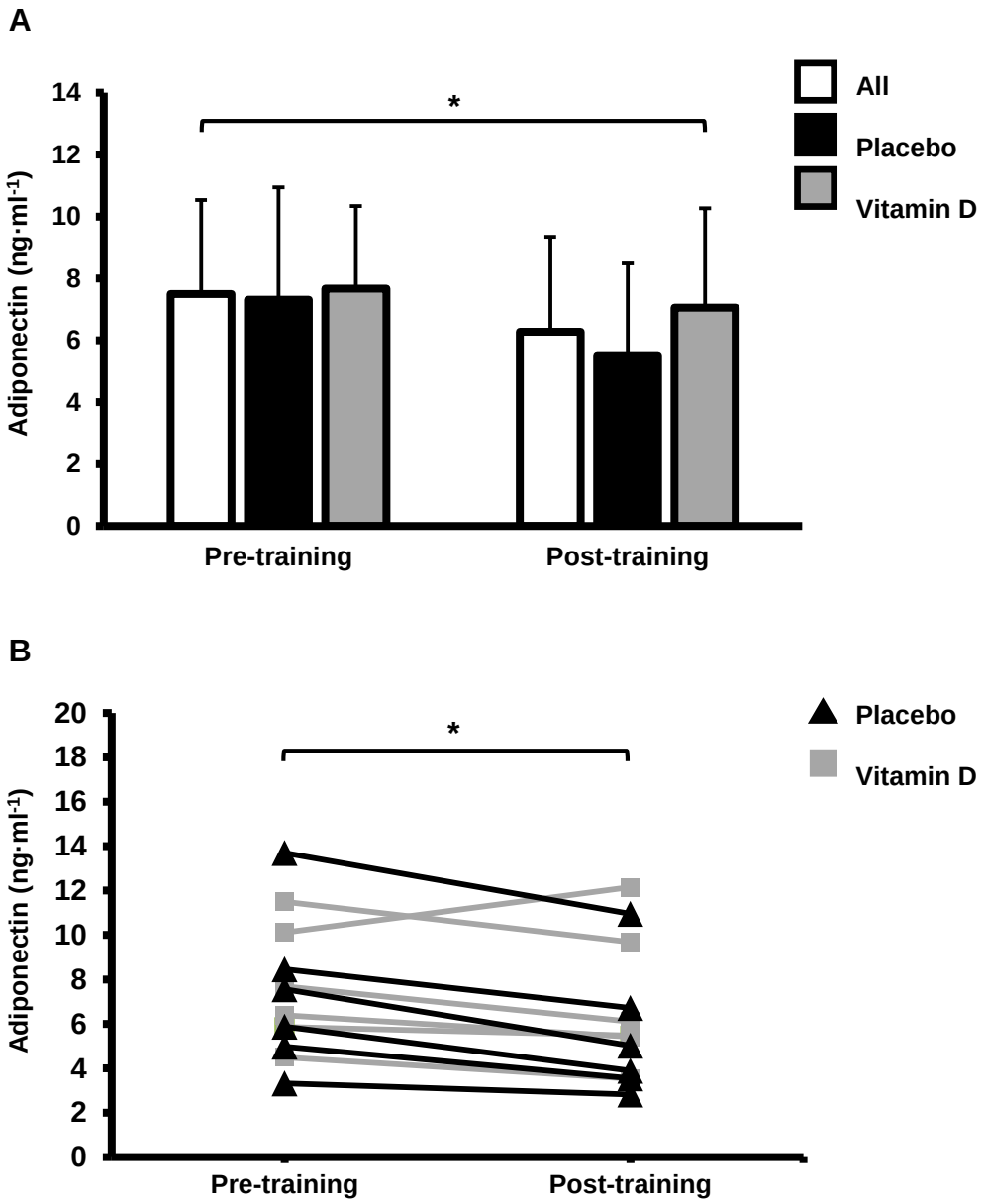

*Figure 11: Group and individual changes for adiponectin: (**A**) baseline and post-intervention adiponectin for all participants (n=12), the placebo group (n=6), and the vitamin D group (n=6); (**B**) adiponectin individual changes (n=12). *Denotes a main effect of time (P<0.05).*

There was no main effect for pre- versus post-training in plasma leptin (F=1.487, *P*=0.251; Figure 12 **A**), and no interaction effect between groups (F=2.932, *P*=0.148, Figure 12 **A**). The individual responses for leptin are also shown in Figure 12 **B**.

