Inter-individual responses to sprint interval training, a pilot study investigating interactions with the sirtuin system

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Abbreviations: ABCA1, ATP-Binding Cassette, Sub-Family A (ABC1), Member 1, CD38, Cyclic ADP-Ribose Hydrolase 1, HDL, high density lipoprotein, HFD, high fat diet, HIC1, Hypermethylated In Cancer 1, LDL, low density lipoprotein, LFD, low fat diet, KanR, Kanamycin Resistance, NAD, Nicotinamide Adenine Dinucleotide, NADSYN1, NAD Synthetase 1, Nampt, Nicotinamide Phosphoribosyltransferase , NNMT, Nicotinamide N-methyltransferase, Nmnat, Nicotinamide Nucleotide Adenylyltransferase , NMRK, Nicotinamide Riboside Kinase , NAPRT1, Nicotinate Phosphoribosyltransferase Domain Containing 1, PARP1, Poly (ADP-Ribose) Polymerase 1, PCA, principal component analysis, PNP, Purine-Nucleoside:Orthophosphate Ribosyltransferase, PSMB6, Proteasome (Prosome, Macropain) Subunit, Beta Type, 61, PPIB, Peptidylprolyl Isomerase B (Cyclophilin B), QPRT Quinolinate Phosphoribosyltransferase, SIRT, sirtuin, T2D, type 2 diabetes, TDO2, Tryptophan 2,3-Dioxygenase, TNF, Tumour Necrosis Factor, UBE2D2, Ubiquitin-Conjugating Enzyme E2D 2 UBCH5B,

Abstract

Sprint intensity interval training (SIT) is reported to improve blood glucose control and may be a useful public health tool. The sirtuins and associated genes are emerging as key players in blood glucose control. This study investigated the interplay between the sirtuin/NAD system and individual variation in insulin sensitivity responses after SIT in young healthy individuals. Before and after 4 weeks of SIT body mass and fat percentage were measured and oral glucose tolerance tests (OGTT) performed in 20 young healthy participants (7 females). Blood gene expression profiles (all 7 mammalian sirtuin genes and 15 enzymes involved in conversion of tryptophan, bioavailable vitamin B3 and metabolic precursors to NAD). NAD/NADP was measured in whole blood. Significant reductions in body weight and body fat post-SIT were associated with altered lipid profiles, NAD/NADP and regulation of components of the sirtuin/NAD system (*NAMPT*, *NMNAT1*, *CD38* and *ABCA1*). Variable improvements in measured metabolic health parameters were evident and attributed to different responses in males and females, together with marked inter-individual variation in responses of the sirtuin/NAD system to SIT.

Keywords: insulin sensitivity, interval exercise, body composition, sirtuin system, inter-individual variation, GeXP

Introduction

Insulin resistance (IR) is a major risk factor for the development of type 2 diabetes (T2D) and people with IR and/or T2D have been found to have 2-4 fold greater risk of CVD with 80% of people with T2D dying from CVD (Capewell et al. 2009). T2D prevalence is rising, with ~400 million global cases (projected to rise to ~600 million cases by 2035), it accounts for >10% of healthcare spending (International Diabetes Federation, 2013). Lack of physical activity and excess weight are the main factors thought to be responsible for this high, and increasing, prevalence. For this reason, and to achieve many of the other health benefits, people are recommended to participate in 150 minutes of moderate or 75 minutes of vigorous intensity exercise per week.

Indeed, moderate intensity exercise (150 min/week) has been shown to be effective in reducing T2D to a greater extent than treatment with metformin (Knowler et al. 2002). Sprint interval training (SIT) has emerged as a strategy that can improve exercise performance, glucose control, insulin sensitivity, mitochondrial capacity, endothelial function and reduce postprandial triacylglycerol in healthy individuals after as little as 2 weeks (Babraj et al. 2009; Burgomaster et al. 2005; Gabriel et al. 2012; Little et al. 2010; Tjønna et al. 2008). A wealth of evidence demonstrates the health benefits of SIT, but inter-individual variation in responses implies interactions with genetics (Timmons et al. 2010) and requires investigation to optimise SIT interventions.

SIRTUIN1 and the enzymes crucial in generating the sirtuin cofactor, nicotinamide adenine dinucleotide (NAD), have recently been implicated in diet and age-induced pathogenesis of T2D (Dong 2012; Haigis and Sinclair 2010; Huynh et al. 2013). The sirtuin/NAD system is

also responsive to altered glucose regulation during the development of diet induced obesity (Drew et al 2016). Increased physical activity is associated with beneficial effects on metabolic health that are associated with altered regulation of the sirtuin system (Koltai et al. 2010). Further research is necessary to decipher the components of the sirtuin system that respond to exercise interventions that improve metabolic health. The sirtuin/NAD system is compromised by interactions with individual genetics (Kilic et al. 2014) since all seven sirtuins (*SIRT1-7*) and NAD biosynthesis enzymes are subject to genetic variation. Sirtuins are dependent on an adequate supply of NAD cofactors for enzymatic activity to regulate cellular processes linking them to the energy status of cells via cellular generation of nicotinamide. NAD generation is dependent on dietary intake of tryptophan and bioavailable niacin, but importantly NAD can be regenerated, independent of diet, by activation of salvage pathways that are upregulated in response to exercise (Hubbard and Sinclair 2014).

Recent technological advances present opportunities to predict health status and benefits conferred by lifestyle interventions using gene expression profiling of whole blood (Drew et al 2012). Considering the difficulties in accessing target tissues in human subjects this presents a strategy warranting consideration for research to identify individual responses to diet and lifestyle interventions. Consequently, our lab has been conducting studies to determine the feasibility of whole blood gene expression profiling to identify responses to interventions in humans (Drew et al 2014). This led to identification of variable levels of *SIRT1* in human whole blood from apparently healthy individuals (Drew et al. 2014, Andraos et al 2016). Further investigation revealed that individuals with low *SIRT1* exhibited higher levels of plasma inflammatory markers (TNFα), deregulated metabolic responses to food consumption (absence of a postprandial *SOCS3* response), elevated *p53* and low levels of high density lipoprotein cholesterol (HDL), factors indicative of reduced health status and

metabolic stress (Drew et al. 2014, Andraos et al 2016). Importantly, these studies have demonstrated stable whole blood gene expression profiles that characterise individual subjects and their individual responses to dietary intervention.

Thus the aim of this study was to determine changes in anthropometric and metabolic measures in response to SIT. Secondly, the study aimed to determine whether these changes were associated with modulation of the sirtuin/NAD system using human whole blood gene expression profiling. We hypothesised that improvements in outcome measures following SIT would be associated with specific changes in components of the sirutin/NAD system.

Materials and Methods

Study population

Twenty recreationally active, but not specifically trained (not engaged in physical training to increase strength, aerobic fitness, speed, fitness or skillful practice for a specific sport or activity), participants aged 18-35 years, non-smokers, free of cardiovascular, metabolic or haematological disorders were recruited. The study was approved by the University of Aberdeen College Ethical Review Board. All participants were fully informed of the aims, risks and discomfort associated with the investigation before providing written informed consent.

Pre-Experimental and Post Training Procedures

Participants were asked to complete a 4 day estimated food diary (2 weekdays and 2 weekend days, on 4 consecutive days) directly preceding the SIT intervention. Recorded food diary data was input into NetWISP dietary software analysis and patterns of daily food and nutrient intake were then determined, with specific reference to macronutrients and micronutrients associated with NAD generation (e.g. niacin). Participants were asked to maintain their habitual dietary intake and physical activity patterns for the duration of the study.

