Obesity, Whole Blood Serotonin and Sex Differences in Healthy Volunteers

Stephanie Hodge a  Brendan P. Bunting b  Edwin Carr c  J.J. Strain a  Barbara J. Stewart-Knox a

a Northern Ireland Centre for Food & Health NICHE, b School of Psychology, University of Ulster, Coleraine, c Department of Chemical Pathology, St Helier Hospital, Carshalton, UK

Key Words
Serotonin • Anthropometric • Waist circumference • BMI • DEXA • Healthy volunteers

Abstract
Background: Obesity is a growing problem throughout Europe, where the rate has more than doubled over the past 20 years. Reduced circulating serotonin may contribute to the development of obesity. This study aimed to explore associations between whole blood (WB) serotonin concentrations and anthropometric measures. Methods: Healthy adult volunteers (N = 68) gave whole blood samples for measurement of WB serotonin, and underwent BMI waist circumference (WC) and waist-to-hip ratio (WHR) assessment as well as DEXA (dual energy X-ray absorptiometry) scans for anthropometric parameters. Student’s t-tests determined differences in WB serotonin and anthropometric measures between sexes. Partial Pearson’s correlations were carried out on anthropometric measures and WB serotonin. Results: For the whole sample, WB serotonin was significantly negatively correlated with BMI, WC, WHR as well as android, gynoid and total % body fat. Analysis by sex showed significant negative correlations between WB serotonin and android, gynoid as well as total fat in males, but not in females. Conclusion: This dichotomy between the sexes implies that there may be sex differences in the way that serotonin interplays with the development of obesity and body fat distribution.

Introduction
Rates of obesity have more than doubled over the past 20 years in most EU countries [1]. Although it is generally accepted that obesity is associated with increased food intake [2] and/or a lack of physical activity [3, 4], it is debated why obesity is on the increase.
Several theories have attempted to explain how psychological processes could lead to obesity. According to Kaplan and Kaplan's [5] psychosomatic theory, obesity arises when people overeat in response to negative mood (affect). More recently, a goal conflict model of hedonic eating has been proposed which holds that excessive intake is driven by the anticipated pleasure of eating [6]. These theories imply that overeating is motivated by the urge to satisfy a psychological need. Such urges could be associated with a neurochemical imbalance in the central (CNS) or peripheral nervous system (PNS). Serotonin (5-HT) is a chemical neurotransmitter that is synthesized in both the serotonergic cells of the CNS and the enterochromaffin cells of the gastrointestinal tract. Serotonin has been shown to be associated with numerous behavioural and psychological factors and is a biochemical marker of mood (affect) [10–12]. Serotonin is just one of many important neurotransmitters in the body, interacting widely with many others. Serotonin interacts with dopamine, a neurotransmitter involved in reward and motivation processes [13]. There is mutual inhibition between the serotonin and dopaminergic systems [14–16]. Serotonin and serotonergic systems throughout the body have been shown to exert an influence on food consumption through control of satiety [17–22] and, thereby, body fat distribution [19].

It is uncertain how central and peripheral serotonergic mechanisms interrelate as serotonin is unable to cross the blood-brain barrier. Owing to the selective nature of the blood-brain barrier, serotonin concentrations in the blood may not necessarily equate to serotonin concentrations or availability in the brain. CNS serotonin (5-HTP) is dependent upon plasma tryptophan crossing the blood-brain barrier. L-tryptophan, an amino acid and precursor to serotonin found naturally in many foods, is converted to 5-hydroxy-L-tryptophan (5-HTP) and then to serotonin in both the CNS and PNS by different isoforms of the enzyme tryptophan hydroxylase [20–25]. Brain tryptophan and serotonin concentrations, however, are not solely determined by plasma tryptophan concentrations, but rather by the ratio of plasma tryptophan to other large neutral amino acids (LNAA's) which compete with it for uptake into the brain. Through consumption of carbohydrates, insulin is released which allows uptake of the LNAA's (with the exception of tryptophan) into skeletal muscle. This 'mopping up' of the other competing amino acids by insulin means tryptophan can pass more easily into the brain [27–29], thus increasing brain serotonin production. Consumption of a protein-rich meal, however, does not appear to have the expected effects of increasing brain tryptophan and serotonin concentrations [28]. In a typical protein meal, tryptophan is the least abundant of all the LNAA's and has to compete with all the other LNAA's before it can cross the blood-brain barrier. Eating a protein-rich meal, therefore, can decrease the plasma tryptophan ratio, even though actual tryptophan concentrations may remain the same as during a carbohydrate-rich meal. This dependence on other amino acid concentrations means that less tryptophan is transported into the brain and less serotonin is produced. It has been theorised that this decrease in transport is why people overeat carbohydrate-rich foods [29]. Increased serotonin production in the brain subsequent to high carbohydrate intake can produce mood-enhancing post-ingestional effects which may further drive intake of such foods and weight gain.