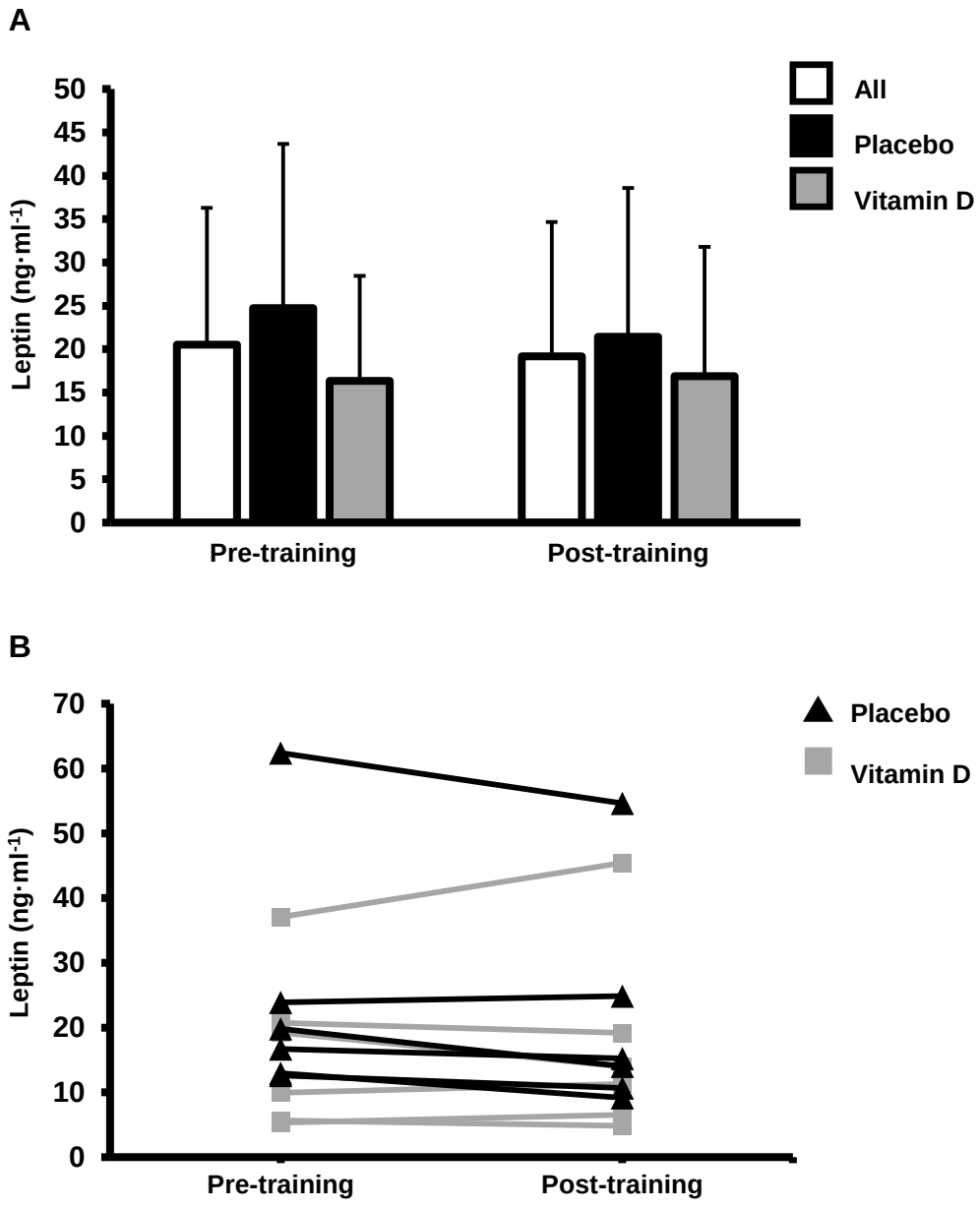

*Figure 12: Group and individual changes for leptin: (**A**) baseline and post-intervention leptin for all participants (n=12), the placebo group (n=6), and the vitamin D group (n=6); (**B**) leptin individual changes (n=12).*

4.6 Lipids

Table 7 shows pre- and post-intervention indices for lipids for all participants and both groups. There was a main effect of time in triglycerides (F=8.798, *P*=0.014), however no interaction effect between groups was observed (F=1.426, *P*=0.260). There was no effect of time on total cholesterol (F=1.237, *P*=0.292), HDL cholesterol (F=1.742, *P*=0.216), or LDL cholesterol (F=2.542, *P*=0.142).