Participants attended the laboratory in the morning after an overnight fast (from 10pm the previous evening) having abstained from strenuous exercise, caffeine and alcohol for 24h. Blood samples and the measurements detailed below were obtained no more than 1 week prior to the SIT intervention and 2 days after the final training session. Body mass and height were measured using standard scales and stadiometers. Subcutaneous body fat was measured at four points on the body (biceps, triceps, suprailiac and subscapular) to the nearest 0.2mm using skinfold callipers (Harpenden) and converted to body fat percentage using the equations of (Durnin and Womersley 1974). Waist/hip ratio was assessed using a tape to measure hip and waist circumferences to the nearest 1cm. An oral glucose tolerance test (OGTT) was performed. Baseline venous blood samples were collected (K3 EDTA and Na Heparin Vacuettes, Greiner Bio-One and Paxgene RNA tube, PreAnalytiX) before ingestion of a 75g glucose bolus (436ml Lucozade Original). Venous blood samples were then collected at 60, 90 and 120 min after the consumption of the 75g glucose bolus. Whole blood collected in Na Heparin tubes were snap frozen prior to NAD assay (see below). Plasma was prepared by centrifugation (Eppendorf Centrifuge 5702/R) at 4000rpm, 4°C for 10 minutes, aliquoted and stored -20°C. Glucose and insulin concentrations were then measured using commercially available assays (see below). Blood collected in the Paxgene tubes was stored according to the manufacturer's instructions prior to total RNA extraction (see below).

Exercise Training

The SIT program was performed using a cycle ergometer and involved 3 sessions per week for 4 weeks. The participants warmed up for 3 mins at a workload of 50 Watts, followed by 4-6 (building from 4 to 6 over the first 6 sessions) maximal 30s sprints at a resistance equivalent to 7.5% of body mass with 3 minutes unloaded cycling between sets. This was followed by a 3 min cool down at 50 Watts. Participants were instructed to perform each sprint maximally with encouragement given during each sprint. The intensity of each bout was not recorded. Once carrying out 6 sprints each session lasted 24 mins. Participants performed this protocol three times each week for a 4-week period and all sessions were supervised.

Glucose and Insulin Analysis

Glucose (Human Glucose, Randox, UK) and insulin (Human Insulin, Mercodia UK) were assayed in plasma using commercially available kits according to the manufacturer's instructions and spectrophotometers, a Camspec M330B and a Biotek, Synergy HT multi-mode microplate reader respectively.

The AUC for both glucose and insulin concentrations during the OGTT were calculated using the trapezoid rule. Insulin sensitivity was estimated using the Cederholm index (Equation 1):

Equation 1: $ISI_{Cederholm} = 75000 + (G_0 - G_{120}) \times 180 \times 0.19 \times BW/120 \text{ X } G_{Mean} \times \log (I_{Mean})$

Where BW = body weight, G_0 and G_{120} = plasma glucose concentration at 0 and 120 minutes ($mmol.l^{-1}$) respectively, and I_{Mean} and G_{Mean} = mean insulin ($mU.l^{-1}$) and glucose ($mmol.l^{-1}$) concentrations during the OGTT respectively.

Lipid profiling

Lipid profiles (total cholesterol, HDL, LDL, triglycerides, Non-HDL and LDL: HDL ratio) of baseline (pre and post SIT) were measured from whole blood samples using commercially available kits (Alere Cholestech LDX® Lipid Profile Cassettes), in accordance with manufacturer's instructions.

Blood expression profiling of sirtuin and NAD biosynthetic enzyme genes

Total RNA was extracted from human whole blood samples collected at baseline (pre and post SIT) and 60 min post OGTT in PAXgene® blood RNA tubes (PreAnalytiX GmbH) (2.5ml) using a PAXgene Blood RNA Kit (Qiagen, Crawley, UK), incorporating DNase digestion. RNA quality was assessed using a NanoDrop Spectrophotometer (NanoDrop Technologies, LabTech International, UK) and the Agilent Bioanalyser (Agilent Technologies, Bracknell, UK). Whole blood total RNA (50 ng) was assayed for sirtuin and NAD biosynthetic enzyme gene targets using the GenomeLab™ GeXP Genetic Analysis System (Beckman Coulter) and our in-house custom designed assay, the hSIRTNADPlex. The hSIRTNADPlex incorporates 25 gene targets, including all 7 mammalian sirtuin genes (*SIRT1-7*), 15 enzymes involved in conversion of tryptophan, niacin, the novel vitamin, nicotinamide riboside, and metabolic precursors to NAD, together with 3 reference genes and a synthetic reference messenger RNA transcript for measuring relative quantitation of gene

expression and reaction efficiencies respectively (Supplementary File S1). Details of the the GenomeLab System for design of in-house custom designed multiplex assays and application to conduct blood gene expression profiling have been published previously (Drew et al. 2011; Drew et al. 2014) and are described in Supplementary File S2. Fragment analysis of the generated hSIRTNADPlex PCR products was conducted using the Beckman Coulter CEQ 8000 GeXP Genetic Analysis system. The raw data generated using the GenomeLab™ GeXP Series Software Suite v11.0 was then exported using the GeXP Fragment Analysis Module and normalized using the GeXP Data Tool.

NAD and NADP assay

NAD and NADP were assessed in whole blood (50ul) at baseline (pre and post SIT) using methods described by Jacobsen and Jacobsen (1997) with modifications as described by Creeke et al. 2007). Spectrophotometric readings were assayed using an MRX plate reader (Dynatech Laboratories).

Bradford Assay

Commercially available reagents (Bradford Reagent, PBS, BSA; Sigma-Aldrich® UK) and 96-well microtiter plates (F96 Maxisorp NUNC immunoplate, VWR) were used to determine total protein content of plasma samples pre and post SIT intervention using standard Bradford assay procedure. Absorbance was measured using a standard curve and plate reader (MRX plate reader, Dynatech Laboratories) at 595nm.

NAMPT and Vitamin B3

Vitamin B3 and NAMPT concentrations were determined via commercially available enzyme-linked immunosorbent assay (ELISA) kits (RayBio® and MyBioSource®, USA) according to the manufacturer's instructions. NAMPT concentrations were subsequently standardised against total protein content assessed by Bradford assay.

Statistics

Plasma glucose and insulin responses to the pre-training and post-training OGTTs were analysed using two-way repeated measures ANOVA with post hoc t-tests. Paired t-tests were used to assess significant changes in anthropometric measures, metabolic biomarkers, gene expression and other relevant markers from pre to post SIT intervention ($p<0.05$). Principal Component Analysis (PCA) and Partial Least Squares (PLS) analyses were performed using SIMCA-P+ 12.0 software (MKS Instruments UK Ltd, Cheshire). PCA was conducted with normalised gene expression and PLS was performed using normalised gene expression data (Y) and anthropometric and metabolic marker data (X). Pearson correlations were calculated using SPSS.

Results

Study participants

Twenty individuals were recruited to participate in the study (13 males and 7 females) and all completed (100% attendance at sessions) the 4 week supervised SIT intervention. The mean ±SD age was 22.8 ±2.8 years, height was 1.77±0.11 m and weight was 73.6±14.3 kg.

Food diaries

Estimated daily macronutrient and niacin intakes were calculated from 14 of the 20 participants (7 female) that returned completed food diaries (Table 1). The data collected indicated mean macronutrient intakes within the normal range recommended intakes prior to study participation. The % of total daily energy ± SEM intakes were fat 33.6 ± 2.3, protein 17.8 ± 0.9 and carbohydrate 44.5 ± 2.5. Individual niacin intake was varied, with mean niacin intake 27.8 ± 3.1 mg/day, ranging from 14.84 – 60.34 mg/day. Participants were asked to maintain their habitual dietary intake patterns for the duration of the SIT intervention.

Anthropometric data

Anthropometric measurements (n=20) pre and post SIT are shown (Table 2). Significant reductions in body mass (p=0.023) and body fat percentage (p=0.0004) were seen after SIT (Table 2). No significant differences were observed in BMI (p=0.07) or waist:hip ratio (p=0.19) post SIT (Table 2). However, review of individual responses to SIT indicated that there was inter-individual variation, with some, but not all participants responding favourably (Figures 1A-D).

