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ORIGINAL ARTICLE

CME

Prevalence and Factors Associated with Brown Adipose Tissue Detected by 18F-fluorodeoxyglucose Positron Emission Tomography/Computed Tomography in Southern Chinese

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ABSTRACT

Objectives: *This study aimed to evaluate the prevalence of brown adipose tissue, as detected by fluorine 18-fluorodeoxyglucose positron emission tomography combined with computed tomography (18F-FDG PET-CT), in a Southern Chinese population. It also set out to determine associated factors with brown adipose tissue, and particularly its relationship with being overweight and having other metabolic disorders such as diabetes mellitus.*

Methods: *We analysed 1765 consecutive 18F-FDG PET-CT scans of 1442 Chinese subjects for the presence of brown adipose tissue. Comparison of variables in subjects with positive and negative brown adipose tissue scans was performed using Student's* t *test. The predictors of observed brown adipose tissue were analysed by multiple logistic regressions. Any $p < 0.05$ was considered statistically significant.*

Results: *Brown adipose tissue was detected in 66 (4.6%) out of 1442 subjects. There were significant differences in age, gender, body mass index, blood glucose levels, and history of diabetes in subjects positive and negative for brown adipose tissue. In the multiple logistic regression test, age and mean monthly temperatures were both independent predictors of the presence of brown adipose tissue ($p \leq 0.001$), which was itself a significant independent predictor of being overweight ($p = 0.001$).*

Conclusions: *Age was the most important factor associated with the prevalence of brown adipose tissue in humans. Lower outdoor temperatures in winter can increase the prevalence of brown adipose tissue, even in sub-tropical climates. There was also an association of brown adipose tissue with being overweight and having higher blood sugar levels, supporting the notion that brown adipose tissue may be a potential therapeutic target for obesity and diabetes.*

Key Words: *Adipose tissue, brown; Chinese; Fluorodeoxyglucose F18; Positron-emission tomography and computed tomography*

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中文摘要

利用18F-FDG PET/ CT檢測中國南方人口褐色脂肪組織的流行率及相關因素

梁紫微、林小玲、王晴兒、孔碧蘭

目的：利用18F-氟代脱氧葡萄糖正電子成像術—電腦斷層掃描（18F-FDG PET-CT）評估中國南方人口中褐色脂肪組織的流行率，並確定與褐色脂肪組織相關的因素，特別是與超重和其他代謝性疾病（如糖尿病）的關係。

方法：分析1442名中國人的1765次18F-FDG PET-CT掃描有褐色脂肪組織的流行率。使用 *t* 檢驗比較分析褐色脂肪組織陽性與陰性兩組受試對象的變量。並用多元logistic回歸分析找出褐色脂肪組織的預測因子。所有$p < 0.05$的差異均具統計學意義。

結果：1442例中，66個（4.6%）有褐色脂肪組織。褐色脂肪組織陽性與陰性兩組受試對象在年齡、性別、身體質量指數、血糖水平和糖尿病史均有顯著差異。多元logistic回歸分析顯示，年齡和每月平均溫度是褐色脂肪組織的獨立預測因子（$p \le 0.001$），而褐色脂肪組織本身就是超重的一個重要獨立預測因子（$p = 0.001$）。

結論：年齡是發生褐色脂肪組織的最重要的相關因素。在冬季，即使是在亞熱帶氣候，較低的室外溫度可以增加褐色脂肪組織的流行率。褐色脂肪組織與超重和較高的血糖水平相關，支持褐色脂肪組織可能是肥胖症和糖尿病的一個潛在治療目標的觀點。

INTRODUCTION

Adipose tissue (fat) can be classified into white adipose tissue and brown adipose tissue (BAT). White adipose cell is a unilocular cell containing a large lipid droplet. It is the primary storage form of energy consisting of triglycerides, and is the more commonly known type of adipose tissue. However, the other type of adipose tissue, BAT, has different functions. Brown adipose cells are multilocular, and contain large quantities of mitochondria. They induce non-shivering thermogenesis to control body temperature and energy expenditure through the expression of uncoupling protein 1 (UCP1),[1] the hallmark BAT protein, which mediates the uncoupling of mitochondrial oxidative phosphorylation from ATP production so that energy is dissipated as heat. Thus, BAT can help burn fat without exercise and may have a role in adult human metabolism, by enabling protection against becoming overweight by increased energy expenditure that in turn avoids cardiovascular disease. It was previously thought that BAT existed only in newborn babies and small animals, and disappeared in human adults. However, the findings of bilateral brown fat in the neck region revealed by positron emission tomography combined with computed tomography (PET-CT) scans indicate that such adipose tissue is also present in adult humans.[2-8] This has stimulated recent interest in the evaluation of BAT, using PET-CT to study its relationship to obesity and cardiovascular risk factors.

