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TITLE

THE EFFECTS OF PHYSICAL ACTIVITY ON BROWN ADIPOSE TISSUE IN HUMANS

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ABSTRACT

Introduction: Brown adipose tissue (BAT) consists of brown adipocytes and its activation is one of the mechanisms that the human body uses to produce heat energy as an adaptation to cold exposure. This process is called "non-shivering thermogenesis" (NST). Increased BAT mass and BAT activity promote energy expenditure suggesting that it may be used against excessive weight and obesity in humans. The aim of this thesis was to investigate the relationship between physical activity levels and BAT mass and its activation in humans. A secondary aim was to examine the relationship between BAT mass, BAT activity and body mass index (BMI) and age, as well as the effects of smoking and cold exposure status on BAT mass and its activation in humans. **Method:** Fourteen male and 6 female cancer patients [age 54.40±19.25, BMI 25.91±4.19, body surface area (BSA) 1.85±0.21, lean body mass (LBM) 57.29±10.72] volunteered to participate in the experiment. The International Physical Activity Questionnaire was used for the measurement of physical activity levels. A personal questionnaire was also used to identify the effects of smoking status and cold exposure on BAT mass and activity. A Positron Emission Tomography and Computed Tomography Siemens Biograph LSO [16-slice] was used for the identification of BAT. **Results:** A low positive relationship between the total METs in a usual week and BAT activity corrected for LBM ($r=0.32$, $p=0.052$) was observed. There is also a moderate negative correlation between BMI and BAT activity, that is: corrected for body weight ($r=-0.42$, $p=0.009$), corrected for BSA ($r=-0.34$, $p=0.035$), and corrected for LBM ($r=-0.47$, $p=0.004$). Additionally, a low negative correlation between age and BAT activity corrected for LBM ($r=-0.32$, $p=0.051$) was detected. Furthermore, a significant main effect of physical activity levels of the last 7 days on BAT activity corrected for BSA ($p= 0.028$) was identified, and a significant main effect of physical activity levels in a usual week on BAT activity corrected for

BSA (p= 0.047) was also identified. Moreover, a main effect of cold exposure on BAT mass corrected for BSA (p= 0.017) was observed. **Conclusions:** Increased levels of physical activity may increase BAT activity. Also, the latter is decreased with age and excessive weight. Finally, individuals exposed in cold more often may have reduced BAT mass.

KEY WORDS

Brown adipose tissue, exercise, physical activity, UCP1

ΠΕΡΙΛΗΨΗ

Εισαγωγή: Ο φαιός λιπώδης ιστός (ΦΛΙ) αποτελείται από φαιά λιποκύταρα και η δραστηριοποίησή του είναι ένας από τους μηχανισμούς που το ανθρώπινο σώμα παράγει θερμότητα ως μία προσαρμογή από την έκθεσή του στο κρύο. Αυτό το φαινόμενο ονομάζεται «θερμογένεση χωρίς ρίγος». Τα αυξημένα επίπεδα της μάζας και της δραστηριότητας του ΦΛΙ προάγουν την ενεργειακή κατανάλωση με αποτέλεσμα την πιθανή μείωση του βάρους και την καταπολέμηση της παχυσαρκίας στον άνθρωπο. Σκοπός της παρούσας διατριβής ήταν να εξετάσει τις επιπτώσεις της φυσικής δραστηριότητας στη μάζα και τη δραστηριότητα του ανθρώπινου ΦΛΙ. Δεύτερος σκοπός ήταν να εξετάσει τη σχέση μεταξύ της μάζας και της δραστηριότητας του ΦΛΙ με το BMI και την ηλικία, καθώς επίσης και τις επιπτώσεις του ενεργητικού καπνίσματος και της έκθεσης στο κρύο στη μάζα και τη δραστηριότητα του ανθρώπινου ΦΛΙ. **Μέθοδος:** Δεκατέσσερις άντρες [14] και έξι [6] γυναίκες καρκινοπαθείς [ηλικίας 54.40±19.25, δείκτη μάζα σώματος (ΔΜΣ) 25.91±4.19, επιφάνεια σώματος (ΕΣ) 1.85±0.21, άλυπης σωματικής μάζας (ΑΣΜ) 57.29±10.72] εθελοντές συμμετείχαν στη μελέτη. Χρησιμοποιήθηκε το διεθνές ερωτηματολόγιο φυσικής δραστηριότητας για να μετρηθούν τα επίπεδα φυσικής δραστηριότητας κάθε ασθενούς. Ένα προσωπικό ερωτηματολόγιο επίσης χρησιμοποιήθηκε για να ερευνηθούν οι επιπτώσεις του ενεργητικού καπνίσματος και της έκθεσης στο κρύο στη μάζα και τη δραστηριότητα του ΦΛΙ. Ελήφθη τομογραφία εκπομπής ποζιτρονίων και αξονική τομογραφία σε τομογράφο Siemens Biograph LSO 16-τομών για τη μέτρηση του ΦΛΙ. **Αποτελέσματα:** Βρέθηκε μία χαμηλή θετική συσχέτιση μεταξύ της συνολικής κατανάλωσης METς σε μία τυπική εβδομάδα και της δραστηριότητας του ΦΛΙ διορθωμένη σε ΑΣΜ (r=0.32, p=0.052). Επίσης βρέθηκε μία μεσαία αρνητική συσχέτιση μεταξύ του ΔΜΣ και της δραστηριότητας του ΦΛΙ, η οποία είναι: διορθωμένη για το βάρος του

σώματος (r=-0.42, p=0.009), διορθωμένη γαι την ΕΣ (r=-0.34, p=0.035), και διορθωμένη για την ΑΣΜ (r=-0.47, p=0.004). Επιπλέον, βρέθηκε μία χαμηλή αρνητική συσχέτιση μεταξύ της ηλικίας των συμμετεχόντων και της δραστηριότητας του ΦΛΙ διορθωμένη για την ΑΣΜ (r=-0.32, p=0.051). Τα αποτελέσματα επίσης έδειξαν στατιστικά σημαντική επίδραση της φυσικής δραστηριότητας των τελευταίων 7 ημερών στη δραστηριότητα του ΦΛΙ διορθωμένη σε ΕΣ (p= 0.028) καθώς επίσης και στατιστικά σημαντική επίδραση της φυσικής δραστηριότητας μίας τυπικής εβδομάδας στη δραστηριότητα του ΦΛΙ διορθωμένη σε ΕΣ (p= 0.047). Επίσης βρέθηκε μία επίδραση της έκθεσης στο κρύο στη μάζα του ΦΛΙ διορθωμένη σε ΕΣ (p= 0.017). **Συμπεράσματα:** Συμπεραίνεται ότι τα αυξημένα επίπεδα φυσικής δραστηριότητας μπορεί να αυξάνουν τη δραστηριότητα του ΦΛΙ. Επιπλέον, ο ΦΛΙ μειώνεται με την ηλικία και με το υπερβολικό βάρος. Τέλος, άτομα που εκτίθενται στο κρύο πιο συχνά μπορεί να μειώσουν τη μάζα του ΦΛΙ.

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ:

Φαιός λιπώδης ιστός, άσκηση, σωματική δραστηριότητα, αποσυζευκτική πρωτεΐνη 1

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LIST OF ABBREVIATIONS

1. BAT (Brown adipose tissue)
2. SNS (Sympathetic nervous system)
3. NST (Non-shivering thermogenesis)
4. UCP1 (Uncoupling protein 1)
5. NAD+ (Nicotinamide adenine dinucleotide)
6. FADH (Flavin adenine dinucleotide)
7. MSTN (Myostatin)
8. ActRIIB (Active of type II receptor)
9. PGC1α (Peroxisom gamma coactivator-1 alpha)
10. NRF1 (Nuclear respiratory factor 1)
11. PPARδ (Peroxisome proliferator activated receptor delta)
12. DIO2 (Type 2 iodothyronine deiondinase)
13. T3 (Triiodothyronine)
14. T4 (Thyroxine)
15. BMI (Body mass index)
16. BF (Body fat)
17. IPAQ (International physical activity questionnaire)
18. PET/CT (Positron emission tomography and computed tomography)
19. ^{18}F-FDG (^{18}-fluoro-deoxy-glocuse)
20. MET (Metabolic equivalent)
21. SUV (Standardized Uptake Volume)
22. MBq (Mega Becquerel)

23. KBq (Kilo Becquerel)

LIST OF SYMBOLS

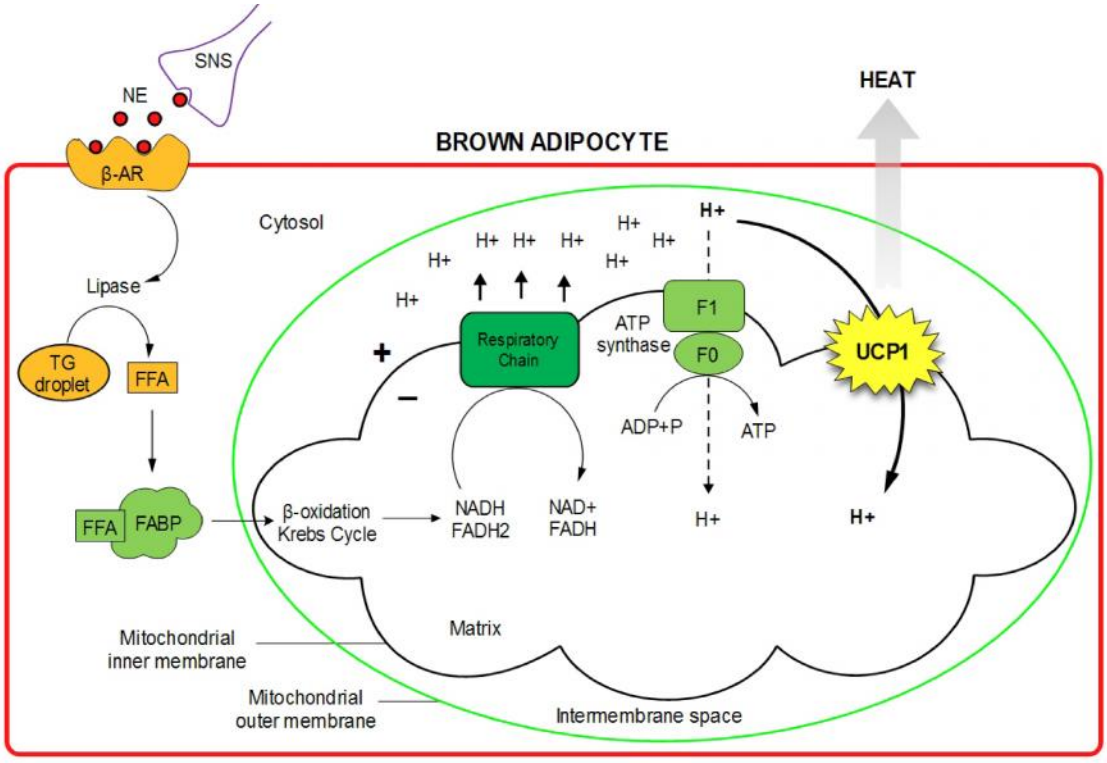
1. Cm^3 (Cubic centimeters)
2. gr (Grammars)
3. kg (Kilograms)
4. °C (Degree of Celsius)
5. % (Percent)
6. º (Degree of)
7. min (Minutes)
8. mRNA (Messenger of Ribonucleic acid)
9. mgHg (Milligram in hydrargyros)
10. mg/dl (Milligrams per deciliter)
11. * (Multiply)
12. + (Add up)
13. = (Equals)
14. / (Divides)
15. m^2 (Square meters)
16. - (Minus and Hyphen)

CHAPTER 1: INTRODUCTION

Brown adipose tissue (BAT) consists of brown adipocytes and its activation is one of the mechanisms used by the human body to produce heat energy. In contrast to white adipose tissue, BAT is located in small amounts in the neck and thorax specifically in the area of the lower neck and collar-bone, as well as along the spine of the chest and abdomen (Farmer, 2009; Mattson, 2010). It is well known that cold exposure triggers sympathetic nervous system (SNS) activation by increasing secretion of catecholamines (Cannon & Nedergaard, 2004). BAT is innervated by SNS through the neurotransmitter norepinephrine and its vascularization is increased in response to cold exposure (Mattson, 2010). BAT activation causes heat production via lipolysis of triglycerides in brown adipocytes as an adaptation to cold exposure in order to support body temperature maintenance (Mattson, 2010). This process is called "non-shivering thermogenesis" (NST) (Figure 1).

NST is activated through the link between norepinephrine and β-adrenergic and this binding results in the activation of lipases which hydrolyze triacylglycerols in free fatty acids. Free fat acids bounding to fatty acids binding proteins and transferred into mitochondria where they undergo β-oxidation and citric acid cycle. Afterwards, nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide ($FADH_2$) coenzymes, are oxidized by the electron transport chain resulting in protons pumped across the mitochondrial membrane. Accordingly, uncoupling protein 1 (UCP1), that is exclusively produced by brown adipocytes, drives protons into mitochondria and heat is produced [figure 1; (Ducharme & Bickel, 2008; Mattson, 2010; Rousset et al., 2004)].

Figure 1: The phenomenon of non-shivering thermogenesis



SNS: *sympathetic nervous system,* ***NE:*** *norepinephrine,* ***β-AR:*** *β-adrenergic receptor,* ***TG:*** *triacylglycerol,* ***FFA:*** *free fatty acids,* ***FABP:*** *fatty-acid-binding protein,* ***H+:*** *electron,* ***UCP1:*** *uncoupling protein 1,* ***ADP:*** *adenosine diphosphate,* ***P:*** *phosphorus,* ***ATP:*** *adenosine-5'-triphosphate,* ***NADH:*** *nicotinamide adenine dinucleotide,* ***$FADH_2$:*** *flavin adenine dinucleotide,* ***F1:*** *The F1 portion of the ATP synthase,* ***F0:*** *the F_O portion of the ATP synthase.*

Evidence revealed that BAT activity is augmented in humans after 2-hour of cold exposure at 16 ºC (van Marken Lichtenbelt et al., 2009). Moreover, BAT activity in adult humans, are more frequent in women than in men, (Cypess et al., 2009). Furthermore, BAT activity is negatively correlated with body mass index (BMI) and total body fat, and it is positively correlated with energy expenditure in humans (van Marken Lichtenbelt, et al., 2009) which suggests that people with excessive weight have lower BAT activity. Additionally, BAT activity is negatively correlated with a change in skin temperature indicating that individuals with higher BAT activity are able to thermoregulate more effectively (van Marken Lichtenbelt,

et al., 2009). However, BAT activity and BAT mass inversely associate with age (Mattson, 2010; Sturkenboom, Franssen, Berkhof, & Hoekstra, 2004) and it is believed that they are reduced approximately after the 13^{th}-15^{th} year of age in puberty (Virtanen & Nuutila, 2011). In this light, it is crucial to keep BAT active if we consider that on average an individual has no more than 100 gr of BAT which when activated is able to produce energy equal to activity of 3-4 kg of white adipose tissue (Virtanen et al., 2009). This is due to the higher oxidative capacity of brown adipocytes due to the size and abundance of mitochondria (Cinti, 2009). Furthermore, studies in mice demonstrated that increased brown fat indicates less weight gain, more insulin sensitivity, lower levels of serum-free fatty acids and lower risk for diabetes type 2 and other metabolic disorders (Cederberg et al., 2001; Kopecky, Clarke, Enerback, Spiegelman, & Kozak, 1995; Kopecky et al., 1996; Seale, Kajimura, & Spiegelman, 2009; Tsukiyama-Kohara et al., 2001). Therefore, increased BAT mass and BAT activity promotes energy expenditure and may be used against excessive weight and obesity in humans. Consequently, it is important to investigate alternative pathways other than cold exposure to keep BAT mass and BAT activity in high levels.