Glucose, insulin and insulin sensitivity

Mean glucose, insulin (Table 3) and glucose concentrations following OGTT (Figure 2A) were not significantly altered in response to SIT. Glucose area under the curve (AUC) did not significantly change post SIT (p=0.34). Mean glucose AUC change post SIT was -15.8 ±

72.9mmol.L^{-1}. However, inter-individual changes were observed (range, -162.9mmol.L^{-1} to +153.2mmol.L^{-1}) in glucose AUC with 13 participants showing a reduction (Figure 2B). Mean insulin AUC was also not significantly reduced (p=0.38) post SIT (Figure 2C). However, despite the lack of significant decreases, mean insulin AUC change was -194.5 ± 971.3mU.L^{-1}, with 14 participants having reduced insulin AUC post SIT (range: -1679mU.L^{-1} to +2302mU.L^{-1}) (Figure 2D). The mean insulin sensitivity increase following 4 weeks SIT was 2.62 ± 13.6mg.mUl^{-1}.min, but this was not significant (p=0.40) (Figure 2E). It was noted that 12 of the 20 participants had increased insulin sensitivity post SIT (range: +25.7mg.mUl^{-1}.min to -23mg.mUl^{-1}.min) (Figure 2F).

Lipid profiling

Lipid profiles, total cholesterol (p=0.42), HDL (p=0.95), LDL (p=0.23), triglycerides (p=0.30), non HDL (0.44) and LDL: HDL ratios (p=0.63) were not significantly altered in response to SIT (n=19) (Table 3) and a wide range of responses observed (data not shown).

Transcriptional responses of SIRT/NAD system to SIT

The hSIRTNADPlex gene expression profiles measured in whole blood total RNA (RIN values 7.0-9.3) pre and post SIT and post OGTT normalised to *UBE2D2*, established as a stably expressed transcript in blood using geNORM (Pattyn et al. 2003), are shown (Figure 3). Small, but significant changes in response to SIT and OGTT were observed (Figure 3). The most prominent responses were up regulation of ABCA1 (0.11 $\pm$ 0.04 $p < 0.05$) and NAMPT (0.16 $\pm$ 0.07 $p < 0.05$) post SIT and glucose pre and post SIT. The up regulation of ABCA1 ($p < 0.05$) in response to glucose was significantly increased post SIT (Figure 3).

NMNAT1 was also up-regulated significantly in response to glucose both pre and post SIT (Figure 3). While SIRT5 and CD38 were down-regulated in response to glucose both pre and post SIT (Figure 3). In addition, HIC1 ($p < 0.05$), SIRT4 ($p < 0.05$), NMRK1 ($p < 0.05$), PSMB6 ($p < 0.05$), PARP1 ($p < 0.05$), SIRT2 ($p < 0.05$), SIRT3 ($p < 0.05$) and SIRT7 ($p < 0.01$) showed significantly differing responses to OGTT pre and post SIT. Inter-individual variation in gene expression was observed in the genes that were shown to be significantly regulated in response to SIT.

Vitamin B3 concentrations

Decreased mean plasma vitamin B3 concentrations following SIT and in response to the OGTT were not significant (Figure 4A) with a wide range of responses observed pre to post SIT levels (range: -1.22μmol.L^{-1} to +0.59μmol.L^{-1}). Thirteen of the 20 participants had reduced levels of vitamin B3 post SIT (Figure 4B).

NAD: NADP ratio analysis

Whole blood (n=11) NAD ($p=0.91$, mean decrease 1.6 ± 50nmol.L^{-1}) and NADP ($p=0.07$, mean increase of 21.8 ± 36.3nmol.L^{-1}) levels did not significantly change post SIT. The NAD: NADP ratio was significantly reduced after 4 weeks of SIT ($p=0.014$) compared with baseline measures (Figure 4C), with a mean decrease of 0.34 ± 0.47 (range: -0.37 to +0.04) (Figure 4D).

NAMPT concentration

NAMPT concentrations did not change significantly post SIT at 0 (p=0.97), 60 (p=0.19) and 120 (p=0.84) minute time points (Figure 4E). Inter-individual variation in NAMPT plasma concentrations were observed ranging from +0.051ng/mg and -0.043ng/mg (Figure 4F).

Correlated SIRT/NAD gene expression and metabolic markers

Expression of *NAMPT*, *CD38*, *ABCA1* and *NMNAT1* were all positively correlated following SIT, while pre SIT expression was not (Table 4). Correlations indicated post SIT *NAMPT* was positively associated with post SIT *ABCA1* (r=0.696, p=0.001) and *NMNAT1* (r=0.660, p=0.002). Additionally, post SIT *ABCA1* was positively associated with post SIT *CD38* (r=0.615, p=0.004) and *NMNAT1* (r=0.689, p=0.001). Post SIT measures of *NMNAT1* demonstrated positive relationships with post SIT measures of *CD38* (r=0.459, p=0.042) and *ABCA1* (r=0.689, p=0.001).

Potential co-regulated expression of *NAMPT*, *CD38*, *ABCA1* and *NMNAT1* was further investigated by determining correlations with metabolic markers pre and post SIT. Notably, plasma glucose and cholesterols were associated with *NAMPT* and *ABCA1* regulation. *NAMPT* and *ABCA1* were positively correlated with post SIT, but not pre SIT fasted glucose (*NAMPT* r = 0.636, p = 0.003; *ABCA1,* r = 0.466, p = 0.038) (Table 5). *NAMPT* and glucose AUC (r = 0.462, p = 0.04) were also positively correlated post SIT, but not pre SIT (Table 5). *NAMPT* was negatively correlated with LDL (r = -0.497, p = 0.03) (Table 5). *NAMPT* expression was also negatively associated with NAD: NADP ratio (r=-0.676, p=0.022) post SIT, but not pre SIT (Table 5).

Principal Component and Partial Least Squares analysis

Principal Component Analysis (PCA) was applied to determine whether there were patterns discernible in the dataset that could be linked to the variable anthropometric and metabolic responses to SIT. PCA conducted on the entire hSIRTNAD gene expression dataset did not reveal obvious patterns. However, focus on the post SIT gene expression profiles resulted in a PCA biplot explaining 44% of the dataset variation (Figure 5A), with clustering of females participants, indicating differing responses of the sirtuin/NAD system to SIT compared to males and in their sirtuin/NAD system responses to SIT (Figure 5A). Partial Least Squares (PLS) analysis indicated associated body composition and metabolic factors distinguishing male and female responses to SIT using normalized hSIRTNADplex gene expression (Y) and anthropometric and metabolic marker data (X) (Figure 5B). The PLS plot identified body fat levels as a factor associated with clustering of female participants (Figure 5B). Glucose and insulin are positioned further from female participants indicating that these factors may also differ in male and female participants in response to SIT (Figure 5B). Notably, *NAMPT* and *ABCA1* expression levels are closely associated with glucose and insulin levels (Figure 5B) reflecting the identified positive correlation post SIT (Table 5) providing further indication that these genes are responsive to glucose and insulin (Figure 4).

Discussion

Supervised SIT has emerged as a strategy to improve exercise performance and health, but as with all exercise there is clear inter-individual variation in responses (Ruchat et al. 2010). In support of previous reports this study observed improvements in body composition (Figure 1), insulin sensitivity and lipid profiles in response to SIT. Interactions with individual genetics may be a factor in individual responses to exercise (Ruchat et al. 2010) and

transcriptional responses of the SIRT/NAD system to SIT were investigated to determine associations with the responses to SIT in blood samples.