Table 7: Blood lipid biomarkers pre- and post-training for all groups

	All (n=12)		**Placebo (n=6)**		**Vitamin D (n=6)**		**Main Effect of Time**	**Interaction Effect Between Groups**
	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training	*P* Value	*P* Value
Cholesterol, $mmol \cdot L^{-1}$	4.74 ± 0.89	4.90 ± 0.71	4.91 ± 0.99	5.17 ± 0.81	4.56 ± 0.83	4.64 ± 0.54	0.292	0.593
Triglycerides, $mmol \cdot L^{-1}$	1.61 ± 0.72	1.45 ± 0.72	1.62 ± 0.75	1.39 ± 0.73	1.61 ± 0.77	1.51 ± 0.76	0.014	0.260
HDL-chol, $mmol \cdot L^{-1}$	1.17 ± 0.26	1.14 ± 0.24	1.21 ± 0.31	1.19 ± 0.31	1.12 ± 0.21	1.10 ± 0.17	0.260	0.860
LDL-chol, $mmol \cdot L^{-1}$	2.83 ± 0.82	3.10 ± 0.56	2.97 ± 0.88	3.35 ± 0.52	2.70 ± 0.82	2.86 ± 0.52	0.142	0.524

HDL-chol, high density lipid cholesterol; LDL-chol, low density lipid cholesterol. All data is presented as mean ± SD.

5.0 Discussion

5.1 Key findings

- All participants, who permanently reside in Scotland, were 25(OH)D_3 deficient at baseline and supplementation with 4000 IU/day increased concentrations but not to sufficient concentration;
- Six weeks of HIIT in an overweight and obese cohort induced an increase in peak $\dot{V}O_2$, with periodisation of training ensuring all sessions were performed in the same HR zone;
- Six weeks of HIIT and 25(OH)D_3 supplementation did not induce a change in fasting glucose or insulin or the insulin sensitivity index in adults with normal glucose tolerance;
- Adiponectin was attenuated after 6 weeks of HIIT in the placebo group and in all participants, however was not detrimentally reduced in the vitamin D group;
- Leptin did not respond to 6 weeks of training and/or 25(OH)D_3 supplementation;
- Plasma triglycerides were reduced in all participants after 6 weeks of HIIT.

5.2 Vitamin D status and effect of supplementation

At baseline all of the participants involved in the current study, who reside in Scotland, were 25(OH)D_3 deficient (<20 ng·ml^{-1}). Major differences in vitamin D status have been found between different regions of the world, primarily attributed to a latitude and 'sun' effect since 80 % of vitamin D intake is through sunlight exposure of a specific wavelength: 290-315 nm (Chapuy *et al.*, 1997; Holick, 2002). Exposure to this strength of UV rays stimulates the conversion of vitamin D to the active form: 25(OH)D_3 (Kumar, 1984). Scotland in particular has been identified as a nation that is likely to suffer from vitamin D deficiency (Hypponen and Power, 2007). This geographical influence is accompanied with a seasonal effect (Bolland *et al.*, 2007), whereby winter months generally depict a higher prevalence of 25(OH)D_3 insufficiency/deficiency (Belenchia *et al.*, 2013). Diet fails to provide an adequate amount of vitamin D to maintain a sufficient status (>30 ng·ml^{-1}), presenting the need for supplementation

(Chapuy *et al.*, 1997). Data collection for the current study was obtained between the months of September to April, therefore the influence of the sun in Scotland is unlikely to facilitate vitamin D synthesis and influence vitamin D status of the participants (Rhodes *et al.*, 2010).

Studies investigating the effects of 25(OH)D_3 supplementation have utilised a range of daily or weekly doses. The relatively high 4000 IU/day (100 µg/day) dose used in the current study was selected based on the susceptibility of overweight/obese adults to be vitamin D deficient (Bolland *et al.*, 2007). Additionally, the dose does not exceed the upper tolerable limit determined by the Institute of Medicine (IOM) in 2010. After the 6 week intervention, there was a 33.5 % increase in 25(OH)D_3 concentration in those consuming 4000 IU/day, which is slightly less of a rise than other studies using the same dose but longer interventions: 12 weeks – 6 months (von Hurst *et al.*, 2010; Harris *et al.*, 2012; Belenchia *et al.*, 2013). Studies involving relatively short supplementation periods of 4 and 8 weeks have supplemented much higher doses of 10,000 IU/day and 50,000 IU/week, respectively, and reported greater increases in 25(OH)D_3 concentration and an improvement in insulin sensitivity (Nazarian *et al.*, 2011; Talaei *et al.*, 2013), however baseline concentrations were elevated in comparison to the present study's findings: 20 ± 7 ng·ml^{-1} (n=8) and 43 ng·ml^{-1} (n=100) compared to 13 ± 3 ng·ml^{-1} (n=12), respectively.