A possible mechanism through which peripheral serotonin may influence obesity is through interaction with leptin, a hormone present in adipose tissue which is thought to regulate (inhibit) appetite, in the regulation of energy and body fat. Subcutaneous injection of serotonin has been shown to decrease circulating leptin and to decrease body fat in rats. Only two human studies appear to have considered peripheral circulating serotonin and obesity. One study [31] found no correlation between waist-to-hip ratio (WHR) and serotonin activity (estimated from prolactin response to D-fenfluramine). The degree to which firm conclusions could be drawn from these results, however, was constrained by the small sample size (N = 19). Another limitation was that WHR alone was used as a measure of
obesity. Waist circumference and DEXA (dual-energy X-ray absorptiometry) are considered better measures of obesity and relate well to visceral fat [32]. The other previous study [33], which included healthy pre- and post-menopausal women, found that serotonin concentrations were inversely associated with fat mass (BMI) (assessed by DEXA), a trend that was most marked in those who were post-menopause, and not on hormone replacement therapy (HRT). There may be sex differences in serotonin mechanisms [34, 35]. Our research, therefore, meets a need to investigate serotonin and various anthropometric outcomes in both sexes of a range of ages. We hypothesise that reduced circulating serotonin will be associated with obesity. This study has explored associations between fasting whole blood (WB) serotonin concentrations and anthropometric measures, including BMI waist circumference (WC), WHR and DEXA, in healthy male and female volunteers of a range of ages and backgrounds.

Material and Methods

Ethical approval was obtained from the University of Ulster Research Ethical Committee (UUREC) in January 2007.

Sampling

Healthy males and females aged between 20 and 66 years (N = 68) were recruited amongst the local population and university staff/students in response to advertising by way of posters displayed in the University of Ulster (all campuses) and in public places, such as libraries and shops, in three local towns. Exclusion criteria included having chronic or acute illness/disease such as coronary heart disease, diabetes and/or stroke and taking medications such as steroids, psychiatric drugs and painkillers currently or for longer than 6 months in the past 5 years. Those who had at any time been diagnosed with clinical depression or other major clinical mental health conditions, those who were or had been pregnant within the previous year or were currently lactating, and those taking certain herbal supplements or other mood-altering substances were also excluded.

Food Frequency Questionnaire

A 7-item food frequency questionnaire was completed by each participant. This instrument included a measure of intake of food and/or fluids (avocados, bananas, plums, pineapple, tomatoes, walnuts and coffee) which may have contributed to changes in concentrations of circulating serotonin. The number of portions consumed over the previous 3 days was recorded.

Whole Blood Serotonin Analysis

For WB serotonin (5-HT) analysis, blood samples were collected by venepuncture into 4 ml purple top (EDTA) blood bottles, from which 2 x 1 ml whole blood was aliquoted into 1.5 ml eppendorf tubes and then mixed with 100 µl of ‘internal solution’ (Sigma-Aldrich, Gillingham, UK) containing 1.1 nmol/l alpha-methyl-5-hydroxy tryptamine malate (based on the method in [36]. Samples were immediately frozen at –70 °C for later batch analysis. Whole-blood samples (N = 68) were then analysed for 5-HT at the St. Helier Hospital, Chemical Pathology Laboratory, Surrey, UK, by means of a high-performance liquid chromatography (HPLC) method, using reagent kit 3030 (Chromsystems Instruments & Chemicals GmbH, Munich, Germany). The frozen blood specimen was thawed on ice, and 50 µl sample was mixed in a reaction vial with 50 µl distilled water and 100 µl Internal Standard. The mixture was then incubated for 10 min at room temperature. An aliquot of 100 µl Precipitation Reagent was then added to the samples to remove excess sodium dodecyl sulphate (a detergent used as a protein solubilisation buffer). The sample was vortex-mixed for 30 s before being incubated for 10 min at 2-8 °C. The sample was then centrifuged for 10 min at 13,000 rpm. An aliquot of 20 µl of the clear supernatant was then injected onto the HPLC column. A calibration curve and controls were run with every batch of samples. For determining intra-assay precision the total coefficient of variation for WB samples containing between 500–1,500 nmol/l 5-HT was approximately 4.0%. The electrochemical detector used was ESA Coulochem 5100A (ESA Analytical, Ltd., Aylesbury, UK) equipped with a 5011A electrode with detection at 0.25 V against the reference electrode.
Anthropometric Parameter Measurement

Body weight was taken using digital scales and height measured using a fixed stadiometer. Waist circumference was measured with a measuring tape at the level of the umbilicus. Hip circumference was measured at the widest part of the hips. A total body DEXA (Lunar Prodigy\textsuperscript{TM}; GE Medical Systems LUNAR, Madison, WI, USA) scan was performed on each volunteer in order to measure the ratio of lean to fat tissue, anthropometric parameters and bone mineral density.