The sirtuin/NAD system consists of 7 sirtuin genes (SIRT1-7) and genes encoding enzymes that are involved in de novo synthesis of NAD from dietary tryptophan and vitamin B3. Additionally, there are a number of enzymes that are involved in recycling NAD to increase and up regulate sirtuin activity in order to maintain homeostasis and respond to various stressors (Ying 2008). This study measured blood profiles of the sirtuin/NAD system pre and post SIT to determine whether SIT led to a shift in the homeostatic sirtuin/NAD profile in each participant as opposed to focusing on acute effects of SIT. Corresponding shifts in other blood factors were considered in parallel two days post the final SIT session. Results of transcriptional profiling of blood from our volunteers indicated interactions with the sirtuin system, with small, but significant down regulation of *CD38* (which depletes NAD) and up regulation of *NMNAT1* and *NAMPT* (rate limiting enzymes in NAD synthesis) and *ABCA1* (a lipid transporter that alters blood cholesterol). This evidence was further supported by correlations showing co-regulated expression of this small gene set (Table 4). Importantly, it is now well recognised that small changes in a gene set within a biochemical pathway are often related to biologically meaningful changes (Subramanian et al. 2005). The correlated gene expression of *NAMPT*, *NMNAT1*, *CD38* and *ABCA1* post SIT (Table 4) indicates that these may be key components of the sirtuin/NAD system responses to exercise training. Indeed, up-regulation of *NAMPT* and *NMNAT1* may be a key response to increased demand for NAD in response to glucose and increased exercise. NAMPT activity elevates synthesis of nicotinamide mononucleotide (NMN), the rate limiting step for NMNAT1 conversion of NMN to NAD (for review see, Garten et al. 2015). However, reduced levels of plasma vitamin B3 post SIT (seen in 13 of the 20 participants) (Figure 4B) may reflect on the

increased demand for these rate-limiting enzymes to recycle NAD precursors. The corresponding down regulation of *CD38* in response to glucose may be a compensatory factor to alleviate increased demand for NAD in regulating the response to elevated plasma glucose. *NAMPT* was negatively correlated with NAD:NADP ratios. This reduction in NAD:NADP ratios post SIT may be a factor prompting the up regulation of *NAMPT* and also the observed up regulation of *NMNAT1*. Reduced NAD:NADP ratios also imply depletion and or increased demand for NAD. This suggests that gaining further positive effects of exercise may be augmented by ensuring dietary sources of tryptophan and vitamin B3 are optimal for the individual. NAMPT has also emerged as player in glucose regulation (Hajianfar et al. 2012; Revollo et al. 2007). Further analysis was conducted to determine whether elevated *NAMPT* gene expression was associated with increased plasma NAMPT since it has been reported that elevated levels of intracellular NAMPT may lead to increased secretion of extracellular NAMPT (Yoon et al. 2015). Variations in extracellular NAMPT plasma levels were observed in the study participants and were not directly correlated with expression of *NAMPT* in blood. This may be attributed to differences in body fat levels in the study participants as studies have indicated that extracellular NAMPT may be primarily secreted from adipose tissue rather than blood cells (Berndt et al 2005; Yoon et al. 2015).

PCA and PLS plots (Figure 5) indicated that the variation in sirtuin/NAD system responses to SIT may be attributed, in part, to sex differences. This is supported by a recent study reporting sex differences in response to 12 weeks of sprint interval training (Bagley et al. 2016). Additionally, it was noted that individual male participants (IDs 1, 6 and 19) appeared to respond differently to SIT in comparison with the remainder of the male cohort (Figure 5)**.** Furthermore, these three male participants had distinctly different responses from each other with respect to favourable improvements in body composition and metabolic markers post

SIT. Different responses were associated with body composition, glucose regulation and cholesterol levels and sirtuin system gene expression profiles. Previous studies have identified differing transcriptional profiles in muscle associated with individuals who demonstrate biological variation in responses to exercise (Keller et al. 2011). Incorporation of measurement of corresponding blood and tissue level sirtuin/NAD system transcriptional profiles in the design of future studies are desirable. Correlated profiles may provide further insights on individual responses to physical activity in tissues that are specifically targeted by training.

The current pilot study was not without limitations. Due to technical issues we were not able to record power output during SIT and inter-individual variations in work could contribute to some of the differences observed. However, participants were asked to give a maximal effort, as the majority of SIT interventions do and rely on. The volunteers were supervised during each session and so any major issues with effort are unlikely. The current study did not account for hormonal differences at different points in the menstrual cycle and future work should consider hormone levels. However, irrespective of potential variation in menstrual status, sirtuin/NAD blood gene expression profiles show less variation when compared to males. It seems unlikely that menstrual status has a large effect. This study did not use a control group. Hence, a comparison with a group not participating in regular SIT sessions was not possible. Responses to SIT were thus compared within individuals using pre-SIT intervention measurements for comparison with post-SIT measurements. Extending SIT studies may facilitate assessments over a period when the study subjects refrain from SIT. It is difficult to provide an appropriate comparable control group for SIT. Monitoring of physical activity levels in the group not participating in SIT would be an important factor to reduce confounding responses during the intervention period. Habitual physical activity

levels were not assessed or whether these changed during the SIT period. All aspects of physical activity (not only the SIT) may alter the sirtuin/NAD system and should be considered in design of future studies.

In conclusion, significant improvements in body composition parameters (body mass and body fat) were achieved in response to SIT, but associated significant improvements in mean levels of metabolic health parameters were not observed (Supplementary File S3). However, it was noted that although mean levels of metabolic health parameters did not reveal significant changes in response to SIT a number of participants did show improved glucose regulation and lipid profiles and associated transcriptional responses in the sirtuin/NAD system. Notably, metabolic improvements in response to SIT were achieved in individuals with poor health parameters measured pre-SIT. Furthermore, differences in female and male participants indicated that sex-specific factors may influence responses to SIT. This pilot study establishes criteria for designing future studies to investigate responses to SIT. In addition to male and female cohorts to determine sex specific effects, measurement of blood and tissue level profiles, the inclusion of a control group not undertaking increased physical activity, gathering additional data on lifestyle factors, such as habitual physical activity, will further inform interpretation of data analysis. Further investigation of the causes of the marked inter-individual variation in observed responses to SIT will be critical in formulating optimal public health messages for SIT and potentially other forms of exercise.

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J.E.D and S.G. designed the study; J.E.D, S.G., A.J.F., T.P.A., E.F, J.W, G.E.H, B.A, S.A.A. S.R.D. and G.W.H researched and interpreted data; J.E.D., S.G. and T.P.A. wrote the manuscript; J.E.D, S.G. and G.W.H discussed/edited the manuscript; all authors critically revised the manuscript for important intellectual content and gave approval of the final version.

None of the authors declares a conflict of interest.

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Table 1 NetWISP dietary analysis of macronutrient and niacin intake of participants prior to SIT

Macro/micronutrient	**Mean ± SEM**	**Recommended Intake**
Fat (% Total Daily Energy Intake)	33.6 ± 2.3	≤35
Protein (% Total Daily Energy Intake)	17.8 ± 0.9	≤15
Carbohydrate (% Total Daily Energy Intake)	44.5 ± 2.5	≥50
Alcohol (% Total Daily Energy Intake)	4.2 ± 1.5	<5
Niacin (mg/day)	27.8 ± 3.1	13-17

Note: data are presented as mean ± standard error of the mean (n=14). Macronutrient intakes are presented as a percentage of total daily energy intake; Niacin intake is presented in milligrams per day. Current recommended daily intakes of macronutrients and niacin are indicated.

Table 2 Anthropometric changes pre and post SIT

Variable	Pre SIT	Post SIT
Body Mass (kg)	73.6 ± 3.2	**72.9 ± 3.1***
BMI (kg/m^2)	23.2 ± 0.6	23.1 ± 0.5
Waist: hip ratio	0.81 ± 0.02	0.79 ± 0.01
Body Fat (%)	21.6 ± 1.4	**20.2 ± 1.3****

Note: data are presented as mean ± standard error of the mean (n=20). Paired t-tests were used at a significance level of $p<0.05$. * indicates $p<0.05$; ** indicates $p<0.01$

Table 3 Plasma lipids, glucose and insulin

Variable	Pre SIT	Post SIT
Total Cholesterol (mmol l^{-1})	3.95 ± 0.157	3.87 ± 0.137
HDL (mmol l^{-1})	1.36 ± 0.052	1.37 ± 0.068
LDL (mmol l^{-1})	2.19 ± 0.150	2.08 ± 0.134
Non-HDL (mmol l^{-1})	2.59 ± 0.179	2.50 ± 0.144
HDL/LDL Ratio	1.69 ± 0.160	1.63± 0.141
TRG (mmol l^{-1})	0.88 ± 0.102	0.93 ± 0.102
Fasting Glucose (mmol l^{-1})	4.92 ± 0.114	4.87 ± 0.120
Fasting Insulin (mU l^{-1})	5.95 ± 1.16	5.53 ± 0.50
AUC Glucose (mmol l^{-1} $2h^{-1}$)	624 ± 19.03	608 ± 19.42
AUC Insulin (mU l^{-1} $2h^{-1}$)	2717 ± 445.94	2523 ± 360.85

Note: Mean levels were not significant ($p<0.05$)

Table 4 Correlation of sirtuin/NAD system genes and pre and post SIT

Gene	**Time**	***NAMPT***		***CD38***		***ABCA1***	
	Point	Pre SIT	Post SIT	Pre SIT	Post SIT	Pre SIT	Post SIT
ABCA1	Pre SIT	0.353	**0.55***	0.434	-0.135		
	Post SIT	-0.15	**0.696****	-0.096	**0.615****		
NMNAT1	Pre SIT	-0.25	0.34	0.208	0.263	0.212	0.18
	Post SIT	-0.35	**0.66****	0.173	**0.459***	0.245	**0.689****

Note: data are presented as R values. Pearson correlations were used at a significance level of $p<0.05$. * indicates $p<0.05$; ** indicates $p<0.01$.