Activated BAT takes up free fatty acids and glucose from the circulation, and hence also the glucose analogue, fluorodeoxyglucose (FDG), the commonest tracer used in PET-CT scans. Several studies analysed large cohorts of clinical subjects that underwent PET-CT with tracer 18F-FDG. The prevalence of BAT ranged from 3.8 to 8.6%.[3,6-12] These retrospective studies were mainly performed in Caucasian subjects, although one was performed in Chinese from Northern China (Beijing) and showed a 3.8% prevalence.[9] Besides retrospective studies, two prospective interventional studies in healthy adults have been performed, whereby brown fat detected on PET/CT was confirmed in one subject selected from the cohort, by obtaining tissue specimens for immunohistochemistry using an anti-UCP1 antibody.[13,14] In the study by van Marken Lichtenbelt et al,[13] BAT was observed in 23 of 24 healthy men; only one obese subject did not show any BAT at room temperature (22°C) and after cold exposure (16°C). The high detection rate of BAT on PET-CT scans further confirmed that it was indeed prevalent in healthy adults and that low temperatures could activate it.

Although there was no significant association between body mass index (BMI) and the presence of BAT in the earlier studies performed by Cohade et al[3] and Truong et al,[4] several recent studies have shown that the presence of brown fat was related to BMI, as well as age, gender, and outdoor temperature, and direct correlations exist between the presence of activated BAT and measures of metabolic activity.[6,8,10,12] Furthermore, a clinical history of diabetes mellitus (DM)[8] and use of beta-blockers[6] are associated with a reduction of brown fat. Studies in healthy subjects showed a similar correlation between the presence of BAT and lower BMIs.[13,14]

This study aimed to evaluate the prevalence of BAT, as detected by 18F-FDG PET/CT, in a Southern Chinese population and to compare any difference from that in Northern Chinese subjects. Cheng et al[9] showed that subjects in Beijing with PET-CT scans showing BAT had a significantly lower BMIs ($p = 0.04$). We also determined the factors associated with the presence of BAT, and in particular its relationship with (i) being overweight taking into account the BMI cut-off for Hong Kong Chinese subjects as ≥23 kg/m^2 (WHO/IASO/IOTF),[15] which is lower than the international cut-off of ≥25 kg/m^2, and (ii) other metabolic disorders such as DM. We also wished to investigate whether the prevalence of BAT may be lower in the sub-tropical warmer climate of Hong Kong than in more temperate climates.

METHODS

We analysed 1765 consecutive 18F-FDG PET-CT scans of 1442 Chinese subjects between January 2009 and December 2009 at the PET-CT Unit of the University of Hong Kong for the presence of BAT. Subjects aged under 18 years and non-Chinese were excluded from this study. For subjects with repeated scans, only the first scan was included for analysis. The vast majority of the scans were performed in cancer patients for clinical management purposes. As this was a retrospective study, IRB approval and consent of patients was not required as all the materials were anonymised. Age, gender, weight, height, BMI, fasting blood glucose level, history of DM, the use of prophylactic steroid cover (for intravenous iodinated CT contrast) and the use of bolus intravenous injection of insulin were recorded. Outdoor temperature was obtained from the website of the Hong Kong Observatory (http://www.weather.gov.hk/wxinfo/pastwx/mws.htm).