It is well known that exercise triggers SNS activity following the same process as cold exposure (Christensen & Galbo, 1983). Given the fact that the latter leads to BAT activation, it would be very interesting to investigate the relationship between exercise and BAT mass plus its activation. Moreover, there is evidence to support that exercise can increase UCP1 mRNA expression (Yamashita et al., 1993) and BAT mass in mice (Rouveix et al., 2007).

Purpose

The aim of this study was to investigate the relationship between physical activity levels and BAT mass and activation in humans. A secondary aim was to examine the effects of BMI, age, smoking status and cold exposure on BAT mass and BAT activity in humans.

Null Hypothesis

Physical activity levels will not be related with BAT mass and BAT activity. BMI and age will not be related with BAT mass and BAT activity. Smoking and cold exposure status will not be related to BAT mass and BAT activity.

Alternative Hypothesis

Physical activity levels will be related to BAT mass and BAT activity. BMI and age will be related to BAT mass and BAT activity. Smoking and cold exposure status will be related to BAT mass and BAT activity.

Research hypothesis

Based on published evidence in mice (Bueno et al., 2011; Fukao et al., 2010; Hu & Wang, 2007; Oh, Kim, Yoon, & Lee, 2007; Rouveix, et al., 2007; Schroeder, Shbiro, Gelber, & Weller, 2010; Seebacher & Glanville, 2010; Sene-Fiorese et al., 2008; Xu et al., 2011) the present research hypothesis is that physical activity levels will be related to BAT mass and BAT activity. Based on previous studies in humans (Sturkenboom, et al., 2004; van Marken Lichtenbelt, et al., 2009) the present research hypothesis is that BMI and age will be related to BAT mass and BAT activity. Finally, based on published evidence in humans (van Marken Lichtenbelt, et al., 2009)

cold exposure status will affect BAT mass and BAT activity and smoking will affect BAT mass and BAT activity due to its relation with human's metabolism (Metsios et al., 2008).

CHAPTER 2: LITERATURE REVIEW

The aim of the literature review was to provide evidence regarding the effects of exercise and/or physical activity on BAT mass and BAT activity in mammals. A secondary aim was to identify evidence from studies in humans concerning the relationship between BAT and physiological factors, through positron emission tomography and computed tomography (PET/CT) measurements. For the literature review process four databases were included in searching procedure (Web of Science, Pub Med Central, Scopus, and Science Direct), and was set as a limit of publications the last 10 years. A comprehensive search using any possible combination of the key words, "brown adipose tissue", "exercise", "physical activity" and "UCP1", was contacted in order to have retrieved the manuscripts for the literature review.

Results of literature review

Exercise and BAT mass

BAT mass may give an index of the tissue's ability for activation, as it is expected that the more the tissue the more its activation. However, BAT mass is only an index of the presence of the tissue and not of its activation due to the fact that BAT's ability for activation must be confirmed by the presence of UCP1 (Tsuboyama-Kasaoka et al., 1998).

It has been reported that wheel running and swimming significantly increased BAT mass in exercised rats compared to sedentary controls (Rouveix, et al., 2007; Schroeder, et al., 2010). It is also supported that wheel running increased formation of BAT in the elderly and middle-aged exercised rats, while it affected the transitive formation of the young exercised rats compared to sedentary controls (Ozden et al., 2004). Nevertheless, another study demonstrated that there was no difference in BAT mass between exercisers and non-exercisers mice (Fukao, et

al., 2010). Two other studies used swimming along with diet to investigate BAT in rats. Sene-Fiorese and colleagues in 2008 reported that swim training significantly increased BAT mass, in both chow and high-fat diets compared to sedentary controls (Sene-Fiorese, et al., 2008). Moreover, Bueno and colleagues in 2011 found that swimming significantly augmented BAT mass of fat exercised rats, compared to inactive controls. However, no differences in BAT appeared in either exercised or sedentary control rats consuming high fat diet (Bueno, et al., 2011).

Exercise and UCP 1 m RNA expression

UCP1 is the only contributor protein in NST (Tsuboyama-Kasaoka, et al., 1998) and thus investigators examined its expression in BAT which gives the ability of the activation of BAT. The present review revealed that there are no studies in humans that examined the effects of exercise on UCP1 and thus are presented herein only studies in mice.

Oh and colleagues in 2007 examined the effects of a 6-week swimming training program, on UCP1 gene expression in both obese and lean rats. A swimming program was performed for 2h/day at 35 °C water temperature in an attempt to avoid an increase of UCP1 due to the cold exposure. The results revealed that exercised female obese significantly improved UCP1 mRNA expression by 15.2% compared to sedentary controls, while a non-significant increase of 5.1% was observed in obese exercised males compared to inactive controls. Furthermore, in the same study swim training significantly enhanced UCP1 amount levels by 51.4% in male obese mice compared to inactive controls. Similarly, exercised lean male mice significantly ameliorated UCP1 mRNA expression levels, compared to lean male sedentary controls (Oh, et al., 2007). Correspondingly, another study supported that treadmill running improved cells expressing UCP1, and increased UCP1 mRNA expression in normal diet mice, compared to inactive

controls (Xu, et al., 2011). Moreover, treadmill running was recently shown to augment UCP1 mRNA expression in mice but only when both exercise and cold stimuli were present (Seebacher & Glanville, 2010). On the other hand, only one study reported that voluntary wheel exercise had no effect on mitochondrial protein content in BAT, in male rodents (Hu & Wang, 2007).

Exercise and relative BAT factors

Several studies examined physiological factors of BAT in an attempt to identify mechanisms that can explain the effects of exercise on BAT function. The present literature review provides evidence which may explain the possible pathways of the effects of exercise on BAT.

Lipogenesis rate and Myostatin

It has been reported that exercise significantly increased BAT lipogenesis rate in rats (Sene-Fiorese, et al., 2008) and BAT adipocyte progenitor cell population in male mice (Xu, et al., 2011). Additionally, increased levels of myostatin (MSTN) is correlated with decreased visceral fat and better insulin sensitivity (Mukherjee et al., 2007; Zhao, Wall, & Yang, 2005) and with enhanced brown adipocyte differentiation and energy expenditure (Tseng et al., 2008). In this light, Bueno and colleagues reported that swimming significantly improved the mRNA expression of MSTN expressed in BAT in mice (Bueno, et al., 2011).

PGC1α, NRF1and PPARδ

Peroxisom gamma coactivator-1 alpha (PGC1α) interacts with the nuclear respiratory factor 1 (NRF1) in order to stimulate mitochondrial biogenesis. Furthermore, peroxisome proliferator activated receptor delta (PPARδ) is also a transcriptional regulator of energy metabolism and its enlarged expression increases energy metabolism (Seebacher & Glanville, 2010). Seebacher and colleagues observed that exercise in 22 °C ambient temperature significantly ameliorated mRNA

concentrations of PGC1α, PPARδ, and NRF1 in BAT, and caused further increase in relative expression of PPARδ in 12 °C (Seebacher & Glanville, 2010).

Type 2 iodothyronine deiondinase

It is well known that SNS triggers NST in BAT and probably also triggers the type 2 iodothyronine deiondinase (DIO2) a selenoenzyme which is established in BAT and generates triiodothyronine (T3) (Martinez de Mena, Scanlan, & Obregon, 2010; Sullo, Brizzi, & Maffulli, 2003). There is also a relationship between SNS and thyroid hormone metabolism in BAT (Sullo, et al., 2003). This relationship indicates a higher serum level of T3 resulting from transformation of thyroxine (T4) to T3 in BAT, and verifies that T3 shaped in BAT promotes NST. Sullo and colleagues in 2003, reported that swimming in rats reduced DIO2 activity in BAT in neutral environmental conditions and after short cold exposure (Sullo, et al., 2003). Similarly, Sullo and colleagues in 2004, reported that swimming in rats decreased levels of T3 stimulated thermogenesis in BAT through changes in DIO2 activity both under basal circumstances and after a 30-minute cold exposure (Sullo, Brizzi, & Maffulli, 2004). Furthermore, Fortunato and colleagues in 2008 found that treadmill exercise significantly decreased DIO2 in BAT 30 min after exercise (Fortunato et al., 2008). This increase was evident for 120 min after the each exercise session. On the other hand, Xu and colleagues reported that DIO2 was significantly increased by exercise in normal diet mice compared to sedentary group on the same diet (Xu, et al., 2011).

Oxidative capacity

Oxidative capacity is also an index that has been examined in order to explain BAT activity due to the fact that NST takes place through brown fat oxidation. Terblanche and colleagues stated that treadmill exercise significantly decreased oxidative capacity in BAT in rats both at rest and

at exhaustion conditions. At rest the exercising rats significantly reduced oxidative capacity of pyruvate, palmitoylcarnitine, succinate, and ferrocytochrome c, compared to sedentary controls. Correspondingly, when exercisers reached exhaustion significantly decreased oxidative capacity of palmitoylcarnitine, succinate, and ferrocytochrome c, compared to sedentary controls (Terblanche, Gohil, Packer, Henderson, & Brooks, 2001).

Evidence in humans

The available literature contains only one study examining humans (86 non obese young healthy Japanese adults) in terms of exercise and BAT. Morita and colleagues investigated whether the Trp64Arg polymorphism in β3-AR gene and the 3826A/G polymorphism in the UCP1 gene were correlated with the energy expenditure and fat oxidation both in resting and during aerobic exercise. The results showed that there was no contribution of UCP1-3826A/G polymorphism in energy expenditure, however, UCP1-3826A/G polymorphism was associated with a reduction of fat oxidation in both resting and during aerobic exercise in male non obese young healthy Japanese adults (Morita, Taniguchi, & Sakaue, 2009). Studies examining the effects of exercise on BAT in the last decade are summarized in table 1.

Table 1: Studies examined the effects of exercise on BAT

Study	Exercise protocol	Effects of exercise on BAT
Schroeder et. al.2010 (mice)	Free wheel running for 23 days at 22±2 °C	Increased BAT mass in fatty females
Rouveix et. al. 2007 (mice)	Free wheel running access for 23h/day	Increased BAT mass
Fukao et. al. 2010 (mice)	Wheel running for 10 weeks	No differences in BAT mass
Ozden et. al. 2004 (mice)	Running for 5-day period	Increased formation of BAT in the elderly and middle-aged rats and affected the transitive formation of the young rats
Sene-Fiorese et. al. 2008 (mice)	Swimming for 8 weeks.	1. Increased BAT mass, 2. Increased lipogenesis rate in BAT
Bueno et al. 2011(mice)	Swimming, for 4 weeks, in 32–36 ◦C and with a weight of 5% body weight attached to the tail of each rat	1. Increased BAT mass 2. Increased MSTN mRNA in BAT
Xu et.al.2011 (mice)	Motorized treadmill for 5 days/week, for 8 weeks.	1. Increased brown cells population, 2. Increased UCP1 mRNA expression, 3. Increased UCP1 mRNA expression and DIO2 levels in normal fed
Oh et.al.2007 (mice)	Swimming 6 weeks in 35 ± 1º C	1. Increased UCP1 mRNA expression in obese females and lean males, 2. Increased UCP1 amount levels in obese males
Seebacher et. al. 2010 (mice)	Wheel running 30 days	1.Increased mRNA concentrations of PGC1a, PPARd, and NRF1, at 22° C, and further increase in expression of PPARd at12°C 2. Increased UCP1 mRNA expression only when both exercise and cold were present
Morita et.al.2009 (Exercise in humans)	One single exercise bout (cycling), 30 min, at 60% VO2 max.	1. No contribution of UCP1-3826A/G polymorphism in energy expenditure (fat oxidation) during exercise 2. Significantly lower level of fat oxidation both at rest and aerobic exercise than that of the Trp/Trp genotype
Hu and Wang 2007 (mice)	Wheel running for 8 weeks	No effects on mitochondrial protein content in BAT
Sullo et. al. 2004 (mice)	Daily swimming for 21 days, in fast flowing water at 25 °C	Decreased T3 induced thermogenesis in BAT through changes in DIO2 activity both basal conditions and cold exposure
Sullo et. al. 2003 (mice)	Swimming in rough water at 25º C, for 10 min until exhaustion	Decreased DIO2 activity in BAT in normal environmental conditions and after short cold exposure
Fortunato et.al. 2008 (mice)	Treadmill for 2–3 days, at 22ºC	Decreased DIO2, 30 min-120 min after exercise session
Terblanche et. al. 2001 (mice)	Treadmill at 15º gradient for 5-6 weeks	Decreased of oxidative capacity both at rest and at exhaustion

***BAT**: Brown adipose tissue, **UCP1**: Uncoupling protein 1, **MSTN**: Myostatin, **APC**: Adipocyte progenitor cells, **DIO2**: Type II iodothyronine deiodinase, **PGC1a**: Peroxisom gamma coactivator-1 alpha, **PPARd**: Peroxisome proliferator activated receptor delta, **NRF1**: Nuclear respiratory factor 1, **T3:** Triiodothyronine.*

Evidence in humans through PET/CT measurements

Scientists have used PET/CT measurements in humans in order to identify the relationship between BAT mass, BAT activity and body composition and metabolism. van Marken Lichtenbelt et. al. in 2009 examined through PET/CT the BAT volume and BAT activity after two hours in cold exposure at 16 °C in 24 healthy males. The results showed that there is a negative correlation between BAT activity and BMI, and also there is a negative correlation between BAT activity and percentage of body fat (% BF) (van Marken Lichtenbelt, et al., 2009). Furthermore, the same study found that BAT activity and BAT volume was lower in overweight and obese individuals than in lean individuals during cold exposure (van Marken Lichtenbelt, et al., 2009). Likewise, a study from the same research group that examined morbidly obese individuals confirmed the negative correlations between BAT activity and BMI and % BF (Vijgen et al., 2011). Additionally, in another study active BAT was identified after two hours of cold exposure in healthy individuals (Virtanen, et al., 2009). In the most recent study, Vijgen et al. examined BAT activity in morbidly obese individuals before and after weight loss induced by bariatric surgery. The results showed that BAT can be recruited in the same regions that can be recruited in lean individuals, and this might show a way to reduce obesity (Vijgen et al., 2012).