Table 5 Correlations between gene expression and metabolic markers pre and post SIT

Variable	*NAMPT*		*ABCA1*	
	Pre SIT	Post SIT	Pre SIT	Post SIT
Fasted Glucose	0.426	**0.636****	0.360	**0.466***
Glucose AUC	0.191	**0.462***	0.360	0.378
LDL	-0.115	**-0.497***	0.004	-0.449
NAD: NADP	0.14	**-0.68***	0.11	-0.004

Note: data are presented as R values. Pearson correlations were used at a significance level of $p<0.05$. * indicates $p<0.05$; ** indicates $p<0.01$. AUC denotes area under the curve.

Figures

Figure 1 Changes in individual anthropometric measures in response to SIT [A] body mass, [B] % body fat [C] BMI [D] waist: hip ratio (n=20).

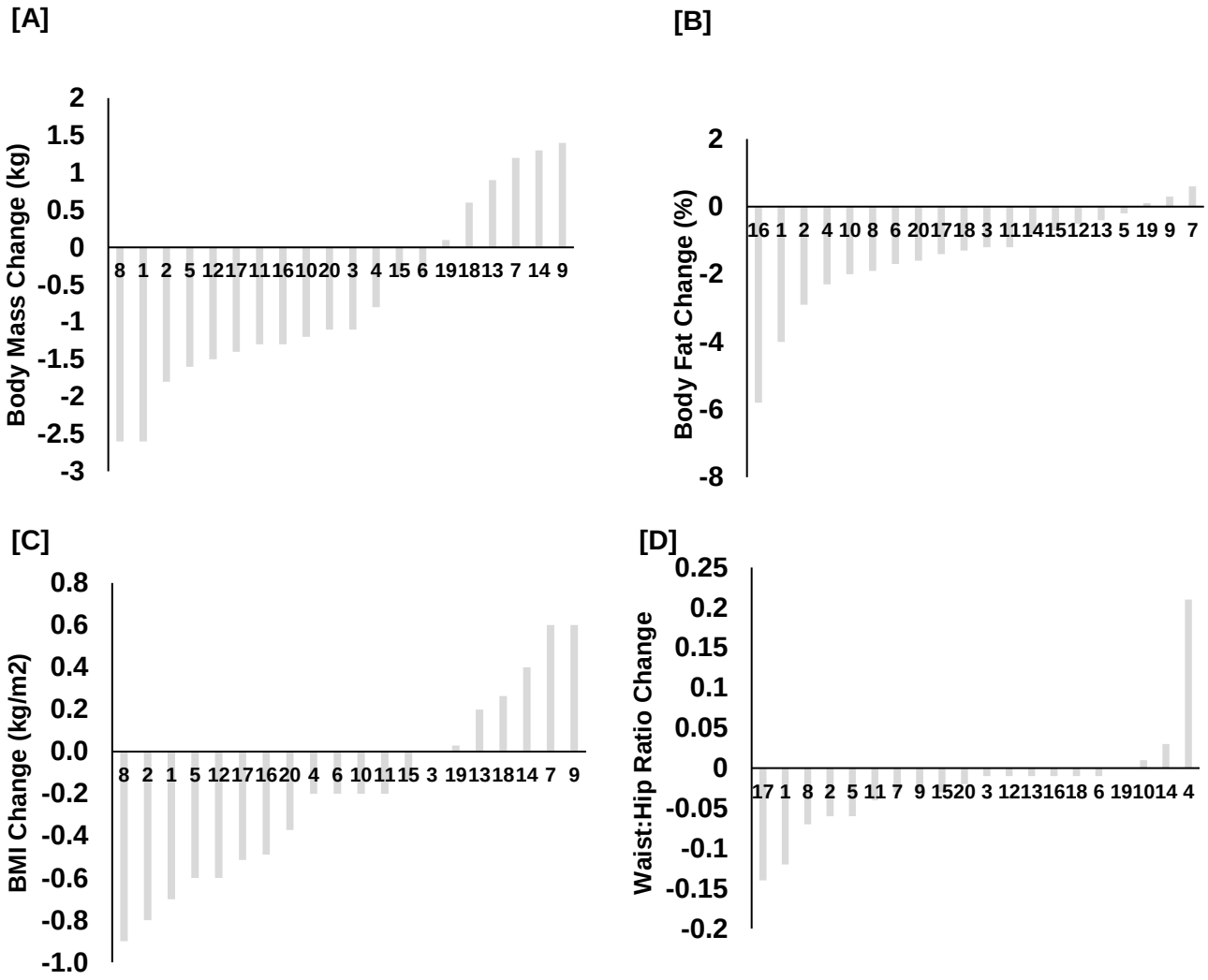
Figure 2 Plasma glucose, insulin and insulin sensitivity responses to SIT. [A] Mean ± SEM glucose area under the curve (AUC) pre and post SIT. [B] Individual glucose AUC changes post SIT. [C] Mean ± SEM insulin AUC pre and post SIT. [D] Individual insulin AUC changes post SIT. [E] Mean ± SEM insulin sensitivity pre and post SIT. [F] Individual insulin sensitivity changes post SIT. Paired t-tests were used on mean levels (n=20) at a significance level of $p<0.05$.

Figure 3 Transcriptional responses of the SIRT/NAD system to SIT. Mean expression for each gene (n=20 $\pm$ SEM) is shown pre-SIT, pre-SIT post OGTT, post SIT and post SIT post OGTT. Gene expression was assessed by hSIRTNADplex assay and normalised to internal reference gene *UBE2D2*. Significant ($p < 0.05$) differences in gene expression comparing pre-SIT to post SIT (a), pre SIT OGGT responses (b), post SIT OGGT responses (c) and pre SIT OGGT to post SIT OGTT responses (d) are indicated.

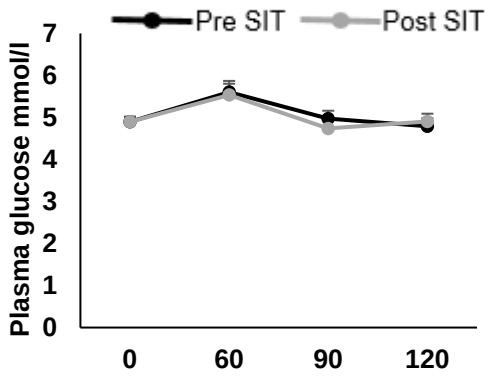
Figure 4 Plasma vitamin B3 and NAD:NADP ratios. [A] Mean ± SEM plasma vitamin B3 pre and post SIT (0) and post OGTT (1 and 2 hours) (n=20). [B] Individual plasma vitamin B3 changes post SIT. [C] Mean ± SEM NAD:NADP ratio pre and post SIT (n=11). [D] Individual NAD: NADP ratio changes post SIT. **[**E] Mean ± SEM plasma NAMPT pre and

post SIT (0) and post OGTT (1 and 2 hours) (n=20). [F] Individual plasma NAMPT changes post SIT. Paired t-tests were used at a significance level of $p<0.05$.