There was no observed effect on insulin sensitivity in the current study. An increase in 25(OH)D_3 concentration alone may not be sufficient to induce an increase in insulin sensitivity or improve glycemic control. Improvements in insulin resistance and fasting plasma insulin have been found when concentrations exceed 30 ng·ml^{-1} (Belenchia *et al.*, 2013), which is the defined concentration of a 'sufficient' status. In the current study, despite a rise in 25(OH)D_3 concentrations to 20 ± 9 ng·ml^{-1} in all participants (n=12) and 22 ng·ml^{-1} in the vitamin D group alone (n=6) after 6 weeks of supplementation, the rise alone may not have been sufficient to improve insulin sensitivity: concentrations must exceed 30 ng·ml^{-1}. However, studies utilising lower doses over longer durations reported increases in 25(OH)D_3 concentration to a sufficient status (>30 ng·ml^{-1}) but were not accompanied with an improvement

in glucose tolerance (Mitri *et al.*, 2011; Breslavsky *et al.*, 2013; Ghavamzadeh *et al.*, 2014; Nader *et al.*, 2014; Ryu *et al.*, 2014). Furthermore, the responder/non-responder theory can account for varying individual responses to supplementation (Saksa *et al.*, 2015). The ingested supplement must be metabolised to the active and transportable form of vitamin D ($25(OH)D_3$), and subsequently converted to $1,25(OH)_2D$ in order to mediate an increase in insulin receptor signalling and thus GLUT4 translocation and glucose uptake (Thorell *et al.*, 1999). Therefore, differing metabolic rates and processes will affect vitamin D metabolism and thus measured systemic $25(OH)D_3$ concentrations.

There are many factors that can influence baseline $25(OH)D_3$ concentration alongside supplementation and thus contribute to the vitamin D status measured: diet – intentional and unintentional, occupation, stress, and sunlight exposure (i.e. vacations) (Wortsman *et al.*, 2000; Blum *et al.*, 2008; Zgaga *et al.*, 2011; Romano *et al.*, 2015) . There were no dietary or activity controls during the 6 week period in the current study, although participants were asked to maintain their normal habitual diet and lifestyle during the study. However, an unintentional change in diet may occur when participating in regular exercise due to appetite alterations and caloric expenditure (Klok *et al.*, 2007; Deighton *et al.*, 2013; Deighton *et al.*, 2014).

Additionally, data collection was not carried out during the summer months, when exposure to the sun in Scotland is more likely, and so the results do not reflect an average annual concentration or consider the seasonal effect.

5.3 Cardiovascular benefits of HIIT

The development of hypertension, impaired glucose tolerance, and adverse lipid profiles are linked to the pathogenesis of T2D (Ferrannini *et al.*, 2005; Gastaldelli, 2011). High intensity intermittent training (HIIT) has been demonstrated to provide improvements and adaptations in parameters of the cardiovascular system (Little *et al.*, 2010; Astorino *et al.*, 2012; Gibala *et al.*, 2012; Weston *et al.*, 2014). The current study found an increase in absolute and relative $\dot{V}O_{2peak}$ and peak power output overall for all participants and the

placebo group alone, although not the vitamin D group. This was accompanied with a decrease in systolic blood pressure, although interestingly this was demonstrated overall for all participants and the vitamin D group, but not the placebo group. The current study found a 8.6 % increase in relative $\dot{V}O_{2peak}$ after 6 weeks of HIIT, which is similar to other HIIT studies over 2-12 weeks that are generally found to be relative to the duration of the training programme (Talanian *et al.*, 2007; Racil *et al.*, 2013; Whyte *et al.*, 2010; Adamson *et al.*, 2014; Gillen *et al.*, 2014; Mancilla *et al.*, 2014), although it should be noted that the protocols differ between studies.