Subjects were fasted for not less than 6 hours before the examination. A dosage of 4.8 MBq/kg body weight of 18F-FDG with individual doses ranging from 144 to 444 MBq were injected intravenously. The subjects remained in the uptake room set at a room temperature of 25°C. Each subject's fasting blood glucose level was measured before the injection of radioactive FDG. If the blood glucose level was >8 mmol/l, a bolus of intravenous insulin was administered (2-4 IU Actrapid HM; Novo Nordisk A/S, Denmark). FDG was injected when the blood glucose level was controlled to ≤8 mmol/l; such injections being at least 1 hour after any injection of insulin (after muscle uptake of glucose/FDG had diminished). Subjects who had a history of asthma, allergy to iodinated contrast medium, drugs, or food were given prophylactic prednisolone 40 mg orally 12 hours and 2 hours before the scan. After the 1-hour uptake time, a whole-body PET-CT scan was obtained with six to seven bed positions in 20 minutes. All scans were acquired with a combined PET-CT scanner (Discovery VCT; 64-MSCT, GE Healthcare Bio-Sciences Corp, Piscataway, NJ, USA; field of view, 50 cm; pixel size, 3.91 mm; spiral CT pitch, 0.984:1; gantry rotation speed, 0.5 s) using a standardised protocol.

All scans were reviewed by the primary author (TML), a specialised PET radiographer supervised by the senior author (PLK), a radiologist, both with over 6 years' experience in PET reporting. The scan was considered positive for the presence of BAT if there was a hypermetabolic region with a maximal standardised uptake value (SUV_{max}) greater than 1.0 g/ml in the PET image with a corresponding density between -250 and -50 Hounsfield units in the CT image.

Data were analysed using the Statistical Package for Social Sciences (version 19.0, Chicago, IL, USA). Comparison between the number of positive and negative BAT scans was carried out using Student's *t* test, with the Bonferroni correction for multiple comparisons ($p < 0.005$) to confirm statistical significance. Multiple logistic regression was then applied to factors found to be significant, in order to assess the independent effect of each factor. The relationship between observed BAT and demographic factors; gender and age, metabolic measures; BMI and fasting blood glucose level; and mean monthly temperature at the time of study of each subject, were analysed. Fasting blood glucose was used as a factor instead of history of DM in the logistic regression. The relationship between the monthly numbers of subjects with BAT and the respective mean monthly outdoor temperature (in degree Celsius) was evaluated using Pearson's correlation coefficient. The association between SUV_{max} as a continuous variable and outdoor temperature, demographic factors and metabolic measures was evaluated by the Spearman correlation coefficient. The BMI cut-off of being overweight for Hong Kong Chinese used in this study was ≥23 kg/m^2 instead of the international cut-off of ≥25 kg/m^2 (WHO/IASO/IOTF[15]). The predictors of overweight in both Hong Kong cut-off and international cut-off were identified using multiple logistic regression. A $p < 0.05$ was considered statistically significant.

RESULTS

BAT was detected in 66 (4.6%) of the 1442 subjects. The prevalence in men and women was 3.5% (27/773) and 5.8% (39/669), respectively, and this difference was statistically significant ($p = 0.034$). BAT was demonstrated mostly in the supraclavicular region and lower neck. Other sites of detectable BAT included the axilla, mediastinal, perivascular, paravertebral, intercostal, and infra-diaphragmatic regions (Figure 1). The SUV_{max} of the detected BAT ranged from 1.3 to 12.9 g/ml (mean ± standard deviation [SD], 4.2 ± 3.0 g/ml).

The monthly mean outdoor temperature in Hong Kong in 2009 ranged from 15.3°C to 29.4°C (mean ± SD, 23.5 ± 4.9°C). BAT was detected in subjects throughout the year. Monthly prevalence was highest at 7.8% (13 of 166) in December and lowest at 1.3% (2 of 152) in July (Figure 2). The monthly prevalence of BAT positivity correlated negatively with the mean monthly temperature by Pearson's correlation ($r = -0.79$; $p < 0.01$).