Procedure

Volunteers were asked to eat their usual diet the day before the study and to refrain from drinking alcohol in the 24 h prior to the study. Informed consent was given in writing on arrival at the study centre on the main study day. The process from arrival at the unit until completion of the tests took no more than 3 h. A verbal explanation of the venepuncture procedure was given to the volunteer. An overnight fasting 4 ml venous blood sample was then collected by a qualified phlebotomist to determine WB serotonin. The volunteer was then given breakfast. Anthropometric measures were then taken, and a total body DEXA scan was carried out. Following the DEXA scan, a social, health and lifestyle questionnaire was completed individually, by self-report, which took between 25 and 45 min.

Statistical Analyses

Demographic data were treated as categorical and reported as percentages. WB serotonin and anthropometric data were treated as continuous. Box plots were created to identify outliers and assess skewness of data. No outliers were removed. Results were expressed as mean values and standard deviations (SD) (table 1). Student’s t tests were employed in order to determine differences in WB serotonin and anthropometric measures between sexes. Partial correlations, using Pearson’s R, were then carried out on anthropometric and WB serotonin (table 2), controlling for sex and age. Data were analysed for the whole sample and for each sex separately. Partial Pearson’s R correlations (controlling for age and sex) were carried out on the frequency of consumption of serotonin-enhancing foods and WB serotonin concentrations. Data analysis was carried out using Statistical Package for the Social Sciences version 17.0 (SPSS, Chicago, IL, USA).

<table>
<thead>
<tr>
<th></th>
<th>Total (N = 65–68)</th>
<th>Males (n = 22–23)</th>
<th>Females (n = 43–45)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age, years</td>
<td>40.5</td>
<td>11.39</td>
<td>38.26</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>26.65</td>
<td>4.97</td>
<td>26.07</td>
</tr>
<tr>
<td>% lean tissue</td>
<td>65.44***</td>
<td>11.41</td>
<td>75.95</td>
</tr>
<tr>
<td>Android region % fat</td>
<td>40.41***</td>
<td>12.24</td>
<td>32.90</td>
</tr>
<tr>
<td>Gynoid region % fat</td>
<td>38.98***</td>
<td>11.43</td>
<td>25.98</td>
</tr>
<tr>
<td>Total % body fat</td>
<td>34.30***</td>
<td>11.37</td>
<td>23.73</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>93.29</td>
<td>12.11</td>
<td>94.41</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>103.4</td>
<td>9.90</td>
<td>102.39</td>
</tr>
<tr>
<td>WHR</td>
<td>0.90</td>
<td>0.06</td>
<td>0.92</td>
</tr>
<tr>
<td>Serotonin, nmol/l</td>
<td>805.62</td>
<td>347.68</td>
<td>734.43</td>
</tr>
</tbody>
</table>

\*p < 0.0, **p < 0.01, ***p < 0.001 (for t-test between sexes).

Where Levene’s Test for Equality of Variances was significant (p < 0.05), equal variances were assumed.
Table 2. Whole blood serotonin and anthropometric parameters

<table>
<thead>
<tr>
<th></th>
<th>Total age and sex controlled</th>
<th>Males age controlled</th>
<th>Females age controlled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 65–68)</td>
<td>(n = 22–23)</td>
<td>(n = 43–45)</td>
</tr>
<tr>
<td>r</td>
<td>p value</td>
<td>r</td>
<td>p value</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>-0.284</td>
<td>-0.366</td>
<td>-0.269</td>
</tr>
<tr>
<td>% lean tissue</td>
<td>0.021*</td>
<td>0.094</td>
<td>0.077</td>
</tr>
<tr>
<td>Android % fat</td>
<td>0.330</td>
<td>0.523</td>
<td>0.217</td>
</tr>
<tr>
<td>Gynoid % fat</td>
<td>-0.004***</td>
<td>-0.550</td>
<td>-0.232</td>
</tr>
<tr>
<td>Total % body fat</td>
<td>-0.315</td>
<td>-0.576</td>
<td>-0.180</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>-0.216</td>
<td>-0.391</td>
<td>-0.174</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>-0.325</td>
<td>-0.405</td>
<td>-0.302</td>
</tr>
<tr>
<td>WHR</td>
<td>-0.322</td>
<td>-0.356</td>
<td>-0.317</td>
</tr>
</tbody>
</table>

*Significant at the p < 0.05 level.
**Significant at the p < 0.01 level.
***Significant at the p < 0.005 level.

