Effects of Developmental and Adult Androgens on Male Abdominal Adiposity

MATTHEW H. McINTYRE,† SUSAN F. LIPSON, AND PETER T. ELLISON
Harvard University, Cambridge, Massachusetts

ABSTRACT  We present results from 42 gay men who completed a survey including self-measurement of waist circumference, height, and weight, in addition to providing saliva samples for the assay of testosterone, and a photocopy of the right hand for the measure of second-to-fourth digit length ratio (2D:4D), proposed as a means of approximating androgenic effects during development. The analyses were conducted as a test of the recent hypothesis, proposed by Abbott et al. ([2002] J Endocrinol 174:1–5), that high prenatal androgen exposure causes greater deposition of fat on the abdomen relative to other depots. We found support for this hypothesis in men, albeit in a limited sample and with self-reported and self-collected data. Am. J. Hum. Biol. 15: 662–666, 2003. © 2003 Wiley-Liss, Inc.

Abdominal adiposity, variously measured by waist circumference, waist-to-hip circumference ratio, and visceral fat volume, commonly presents with metabolic disorders related through insulin resistance, including diabetes, coronary heart disease, and polycystic ovarian syndrome (Goudas and Dumesic, 1997; Holte et al., 1994; Morin-Papunen et al., 2000; Pasquali et al., 1993). Recent evidence has suggested that excessive abdominal adiposity may be the primary cause of metabolic dysfunction in many cases (Hanley et al., 2002; Okosun, 2000; Okosun et al., 2000; Rexrode et al., 1998). While abdominal adiposity is strongly correlated with total adiposity, Abbott et al. (2002b) recently proposed that prenatal androgen exposure in females promotes abdominal deposition specifically (Eisner et al., 2003; Abbott et al., 1998, 2002a). This proposal is consistent with well-established sex differences in measures of abdominal adiposity. Prenatal androgen effects may also promote a pattern, later in life, of abdominal adipose deposition in men.

Adult testosterone exposure in men, by contrast, tends to reduce abdominal adiposity (Garaulet et al., 2000; Munzer et al., 2001; Tsai et al., 2000; Vermeulen et al., 1999; Rebuffé-Scrive et al., 1991). The effect is mediated at multiple levels. Testosterone alters body composition by promoting lean mass over fat mass (Bhasin et al., 2001; van den Beld et al., 2000; Vermeulen et al., 1999; Bross et al., 1998). Testosterone also reduces serum adipose measures, including triglycerides, and cholesterol or, more particularly, low-density lipoprotein molecular size and relative concentration (Bhasin et al., 2001; Dobs et al., 2001; Haffner et al., 1996). Finally, testosterone inhibits lipogenesis in abdominal adipocytes (De Pergola, 2000; Garaulet et al., 2000; Rebuffé-Scrive et al., 1991).

Little is known about the effects of prenatal androgens in humans because of the difficulty of direct measurement. Manning et al. (1998) have proposed that the ratio of the second to fourth digits (2D:4D) may be a proxy of prenatal androgen exposure, such that low 2D:4D is associated with high prenatal androgen exposure. It has been robustly established that females have higher 2D:4D than males both before and after puberty (Manning et al., 1998; Peters et al., 2002; Williams et al., 2000; MacFadden and Schubel, 2002). A homologous sex difference has also been described in mice (Brown et al., 2002b). Furthermore, low 2D:4D is associated with the prenatal hyperandrogen condition, congenital adrenal hyperplasia (Brown et al., 2002a; Ökten et al., 2002). It has already been reported that 2D:4D is correlated with waist-to-hip circumference ratio among women (Manning et al., 2000;
Manning, 2002, p. 33). In this article we employ 2D:4D to explore proposed prenatal androgen effects on abdominal adiposity in middle-aged men. In addition, we consider the association of abdominal adiposity with adult salivary testosterone level.

SUBJECTS AND METHODS

Subjects

The results presented here are secondary findings from a mail-in survey of gay men designed to investigate the relationship among androgens, sexuality, and gender role. About 189 members of the Harvard Gay and Lesbian Caucus (HGLC), an alumni organization, were contacted by mail, that is, all who identified themselves as “gay men,” who provided contact addresses for general use by other members, and who lived in the United States but outside the Boston area. Of those contacted, 54 (28.6%) participated. A comparison of the ages of respondents to this survey with respondents to a general membership survey by the HGLC (of both men and women) reveals that our participants may be somewhat older (see Table 1). We cannot assess whether this sample may be biased in other, more salient ways.