Discussion of literature review

Exercise and BAT mass

Generally speaking, it has been recognized by the majority of the studies that exercise increases BAT mass in mice (Bueno, et al., 2011; Ozden, et al., 2004; Rouveix, et al., 2007; Schroeder, et al., 2010; Sene-Fiorese, et al., 2008). Increased BAT mass may contribute in higher NST which may decrease of feeding effectiveness leading to decreased body weight (Schroeder, et al., 2010). Due to the specific activity of BAT, that is, to produce heat in order to maintain body

temperature which increases energy expenditure under low environmental temperatures, this might be a mechanism against obesity (Cannon & Nedergaard, 2004; Redinger, 2009). Furthermore, the increased BAT mass may have an anti-inflammatory effect; however this may be possible in combination with the improvement of adiponectin concentration in muscles and the decrease of white adipose tissue levels (Fukao, et al., 2010). The age-depended effects of exercise on BAT mass indicate that young mice ameliorate smaller amount of their BAT levels, than middle-age and elderly (Ozden, et al., 2004). On the whole, the existing evidence suggests that a period of endurance exercise, between 4-10 weeks is required to increase BAT mass in mice. However, due to the lack of evidence in humans, further investigation is essential in order to clarify if exercise increases BAT mass in humans and which type and duration of exercise is the most appropriate for this purpose.

Exercise and UCP 1 m RNA expression

The majority of the evidence demonstrates that exercise increases UCP1 mRNA expression in BAT in mice (Oh, et al., 2007; Seebacher & Glanville, 2010). Swimming and running ameliorated UCP1 mRNA expression in BAT both in male and female rats, equally in obese and lean rats (Oh, et al., 2007). Therefore, an aerobic exercise intervention either swimming or running between 4-8 weeks is needed in order to increase UCP1 mRNA expression in mice. The enlarged UCP1 mRNA expression in BAT improves fatty acid oxidation and may promote the energy metabolism which may prevent dyslipidemia (Oh, et al., 2007) and obesity in mice (Ricquier, 2005). However, the amplified UCP1 mRNA expression by exercise is under discussion due to the finding from Seebacher and Glanville, that is, UCP1 mRNA expression was increased when both exercise and cold were present (Seebacher & Glanville, 2010). It seems that a threshold in ambient temperature is needed below which UCP1 mRNA expression can be

increased. However exercise could be required, as well in addition to low temperature, so that UCP1 mRNA is increased.

The mechanisms by which exercise stimulates UCP1 mRNA expression remain unknown. It appears that UCP1 mRNA expression is increased in regular exercisers. Consequently, this may lead to improved thermogenesis of exercisers compared to sedentary individuals which consequently causes higher energy expenditure (Seebacher & Glanville, 2010). Yet, reduced UCP1 mRNA expression may indicate decreased mitochondrial capacity leading to lower energy expenditure (Seebacher & Glanville, 2010). In addition, exercise in cold ambient temperatures may augment metabolic rate more than that occurring in warm environmental conditions (Seebacher & Glanville, 2010). Additionally, a possible increased of UCP1 mRNA expression enhances the energy coming from fatty acids oxidation as heat, instead of being deposited as white adipose tissue. Thus, the enlarged energy expenditure may lessen obesity (Seebacher & Glanville, 2010; Wang et al., 2003).

Exercise and relative BAT factors

Lipogenesis rate, Myostatin and oxidative capacity

The ameliorated lipogenesis in BAT may come from the glycogen lessening during and after an exercise session, which activates the metabolism of fatty acids. Another reason may be that during the recovery period after exercise, glycerol is required for glycogen repositioning (Bernardes, Manzoni, Souza, Tenório, & Dâmaso, 2004; Gauthier, Couturier, Latour, & Lavoie, 2003; Sene-Fiorese, et al., 2008). Besides, the increase of brown adipocyte progenitor cells affected by exercise indicates better insulin sensitivity and glucose homeostasis (Xu, et al., 2011). Furthermore, a study revealed that the exercise intervention caused decreased oxidative capacity in BAT (Terblanche, et al., 2001). This may had been due to the greater lipogenetic rate

in BAT, which indicates plenty of lipids containing fatty acids. The possible reason of this reduction of the oxidative capacity is the high concentrations of peroxides and hydroperoxides which are formed by the better mitochondrial activity that exercise causes (Terblanche, et al., 2001). However, the reduction of oxidative capacity in BAT due to exercise may be considered as a beneficial effect because of lower rise in body temperature during exercise, mostly in trained animals (Gohil, Henderson, Terblanche, Brooks, & Packer, 1984). Therefore, the relative drop in oxidative capacity in BAT may result in the extrusion of a higher amount of fatty acids (Terblanche, et al., 2001).

Reduced levels of MSTN, which is phosphorylated by the active of type II receptor (ActRIIB), is described as a harmful regulator in skeletal muscle (Bueno, et al., 2011; McPherron & Lee, 1997). On the other hand, over expression of MSTN leads to decreased visceral fat and better insulin sensitivity (Mukherjee, et al., 2007; Zhao, et al., 2005). Likewise, higher MSTN pro peptide expression is correlated with immune system activation in mice (Lyons, Haring, & Biga, 2010; Wilkes, Lloyd, & Gekakis, 2009), and is associated with enhanced brown adipocyte differentiation and energy expenditure (Tseng, et al., 2008). Therefore, the increased mRNA expression of MSTN and decreased mRNA expression of its receptor ActRIIB in BAT provoked by exercise may indicate an involvement of MSTN in energy homeostasis (Bueno, et al., 2011).

PGC1α, NRF1and PPARδ

Decreased expression of PGC1α and PPARδ is associated with increased possibility of insulin resistance and type 2 diabetes (Hoehn et al., 2010). Additionally, reduced expression of PPARδ indicates damage of glucose and fatty acid metabolism which also leads to increase risk of obesity and type 2 diabetes (Nunn, Bell, & Guy, 2009; O'Gorman & Krook, 2008; Wang, et al., 2003). In addition, lower expression of NRF1 has as a result lower levels of glucose transporter

4, and augments the risk of type 2 diabetes (Baar et al., 2002; Holloszy, 2008). On the other hand, increased expression of PGC1α is crucial because PGC1α regulates NST in BAT (Cannon & Nedergaard, 2004; Puigserver & Spiegelman, 2003) by increasing the expression of UCP1 (Cannon & Nedergaard, 2004; Lowell & Spiegelman, 2000). Exercise augments expression of PGC1α, PPARδ and NRF1 in BAT in mice and this may lead to more thermogenic capacity which possibly ends to increase energy expenditure and in line may lead to a lower risk for obesity. Therefore, exercise is characterized as an essential method to enhance expression of PGC1α, PPARδ and NRF1 in BAT in mice (Seebacher & Glanville, 2010).

Type 2 iodothyronine deiondinase

The effect of exercise on the deiodinating activity in BAT may play a central role in thyroid hormone regulation in BAT although the thermogenesis mechanisms related to increased levels of T4 to T3 conversion are unclear. There is evidence that reduced levels of DIO2 in BAT are attributed to exercise (Fortunato, et al., 2008; Sullo, et al., 2003, 2004). DIO2 regulates T3 production through removal of specific iodine atoms from the originator molecule thyroid T4 or T3 itself. Therefore, reduced levels of DIO2 in BAT indicate decreased levels of T4 and T3 which participate in energy metabolism. Thus, this mechanism is probably important for the control of BAT thermogenesis after exercise (Fortunato, et al., 2008). Furthermore, thermogenesis during exercise may activate mechanisms involved in the reduction in T4 and T3 hormone levels in order to avoid major increase in body temperature through changes in energy metabolism (Fortunato, et al., 2008).

Thyroid hormone activity has a close connection with SNS. In addition, DIO2 activity in BAT is under SNS control that motivates the thermogenic response in BAT (Silva, Mellen, & Larsen, 1987). The mechanisms involved in decreasing of DIO2 in BAT may be the same as

those involved in the reduction of thyroid hormones (Sullo, et al., 2003). Additionally, the lower levels of DIO2 after exercise in cold environment may be explained by the lower sympathetic activity (Sullo, et al., 2004). However, Xu and colleagues reported the opposite, that is, DIO2 levels were increased in BAT after exercise (Xu, et al., 2011), giving the perception that the mechanisms involved in the effects of exercise on DIO2 levels in BAT must be further investigated.

Evidence in humans

Information showing the effects of exercise on BAT in humans is very limited. In this literature review, evidence is provided from only one study that examined humans. This study demonstrates that there is no contribution of UCP1-3826A/G polymorphism in energy expenditure, and in fat oxidation during aerobic exercise (Morita, et al., 2009). Nevertheless, at that study there was no exercise intervention, but only one single exercise bout for 30 min, and consequently this may explain the results. To date there is no other study that examined the relationship between exercise and BAT in humans.

Evidence in humans through PET/CT measurements

PET/CT were used for identifying cancerous tumors or other pathologic hypermetabolic tissues in humans, however it was reported that some areas showed intense ^{18}F-FDG uptake indicated a physiologic activity. Several studies assumed that those areas reflect the metabolic activity of brown fat (Cohade, Osman, Pannu, & Wahl, 2003; Hany et al., 2002). Indeed, studies conducted for other reasons that used PET/CT indicated that BAT was active in adults' humans (Hany, et al., 2002; Nedergaard, Bengtsson, & Cannon, 2007; Yeung, Grewal, Gonen, Schoder, & Larson, 2003). Afterwards, PET/CT measurements were used exclusively for the identification of BAT in humans. It is clear that BAT activity is triggered from acute cold exposure in humans as

indicated by several studies (van Marken Lichtenbelt, et al., 2009; Vijgen, et al., 2012; Vijgen, et al., 2011; Virtanen, et al., 2009). Furthermore, PET/CT measurements revealed that the more the BMI in humans the less the BAT activity (van Marken Lichtenbelt, et al., 2009), as well as increased BAT activity indicates increased energy expenditure (van Marken Lichtenbelt, et al., 2009). However, to date no study examined the relationship between exercise and/or physical activity and BAT in humans through PET/CT measurements.

Conclusions of literature review

In conclusion, exercise appears to increase BAT mass and UCP1 mRNA expression in mice giving the ability for more effective NST. However, evidence from mice (Seebacher & Glanville, 2010) suggest that in order to increase UCP1 expression the presence of both exercise and cold is necessary. Exercise may ameliorate NST through the stimulation of relative BAT factors and can increase metabolic activity. Therefore, exercise can increase energy expenditure through BAT activity, yet only evidence from mice can support this mechanism.

Due to the lack of evidence in humans, further exploration is required to verify the existing mechanisms regarding the effects of exercise on BAT in humans. Furthermore, the negative relationship between BMI and BF with BAT activity in humans may indicate that higher physical activity and exercise levels which usually designate a lower BMI and lower BF may lead to a possible relationship between physical activity and/or exercise and BAT activity in humans. However, an examination with PET/CT requires an injection to the examined humans of the ^{18}-fluoro-deoxy-glocuse (^{18}F-FDG), which exposes people to radiation. Thus, must be found alternative methods to identify BAT in humans such as to examine people who would take PET/CT examination for other reasons like cancer patients.

CHAPTER 3: METHOD

Participants

Approval was obtained from the Ethics Committee of the Department of Physical Education and Sport Sciences, of Thessaly University, and from the Director of PET/CT department of the "HYGEIA Hospital". Participants visited once the "HYGEIA Hospital" after an appointment between 08:15 am and 13:00 pm. Following a previous study using similar methodology (van Marken Lichtenbelt, et al., 2009) the minimum required sample size was estimated to 20 individuals. Therefore written informed consent was received from 14 males and 6 females cancer patients [age 54.40±19.25, body mass index (BMI) 25.91±4.19, body surface area (BSA) 1.85±0.21, lean body mass (LBM) 57.29±10.72] who volunteered to participate in the experiment. For the present study cancer patients were recruited due to the fact that those patients would have taken PET/CT examination for the identification of cancer tumorous and thus they would not have been further exposed to radiation for the identification of BAT. Exclusion criteria included previous medications that affected the metabolism of the participants or alter BAT such as β-blockers. Furthermore, patients with chronic health problems that affected their metabolism were excluded from the study as well as patients with history of diabetes mellitus or hyper metabolic disease were also excluded. Moreover, patients that their blood glucose prior to examination was >150 mg/dl were excluded from the study. Additionally, we instructed the participants to refrain from any kind of food at least 6 hours prior to examination.

Measurements procedure

Medical history was obtained from a team of physicians in order to identify patients' health status, clarify the clinical question to be answered by the examination and give them their

approval to take the examination. Afterwards, participants completed the physical activity questionnaire and the personal questionnaire (Appendix c). Patients were hydrated and measurements were taken for height, weight, blood pressure and serum glucose levels and a venous catheter was placed to the arm of each patient. Thereafter, individuals were positioned to a dark room in a supine position. Subsequently, if participants' systolic blood pressure was >110 mgHg they received furosemide (lasix) as a diuretic. In addition, all participants received diazepam (stedon) in order to avoid excessive muscle activity during the examination that could have affected the BAT measurements (Barrington & Maisey, 1996). Finally, ^{18}F-FDGwas administered to the patients intravenously, and then they remained for 1 hour in a supine position into the dark, quiet, warm room. The dose of ^{18}F-FDG was 347.450±23.315 mega becquerel (MBq) dependent on the weight of each participant. One hour after the intravenous administration of the radiopharmaceutical, CT was firstly performed and then PET data were received (4 min per bed, distance covered per bed 15 cm, 6-8 beds depending on patients' height) at a Siemens Biograph LSO 16-slice device. Images were obtained from the base of the skull to the upper third of the thigh. Data were reconstructed at three levels, were corrected for attenuation and finally PET and CT images were fused.