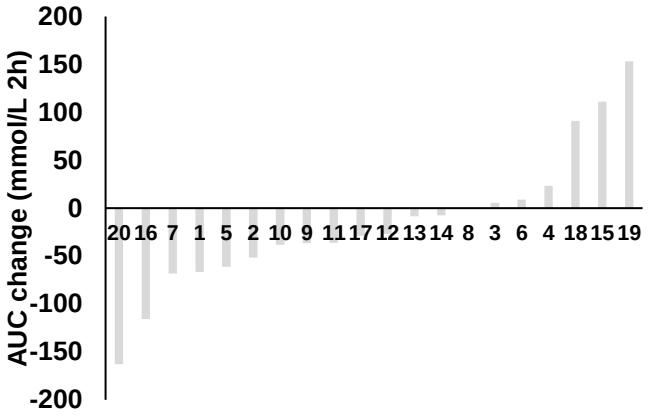
Figure 5 Principal component (PCA) and partial least squares (PLS) analyses of *UBE2D2* normalised post SIT hSIRTNADplex gene expression and anthropometric and metabolic markers. Female (Δ) and male (▲) participants are indicated. [A] PCA biplot post SIT. The PCA biplot permits visualisation of inherent clustering patterns of individuals and associated gene expression levels (denoted by ● and gene symbol). The measurements all fall within the Hotelling T2 95% confidence limit. [B] PLS analysis coefficients of weights of gene expression data (Y) and anthropometric and metabolic markers (X) post SIT. The anthropometric and metabolic markers are indicated in grey font. The PLS permits visualisation of associations between individuals and anthropometric and metabolic markers.


[A]
Body Mass Change (kg)
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
8 1 2 5 12 17 11 16 10 20 3 4 15 6 19 18 13 7 14 9
[B]
Body Fat Change (%)
2
0
-2
-4
-6
-8
16 1 2 4 10 8 6 20 17 18 3 11 14 15 12 13 5 19 9 7
[C]
BMI Change (kg/m2)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
8 2 1 5 12 17 16 20 4 6 10 11 15 3 19 13 18 14 7 9
[D]
Waist:Hip Ratio Change
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
17 1 8 2 5 11 7 9 15 20 3 12 13 16 18 6 19 10 14 4

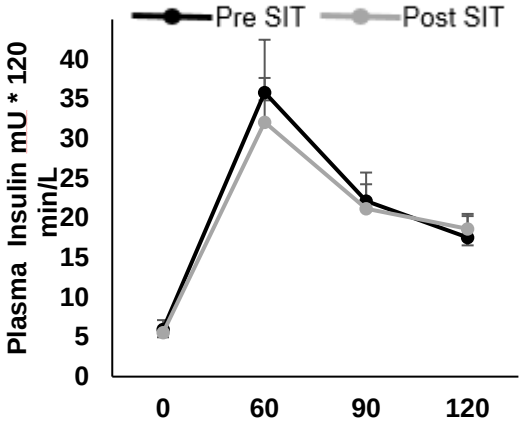
[A]


Pre SIT
Post SIT
Plasma glucose mmol/l
7
6
5
4
3
2
1
0
0
60
90
120

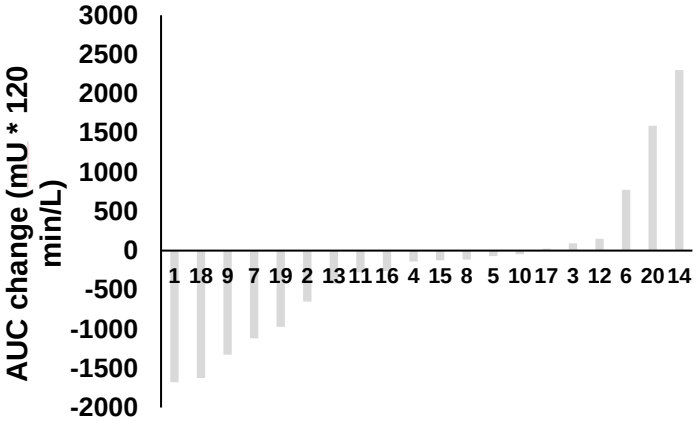
[B]


AUC change (mmol/L 2h)
200
150
100
50
0
-50
-100
-150
-200
20 16 7 1 5 2 10 9 11 17 12 13 14 8 3 6 4 18 15 19

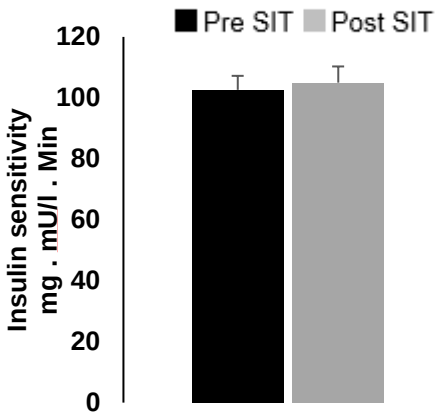
[C]


Pre SIT
Post SIT
Plasma Insulin mU * 120 min/L
40
35
30
25
20
15
10
5
0
0
60
90
120

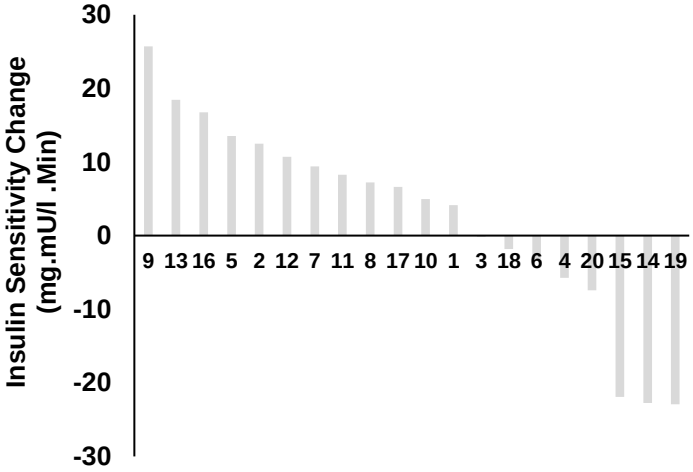
[D]