There is a responder/non-responder theory associated with exercise, with endurance training and interval training demonstrating differential responses and adaptations (Sisson *et al.*, 2009; Scharhag-Rosenberger *et al.*, 2012), with an observed heterogeneous response to HIIT (Higgins *et al.*, 2014). This may suggest that different exercise models and intensities may provide different adaptations and responses on an individual-specific level, which can be relevant for underpowered findings. Systolic blood pressure has been shown to be influenced by both acute and regular recurrent exercise and vitamin D status (Roberts *et al.*, 2002; Tomaschitz *et al.*, 2010; Astorino *et al.*, 2012). The association between blood pressure and insulinemia may work in a feedback loop mechanism, with a cause and effect relationship between the two factors modified by exercise (Roberts *et al.*, 2013).

Reductions in waist and hip circumference and in the waist-to-hip ratio have been found after exercise training interventions (Dunstan *et al.*, 2002; Whyte *et al.*, 2010; Mitranun *et al.*, 2014). However, the current study did not observe any change in body mass or waist and hip circumference.

5.4 HIIT and vitamin D supplementation on insulin sensitivity

It was hypothesised that 6 weeks of HIIT would induce an increase in insulin sensitivity, likely to be attributed to an improvement in hepatic or skeletal muscle insulin-mediated metabolic pathways. An improvement in $\dot{V}O_{2peak}$ has been shown to directly and indirectly induce an increase in insulin sensitivity through skeletal muscle and hepatic metabolism in overweight and obesity

(Mancilla *et al.*, 2014; Cocks *et al.*, 2015). However, the finding that glucose tolerance remained unchanged after 6 weeks of HIIT contrasts with other studies utilising the same training protocol in overweight and obese adults with and without T2D for 2 and 12 weeks, respectively (Little *et al.*, 2011; Mancilla *et al.*, 2014). Mechanisms underpinning the improvement in insulin sensitivity, such as the protein content of GLUT4, the translocation of GLUT4 in hepatic and skeletal muscle cells, and an increase in resting muscle glycogen have been demonstrated after only 2 weeks of HIIT using the same protocol (Little *et al.*, 2010; Little *et al.*, 2011), however, muscle tissue samples were not analysed in the present study. These studies investigated different populations compared to the present study: healthy and overweight adults with T2D.

One of the contributors to the pathogenesis of T2D is chronic hyperglycaemia implying that a reduction in fasting glucose could lead to the prevention of a dangerously high glucose state (Gastaldelli *et al.*, 2000; Ferrannini *et al.*, 2005). The current study observed no effect of training on fasting glucose or insulin, when combined with 25(OH)D_3 supplementation or a placebo. All participants had normal fasting glucose and glucose tolerance at baseline, therefore an alteration would not necessarily indicate a health benefit. Similarly, a decrease in glucose AUC (obtained from the response to an oral glucose tolerance test) may not indicate a sign of improved insulin sensitivity since the main role of insulin is to regulate glucose homeostasis. A better indicator of insulin sensitivity and secretion may be insulin AUC and the calculation of whole body insulin sensitivity using Insulin Sensitivity Index (Matsuda and DeFronzo, 1999) and HOMA-IR. Although the current study observed no effect on insulin sensitivity, calculated using both methods, improvements have been reported following investigations using the same protocol for only 2 weeks (Hood *et al.*, 2011).

It has been proposed that diet alone or diet combined with regular exercise are stronger determinants of insulin sensitivity rather than exercise alone (Tamura *et al.*, 2005; Larson-Meyer *et al.*, 2006). The methodology of the current study involved no dietary adjustments other than supplementation with 25(OH)D_3.

It is important to consider the timing of the post-training OGTT as it will determine whether the results demonstrate an acute response of the last

training session or the chronic adaptation to the training and/or supplementation. In the current study, the post-intervention OGTT was performed 72 h after the cessation of training and course of supplements/placebo to prevent any residual effects of the last HIIT session on blood markers. Contrasting methodology has analysed insulin sensitivity via an OGTT or the gold standard hyperinsulinemic euglycaemic clamp at 15 h – 72 h post-training with differing results in indices of insulin sensitivity and glucose control (Richards *et al.*, 2010; Whyte *et al.*, 2010). It could also be hypothesised that engagement in regular exercise is required to induce the acute metabolic response and subsequently maintain the residual effect, as opposed to providing chronic adaptations. Studies that have performed the post-training OGTT at 24 h after the final HIIT bout may reflect the combined impact of acute and chronic exercise training (Rogers, 1989). Therefore, the lack of effect on ISI and HOMA-IR in the current study at 72 h post-cessation of training could be attributed to the loss of the acute molecular response, however Metcalfe *et al.* (2012) reported an increase in insulin sensitivity when the post-OGTT was also performed 72 h after the final training session. Conversely, the difference between the response to a 75 g OGTT at 24 h and 72 h post-training has been demonstrated by Whyte *et al.* (2010) whereby they reported an increase in the Insulin Sensitivity Index after 24 h but the impact was lost at 72 h.