Measurements of physical activity levels and personal questionnaire

Physical activity levels was measured through International Physical Activity Questionnaire-short form (IPAQ) using both the usual week and the last 7-day forms (Appendix c), which have been validated for the Greek population (Papathanasiou et al., 2009), and they were used in the past for clinical populations (Metsios et al., 2007; Van Dyck et al., 2011). There is no difference between the usual week and the 7-day form of IPAQ except that each questionnaire refers to the physical activity of a usual week and the last 7-day, respectively. Both questionnaires are self-

reported and were completed by the participants. The obtained data were calculated and transformed as metabolic equivalent (MET)-minutes per week, and categorized in low, moderate and high level of physical activity as indicated by the Guidelines for Data Processing and Analysis of the IPAQ, November 2005 (Ainsworth et al., 2000; Karolinska, 2012).

Physical activity categories

Individuals who did not meet the criteria for moderate and/or high levels of physical activity were considered to engage in low levels of physical activity (Karolinska, 2012). The criteria for "moderate" physical activity levels were: a) 3 or more days of vigorous-intensity activity of at least 20 min per day OR b) 5 or more days of moderate-intensity activity and/or walking of at least 30 min per day OR c) 5 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum total physical activity of at least 600 MET-min per week. Individuals who met at least one of the above criteria defined as accumulating a minimum level of activity and therefore classified as "moderate" (Karolinska, 2012). The criteria for "high" physical activity levels were: a) vigorous-intensity activity on at least 3 days achieving a minimum total physical activity of at least 1500 MET-min per week OR b) 7 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum total physical activity of at least 3000 MET-min per week (Karolinska, 2012).

METs calculation

The MET calculation was performed according to the MET equations for the IPAQ (Karolinska, 2012):

a) Walking (Low)= 3.3 METs*minutes of walking*days of activity,

b) Moderate intensity = 4 METs *minutes of walking*days of activity, and

c) High intensity = 8 METs*minutes of walking*days of activity.

Total MET values calculated according to the equation (Karolinska, 2012):

MET-minutes per week = Walking (METs*min*days) + Moderate (METs*min*days) + Vigorous (METs*min*days).

Values less than 10 min were not included in the calculation of summary scores, due to the guidance that episodes or bouts of at least 10 minutes are required to achieve health benefits (Karolinska, 2012). Values more than 180 min are truncated according to the rules by the calculation guidance (Karolinska, 2012). The physical activity variables that were used for the statistical analysis are presented in table 2.

Table 2: Physical activity variables were used for statistical analysis

Values	Units
Physical activity last 7 days	Categories: Low, Moderate, High
Total METs last 7 days	METs
Physical activity usual week	Categories: Low, Moderate, High
Total METs usual week	METs

Personal questionnaire

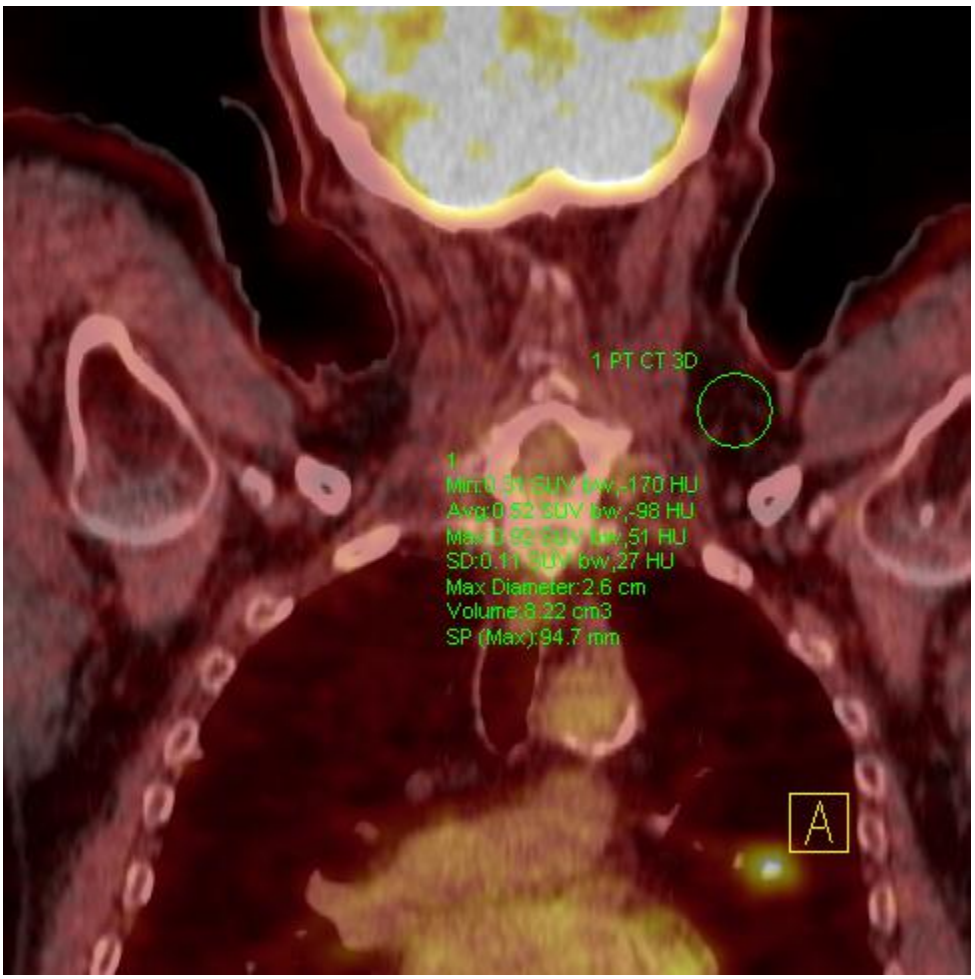
Prior to the PET/CT measurements, participants filled a personal questionnaire (Appendix c) in order to collect information on smoking and cold exposure status. The questionnaire is self-reported and was built by the investigator of the study based on similar questionnaires that examine cold exposure or smoking status.

Measurements of BAT

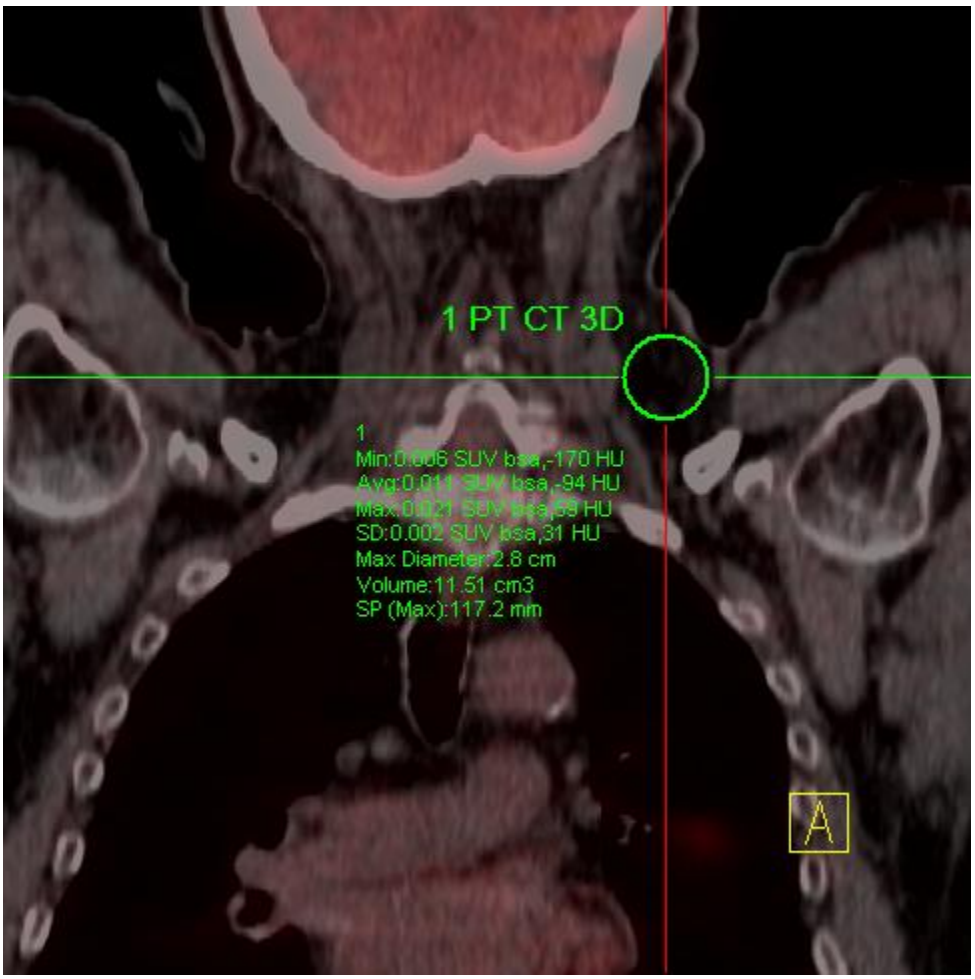
Examination procedure

Measurements of BAT were obtained from both the left and the right side for supraclavicular area and along the spine area, respectively. A sphere volume of interest was drawn at the area of interest and attention was paid so that other hypermetabolic tissues were not included. All PET/CT studies were interpretated by two independent experienced physicians. In case that the examined area was comprised by cancerous tumors or other pathologic hypermetabolic tissues this participant was excluded from the study. An average from the maximum values (Adams, Turkington, Wilson, & Wong, 2010) of the left and right side measurements was calculated for the supraclavicular area and along the spine area, respectively, in order to estimate the final values for BAT volume (cm^3) and BAT activity in Standardized Uptake Volume (SUV) units. Subsequently, the values of supraclavicular area and along the spine area were added up for BAT volume (cm^3) and BAT activity (SUV), respectively, in order to estimate the total values for each participant. Representative pictures from identified BAT in supraclavicular area corrected for body weight (BW), BSA, and LBM are displayed in pictures 1, 2, and 3, respectively.

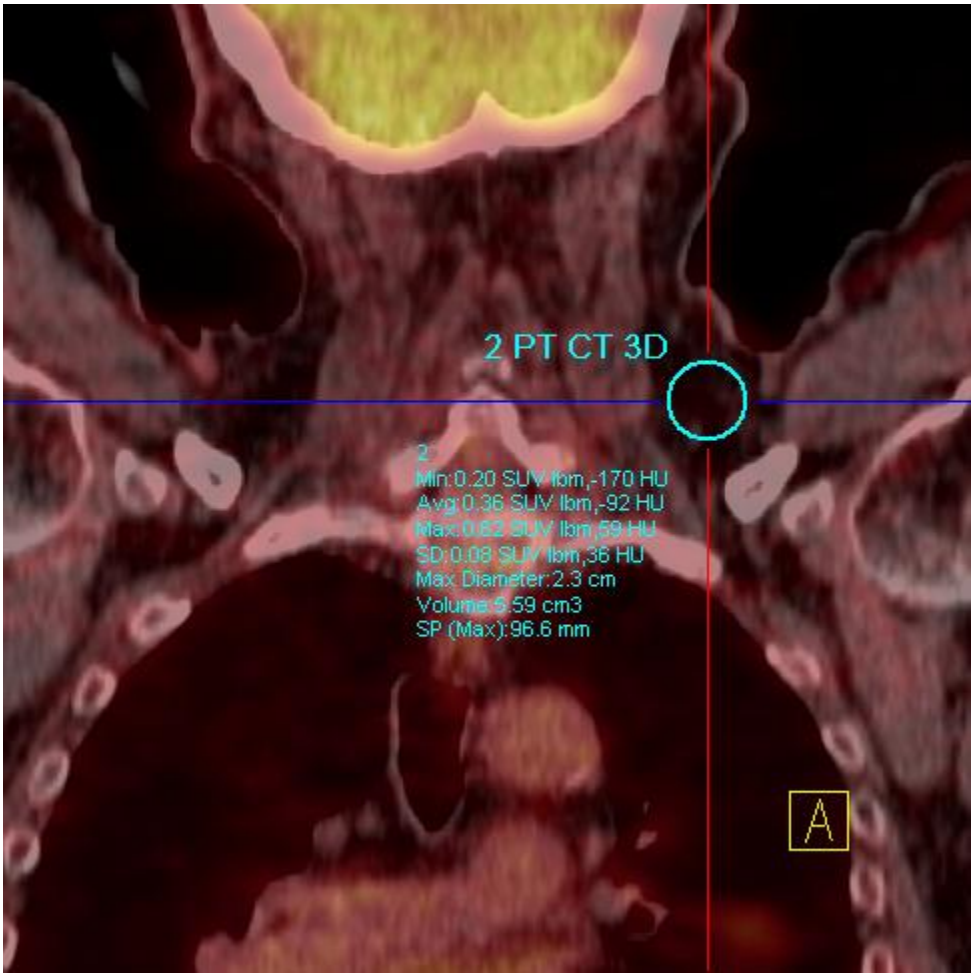
Picture 1: Identified BAT corrected for BW (supraclavicular)



Picture 2: Identified BAT corrected for BSA (supraclavicular)



Picture 3: Identified BAT corrected for LBM (supraclavicular)



BAT activity values

BAT activity SUV transformed to kilo becquerel (KBq) units (Kim, Gupta, Chandramouli, & Alavi, 1994; Sugawara, Zasadny, Neuhoff, & Wahl, 1999) following standard methodology (Vijgen, et al., 2012) and the study that defined values for the Siemens PET/CT software (Fletcher et al., 2008). Based on previous studies that used three corrections for radioactivity values we included in the study those three corrections that are, corrected for BW, for BSA and for LBM (Kim, et al., 1994; Kinahan & Fletcher, 2010; Krak et al., 2003; Sugawara, et al., 1999; Wahl, Jacene, Kasamon, & Lodge, 2009).

SUV units were transformed to KBq units corrected for BW (Fletcher, et al., 2008; Sugawara, et al., 1999) according to the equation:

SUV= r (KBq)/ [α'(injected ^{18}F-FDG in KBq)/ weight (gr)]

Similarly, SUV units were transformed to KBq corrected for BSA (Kim, et al., 1994; Sugawara, et al., 1999) according to the equation:

SUV= r (KBq)/ [α'(injected ^{18}F-FDG in KBq)/ BSA (m^2)]

Likewise, SUV units were transformed to KBq corrected for LBM (Kim, et al., 1994; Sugawara, et al., 1999) according to the equation:

SUV= r (KBq)/ [α'(injected ^{18}F-FDG in KBq)/ LBM (Kg)]

For the calculation of BSA and LBM for each participant the Siemens PET/CT software equations were used (Sugawara, et al., 1999), that are:

$BSA=BW^{0.425} * Height\ (H)^{0.725} * 71.84$ for both male and female, and

$LBM^{male}=1.10 * BW-120 * (BW/H)^2$, and

$LBM^{female} = 1.07 * BW-148 * (BW/H)^2$

The BAT variables were used for statistical analysis are presented in table 3.