Of the 54 respondents, 12 were excluded from all analyses presented in this article for one or more of the following reasons:

the photocopy of their right hand used to measure 2D:4D was not provided or of poor quality; saliva samples were not provided, were contaminated, or were of insufficient volume for assay; or anthropometric measurements were not reported.

Measures

Participants were given a survey including several subparts. They also took anthropometric measurements, including waist circumference, from themselves according to written instructions. They reported their own heights and weights. The accuracy or reliability of these self-measures cannot be determined. Table 2 gives descriptive statistics of the measures used in this article (from included subjects only).

Participants were also asked to provide a photocopy of the right hand, from which finger lengths were measured (proximal flexion from tip), and 2D:4D calculated. All fingers were remeasured by a second person, yielding an interrater correlation of 0.80 for 2D:4D. Manning (2002, p. 4) has shown that 2D:4D measures taken from photocopies are correlated (r = 0.88) with 2D:4D measures taken by caliper.

This research was conducted with the approval of both Harvard University’s institutional review board and the chair of the HGLC.

Salivary testosterone assay

Subjects also returned three saliva samples by mail for salivary testosterone assay. Subjects were asked to collect these samples on separate days, immediately after waking. Saliva samples were assayed for testosterone in the Reproductive Ecology Laboratory, Harvard University, using a modified application of the 125I double antibody kit produced by Diagnostic Systems Laboratories (Webster, TX). Sample and standard rea-

TABLE 1. Comparison of participant ages with ages of respondents to a general membership survey of the same population

<table>
<thead>
<tr>
<th>Age group</th>
<th>HGLC survey</th>
<th>Present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>18–29</td>
<td>9% (48)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>30–39</td>
<td>32% (174)</td>
<td>22% (12)</td>
</tr>
<tr>
<td>40–49</td>
<td>26% (140)</td>
<td>30% (16)</td>
</tr>
<tr>
<td>50–59</td>
<td>20% (109)</td>
<td>22% (12)</td>
</tr>
<tr>
<td>60–69</td>
<td>8% (44)</td>
<td>22% (12)</td>
</tr>
<tr>
<td>70+</td>
<td>4% (22)</td>
<td>4% (2)</td>
</tr>
</tbody>
</table>

Data from HGLC Newsletter, February 2003.

TABLE 2. Descriptive statistics of measures employed in regression analyses

<table>
<thead>
<tr>
<th>Measures</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (years)</td>
<td>50.0</td>
<td>48.0</td>
<td>31–76</td>
<td>11.5</td>
</tr>
<tr>
<td>weight (kg)</td>
<td>78.3</td>
<td>77.6</td>
<td>63.5–122.5</td>
<td>10.7</td>
</tr>
<tr>
<td>waist circ. (cm)</td>
<td>95.2</td>
<td>91.4</td>
<td>73.7–134.6</td>
<td>11.1</td>
</tr>
<tr>
<td>2D:4D</td>
<td>0.973</td>
<td>0.963</td>
<td>0.933–1.05</td>
<td>0.001</td>
</tr>
<tr>
<td>testosterone (pmol/L)</td>
<td>410.5</td>
<td>408.25</td>
<td>63.5–920.8</td>
<td>206.7</td>
</tr>
</tbody>
</table>
tions were run in duplicate. Substrate (150 µl) was pipetted into borosilicate tubes, 100 µl of sample, and 50 µl of buffered saline or, for the standard reactions, a 400 pg/ml standard solution was added in volumes of 2, 5, 12, 30, 75, and 150 µl, with volumes of buffered saline adjusted to yield 150 µl total volume. Antiserum, diluted 1:3 (100 µl), and undiluted tracer (200 µl) were added to sample and standard tubes. Reactions incubated overnight for at least 18 hours, after which precipitating reagent (400 µl) was added and tubes were centrifuged and aspirated. The assays were sensitive to 14 pmol/L testosterone.