Subjects with BAT positivity were 19 to 68 (mean ± SD, 44 ± 14) years old. BAT was significantly more common in those who were younger (44 ± 14 vs. 61 ± 14 years old; $p < 0.001$) and in female (59% vs. 46%, $p < 0.05$) subjects. BAT also existed more frequently in subjects with lower BMI (21.2 ± 3.1 kg/m^2 vs. 22.4 ± 3.7 kg/m^2, $p < 0.01$) and lower fasting blood glucose levels (5.9 ± 0.9 mmol/l vs. 6.4 ± 1.6 mmol/l; $p < 0.01$). Also, BAT was detected only in subjects with no history of DM (0% vs. 10%, $p < 0.01$). Moreover, lower outdoor temperature (21.6 ± 4.6C° vs. 23.4 ± 4.0C°; $p < 0.005$) resulted in higher prevalence of detected BAT. By using the Bonferroni correction ($p < 0.005$), there were still significant differences for age and monthly temperatures between the groups with positive and negative BAT (Table 1). Using multiple logistic regression, age and mean monthly temperatures were both significant independent predictors of the presence of BAT ($p \leq 0.001$) [Table 2]. Among BAT-

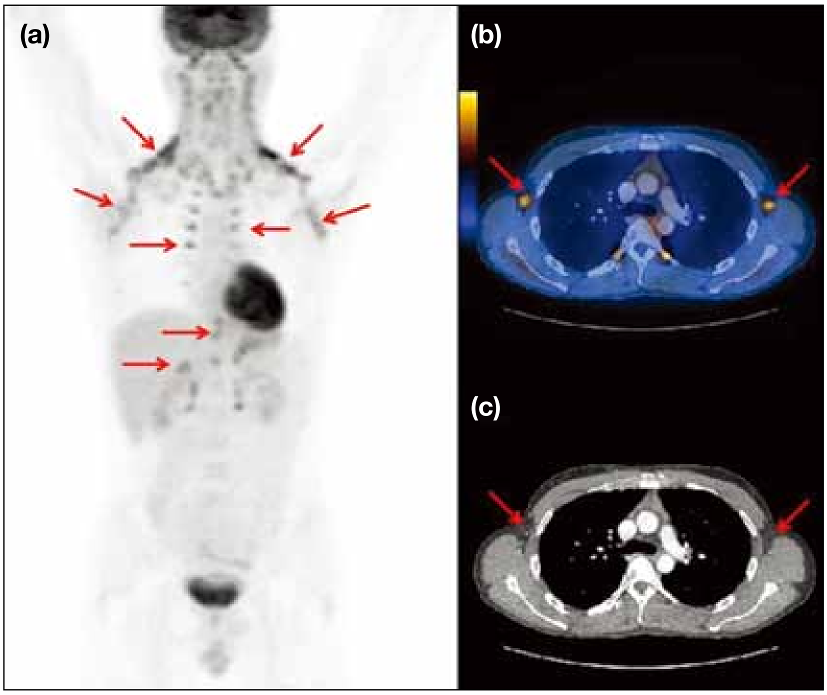

Figure 1. Positron-emission tomography/computed tomography (PET/CT) image shows distribution of whole-body brown adipose tissue. (a) Maximum intensity projection (MIP) PET image in a 28-year-old man with nasopharyngeal carcinoma evaluated before radiotherapy shows hypermetabolic uptakes in supraclavicular region, axillae, paravertebral intercostal spacing of thoracic spine, para-aortic region and perinephric region (arrows). Fusion of (b) PET/CT image and (c) axial CT shows bilateral increased uptakes in axillae (arrows).