Table 3: BAT variables were used for statistical analysis

Values	Units
Total BAT volume corrected for BW (TVBW)	Cm^3
Total BAT volume corrected for BSA (TVBSA)	Cm^3
Total volume corrected for LBM (TVLBM)	Cm^3
Total SUV corrected for BW (TSUVBW)	SUV
Total SUV corrected for BSA (TSUVBSA)	SUV
Total SUV corrected for LBM (TSUVLBM)	SUV
Radioactivity corrected for BW(rKBqBW)	KBq
Radioactivity corrected for BSA (rKBqBSA)	KBq
Radioactivity corrected for LBM (rKBqLBM)	KBq

Statistical Analysis

The statistical analysis was completed using the statistical package of social sciences 18.0 (SPSS 18.0) and the level of significance was set at $P<0.05$. All continuous variables were tested for normal distribution. Exploratory analysis revealed abnormal distribution for some continuous variables and, therefore, non-parametric tests were adopted. Non-parametric correlation (Kendall's tau-b) was performed so as to identify the relationship between physical activity levels (total METs both the last 7 days and a usual week) and BAT mass, BAT activity, BMI, as well as age. Kruskal-Wallis analysis of variance was used to detect any effects of physical activity (low, moderate, and high; both the last 7 days and a usual week) on BAT mass and activity. Furthermore, in order to analyze data for the study's secondary aim Kruskal-Wallis analysis of variance was also used to identify a potential effect of smoking status and cold exposure on BAT mass and BAT activity. Subsequently, post-hoc Mann-Whitney U tests were used to specify the effects were found from Kruskal-Wallis analyses.

CHAPTER 4: RESULTS

The results revealed no relationship between the total METs expenditure in the last 7 days and BAT mass and BAT activity ($p>0.05$). Non-parametric correlation showed that the total METs expenditure in a usual week and BAT activity had a tendency to be related, yet the correlation was not statistically significant (Figures 2 and 3; $p>0.05$). Additionally, it seems that there is a positive relationship between the total METs expenditure in a usual week and BAT activity corrected for LBM ($r=0.32$, $p=0.05$) (fig.4).

Figure 2: Activity of BAT (KBq) corrected for BW in relation to total METs usual week.

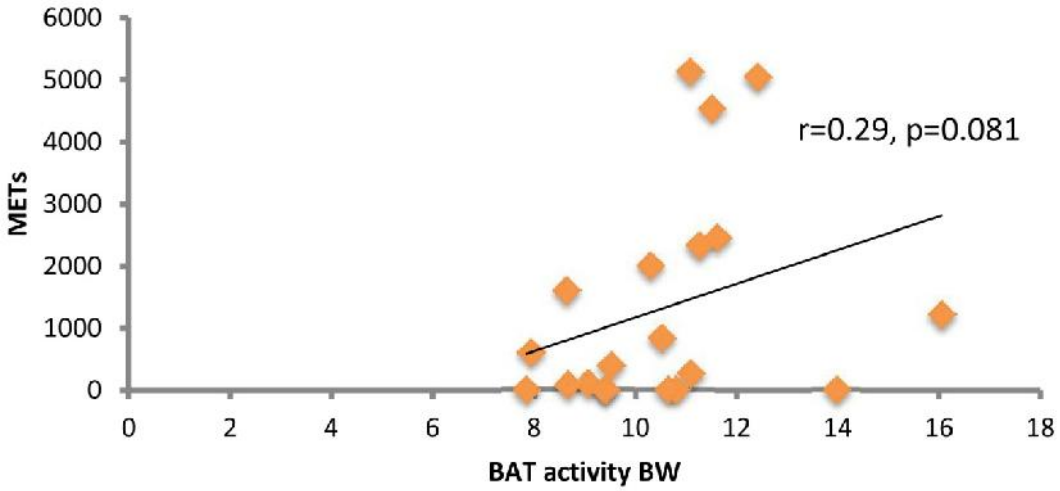


Figure 3: Activity of BAT (KBq) corrected for BSA in relation to total METs usual week.

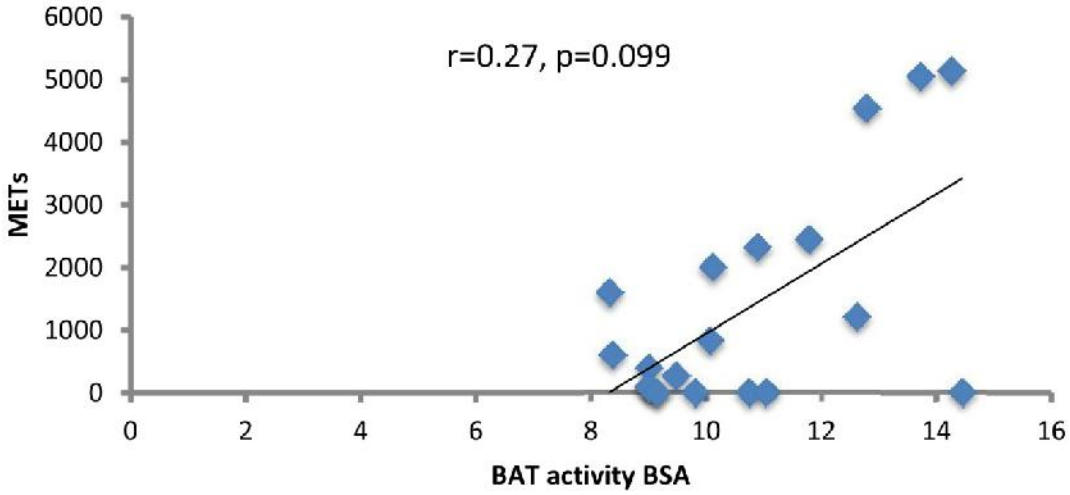
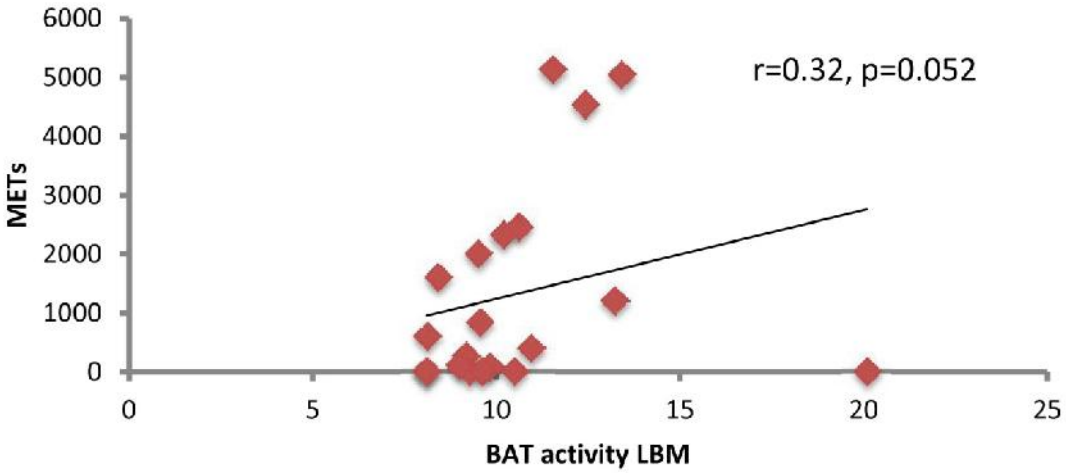


Figure 4: Activity of BAT (KBq) corrected for LBM in relation to total METs usual week.



The results also showed that there is a moderate negative correlation between BMI and BAT activity when corrected for BW (r=-42, p=0.09) (fig. 5), for BSA (r=-34, p=0.035) (fig. 6), and for LBM (r=-0.047, p=0.04) (fig. 7).

Figure 5: Activity of BAT (KBq) corrected for BW in relation to BMI.

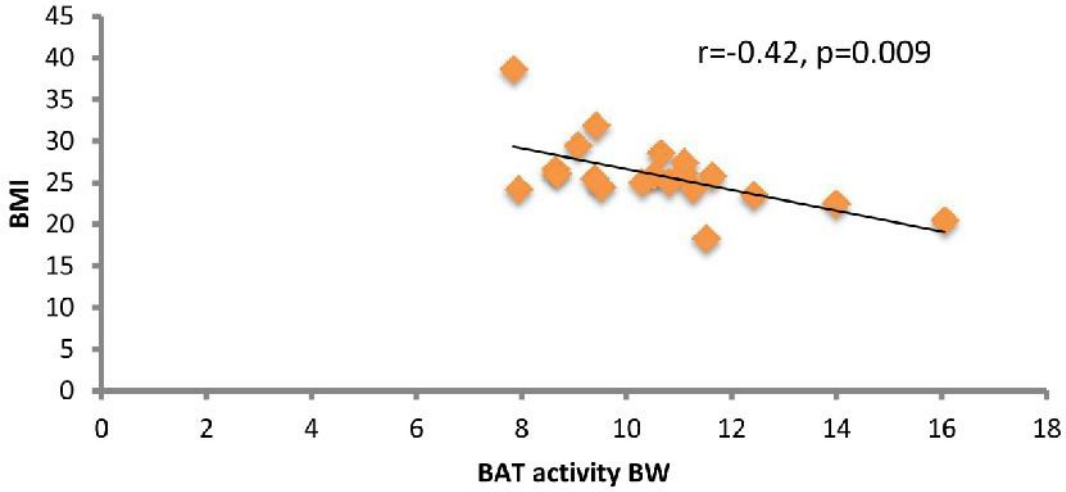


Figure 6: Activity of BAT (KBq) corrected for BSA in relation to BMI.

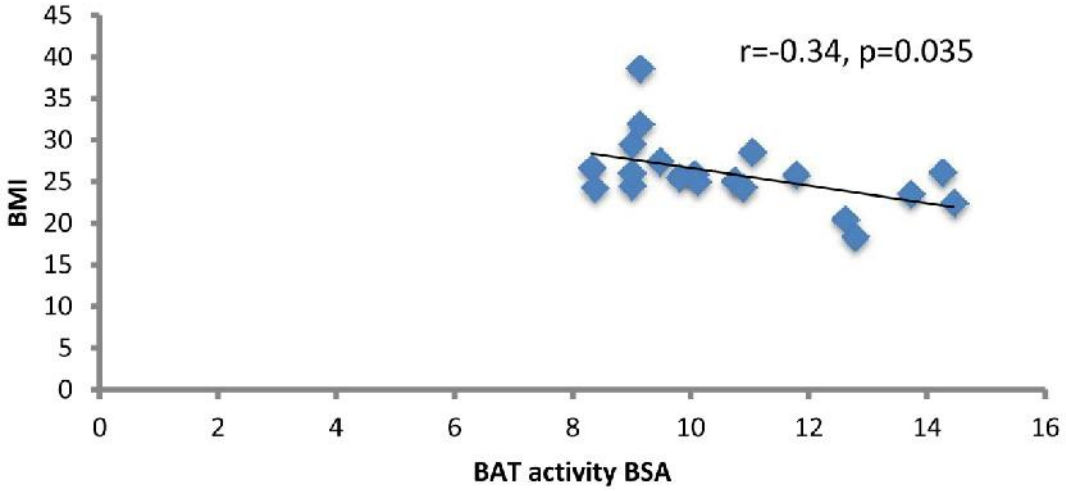
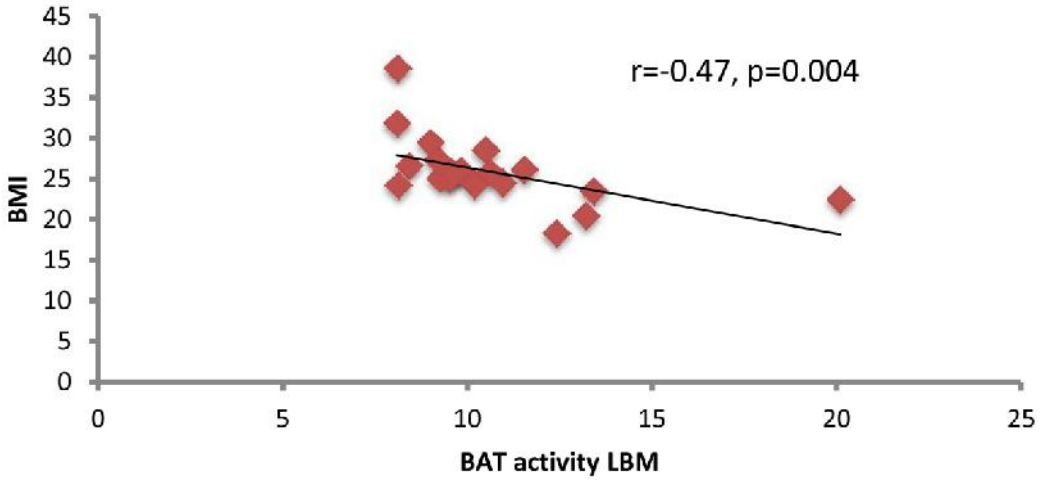


Figure 7: Activity of BAT (KBq) corrected for LBM in relation to BMI.



The non-parametric correlation also displayed a negative correlation between age and BAT activity corrected for LBM (r=-0.32, p=0.051) (fig. 10). However, there was no relationship between age and BAT activity corrected for BW and BSA (p>0.05) (fig. 8 and 9).

Figure 8: Activity of BAT (KBq) corrected for BW in relation to age.

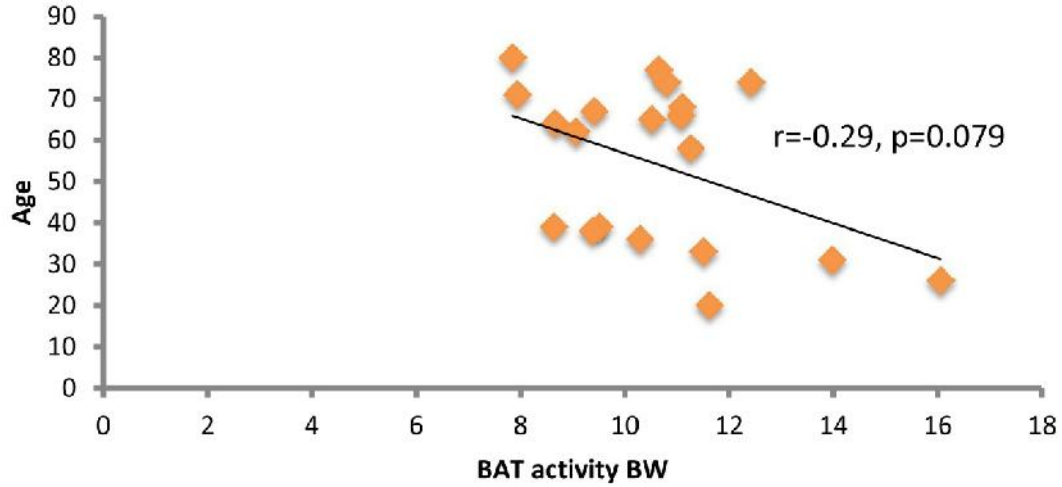


Figure 9: Activity of BAT (KBq) corrected for BSA in relation to age.