Samples were assayed in three lots. Reaction parameters of the three assay lots were similar. Interassay coefficients of variation were 12.2% for low pools and 7.7% for high pools. The intraassay coefficient of variation was 4.6%. Concentration values were only used if the duplicate coefficients of variation were less than 20%. The sample concentrations used are the averages of duplicates.

Samples from individuals giving multiple samples were allotted to different assays in order to distribute interassay error. The total number of samples from 48 individuals was 111, 39 in the first assay, 41 in the second assay, and 31 in the third assay. Of 48 subjects with at least one saliva sample, 10 provided one sample, 13 provided two samples, and 25 provided three samples. The testosterone variable used in our analyses is the natural log of the average sample concentration (hereafter, “ln(testosterone)”).

RESULTS

Multiple regression analyses were performed to assess the influence of markers of androgen production (testosterone and 2D:4D) on waist circumference (WC), our measure of abdominal adiposity. Weight and age strongly predict WC, presumably because they are markers of total body composition and fat mass. Natural logged weight and age together explain about 75% of the variance in WC (see Table 4), although much of the variance explained by age covaries with both ln(testosterone) and 2D:4D. Table 3 shows the simple correlations among the employed variables. The relationship between ln(age) and ln(testosterone) is expected. However, the relationship between ln(age) and 2D:4D is unexpected (Manning, 2002) and possibly spurious. None of the correlations suggests a serious collinearity problem among independent variables. The results of multiple regression analysis should be informative. Table 4 shows the results of five multiple regressions on the dependent variable WC. Adding androgen markers as predictors increases the explained variance in WC by about 10% over ln(weight) and ln(age) alone.

**Interpretations of coefficients**

Recall that 2D:4D is proposed as an *inverse* indicator of prenatal androgen production. Therefore, to interpret partial regression coefficients with 2D:4D as prenatal androgen production, the signs should be reversed. As expected, effects of adult and prenatal androgens are in opposite directions, with adult androgens associated with reduced WC and prenatal androgens with increased WC.

In the fifth analysis (Table 4), we introduce the interaction between 2D:4D and weight. We interpret the interaction as follows. Prenatal androgens modify the relationship between total fat mass (approximated by weight) and abdominal fat mass (approximated by WC). Specifically, prenatal androgens increase the proportion of abdominal fat with increasing weight. The interaction therefore reflects the bias toward deposition of fat on the waist relative to other regions.

**DISCUSSION**

While these results come from a small, select sample of middle-aged men, they are particularly interesting in light of the recent hypothesis proposed by Abbott et al. (2002b)
that prenatal androgens promote the abdominal fat depots which in excess may disrupt glucose homeostasis. We have no reason to believe that fat depositional processes would operate differently in gay and straight men. However, the specificity of the sample warns us to take these data as more analogous to a case study than to an epidemiological study. In particular, it has been suggested that the 2D:4D of gay men may differ from those of broader populations of men (Robinson and Manning, 2000; Lippa, 2003). It is unclear how this or other differences may attenuate the generality of the results presented in this article.

Because the measures employed in these analyses do not directly reflect body composition or adiposity (or for that matter, prenatal androgens in the case of 2D:4D), the greater concern is with properly interpreting regression coefficients. Can we be confident that weight and WC in this sample are reflections of adiposity?

We have attempted in this study, using statistical interactions, to distinguish effects of androgens on total fat mass from effects on the pattern of fat deposition, specifically abdominal deposition bias. This was only successful for prenatal androgens (2D:4D) for which the interaction with weight, interpreted as abdominal deposition bias, was significant. Prenatal androgens appear to promote abdominal deposition, both specifically and perhaps by increasing total fat mass. Because these findings are correlational, it is difficult to know whether interaction effects can be causally interpreted or reflect spurious correlations among the variables. Adult androgens appear to reduce abdominal adiposity, whether through direct effects on abdominal tissue or otherwise. The latter finding is unsurprising given the strength of previous findings, including Munzer et al. (2001) and Rebuffé-Scrive et al. (1991).

These results provide suggestive and preliminary evidence of a role of prenatal androgens in the abdominal fatness of middle-aged men. The factors mediating this relationship remain unknown, but are likely to include direct effects on android adipocytes and development of the android depots.

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LITERATURE CITED