5.5 Adiponectin and leptin

The current study reports an interesting finding: adiponectin concentration was unaltered in the vitamin D group alone, but decreased in all participants and the placebo group alone. Both exercise and supplementing with $25(OH)D_3$ have been shown to upregulate adiponectin concentrations and decrease the presence of chronic inflammation. Excessive fat deposition has also been associated with a decline in adiponectin concentrations and the development of T2D (Kadowaki *et al.*, 2006; Balducci *et al.*, 2010; Chen *et al.*, 2015). Therefore, it is hypothesised that adiponectin concentrations will generally be lower in overweight and obese individuals than those with a BMI regarded as healthy (19-25 $kg \cdot m^2$). Body mass did not change during the 6 week

intervention and thus any changes in adiponectin or leptin were not attributed to changes in body mass.

Although the current study did not observe an increase in adiponectin, vitamin D_3 supplementation alone has been found to induce an increase in adiponectin in adolescents and adults with and without T2D (Belenchia *et al.*, 2013; Breslavsky *et al.*, 2013). The decline in adiponectin observed in participants consuming the placebo may be attributed to unaltered 25(OH)D_3 concentration and the effect of regular HIIT. Exercise-induced skeletal muscle damage frequently occurs following strenuous exercise, specifically HIIT, particularly in those unaccustomed to exercise such as an inactive population. The mechanical disruption of the contracting fibres in skeletal muscle can initiate the inflammatory protein cascade (Pedersen and Hoffman-Goetz, 2000), which would in turn could affect the secretion of adiponectin. However, the post-OGTT was performed 72 hours after the last HIIT session to avoid the acute effects of exercise influencing blood markers.

The current study found no change in leptin concentration after 6 weeks of HIIT with or without 25(OH)D_3 supplementation, which is similar to a recent supplementation study (Breslavsky *et al.*, 2013). Vitamin D_3 supplementation alone, even a low dose of 400 IU/day, has been shown to increase leptin concentration after 14 weeks, however this was in adults with established impaired glucose tolerance (Ghavamzadeh *et al.*, 2014). In contrast, a reduction in leptin concentration and the leptin-to-adiponectin ratio was reported following 6 months of supplementation with 4000 IU/day in obese adults with normal glucose tolerance (Belenchia *et al.*, 2013). The reduction in leptin was associated with an elevation in 25(OH)D_3 concentration to the classified 'sufficient' status (Belenchia *et al.*, 2013). As previously discussed, alterations in blood markers and insulin sensitivity may only be associated with the presence of a 'sufficient' vitamin D status (>30 $ng \cdot ml^{-1}$).

Independent of vitamin D status, exercise has also been demonstrated to affect leptin due to an improvement in $\dot{V}O_{2peak}$ (Bouassida *et al.*, 2010), with reductions in leptin observed alongside increases in adiponectin (Trapp *et al.*, 2008). However, the current study did not induce a reduction in leptin in overweight and obese adults despite the increase in $\dot{V}O_{2peak}$. Interestingly, in

light of the attenuation of leptin, Trapp *et al*. (2008) also reported a reduction in insulin resistance and fasting insulin that was accompanied by a decrease in total body mass. It could be hypothesised that the lack of alteration of leptin in response to HIIT could be due to an unaltered BMI and body mass. However, this creates a paradox as an increase in leptin is desired to promote a reduction in appetite through the feeling of satiety and thus induce weight loss through caloric deficit (Klok *et al.*, 2007; Deighton *et al.*, 2013; Deighton *et al.*, 2014).