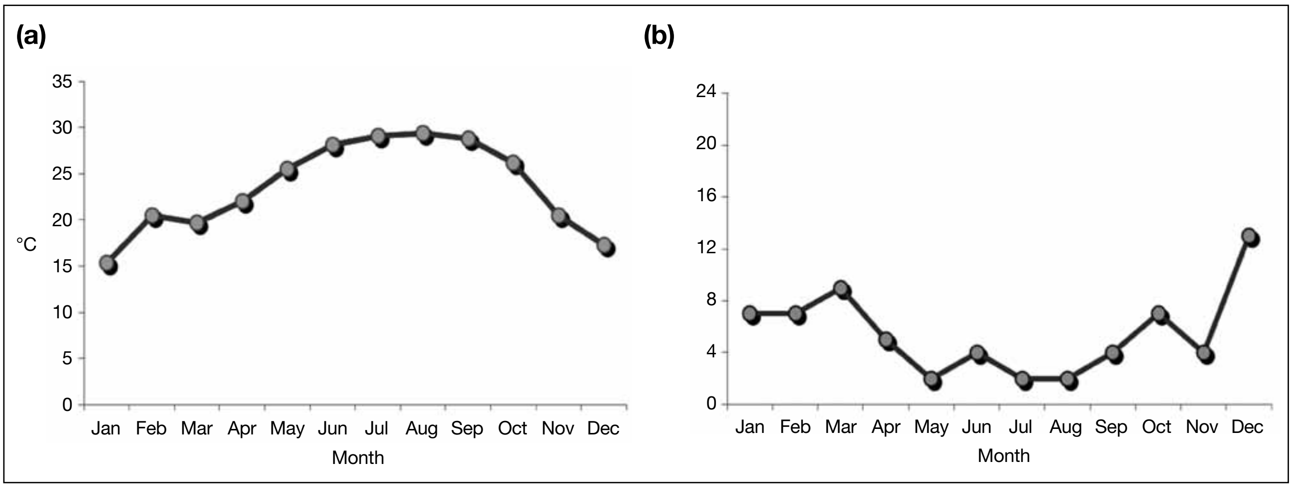

Figure 2. (a) The trend of mean monthly temperature and (b) the number of monthly brown adipose tissue–positive subjects over the months of 2009.

Table 1. Characteristics of subjects with and without detected brown adipose tissue (BAT) in positron-emission tomography/computed tomography scans.

Detection of fluorine 18-fluorodeoxyglucose BAT	Mean ± standard deviation or %	
	Without BAT (n = 1376)	With BAT (n = 66)
Age (years)	61.4 ± 14.2	43.7 ± 13.5*
Weight (kg)	57.9 ± 11.2	55.2 ± 8.7
Height (cm)	160.7 ± 8.7	161.6 ± 7.8
Body mass index (kg/m²)	22.4 ± 3.7	21.2 ± 3.1†
Fasting blood glucose (mmol/l)	6.4 ± 1.6	5.9 ± 0.9†
Gender (male %)	54	41†
Diabetes mellitus (%)	10	0†
Cancer (%)	97	100
Steroid used (%)	14	15
Intravenous insulin used (%)	3	0
Mean monthly temperature at study (°C)	23.4 ± 4.7	21.6 ± 4.6*

* Using Bonferroni correction, $p < 0.005$ between subjects with and without BAT (ANOVA / ANCOVA / chi-square / X^2 tests as appropriate).
† $p < 0.05$ between subjects with and without BAT (ANOVA / ANCOVA / chi-square / X^2 tests as appropriate).

Table 2. Predictors of the prevalence of brown adipose tissue (BAT) in positron-emission tomography/computed tomography scans.

Variable	Odds ratio	p Value*
Decreasing age	0.925	<0.001
Low body mass index	0.943	0.138
Low fasting blood glucose†	0.842	0.127
Female gender	0.604	0.067
Low mean temperature	0.910	0.001

* Based on multiple logistic regressions.
† Fasting blood glucose is used as a factor instead of diabetes mellitus (DM) as statistical error may arise from the entry of '0' subjects having DM in the positive BAT group.

positive subjects, the association between SUV_{max} and characteristics of the subjects were analysed as shown in Table 3. Only the relationship between age and SUV_{max} was found to be significantly correlated ($p < 0.001$). Furthermore, the presence of BAT was a significant independent predictor of being overweight using both the Hong Kong Chinese cut-off (≤23 kg/m²) [$p = 0.001$] and international cut-off (≤25 kg/m²) [$p < 0.05$], but not for DM (Tables 4 and 5).

DISCUSSION

Many studies have indicated that the prevalence of BAT detected by 18F FDG PET-CT scan depends on age, gender, BMI, and outdoor temperature.[6,8,10,12] In these studies involving large cohorts, data were collected in relatively cold temperate climates (Beijing,[9] Canada,[8] Italy,[10] the United Kingdom,[7] and the United States[3,4,6,11,12]). However, to date there has been no report of the prevalence of BAT in areas with warmer sub-tropical climates like Hong Kong. Moreover, whether the prevalence of BAT in Southern Chinese populations

Table 3. Relationships between intensity of brown adipose tissue (BAT) fluorine 18-fluorodeoxyglucose uptake and subject characteristics.