Results

Sample Description

Demographic Characteristics

66.2% of the participants were female. Age ranged between 20 and 66 years, with a mean age of 40.5 years. Males (n = 23) had a mean age of 38.3 years; females (n = 45) had a mean age of 41.6 years. Among females (n = 44), contraceptive pills or HRT were used by 12.8% (n = 5). Independent samples t-test (t = 1.847, p = 0.125) showed that there was no significant difference in WB serotonin concentrations between females who were using HRT/contraceptive pills and those who do not. Education level was as follows: no formal education (4.4%); primary or secondary (25%); vocational or higher vocational (26.5%); and university degree or higher (42.6%). Socio-economic class (NS-SEC) was as follows: higher professional (13.2%); lower professional or higher technical (22.1%); intermediate (19.1%); self-employed (small companies); lower supervisory or technical (8.8%); semi-routine and routine (11.8%); never worked or unemployed (2.94%); retired (5.9%); and, students (16.8%).

There was no significant correlation between the frequency of consumption of serotonin-enhancing foods, measured as total number of serotonin-enhancing food or drink portions in the 3 days prior to the study day, and WB serotonin concentrations (r = 0.063, p = 0.623).

Anthropometric Measures

Percentage lean tissue was significantly higher in males (75.95%) than in females (60.06%) (df = 42.2, p < 0.001) (table 1). Total body fat was significantly higher in females (39.70%) than in males (23.73%) (df = 43.9, p < 0.001). Females (44.24%) showed higher amounts of android fat in these regions than males (32.90%) df = 66.0, p < 0.001). Females (45.62%) had higher amounts of gynoid fat than males (25.98%) (df 0 40.2, p < 0.001).
There were no significant differences in WB serotonin concentrations between the sexes (table 1).

**Associations between WB Serotonin and Anthropometric Measures**

Analysis of the whole sample showed significant negative correlations between WB serotonin and body weight ($r = -0.260$, $p = 0.037$), BMI ($r = -0.284$, $p = 0.021$), waist circumference ($r = -0.325$, $p = 0.008$), WHR ($r = -0.322$, $p = 0.009$), gynoid % body fat ($r = -0.315$, $p = 0.010$), android % fat ($r = -0.346$, $p = 0.004$) as well as total % fat ($r = -0.345$, $p = 0.005$). There was a positive correlation ($r = 0.33$, $p = 0.008$) between WB serotonin and % lean tissue (table 2).

**Associations between WB Serotonin and Anthropometric Measures by Sex**

In males negative correlations were found between WB serotonin and total % body fat ($r = -0.532$, $p < 0.05$), android ($r = -0.55$, $p < 0.01$) as well as gynoid ($r = 0.576$, $p < 0.01$) body fat. Males also showed a positive correlation between WB serotonin and % lean tissue ($r = 0.523$, $p < 0.05$). Females showed a negative correlation between WB serotonin and waist circumference ($r = -0.302$, $p < 0.05$) as well as WHR ($r = -0.317$, $p < 0.05$) (table 2). After controlling for HRT and use of contraceptive pills, both waist circumference ($r = -0.290$, $p = 0.063$) and WHR ($r = -0.286$, $p = 0.067$) were not found to be significantly correlated with serotonin in females. Controlling for HRT and oral contraceptive use did not alter any other variable associations.

**Discussion**

We hypothesised that circulating serotonin may contribute to the development of obesity. In keeping with this theory, data for the whole sample showed that lower WB serotonin was associated with greater body fat and lesser percentage lean mass. This finding agrees with those from rodent studies [33] and implies that peripheral serotonin plays a role in the development of obesity. Nonetheless, there appeared to be large inter-individual variation in WB serotonin concentrations. Peripheral serotonin may be measured in WB, serum or alternatively in platelet-poor plasma. In our study we determined WB serotonin [41]. There are several different assay methods, including ELISA, HPLC by electrochemical or fluorimetric detection and liquid chromatography-mass spectrometry (LC/MS), for measuring serotonin. HPLC assays by electrochemical detection, which was the method used in our study, are considered the ‘gold standard’ for the determination of serotonin concentrations [33]. The variation was unlikely to be a consequence of measurement error. It is possible that such variation could have resulted from differences in food intake during the days prior to measurement. The frequency with which foods, which might have affected WB serotonin concentrations, were consumed over the previous 3 days prior to study, however, was not associated with serotonin concentrations. Alternatively, individual differences in WB serotonin concentrations may have arisen because of variation in the time of year in which WB serotonin sampling occurred. Evidence that serotonin concentrations vary by season is contradictory. Whereas one study [37] found low serotonin values in healthy volunteers during the summer as compared to the rest of the year, a large study also of healthy volunteers [38] (N = 500 approximately) found no substantial seasonal variations
in WB serotonin. The SD in WB serotonin observed in the current study concur with those reported in other studies of healthy individuals [38].