5.6 Impact on lipid profile and metabolism

Changes in lipids have independently been associated with improvements in insulin sensitivity, with visceral fat deposition and circulating blood triglyceride content affecting cell-specific sensitivity to the action of insulin and glucose regulation (Larson-Meyer *et al.*, 2006). The present study observed a reduction in triglycerides in the group as a whole (n=12). Sprint interval training over 6 weeks involving 4 Wingates with 4 min recovery periods, has also reported a reduction in triglyceride concentration and post-prandial AUC triglyceride response following a high fat meal test (Freese *et al.*, 2015). The attenuation in triglycerides may be attributed to the higher intensity of the exercise model, which induces an up-regulation in lipolysis and thus a clearance in circulating triglycerides in the blood (Herd *et al.*, 1985; Herd *et al.*, 2001). Low HDL cholesterol concentration and elevated triglyceride concentration is associated with insulin resistance, therefore a reduction in triglycerides may favour improvements in glucose tolerance and sensitivity to insulin action (Couillard *et al.*, 2001).

5.7 Adherence to HIIT

The protocol was adhered to by all volunteers in the study with positive verbal feedback. Despite the study not involving an alternative model of exercise for comparison, the 100 % adherence to all 18 training sessions and the 10 intervals within a single session, suggest that HIIT is a feasible exercise model that can be adhered to by an overweight and obese population that habitually

lead an inactive lifestyle. However, all HIIT sessions were performed in a laboratory setting under supervision and thus adherence may be attributed to a 'personal training effect'. However, it has been shown that HIIT has been adhered to when not supervised by obese adults with pre-diabetes following a supervised training phase (Jung *et al.*, 2015). In the latter study the participants also received behavioural counselling during the training phase sessions, which may have aided autonomy. This preliminary study was successful in providing scope for HIIT as an exercise model that can be adhered to over the short-term beyond the bounds of a laboratory setting. There has been contrasting studies investigating the affective response of exercise intensity on adherence and the potential risks involved in specific population groups, i.e. obese and elderly (Mediano *et al.*, 2014). The time-efficient attraction of interval training (Stutts, 2002) and physiological benefits, alongside adherence (Jung *et al.*, 2014; Jung *et al.*, 2015) and enjoyment (Guiraud *et al.*, 2011), places HIIT as a form of exercise that has potential to provide health benefits.

5.8 Conclusions

The findings of the current study demonstrate the effectiveness of HIIT to increase peak physical capacity and power output, and induce a reduction in systolic blood pressure that could reduce hypertension. These cardiovascular benefits were demonstrated following only 6 weeks of HIIT training using the relatively short protocol of 10 x 1 min at 89 ± 5 % of HR_{peak} interspersed with 1 min active recovery. Although there was no effect of HIIT and 25(OH)D_3 supplementation on insulin sensitivity, there was an observed decrease in plasma triglycerides, demonstrating an impact on the lipid profile. Additionally, there was an interesting finding in adiponectin in response to the intervention, with an overall reduction in all participants and the placebo group but not the vitamin D group alone. The combined influence of HIIT and 25(OH)D_3 supplementation on the inflammatory profile and related proteins and hormones, including adiponectin and leptin, should be investigated following longer exposure to both interventions. The findings from the current study reject the hypothesis that HIIT and 25(OH)D_3 combined will improve insulin sensitivity in overweight and obese males and females, however HIIT alone

did induce cardiovascular and metabolic adaptations, although not accompanied with an impact on the insulin profile.

5.9 Issues, limitations, and future research

The current investigation is underpowered and limited by sample size (n=12) and it is therefore recommended that larger studies are undertaken in the future. Future research should analyse the combined effect of 25(OH)D_3 supplementation and HIIT on the inflammatory profile, specifically proteins and hormones involved in the pathogenesis of insulin resistance. In addition to the effects on physiological and metabolic parameters, the effect of HIIT and 25(OH)D_3 supplementation should be investigated in relation to hormones that mediate satiety and hunger: ghrelin and leptin. A vitamin D supplementation group should also be included in future studies to allow further comparison.

It is important to establish the efficacy of this type of training in an unsupervised 'real-world' scenario. Future studies should investigate adherence to HIIT training following an intervention or a 'training phase', as utilised by Jung *et al*. (2015), compared to moderate intensity endurance training in an overweight and obese population.

The present study also only included 3 females, which limits the capacity to look at gender differences. However, due to hormonal differences between genders and evidence of differential beta-cell function and insulin secretion rates in females compared to males (Macotela *et al.*, 2009), it is important to investigate gender differences in response to both HIIT and 25(OH)D_3 supplementation: combined and independently. In addition, the adaptations to different exercise training intensities should be investigated in order to recommend exercise protocols specific to gender and thus effectively provide health benefits.