Variable	Mean ± standard deviation (range) or %	*r*	p Value
Ambient temperature (°C)	21.6 ± 4.6 (15.3-29.4)	-0.19	0.12
Age (years)	43.7 ± 13.5 (19-68)	-0.42	<0.001
Sex (male %)	41	0.11	0.38
Height (cm)	161.6 ± 7.8 (146-180)	0.23	0.06
Weight (kg)	55.2 ± 8.7 (36.4-80)	-0.03	0.79
Fasting glucose level (mmol/l)	5.9 ± 0.9 (4.5-8.3)	0.06	0.66
Steroid covered (%)	15	0.10	0.41
Body mass index (kg/m^2)	21.1 ± 3 (14.8-31.1)	-0.21	0.09

Table 4. Characteristics of subjects with body mass indexes (BMIs) of <23 kg/m^2 and ≥23 kg/m^2.

Characteristic	Mean ± standard deviation (range) or %	
	BMI <23 kg/m^2 (n = 854)	BMI ≥23 kg/m^2 (n = 588)
Age (years)	59.7 ± 15.2	61.8 ± 13.6*
Weight (kg)	51.9 ± 8.0	66.3 ± 9.2*
Height (cm)	161.2 ± 8.6	160.1 ± 8.6*
Brown adipose tissue (%)	6	2*
Fasting blood glucose (mmol/l)	6.3 ± 1.5	6.5 ± 1.7
Sex (male %)	54	54
Diabetes mellitus (%)	9	11
Cancer (%)	96	98
Steroid used (%)	14	13
Intravenous insulin used (%)	2	3

* $p < 0.05$ between subjects with BMI <23 kg/m^2 and BMI ≥23 kg/m^2 (ANOVA / ANCOVA / chi-square / X^2 tests as appropriate).

Table 5. Predictors of overweight as defined by body mass index (BMI).*

Variable	Predictor of BMI ≥23 kg/m^2		Predictor of BMI ≥25 kg/m^2	
	Odds ratio	p Value†	Odds ratio	p Value†
Age	1.007	0.085	1.005	0.248
Brown adipose tissue	0.346	0.001	0.410	0.043

* Weight and height are excluded as factors because they are components of BMI and highly correlated.
† Based on multiple logistic regressions.

differs from that in the Northern Chinese is also unknown.

In the present study of 1442 Chinese subjects residing in Hong Kong, the prevalence of BAT was 4.6%, which is in keeping with the 3.8% rate in the Northern Chinese.[9] The result is within the range of other populations with a prevalence of 3.8 to 8.6%.[3,6-12] In a recent study, no significant difference in the prevalence of BAT was noted between different ethnic groups, namely, white, black, Hispanic, and Asian.[12] In actual fact, these figures reflect only the minimal estimation of the BAT frequency in humans, as PET-CT scans only demonstrate activated BAT which takes up 18F-FDG. The role of BAT in newborn babies is to maintain the energy balance and burn fatty acid into heat to keep warm. The existence of BAT in human adults suggests that BAT still has a role in adults to balance energy intake and expenditure by thermogenesis.

Hong Kong is situated on the southeast coast of China which is within the sub-tropical region. In sub-tropical climates, there are distinct seasons but a relatively warm winter. The outdoor temperature exceeds 31°C in summer (June to August) and does not commonly drop below 10°C in winter (December to February). Outdoor workers in Finland were found to have much more BAT in biopsy specimens compared with indoor workers of the same age.[16] van Marken Lichtenbelt et al[13] and Saito et al[14] studied cold-activated BAT and further confirmed that low prevailing temperatures increase the detection of BAT in healthy subjects. In 2009, the monthly mean outdoor temperature in Hong Kong was between 15.3°C and 29.4°C. The relatively high outdoor temperature in Hong Kong when compared to the other studies from Canada, Italy, the United Kingdom, and the United States may explain the relatively low prevalence of BAT. Even in our climate however, we found a relationship between the presence of BAT within the relatively narrow temperature range ranges prevailing ($r = -0.79$; $p < 0.01$), which was comparable to a report by Cohade et al[3] showing a similar relationship with an $r = -0.62$. Whilst cold exposure can increase the prevalence of BAT, BAT was nevertheless detected in two of 152 subjects in July 2009, when the mean temperature was 29.1°C. This suggests that factors other than temperature can determine the presence of BAT.