Our findings also implied that there are sex differences in the way that circulating serotonin interplays with body fat distribution. Associations between WB serotonin and anthropometric measures differed among males (who showed significant negative correlations for body fat measures) and females (who showed no significant correlations for anthropometric and DEXA-scanned body fat measures). Both lean mass (positive correlation) and fat mass (negative correlation) appeared to be significantly correlated with serotonin in males. Although these correlations were in the same direction in females, these relationships were not significant. This finding appears contradictory to findings from a previous study of females (N = 275) [33] which found that both lean mass and total fat mass were significantly negatively correlated with serum serotonin. When hormonal status (pre-menopausal, post-menopausal on HRT or post-menopausal not on HRT) was considered, differences in the direction of the relationship between serum serotonin and lean mass were seen. Higher serum serotonin was associated with lower lean mass in those who were post-menopausal, but unrelated to lean mass in those who were pre-menopausal [33]. A possible limitation of our study, therefore, was that the stage of the menstrual cycle was not recorded in the females of childbearing age. That fluctuating oestrogen concentrations can influence serotonin activity [35] may account for the weaker correlations found in our female data. In our study, the mean female age was 41.64 ± 11.8 years, and, although we did not record menopausal status, we might still infer from our (younger) sample that a higher percentage was pre-menopausal than in the previously cited study by Modder and colleagues [33] (mean sample age 57.9 ± 17.7 years, with 32.7% pre-menopausal). Previous studies of sex differences in serotonin concentrations have reported mixed findings [39, 40]. There was no apparent difference in WB serotonin between the sexes and which may have explained individual differences in associations between WB serotonin and body fat observed in our study. It is also possible that the larger proportion of females (n = 45) relative to males (n = 23) may have biased results. It is clear that some correlations were stronger when the whole sample was taken into account rather than each sex taken separately.

A further potential limitation which may be levelled at this study is that, compared to the general population of Northern Ireland, our sample contained a greater percentage of highly educated people. According to the Northern Ireland Census 2001 [42], those with a first degree or higher represent 15.8% of the population. In our sample this number was 42.6%. This higher proportion of well-educated individuals could potentially have affected the outcomes. Education tends to be negatively correlated with patterns of obesity, particularly among women, in countries such as the UK and others within Europe (those falling into the ‘high’ HDI (Human Development Index) WHO category [43].

**Conclusion**

Although these cross-sectional correlations cannot specify cause and effect, the findings imply that serotonin, which is a putative marker of mood [10, 11], could be an antecedent and/or a consequence of obesity. As hypothesised [19], it is possible that people are becoming obese through eating excessively in order to enhance mood which may be associated with serotonin. Similarly, the hedonic theory of eating [6] which holds that obesity is a consequence of overeating in anticipation of the pleasure of eating could explain our data. It is also possible that serotonin, in tandem with dopamine, could be at least partly responsible for this hedonic behaviour as both neurotransmitters are known to contribute towards controlling attention, reinforcement and reward. The degree to which these biochemical
markers of mood translate into behaviour, however, is not well established. It is also possible, as previous studies of rodent CNS [44] and peripheral [33] serotonin have suggested, that leptin interacts with serotonin in the aetiology of obesity. Future research should consider possible associations between peripheral serotonin and leptin. Meanwhile, these data suggest that treatments which enhance serotonin may also have potential to treat obesity.

Acknowledgements

This research was conducted as part of a Department of Education and Learning (DEL) for Northern Ireland funded PhD studentship. Additional funding was provided by the Health Research Board (HRB) Ireland.

Disclosure Statement

The authors are unaware of any conflicts of interest relating to their research.

References

12. Flory JD, Manuck SB, Matthews KA, Muldoon MF: Serotonergic function in the central nervous system is associated with daily ratings of positive mood. Psychiatr Res 2004;129:11–19.