6.0 References

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7.0 Appendices

Appendix 1 A

Physiology Screening Questionnaire

Please read the following carefully and answer all the questions truthfully. Information will be treated with the strictest confidence.

This is a strictly private confidential document.

Name: _____

Date of birth: _____

Please delete as appropriate:

Have you ever had a cardiac-related issue such as a heart attack, hypertrophic cardiomyopathy, congenital abnormality, heart valve defect, heart failure or heart rhythm disturbance? Yes/No

Have you ever received treatment for a heart problem such as heart surgery, the fitting of a pacemaker/defibrillator, coronary angioplasty or heart transplantation? Yes/No

Are you currently taking medication for your heart? Yes/No

How many units of alcohol do you consume a week?

N.B One alcohol unit is measured as 10ml or 8g of pure alcohol. This equals one 25ml single measure of whisky (ABV 40%), or a third of a pint of beer (ABV 5-6%) or half a standard (175ml) glass of red wine (ABV 12%).

_____ units

Do you currently or have you ever suffered from any of the following:

Arthritis, osteoporosis or any other bone or joint problem?	Yes/No
Asthma, bronchitis or any other respiratory problem?	Yes/No
Coagulation disorders?	Yes/No
Diabetes (Type I or Type II)?	Yes/No
Epilepsy?	Yes/No
Hypertension (High Blood Pressure)?	Yes/No

Liver or gastrointestinal problems?	Yes/No
Kidney problems?	Yes/No
Infectious disease such as HIV, hepatitis or glandular fever?	Yes/No
Autoimmune disease?	Yes/No
Any peripheral or central nervous system disease? (e.g. Alzheimer's, Meningitis, Huntington's, Parkinson's, Tourette's)	Yes/No

Do you experience any of the following:

Chest discomfort with exertion?	Yes/No
Unreasonable breathlessness?	Yes/No
Dizziness, fainting, blackouts?	Yes/No
Palpitations or skipped heart beats?	Yes/No
Unusual levels of fatigue?	Yes/No

Please indicate if any of the following are true:

You have a close blood male relative (father or brother) who has had a heart attack before the age of 55 or a close female relative (mother or sister) who has had a heart attack before the age of 65?	Yes/No
You have elevated levels of cholesterol or are on lipid lowering medication?	Yes/No
You are a cigarette smoker	Yes/No
You have elevated levels of blood glucose?	Yes/No
You are completely inactive (do not take part in 20 minutes of moderate physical activity such as walking, 3 times per week)?	Yes/No
You have suffered a stroke or major cardiac event?	Yes/No
You have been bedridden in the past 3 months?	Yes/No
You have suffered from an infectious disease in last 6 weeks?	Yes/No

Approximately how many hours in each 24-hour day do you usually spend sitting? _____ hours

Approximately how many hours in each 24-hour day do you usually spend watching a screen (i.e. TV, iPad, computer monitor) _____ hours

Are you currently taking any medications?

If Yes please give details:

Have you any other conditions that may be relevant to an individual undertaking strenuous exercise?

If Yes please give details:

Physical Measurements (to be completed by researcher):

Blood Pressure:_____ mmHg

BMI: _____ kg/m^2

Declaration:

I have understood all of the questions put to me and that my answers are correct to the best of my knowledge. I understand that this information will be treated with the strictest confidence.

Name of participant: _____

Signature of participant: _____

Date: _____

Name of researcher: _____

Signature of researcher:_____

Date: _____

Appendix 1 B

Informed Consent Form

TITLE: The combined effect of high intensity intermittent training and vitamin D supplementation on insulin sensitivity in overweight and obese males and females

Declaration for consent

You have consented to take part in the research study at Edinburgh Napier University, Sighthill Campus.

If any of these factors apply please do not sign and give consent to take part in the study. You do not have to say which factors apply.

- Diabetic
- Current smoker (or a smoker who has quit less that 15 months ago)
- Use of a tanning bed or undergoing UV light therapy
- Use of vitamin D supplements or any multivitamins containing vitamin D
- Known cardiovascular disease or autoimmune diseases
- Participants who have experienced infectious disease within 6 weeks

I have read and understood the information sheet and this consent form.
I have had an opportunity to ask questions about my participation.
I understand that I am under no obligation to take part in this study.
I understand that I have the right to withdraw from this study at any stage without giving any reason.
I agree to participate in this study.

Name of participant: _____
Signature of participant: _____
Date: _____

Name of researcher: _____
Signature of researcher:_____
Date: _____