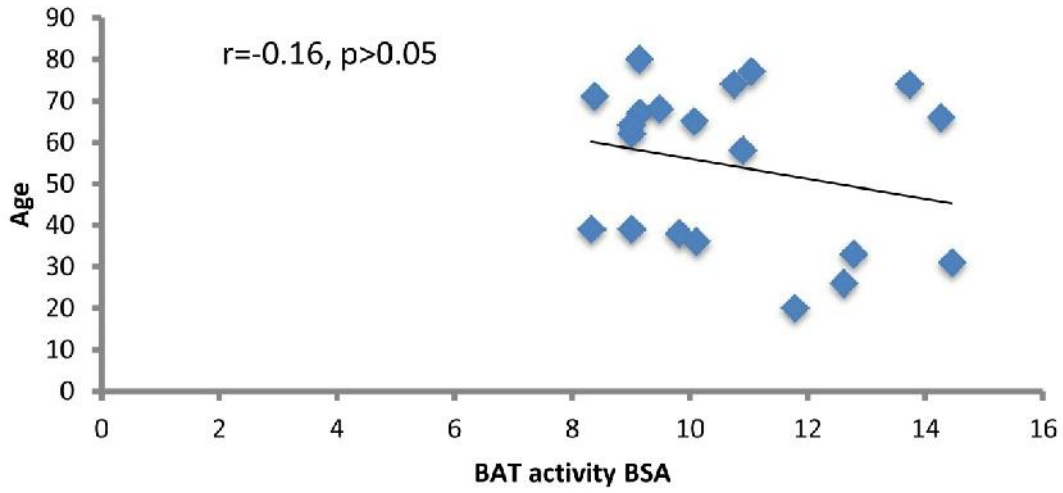
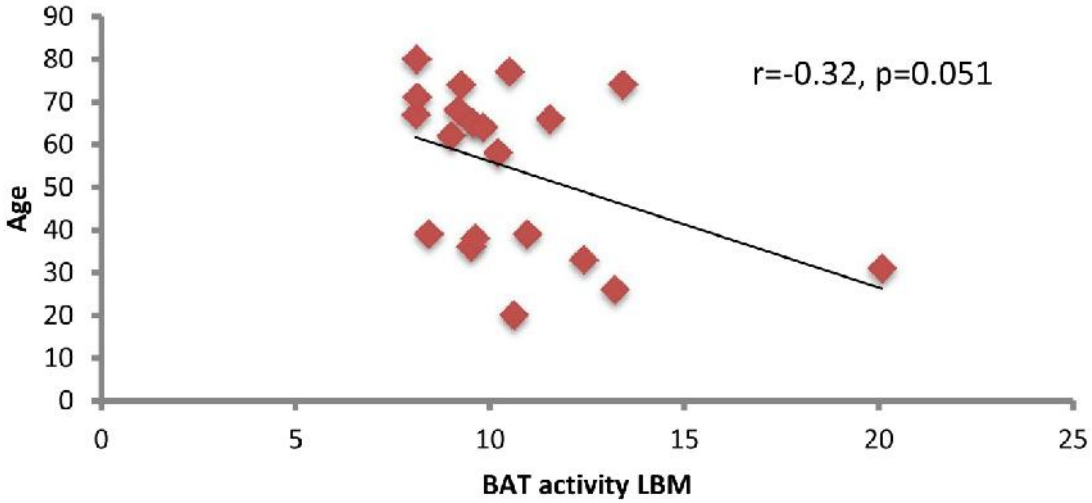


Figure 10: Activity of BAT (KBq) corrected for LBM in relation to age.



The Kruskal-Wallis analyses detected a significant main effect of physical activity levels of the last 7 days on BAT activity (KBq) corrected for BSA (p= 0.028) (table 4), and a significant main effect of physical activity levels in a usual week on BAT activity (KBq) corrected for BSA (p= 0.047) (table 4), as well as a significant main effect of physical activity levels in a usual week on BAT activity (SUV) corrected for BSA (p=0.028) (table 4). The post-hoc Mann-Whitney U tests indicate that participants that engaged in high levels of physical activity in the last 7 days had higher levels of BAT activity corrected for BSA than those engaged in low levels of physical activity (p=0.021). Furthermore, participants that engaged in high levels of physical activity in a usual week had higher levels of BAT activity (KBq) corrected for BSA than those engaged in low levels of physical activity (p=0.011), as well as they had higher levels of BAT activity (SUV) corrected for BSA than those engaged in moderate levels of physical activity (p=0.028) (table 4). Therefore, the results indicate that individuals engaged in increased levels of physical activity demonstrate more the BAT activity.

Table 4: Effects of physical activity levels on BAT activity corrected for BSA

Effects of physical activity levels on BAT activity (BSA)

	Physical Activity (Last 7 days)		Physical Activity (Usual week)	
	(KBq) ∞	(SUV) ≠	(KBq) ¥	(SUV) £
Low	10.01±1.65 *	0.054±0.006	9.93±1.61 #	0.055±0.006
Moderate	11.33±0.62	0.059±0.005	10.46±3.03	0.047±0.005 †
High	13.58±0.74 *	0.062±0.008	12.25±1.62 #	0.060±0.006 †

∞ Significant effect of physical activity levels last 7 days on BAT activity (KBq) p=0.028.

* Significant differences between low and high levels of physical activity last 7 days in BAT activity (KBq), p=0.021.

¥ Significant effect of physical activity levels usual week on BAT activity (KBq) p=0.047.

Significant differences between low and high levels of physical activity usual week in BAT activity (KBq), p=0.011.

£ Significant effect of physical activity levels usual week on BAT activity (SUV) p=0.028.

† Significant differences between moderate and high levels of physical activity usual week in BAT activity (SUV), p=0.046.

≠ No effect of physical activity levels last 7 days on BAT activity (SUV) p>0.05.

In order to assess the secondary aim of this study the effects of smoking status and cold exposure on BAT mass and BAT activity were examined. The results showed a main effect of cold exposure on BAT mass corrected for BSA (p= 0.017) (Table 5). The post-hoc Mann-Whitney U tests indicate that participants who were exposed to cold some times a day they had lower BAT mass corrected for BSA than those who exposed to cold once a week (p=0.030) and those who exposed to cold once or less a month (p=0.030) (Table 5). On the other hand, no relationship between BAT mass and activity and smoking status was found (p>0.05).

Table 5: Effects of cold exposure and smoking on BAT mass (cm^3).

Effects of cold exposure and smoking on BAT mass (cm^3) corrected for BSA

Cold exposure *				
Continuously	Some times α day	Once a day	Once a week	Once or less a month
14.39±1.57	12.06±2.01#	---	17.45±2.82#	16.23±1.10#
Smoking				
Yes			No	
13.24±1.50			13.85±2.87	

* Significant effect of cold exposure on BAT mass corrected for body surface area p=0.017.

Participants who were exposed to cold some times a day they had lower BAT mass than those who exposed to cold once a week p=0.030 and once or less a month p=0.030.

No effect of smoking on BAT mass

CHAPTER 5: DISCUSSION

The main aim of this thesis was to investigate the relationship between physical activity levels and BAT mass and BAT activity in humans. The IPAQ along with the PET/CT measurements revealed that physical activity was positively associated with BAT activity, confirming the research hypothesis. However, the results showed that there is no relationship between physical activity levels and BAT mass. Therefore, the research hypothesis was confirmed in part as the results indicate that individuals engaged in increased levels of physical activity demonstrate more the BAT activity and thus as far as the BAT activity is concerned the null hypothesis was rejected.

This is the first study that examined the relationship between physical activity levels and BAT in humans. The results are in agreement with experiments in mice showing that exercise increases BAT activity (Hu & Wang, 2007; Oh, et al., 2007; Xu, et al., 2011). This increased BAT activity which ameliorates energy expenditure in humans could be used against excessive weight and obesity (Schroeder, et al., 2010; van Marken Lichtenbelt, et al., 2009). In a previous study (van Marken Lichtenbelt, et al., 2009) participants were exposed to cold 2 hours prior the examination with PET/CT and this increased their NST and subsequently increased their energy expenditure. The present study demonstrates that both acute (last 7 days) and chronic (usual week) physical activity can increase BAT activity which can, in turn, increase energy expenditure. Therefore, humans may not need a cold exposure in order to increase their BAT activity. This is relatively in contrast to a previous study in mice indicating that both exercise and cold exposure are needed in order to increase UCP1 mRNA expression which is the main contributor in NST (Seebacher & Glanville, 2010).

A secondary aim of this thesis was to examine the relationship between BAT mass/activity and BMI and age, as well as the effects of smoking status and cold exposure on BAT mass and activity. Some – but not all (van Marken Lichtenbelt, et al., 2009) – previous studies suggested that BAT mass is likely to be decreased with age (Sturkenboom, et al., 2004). The present study showed that BAT mass may decrease with the age, confirming the research hypothesis regarding the relationship between BAT mass and age. On the other hand, no relationship between BAT activity and age was detected and therefore this research hypothesis was rejected. In addition, no relationship between BAT and smoking status was found and thus this research hypothesis was rejected as well.

The present study also confirms evidence from previous studies indicate that individuals with increased BMI demonstrate less the BAT activity than individuals with normal BMI (van Marken Lichtenbelt, et al., 2009; Virtanen, et al., 2009). This finding confirmed the present research hypothesis that there is a relation between BAT activity and BMI. In addition, it was also revealed that people exposed to cold more often during their normal life may have lower BAT mass than those who avoid cold exposure, and consequently this research hypothesis was rejected. This finding is not in accordance with evidence showing that acute cold stimuli is necessary in order to activate existing BAT in humans (Cannon & Nedergaard, 2012; Ouellet et al., 2012).

Limitations

A limit of the present study is that we examined cancer patients. Despite criteria adopted to ensure that the participants did not take medicines that affected metabolism, our findings may reflect that measurements were conducted in a clinical sample. Previous studies have also examined BAT activity through PET/CT in clinical populations including cancer patients in

order to compare the results either with cold exposure status and age or with various physiological indices (Baba, Jacene, Engles, Honda, & Wahl, 2010; Cypess, et al., 2009). These studies accepted the values of BAT activity that were retrieved from the PET/CT measurements if the examined area of the patients had no gross lesions or abnormalities, even though sometimes the BAT activity may be indistinguishable from uptake in tumors or lymph nodes (Baba, et al., 2010; Cypess, et al., 2009). In order to minimize the effect that this limitation had on our results, all areas that were examined in each patient were verified by a physician through the PET/CT software to ensure that they did not include a cancerous tumor. In case that the examined area was comprised by cancerous tumors or other pathologic tissue, the participant was excluded from the study. A different limitation of the present study is that no other physiological factors such as UCP1 mRNA expression in BAT as well as energy expenditure of the patients were examined in order to compare them with the obtained evidence. Furthermore, there is no evidence demonstrate the effects of chemotherapy, radiotreatment, diazepam and furosemide on brown fat metabolism, and therefore the results of the present study could have affected from those factors. Finally, based on previous studies we used three corrections for radioactivity values that are, corrected for BW, for BSA and for LBM, but this is the first time that those corrections were used for BAT values.

CHAPTER 6: CONCLUSIONS

Measurements of BAT mass and activity, engagement in physical activity, exposure to cold and smoking status in 20 cancer patients demonstrated that:

- Increased levels of physical activity may increase BAT activity.
- BAT activity is negatively related to age and body mass.
- Frequent exposure to cold may decrease BAT mass, but this does not necessarily result in lower BAT activity.
- There is no relationship between smoking status and either BAT mass or activity.

CHAPTER 7: REFERENCES

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APPENDICES

APPENDIX A:

University of Thessaly

Department of Physical Education and Sport Sciences

Master of Science Program: **"Exercise and Health"**

Evaluation Criteria of Master of Science Thesis

Student's name: **Petros Dinas**

Title: **The effects of physical activity on brown adipose tissue in humans**

Supervisor: **Professor Yiannis Koutedakis**

First committee member: **Associate Professor Athanasios Jamurtas**

Second committee member: **Assistant Professor Panagiotis Georgoulias**

Introduction (Aim of the study, importance of the study, terminology, limitations)

(/5)

Literature review (Does the introductory paragraph describe the contents of the literature review and the effectiveness of the subchapters, uses of thematic sub, development from the general to the specific parts, detailed critical analyses of previous studies with emphasis to new

evidence, provides detailed information about the aim and scope of the study, aim of the study and hypothesis).

(/25)

Method (Does method section describe the data collection? Does method section describe in details the method, instruments, and procedures? Does method section describe the power and the reliability of the measurements? Is the statistical analyses appropriate?)

(/20)

Results (Are they reasonably presenting tables and figures? Does results section emphasize to the most important results and comments?)

(/15)

Discussion (Does discussion section emphasize to the new evidence of the study? Does it correlate the new evidence with the previous data? Does discussion section refer to the possible clinical implications and/or to the effects of the results on physical education and sport sciences?)

(/20)

Conclusions-proposals (Is the aim of the study presenting in terms of the hypothesis? Are they presenting recommendations for future research?)

(/5)

Structure of the manuscript

(/5)

Grammar, spelling, APA style

(/5)

Grade: .....%

Completed after discussion of the examiners

Final comments: .....

.....

Final Grade: .....%

Signatures: Supervisor: .....

First committee member: ……

Second committee member: …….

APPENTIX B: Meeting Book

Student's name: **Petros Dinas**

1st meeting

Date: 21-9-2011. Time: 12:00 p.m. Supervisor's signature: ….....

Objective: Proposing graduate thesis. Student's signature: .....

Next objective: Improvement of the proposal and resubmission to the supervisor

2ed meeting

Date: 5-10-2011. Time: 13:30 p.m. Supervisor's signature: .....

Objective: Reexamination of the thesis proposal. Student's signature: .....