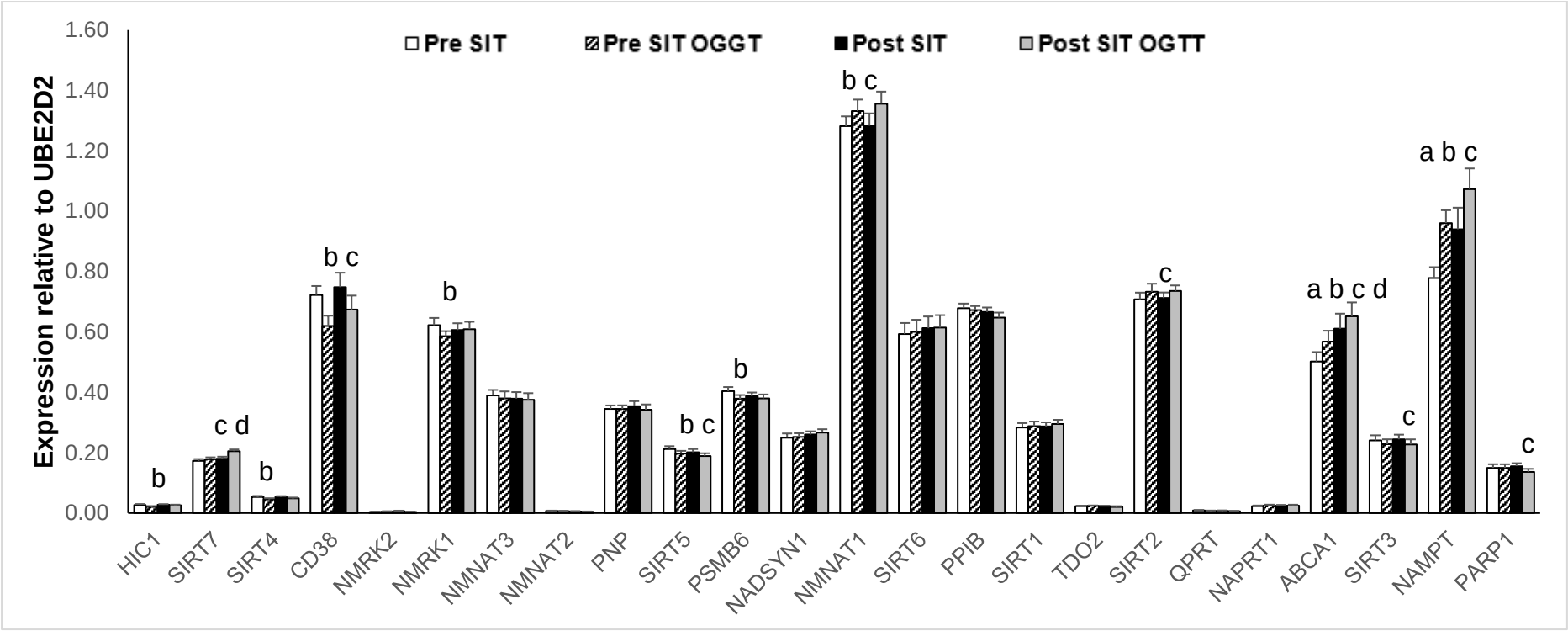

AUC change (mU * 120 min/L)
3000
2500
2000
1500
1000
500
0
-500
-1000
-1500
-2000
1 18 9 7 19 2 13 11 16 4 15 8 5 10 17 3 12 6 20 14

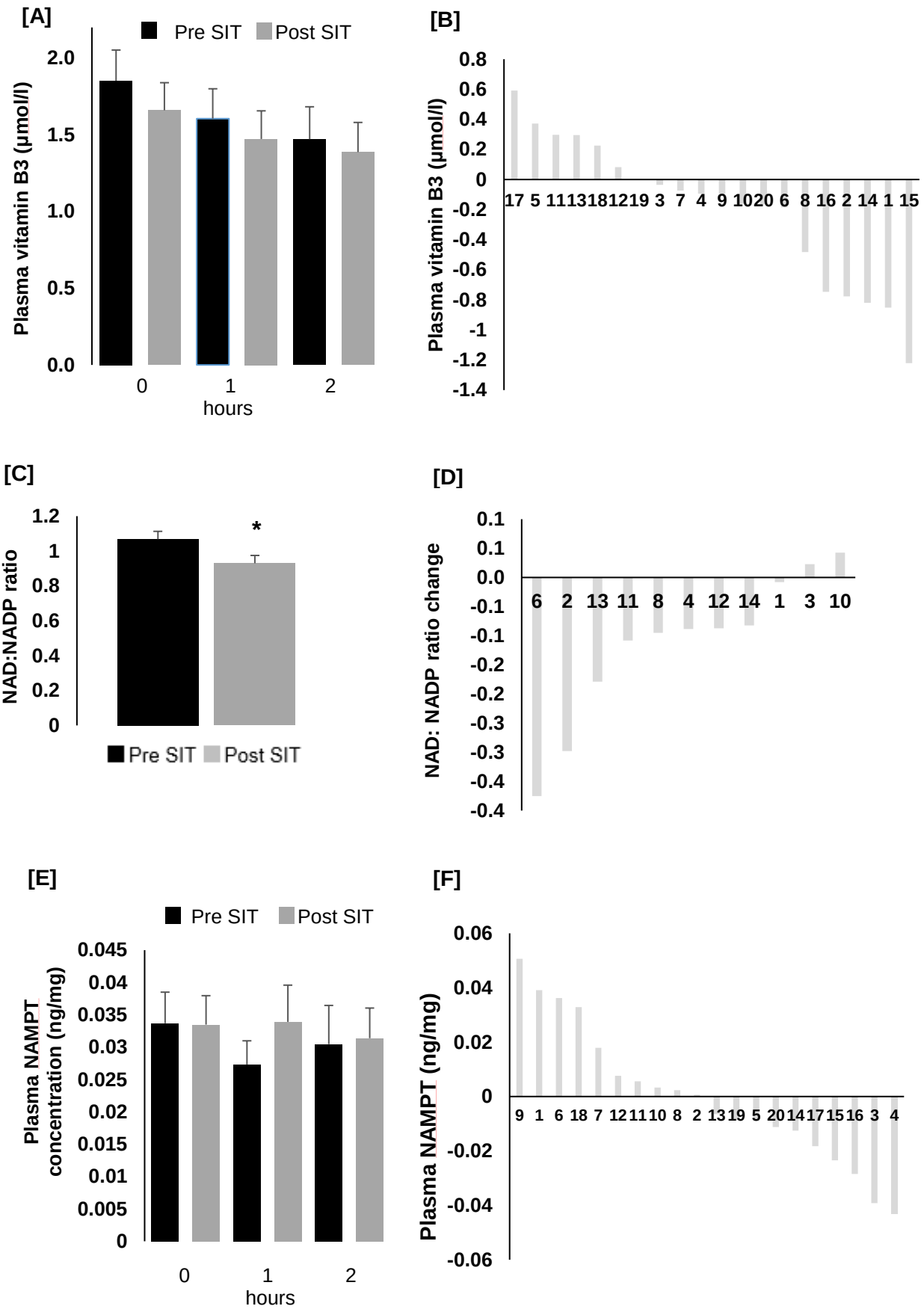
[E]


Pre SIT
Post SIT
Insulin sensitivity mg . mU/l . Min
120
100
80
60
40
20
0

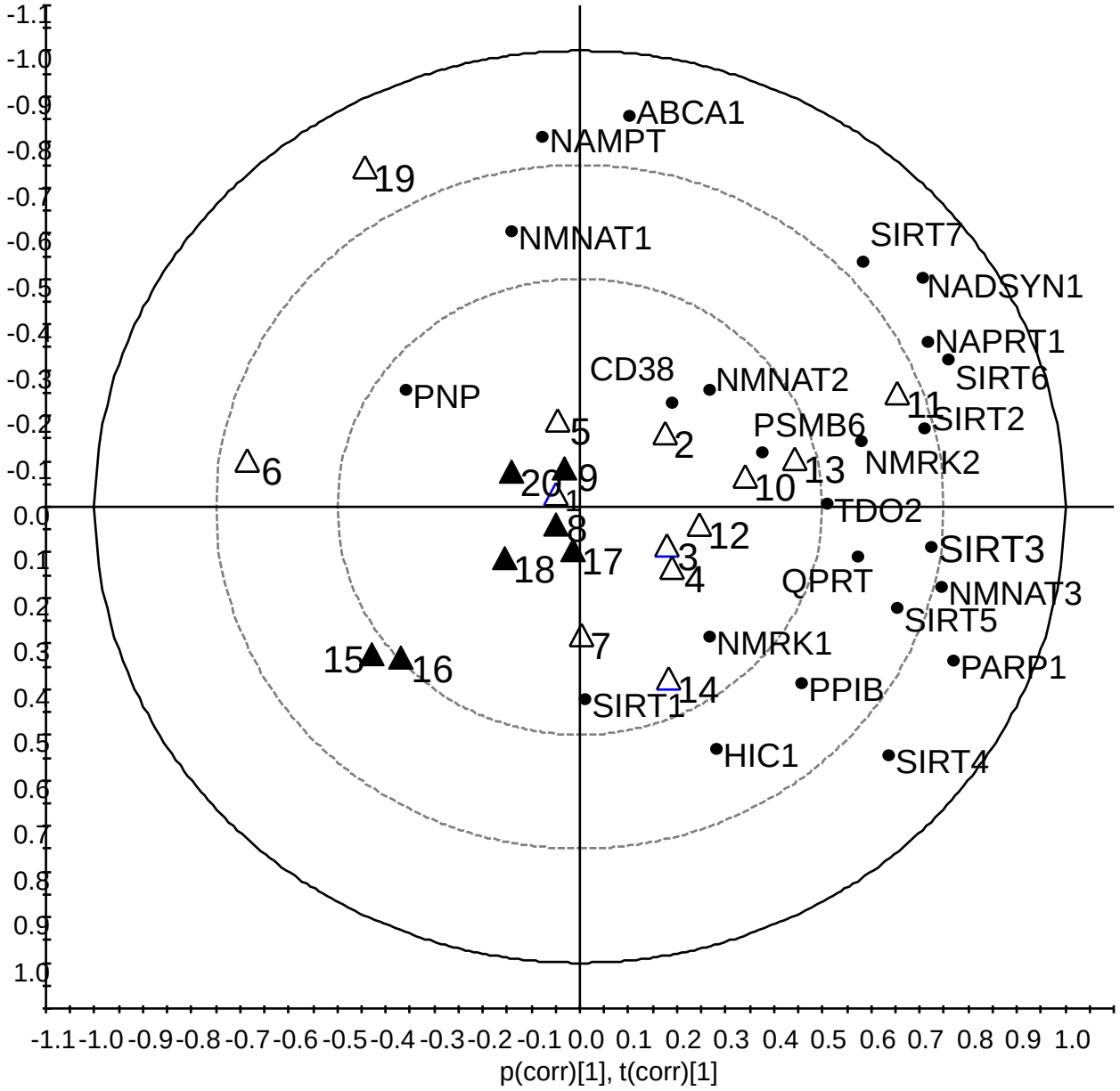
[F]