In the Asian population, the BMI cut-off for 'overweight' is lower than the international cut-off as it has been suggested that the percentage of fat leading to health risk is probably lower than that in European populations. It has also been found that the Asian populations have a higher percentage of body fat at a low BMI.[17] In epidemiological studies using the same BMI cut-off value for overweight as the international standard, Asians were found to be at higher risk of type 2 DM and cardiovascular disease, for which reason a lower BMI cut-off was suggested for them. The overweight cut-off value for Hong Kong Chinese was suggested as 23 kg/m^2.[18] Using this cut-off value, BAT remained a predictor of overweight in this study, suggesting that the association between BMI and presence of brown fat also applies in our local population.

Age has been found to be the most important factor associated with the prevalence of BAT in humans, and our results concurred with this. Notably, we found age to be an independent predictor for the detection of BAT in our cohort. We also found an inverse correlation between SUV_{max} and age ($r = -0.42$; $p < 0.001$) that was consistent with previous findings,[7,8,10,12] and also found a trend towards lower SUV_{max} in older compared to younger subjects. SUV_{max} can provide an estimate of the tissue's metabolic activity since it represents the glucose utilisation, and suggests that in older subjects BAT seems to be less metabolically active. The decrease in BAT activity in the elderly may be due to the atrophy of adipose tissue during ageing. The loss of BAT derived energy expenditure with ageing may therefore lead to obesity. A study in rodents also showed a loss of functional BAT in older animals, as manifested by decreased non-shivering thermogenesis.[19] Another suggestion for the decline in functional BAT with age is related to decreasing secretion of sex hormones. The activity of BAT is determined by adrenergic agonists and may be controlled by the balance of the stimulatory effort of sex hormones and the inhibitory effects of cortisol. The inhibitory effect of cortisol predominates in the elderly owing to the reduction in their circulating sex hormone levels, consistent with the decline of active BAT in the elderly.[20]

Evidently, like muscles, BAT can be activated by insulin,[21] as it originates from similar cells.[22] In that study, insulin was infused into subjects at 1 mU/kg/min with 20% glucose to maintain normoglycaemia during the examination. We did not find any effect of insulin on BAT in our patients who required insulin to reduce blood glucose levels. In our study however, only small bolus doses were injected intravenously instead of as an infusion, and 18F FDG was administered 1 hour after so as to reduce muscle uptake.

In this study, as in Jacene et al's study,[11] the fasting blood glucose level was lower in subjects with BAT, although it was not found to be an independent predictor. Furthermore, in our cohort, no BAT-positive subjects had a history of DM. However, since there were '0' subjects having DM in the BAT-positive group, such an entry in the multiple logistic regression analysis may not be statistically valid. As indicated by Ouellet et al,[8] the presence of BAT may be protective against DM, and our findings also support this notion.

In clinical practice, hypermetabolic BAT on PET-CT image may lead to false positivity for malignancy and therefore, a reduction in diagnostic accuracy. Indeed BAT uptake was not uncommonly mistaken as tumour, lymph node, or other pathological conditions, before the advent of the hybrid PET-CT scanner.[4,23] Studies have evaluated the effectiveness of reducing BAT uptake in clinical PET scans by methods, including keeping warm, eating high fat and very low carbohydrate meals,[24] and administration of propranolol,[25] fentanyl and diazepam.[26] With the introduction of the PET-CT scanner after 2001, the differentiation between BAT and pathological / malignant lesions became more accurate as the images could be evaluated for anatomical correlation together with fusion CT.

Important limitations of this study included its retrospective nature, and no deliberate involvement of normal healthy controls. Another drawback was that only the activated BAT was detectable by PET-CT scan, as it was not feasible to measure inactive BAT.

CONCLUSION

We found the prevalence of BAT detected on PET-CT scans to be 4.6% in the Southern Chinese population, which was similar to that reported in Northern Chinese subjects. The prevalence of BAT in the sub-tropical warmer climate of Hong Kong was comparable to that encountered in studies performed in other temperate climates. In keeping with other retrospective studies, we found age, gender, BMI, blood glucose level, history of DM, and average monthly temperatures to be factors associated with BAT detection. The association of BAT with normal BMI (≤23 kg/m^2) and lower blood sugar levels support the notion that BAT may be a potential therapeutic target for the management of subjects who are overweight and / or diabetic.

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