Next objective: Submission of the required papers for the thesis proposal to the department's bioethics committee

3d meeting

Date: 23-11-2011. Time: 17:30 p.m. Supervisor's signature: …….....

Objective: Examination of the required papers for the thesis proposal. Student's signature:

…….....

Next objective: Submission of the required papers for the thesis proposal to the department's bioethics committee, data collection, approval of the thesis proposal from the department's bioethics committee.

4th meeting

Date: 23-1-2012. Time: 13:00 μ.μ. Supervisor's signature: …….....

Objective: Analysis of the data collection. Student's signature: .....

Next objective: Data collection

5th meeting

Date: 20-2-2012. Time: 14:00 p.m. Supervisor's signature: …….....

Objective: Analysis of data collection, potential problems. Student's signature:

…….....

Next objective: Data collection.....

6th meeting

Date: 15-3-2012. Time: 17:30 p.m. Supervisor's signature: …….....

Objective: Analysis of data collection, potential problems. Student's signature:

…….....

Next objective: Data collection.....

7th meeting

Date: 23-4-2012. Time: 14:00 p.m. Supervisor's signature: …….....

Objective: Discussion about the final data. Student's signature: .....

Next objective: Data analysis

8th meeting

Date: 15-5-2012. Time: 12:00 p.m. Supervisor's signature: ….....

Objective: Data analysis. Student's signature: …….....

Next objective: Preparation of master thesis text

9th meeting

Date: 30-5-2012. Time: 13:00 p.m. Supervisor's signature: …….....

Objective: Corrections of the thesis text. Student's signature: …….....

Next objective: Final decision for the text of the thesis

10th meeting

Date: 15-6-2012. Time: 10.00 a.m. Supervisor's signature: …….....

Objective: Final decision for the thesis. Student's signature: …….....

Next objective: Approval

APPENDIX C: Manuscript of consent form and questionnaires

Κωδικός

_____

Ημερομηνία: / / 201

Έντυπο συναίνεσης δοκιμαζόμενου σε ερευνητική εργασία

Τίτλος Ερευνητικής Εργασίας: Η επίδραση της φυσικής δραστηριότητας στο ανθρώπινο φαιό λιπώδη ιστό.

Επιστημονικός Υπεύθυνος: Δρ. Κουτεντάκης Ιωάννης (τηλ. 2431047056, email: y.koutedakis@pe.uth.gr)

Επιστημονικός Σύμβουλος: Δρ. Φλουρής Ανδρέας, (τηλ. 2431500601, email:aflouris@cereteth.gr)

Ερευνητές: Ντίνας Πέτρος (τηλ. 6974010118, email: petros.cd@gmail.com)

1. Σκοπός της ερευνητικής εργασίας

Σκοπός της μελέτης είναι να εξετάσει τη σχέση της φυσικής δραστηριότητας (άσκηση) με τη δραστηριότητα του ανθρώπινου φαιού λιπώδη ιστού.

2. Διαδικασία

Κατά τη διάρκεια της εξέτασης με τον Τομογράφο Εκπομπής Ποζιτρονίων (ΤΕΠ) και της Αξονικής Τομογραφίας (ΑΤ) [ΤΕΠ/ΑΤ] του νοσοκομείου «Υγεία» θα ανιχνευθεί η ποσότητα του ενεργού Φαιού Λιπώδη Ιστού στο σώμα σας ο οποίος βρίσκεται στην περιοχή του στήθους. Αφού υπογράψετε το παρόν έντυπο δίνοντας τη συγκατάθεσή σας να συμμετάσχετε στη μελέτη θα σας ζητηθεί να συμπληρώσετε δύο σύντομα ερωτηματολόγια φυσικής δραστηριότητας πιστοποιημένα για τον ελληνικό πληθυσμό. Επίσης θα συμπληρώσετε μια φόρμα που θα μας παρέχει πληροφορίες σχετικά με παράγοντες που επηρεάζουν τη λειτουργία του φαιού λιπώδη ιστού. Η όλη διαδικασία θα πραγματοποιηθεί μόνο μία φορά.

3. Κίνδυνοι και ενοχλήσεις

Από τη συγκέντρωση των δεδομένων της μελέτης δεν εκτίθεστε σε κανένα παραπάνω κίνδυνο εκτός αυτών της συνηθισμένης εξέτασης με τον ΤΕΠ/ΑΤ.

4. Προσδοκώμενες ωφέλειες

Με την συμμετοχή σας θα λάβετε πληροφορίες για την ενεργοποίηση του Φαιού Λιπώδη Ιστού στο σώμα σας ο οποίος είναι υπεύθυνος για την παραγωγή θερμότητας. Επίσης θα σας δοθούν πληροφορίες σχετικά με τα επίπεδα της φυσικής σας δραστηριότητας. Οι πληροφορίες από τη συγκεκριμένη μελέτη θα βοηθήσουν να κατανοήσουμε αν ενεργοποιείται ή όχι με τη φυσική δραστηριότητα ο Φαιός Λιπώδης Ιστός. Αυτό θα μας οδηγήσει σε συμπεράσματα σύμφωνα με τα οποία μπορούμε να συνδέσουμε ή όχι την ενέργεια του παραπάνω ιστού με διάφορες φυσιολογικές λειτουργίες στον άνθρωπο.

5. Δημοσίευση δεδομένων – αποτελεσμάτων

Η συμμετοχή σας στην έρευνα συνεπάγεται ότι συμφωνείτε με την μελλοντική δημοσίευση των αποτελεσμάτων της, με την προϋπόθεση ότι οι πληροφορίες θα είναι ανώνυμες και δε θα αποκαλυφθούν τα ονόματα των συμμετεχόντων. Τα δεδομένα που θα συγκεντρωθούν θα κωδικοποιηθούν με αριθμό, ώστε το όνομα σας να μην φαίνεται πουθενά.

6. Πληροφορίες

Μη διστάσετε να κάνετε ερωτήσεις γύρω από το σκοπό ή την διαδικασία της μελέτης. Αν έχετε οποιαδήποτε αμφιβολία ή ερώτηση ζητήστε μας να σας δώσουμε διευκρινίσεις.

7. Ελευθερία συναίνεσης

Η συμμετοχή σας στην εργασία είναι εθελοντική. Είστε ελεύθερος-η να μην συναινέσετε ή να διακόψετε τη συμμετοχή σας όποτε το επιθυμείτε χωρίς καμία επίπτωση στις εξετάσεις που θα κάνετε στο νοσοκομείο «Υγεία» .

8. Δήλωση συναίνεσης

Διάβασα το έντυπο αυτό και κατανοώ τις διαδικασίες που θα ακολουθήσω. Συναινώ να συμμετάσχω στην ερευνητική εργασία.

Ημερομηνία: __/__/__ Υπογραφή ερευνητή Ονοματεπώνυμο

και υπογραφή παρατηρητή

Ονοματεπώνυμο και

υπογραφή συμμετέχοντος

Σύντομο Διεθνές Ερωτηματολόγιο Φυσικής Δραστηριότητας

Τελευταίες 7 ημέρες

Οι παρακάτω ερωτήσεις αφορούν το χρόνο που έχετε αφιερώσει για κάποια σωματική δραστηριότητα **τις τελευταίες 7 ημέρες**. Περιλαμβάνουν ερωτήσεις σχετικά με δραστηριότητες που κάνατε κατά την εργασία σας, στις μετακινήσεις σας, στις δουλειές του σπιτιού, του κήπου και στον ελεύθερο χρόνο σας για ψυχαγωγία, άσκηση ή άθληση. Σας παρακαλώ να απαντήσετε όλες τις ερωτήσεις, ακόμα και εάν πιστεύετε ότι δεν είστε ένα σωματικά δραστήριο άτομο.

Πριν απαντήσετε τις ερωτήσεις 1 και 2, σκεφτείτε όλες τις **έντονες** σωματικές δραστηριότητες που κάνατε κατά **τις τελευταίες 7 ημέρες.** Μια έντονη σωματική δραστηριότητα αναφέρεται σε δραστηριότητες που απαιτούν έντονη σωματική προσπάθεια και σας κάνουν να αναπνέετε σημαντικά δυσκολότερα από ότι συνήθως. Σκεφθείτε μόνο τις **έντονες** σωματικές δραστηριότητες που κάνατε και είχαν διάρκεια **μεγαλύτερη από 10 λεπτά** κάθε φορά.

1. **Κατά τις τελευταίες 7 ημέρες, πόσες ημέρες κάνατε κάποια σωματική δραστηριότητα, όπως σκάψιμο, έντονη άσκηση με βάρη, τρέξιμο σε διάδρομο με κλίση, γρήγορο τρέξιμο, aerobics, γρήγορη ποδηλασία, γρήγορη κολύμβηση, τένις μονό, αγώνας σε γήπεδο (ποδόσφαιρο, basketball, volleyball, handball);**

   ..... ημέρες ανά εβδομάδα

   αν δεν κάνατε έντονες σωματικές δραστηριότητες, σημειώστε Χ εδώ ☐ και προχωρήστε στην ερώτηση 3.

2. **Τις ημέρες που κάνατε κάποια έντονη σωματική δραστηριότητα πόση ώρα αφιερώνατε συνήθως;**

   ..... λεπτά ανά ημέρα δεν γνωρίζω/δεν είμαι βέβαιος ☐

Πριν απαντήσετε τις ερωτήσεις 3 και 4, σκεφτείτε όλες τις **μέτριας έντασης** σωματικές δραστηριότητες που κάνατε κατά **τις τελευταίες 7 ημέρες.** Μια μέτριας έντασης σωματική δραστηριότητα αναφέρεται σε δραστηριότητες που απαιτούν μέτρια σωματική προσπάθεια και σας κάνουν να αναπνέετε κάπως δυσκολότερα από ότι συνήθως. Σκεφθείτε μόνο τις **μέτριας έντασης** σωματικές δραστηριότητες που κάνατε και είχαν διάρκεια **μεγαλύτερη από 10 λεπτά** τη φορά.

3. **Κατά τις τελευταίες 7 ημέρες, πόσες ημέρες κάνατε κάποια μέτρια σωματική δραστηριότητα, όπως το να σηκώσετε και να μεταφέρετε ελαφρά βάρη (μικρότερα από 10 κιλά), συνολική καθαριότητα του σπιτιού, ήπιες ρυθμικές ασκήσεις σώματος, ποδηλασία αναψυχής με χαμηλή ταχύτητα, χαλαρή κολύμβηση; <u>Σας παρακαλώ να μη συμπεριλάβετε το περπάτημα</u>**.

   ..... ημέρες ανά εβδομάδα

   αν δεν κάνατε μέτριας έντασης σωματικές δραστηριότητες, σημειώστε Χ εδώ ☐

   και προχωρήστε στην ερώτηση 5.

4. **Τις ημέρες που κάνατε κάποια μέτρια σωματική δραστηριότητα πόση ώρα αφιερώνατε συνήθως;**

   ..... λεπτά ανά ημέρα δεν γνωρίζω/δεν είμαι βέβαιος ☐

Πριν απαντήσετε στις ερωτήσεις 5 και 6, σκεφτείτε το χρόνο που περπατήσατε κατά **<u>τις τελευταίες 7 ημέρες.</u>** Να συμπεριλάβετε το περπάτημα στο χώρο της εργασίας σας, στις μετακινήσεις σας και στον ελεύθερο χρόνο σας για ψυχαγωγία, άσκηση ή άθληση.

5. **Τις τελευταίες 7 ημέρες, πόσες ημέρες περπατήσατε για περισσότερο από 10 συνεχόμενα λεπτά;**

   ..... ημέρες ανά εβδομάδα

   αν δεν περπατήσατε καμία ημέρα περισσότερο από 10 συνεχόμενα λεπτά,

   σημειώστε Χ εδώ ☐ και προχωρήστε στην ερώτηση 7.

6. **Τις ημέρες που περπατήσατε, για περισσότερο από 10 συνεχόμενα λεπτά, πόση ώρα περάσατε περπατώντας;**

   ..... λεπτά ανά ημέρα δεν γνωρίζω/δεν είμαι βέβαιος ☐

7. **Πόσο χρόνο περάσατε καθισμένοι σε μία συνηθισμένη μέρα κατά τη διάρκεια των τελευταίων 7 ημερών; Ο χρόνος αυτός μπορεί να περιλαμβάνει το χρόνο που περνάτε καθισμένοι στο σπίτι, στο γραφείο, όταν επισκέπτεστε φίλους, όταν διαβάζετε, μελετάτε ή βλέπετε τηλεόραση, <u>αλλά δεν περιλαμβάνει τον ύπνο</u>.**

   ..... ώρες ανά ημέρα δεν γνωρίζω/δεν είμαι βέβαιος ☐

ΠΡΟΣΩΠΙΚΟ ΕΡΩΤΗΜΑΤΟΛΟΓΙΟ

1. **Φύλο**: Άνδρας ☐ Γυναίκα ☐

2. **Ηλικία:**_____ **΄Υψος:**_____ **Βάρος:**_____

3. **Πάσχετε από κάποια χρόνια νοσήματα;** Ναι ☐ Όχι ☐

Αν «ναι» παρακαλώ περιγράψτε παρακάτω πια είναι αυτά:

.....

.....

4. **Καπνίζετε αυτή την περίοδο;** Ναι ☐ Όχι ☐

Αν απαντήσατε «Όχι» στην ερώτηση 4, παρακαλώ προχωρήστε στην ερώτηση 9.

Αν απαντήσατε «Ναι» στην ερώτηση 4,παρακαλώ απαντήστε τις παρακάτω ερωτήσεις:

5. **Τσιγάρα** Πόσα τη ημέρα; ____ Για πόσα χρόνια; ____

6. **Στριφτό** Πόσα τη ημέρα; ____ Για πόσα χρόνια; ____

7. **Πούρα** Πόσα τη ημέρα; ____ Για πόσα χρόνια; _____

8. **Πίπα** Πόσες φορές τη μέρα; ____ Για πόσα χρόνια; ___

9. **Είστε πρώην καπνιστής/στρια;** Ναι ☐ Όχι ☐

Αν απαντήσατε «Ναι» στην ερώτηση 9, παρακαλώ απαντήστε τις παρακάτω ερωτήσεις:

10. Πόσων ετών ήσασταν όταν ξεκινήσατε το κάπνισμα;.....

11. Σε ποια ηλικία σταματήσατε;.....

12. Πόσο συχνά εκτίθεστε στο κρύο; Παρακαλώ δώστε μόνο μια απάντηση.

Συνέχεια ☐ Μερικές φορές την ημέρα ☐ Μία φορά την ημέρα ☐

Μία φορά την εβδομάδα ☐ Μία φορά ή λιγότερες το μήνα ☐

13. Πόσο συχνά αισθάνεστε ότι κρυώνετε; Παρακαλώ δώστε μόνο μια απάντηση.