Insulin Sensitivity Change (mg.mU/l .Min)
30
20
10
0
-10
-20
-30
9 13 16 5 2 12 7 11 8 17 10 1 3 18 6 4 20 15 14 19

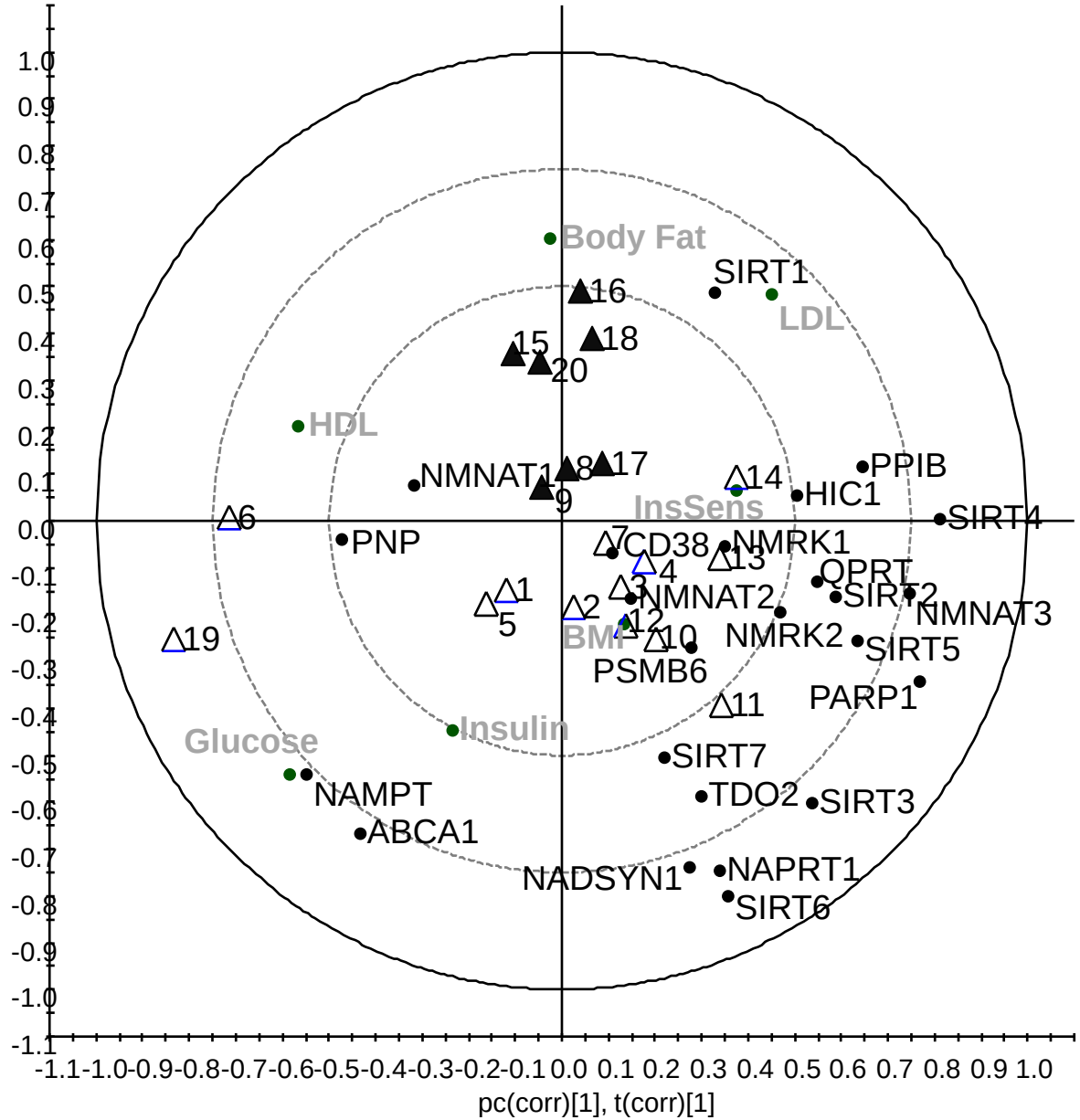

Pre SIT
Pre SIT OGGT
Post SIT
Post SIT OGTT
Expression relative to UBE2D2
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
b
c d
b
b c
b
b c
b
b c
c
a b c d
c
a b c
c
HIC1
SIRT7
SIRT4
CD38
NMRK2
NMRK1
NMNAT3
NMNAT2
PNP
SIRT5
PSMB6
NADSYN1
NMNAT1
SIRT6
PPIB
SIRT1
TDO2
SIRT2
QPRT
NAPRT1
ABCA1
SIRT3
NAMPT
PARP1


[A]
Pre SIT
Post SIT
Plasma vitamin B3 (µmol/l)
2.0
1.5
1.0
0.5
0.0
0
1
2
hours
[B]
Plasma vitamin B3 (µmol/l)
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
17 5 11 13 18 12 19 3 7 4 9 10 20 6 8 16 2 14 1 15
[C]
NAD:NADP ratio
1.2
1
0.8
0.6
0.4
0.2
0
*
Pre SIT
Post SIT
[D]
NAD: NADP ratio change
0.1
0.1
0.0
-0.1
-0.1
-0.2
-0.2
-0.3
-0.3
-0.4
-0.4
6 2 13 11 8 4 12 14 1 3 10
[E]
Pre SIT
Post SIT
Plasma NAMPT concentration (ng/mg)
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0
1
2
hours
[F]
Plasma NAMPT (ng/mg)
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
9 1 6 18 7 12 11 10 8 2 13 19 5 20 14 17 15 16 3 4

[A]


ABCA1
NAMPT
19
NMNAT1
SIRT7
NADSYN1
NAPRT1
SIRT6
CD38
NMNAT2
PNP
11
SIRT2
PSMB6
5
2
6
NMRK2
13
20
9
10
1
TDO2
8
12
SIRT3
18
17
3
4
QPRT
NMNAT3
SIRT5
7
NMRK1
15
16
PARP1
14
PPIB
SIRT1
HIC1
SIRT4
p(corr)[1], t(corr)[1]

[B]


Body Fat
SIRT1
16
LDL
15
18
20
HDL
NMNAT1
8
17
14
PPIB
9
HIC1
InsSens
6
SIRT4
PNP
7
CD38
NMRK1
4
13
QPRT
3
1
NMNAT2
SIRT2
2
12
NMNAT3
5
BMI
10
NMRK2
19
SIRT5
PSMB6
PARP1
11
Insulin
Glucose
SIRT7
NAMPT
TDO2
SIRT3
ABCA1
NADSYN1
NAPRT1
SIRT6
pc(corr)[1], t(corr)[1]