Συνέχεια ☐ Μερικές φορές την ημέρα ☐ Μία φορά την ημέρα ☐

Μία φορά την εβδομάδα ☐ Μία φορά ή λιγότερες το μήνα ☐

14. Τι κάνετε για να προφυλαχτείτε από το κρύο; Παρακαλώ δώστε μόνο μια απάντηση.

Τίποτα το ιδιαίτερο ☐ Αποφεύγω να βγαίνω έξω από το σπίτι ☐ Άλλα ☐

15. Παρακαλώ βαθμολογήστε από το 0 έως το 10 το πόσο κρυώνετε όταν κάνετε τις παρακάτω δραστηριότητες (10= μεγαλύτερη ενόχληση).

Δουλειές του σπιτιού; ..... Όταν ντύνεστε και ξεντύνεστε; .....

Στην δουλειά σας; ..... Κάνοντας το χόμπι σας; .....

16. Παρακαλώ βαθμολογήστε από το 0 έως το 10 το πόσο κρυώνατε τις τελευταίες 6 ώρες (10= μεγαλύτερη ενόχληση)

.....

17. Παρακαλώ βαθμολογήστε από το 0 έως το 10 τη σωματική σας δραστηριότητα τις τελευταίες 6 ώρες (10= περισσότερη σωματική δραστηριότητα)

.....

18. **Τους τελευταίους 6 μήνες παίρνατε κάποια φάρμακα;** Ναι ☐ Όχι ☐

Αν απαντήσατε «Όχι» στην ερώτηση 18, παρακαλώ προχωρήστε στην ερώτηση 20.

Αν απαντήσατε «Ναι» στην ερώτηση 18, ποια φάρμακα παίρνατε;

.....

.....

.....

19. **Συνεχίζετε ακόμη και σήμερα να παίρνετε τα ίδια φάρμακα;** Ναι ☐ Όχι ☐

Αν απαντήσατε «Ναι» στην ερώτηση 19, ποια φάρμακα συνεχίζετε να παίρνετε;

.....

.....

.....

20. **Κάνετε χρόνια χρήση κάποιων φαρμάκων;** Ναι ☐ Όχι ☐

Αν απαντήσατε «Ναι» στην ερώτηση 20, ποια φάρμακα παίρνετε και για πόσο χρονικό διάστημα;.....

.....

.....

.....

21. **Αν θα σας ενδιέφερε να έρθουμε σε επαφή μαζί σας για να συλλέξουμε περισσότερες πληροφορίες παρακαλούμε σημειώστε τον αριθμό του τηλεφώνου σας.**

_____

Σύντομο Διεθνές Ερωτηματολόγιο Φυσικής Δραστηριότητας

Μία συνηθισμένη εβδομάδα

Οι παρακάτω ερωτήσεις αφορούν το χρόνο που έχετε αφιερώσει για κάποια σωματική δραστηριότητα **κατά τη διάρκεια μιας συνηθισμένης εβδομάδας**. Περιλαμβάνουν ερωτήσεις σχετικά με δραστηριότητες που κάνατε κατά την εργασία σας, στις μετακινήσεις σας, στις δουλειές του σπιτιού, του κήπου και στον ελεύθερο χρόνο σας για ψυχαγωγία, άσκηση ή άθληση. Σας παρακαλώ να απαντήσετε όλες τις ερωτήσεις, ακόμα και εάν πιστεύετε ότι δεν είστε ένα σωματικά δραστήριο άτομο.

Πριν απαντήσετε τις ερωτήσεις 1 και 2, σκεφτείτε όλες τις **έντονες** σωματικές δραστηριότητες που κάνατε **κατά τη διάρκεια μιας συνηθισμένης εβδομάδας.** Μια έντονη σωματική δραστηριότητα αναφέρεται σε δραστηριότητες που απαιτούν έντονη σωματική προσπάθεια και σας κάνουν να αναπνέετε σημαντικά δυσκολότερα από ότι συνήθως. Σκεφθείτε μόνο τις **έντονες** σωματικές δραστηριότητες που κάνατε και είχαν διάρκεια **μεγαλύτερη από 10 λεπτά** κάθε φορά.

1. **Κατά τη διάρκεια μιας συνηθισμένης εβδομάδας, πόσες ημέρες κάνατε κάποια σωματική δραστηριότητα, όπως σκάψιμο, έντονη άσκηση με βάρη, τρέξιμο σε διάδρομο με κλίση, γρήγορο τρέξιμο, aerobics, γρήγορη ποδηλασία, γρήγορη κολύμβηση, τένις μονό, αγώνας σε γήπεδο (ποδόσφαιρο, basketball, volleyball, handball);**

   ..... ημέρες ανά εβδομάδα

   αν δεν κάνατε έντονες σωματικές δραστηριότητες, σημειώστε Χ εδώ ☐ και προχωρήστε στην ερώτηση 3.

2. **Τις ημέρες που κάνατε κάποια έντονη σωματική δραστηριότητα πόση ώρα αφιερώνατε συνήθως;**

   ..... λεπτά ανά ημέρα δεν γνωρίζω/δεν είμαι βέβαιος ☐

Πριν απαντήσετε τις ερωτήσεις 3 και 4, σκεφτείτε όλες τις **μέτριας έντασης** σωματικές δραστηριότητες που κάνατε κατά **τη διάρκεια μιας συνηθισμένης εβδομάδας.** Μια μέτριας έντασης σωματική δραστηριότητα αναφέρεται σε δραστηριότητες που απαιτούν μέτρια σωματική προσπάθεια και σας κάνουν να αναπνέετε κάπως δυσκολότερα από ότι συνήθως. Σκεφθείτε μόνο τις **μέτριας έντασης** σωματικές δραστηριότητες που κάνατε και είχαν διάρκεια **μεγαλύτερη από 10 λεπτά** τη φορά.

3. **Κατά τη διάρκεια μιας συνηθισμένης εβδομάδας, πόσες ημέρες κάνατε κάποια μέτρια σωματική δραστηριότητα, όπως το να σηκώσετε και να μεταφέρετε ελαφρά βάρη (μικρότερα από 10 κιλά), συνολική καθαριότητα του σπιτιού, ήπιες ρυθμικές ασκήσεις σώματος, ποδηλασία αναψυχής με χαμηλή ταχύτητα, χαλαρή κολύμβηση; <u>Σας παρακαλώ να μη συμπεριλάβετε το περπάτημα</u>**.

   ..... ημέρες ανά εβδομάδα

   αν δεν κάνατε μέτριας έντασης σωματικές δραστηριότητες, σημειώστε Χ εδώ ☐ και προχωρήστε στην ερώτηση 5.

4. **Τις ημέρες που κάνατε κάποια μέτρια σωματική δραστηριότητα πόση ώρα αφιερώνατε συνήθως;**

   ..... λεπτά ανά ημέρα δεν γνωρίζω/δεν είμαι βέβαιος ☐

Πριν απαντήσετε στις ερωτήσεις 5 και 6, σκεφτείτε το χρόνο που περπατήσατε **<u>κατά τη διάρκεια μιας συνηθισμένης εβδομάδας.</u>** Να συμπεριλάβετε το περπάτημα στο χώρο της εργασίας σας, στις μετακινήσεις σας και στον ελεύθερο χρόνο σας για ψυχαγωγία, άσκηση ή άθληση.

5. **Κατά τη διάρκεια μιας συνηθισμένης εβδομάδας, πόσες ημέρες περπατήσατε για περισσότερο από 10 συνεχόμενα λεπτά;**
   ..... ημέρες ανά εβδομάδα

   αν δεν περπατήσατε καμία ημέρα περισσότερο από 10 συνεχόμενα λεπτά, σημειώστε Χ εδώ ☐ και προχωρήστε στην ερώτηση 7.

6. **Τις ημέρες που περπατήσατε, για περισσότερο από 10 συνεχόμενα λεπτά, πόση ώρα περάσατε περπατώντας;**

   ..... λεπτά ανά ημέρα δεν γνωρίζω/δεν είμαι βέβαιος ☐

7. **Πόσο χρόνο περάσατε καθισμένοι σε μία συνηθισμένη μέρα κατά τη διάρκεια μιας συνηθισμένης εβδομάδας; Ο χρόνος αυτός μπορεί να περιλαμβάνει το χρόνο που περνάτε καθισμένοι στο σπίτι, στο γραφείο, όταν επισκέπτεστε φίλους, όταν διαβάζετε, μελετάτε ή βλέπετε τηλεόραση, <u>αλλά δεν περιλαμβάνει τον ύπνο</u>.**

   ..... ώρες ανά ημέρα δεν γνωρίζω/δεν είμαι βέβαιος ☐

Τέλος ερωτηματολογίου. Σας ευχαριστούμε για τη συμμετοχή σας

Υπεύθυνη Δήλωση

Ο κάτωθι υπογεγραμμένος Πέτρος Ντίνας ΑΕΜ: 04/2010, μεταπτυχιακός φοιτητής του τμήματος Επιστήμης Φυσικής Αγωγής και Αθλητισμού του Πανεπιστημίου Θεσσαλίας του προγράμματος "Άσκηση και Υγεία"

δηλώνω υπεύθυνα ότι αποδέχομαι τους παρακάτω όρους που αφορούν:

(α) στα πνευματικά δικαιώματα της Μεταπτυχιακής Διπλωματικής Εργασίας (ΜΔΕ) μου με τίτλο «Η επίδραση της φυσικής δραστηριότητας στο ανθρώπινο φαιό λιπώδη ιστό» (β) στη διαχείριση των ερευνητικών δεδομένων που θα συλλέξω στην πορεία εκπόνησής της:

1. Τα πνευματικά δικαιώματα του τόμου της μεταπτυχιακής διατριβής που θα προκύψει θα ανήκουν σε μένα. Θα ακολουθήσω τις οδηγίες συγγραφής, εκτύπωσης και κατάθεσης αντιτύπων της διατριβής στα ανάλογα αποθετήρια (σε έντυπη ή/και σε ηλεκτρονική μορφή).

2. Η διαχείριση των δεδομένων της διατριβής ανήκει από κοινού σε εμένα και στον κύριο επιβλέποντα καθηγητή κ.Κουτεντάκη Ιωάννη.

3. Οποιαδήποτε επιστημονική δημοσίευση ή ανακοίνωση (αναρτημένη ή προφορική), ή αναφορά που προέρχεται από το υλικό/δεδομένα της εργασίας αυτής θα γίνεται με συγγραφείς εμένα τον ίδιο, τον κύριο επιβλέποντα ή/και άλλους ερευνητές (πχ μέλη της τριμελούς συμβουλευτικής επιτροπής, συνεργάτες κλπ), ανάλογα με τη συμβολή τους στην έρευνα και στη συγγραφή των ερευνητικών εργασιών.

4. Η σειρά των ονομάτων στις επιστημονικές δημοσιεύσεις ή επιστημονικές ανακοινώσεις θα αποφασίζεται από κοινού από εμένα και τον κύριο επιβλέποντα της εργασίας, πριν αρχίσει η εκπόνησή της. Η απόφαση αυτή θα πιστοποιηθεί εγγράφως μεταξύ εμού και του κύριου επιβλέποντος καθηγητή.

Τέλος, δηλώνω ότι γνωρίζω τους κανόνες περί δεοντολογίας και περί λογοκλοπής και πνευματικής ιδιοκτησίας και ότι θα τους τηρώ απαρέγκλιτα καθ' όλη τη διάρκεια της φοίτησης και κάλυψης των εκπαιδευτικών υποχρεώσεων μου που προκύπτουν από το ΠΜΣ Άσκηση και Υγεία/τμήματος Επιστήμης Φυσικής Αγωγής και Αθλητισμού του Πανεπιστημίου Θεσσαλίας και καθ' όλη τη διάρκεια των διαδικασιών δημοσίευσης που θα προκύψουν μετά την ολοκλήρωση των σπουδών μου.

22- 6 –2012

Ο δηλών

Πέτρος Ντίνας

Υπεύθυνη Δήλωση

Ο κάτωθι υπογεγραμμένος Πέτρος Ντίνας, ΑΕΜ:04/2010 μεταπτυχιακός φοιτητής του τμήματος Επιστήμης Φυσικής Αγωγής και Αθλητισμού του Πανεπιστημίου Θεσσαλίας του προγράμματος Άσκηση και Υγεία

δηλώνω υπεύθυνα ότι αποδέχομαι τους παρακάτω όρους που αφορούν

τα πνευματικά δικαιώματα και τη διαχείριση του επιστημονικού υλικού ή των ερευνητικών δεδομένων που θα έχω στη διάθεσή μου κατά την πορεία της συμμετοχής μου στο ερευνητικό έργο με τίτλο "Η επίδραση της φυσικής δραστηριότητας στο ανθρώπινο φαιό λιπώδη ιστό"

και του οποίου επιστημονικώς υπεύθυνος είναι ο Καθηγητής Κουτεντάκης Ιωάννης.

1. Τα πνευματικά δικαιώματα του έργου αυτού μετά την αποπεράτωσή του ανήκουν στον επιστημονικώς υπεύθυνο.
2. Οποιαδήποτε επιστημονική δημοσίευση ή ανακοίνωση (αναρτημένη ή προφορική), ή αναφορά που προέρχεται από το υλικό/ δεδομένα της εργασίας αυτής και η πιθανή συμμετοχή του ονόματός μου σε αυτήν αποφασίζεται εκ των προτέρων από εμένα και τον επιστημονικώς υπεύθυνο του έργου και αυτό θα πιστοποιηθεί εγγράφως μεταξύ εμού και του υπεύθυνου.
3. Η σχετική σύμβαση εργασίας και οι υποχρεώσεις μου από τη συμμετοχή μου στο έργο αυτό, ρυθμίζονται με βάση τους κανόνες και τις αρχές της Επιτροπής Ερευνών του ΠΘ.

Τέλος, δηλώνω ότι γνωρίζω τους κανόνες περί δεοντολογίας στην έρευνα της σχετικής με το συγκεκριμένο ερευνητικό έργο περιοχής, τους κανόνες περί λογοκλοπής και πνευματικής ιδιοκτησίας και ότι θα τους τηρώ απαρέγκλιτα καθ' όλη τη διάρκεια της συμμετοχής μου στο έργο αυτό και κατά την πιθανή μου συμμετοχή σε διαδικασίες δημοσίευσης που θα προκύψουν μετά το τέλος του έργου.

22-6-2012

Ο δηλών

Πέτρος Ντίνας