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Muscle-building supplement use and increased risk of testicular germ cell cancer in men from Connecticut and Massachusetts

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Background: No analytic epidemiological study has examined the relationship between use of muscle-building supplements (MBSs) and testicular germ cell cancer (TGCC) risk.

Methods: We conducted a population-based case–control study including 356 TGCC cases and 513 controls from Connecticut and Massachusetts.

Results: The odds ratio (OR) for ever use of MBSs in relation to risk of TGCC was significantly elevated (OR = 1.65, 95% confidence interval (CI): 1.11–2.46). The associations were significantly stronger among early users, men with more types of MBSs used, and longer periods of use.

Conclusions: MBS use is a potentially modifiable risk factor that may be associated with TGCC.

Testicular germ cell cancer (TGCC) is the most common solid malignancy in men aged 15–39 years (Ferlay *et al*, 2012). The age-adjusted incidence of TGCC in the United States (US) based on data from the Surveillance Epidemiology and End Results (SEER) program has been increasing, growing from 3.7 out of 100 000 in 1975 to 5.9 out of 100 000 in 2011 (SEER Program, 2014).

Cryptorchidism, abnormal development of the testicles, and family history are known risk factors for TGCC (Greene *et al*, 2010; Schnack *et al*, 2010; Lip *et al*, 2013), but these known factors cannot explain the increasing trend of TGCC given that only a relatively small percentage of cases have a history of cryptorchidism (~10%) (Ferguson and Agoulnik, 2013).

Use of performance-enhancing substances has become increasingly popular in the US population (Andres *et al*, 1999; Froiland *et al*, 2004; Bemben and Lamont, 2005). Some previous evidence has indicated that certain ingredients of muscle-building supplements (MBSs) may be related to testicular damage (Yu and Deng, 2000; Han *et al*, 2013; National Center for Biotechnology Information, 2014). It is therefore biologically plausible that MBS use could be associated with the risk of TGCC. A case series that evaluated MBS use among 129 TGCC cases in the United States observed that a relatively high percentage of cases (~20%) had used some form of supplements, but no control group was included in this study for comparison (Chang *et al*, 2005). To better understand the

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role of MBS use on the risk of TGCC, we conducted a population-based case–control study in Connecticut (CT) and Massachusetts (MA).

MATERIALS AND METHODS

Study population. Subjects for this population-based case–control study were recruited between 2006 and 2010 among male residents of CT and MA. The incident cases included newly diagnosed patients with TGCC (International Classification of Diseases for Oncology Morphology Codes 9906–9910) identified using the Yale Comprehensive Cancer Center's Rapid Case Ascertainment Shared Resource (RCA) and the Massachusetts Cancer Registry over the same time period.

The eligibility criteria for cases in the study included having a histologically confirmed TGCC (Stage 0–IV) diagnosed during 2006–2010, no previous cancer diagnoses except for non-melanoma skin cancer, being a male resident of CT or MA and between the ages of 18–55 at diagnosis, alive and competent to answer questions at the time of interview, and able to speak English in order to complete the interview. Population-based controls were identified among English-speaking male residents of CT and MA between the ages of 18–55 at the time of the interview, using random digit dialing. Controls were frequency-matched to cases sampling on the basis of age categories, and individuals with a previous history of cancer with the exception of non-melanoma skin cancer were excluded as potential controls. The study was approved by the Institutional Review Boards of Yale University, the Connecticut Department of Public Health Human Investigation Committee, the Harvard School of Public Health Human Subject Committee, the Massachusetts Department of Public Health, Dana Farber Cancer Institute, and the 28 participating hospitals in Connecticut.

Data collection. All subjects included in the study completed an in-person and standardised, structured questionnaire implemented by trained study interviewers. A total of 356 cases and 513 controls were included in the present study with a participation rate of 57.4% for the cases and 47.8% for the controls. The interview included questions about a wide variety of characteristics suspected to be associated with the risk of TGCC, including MBS. MBS use was defined as use for at least once a week for $\geqslant 4$ consecutive weeks. The interview included an assessment of 30 different types of MBS powders or pills. The major ingredients, including creatine, protein, and androstenedione or its booster, were abstracted according to the product ingredients.

Statistical analysis. Unconditional logistic regression models were used to evaluate the associations between the use of MBS and the risk of TGCC. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated for ever *vs* never MBS use and for several additional metrics in relation to risk of TGCC. These metrics included age at first use, number of MBS products used, and duration of use. Polytomous logistic regression models and then hierarchical coefficients tests were used to evaluate the associations between the use of MBS and the risk of TGCC by subtypes (seminoma and non-seminoma, each *vs* controls). $P<0.05$ was the criterion of statistical significance, and all statistical tests were two sided. Statistical analyses were conducted using Stata Version 10.0 software (Stata, College Station, TX, USA).

RESULTS

As shown in Table 1, the cases were slightly younger and more likely to be white than the controls. The prevalence of cryptorchidism and injury to the testes or groin was higher in cases than that in controls. However, years of education, prevalence of tobacco smoking, prevalence of alcohol drinking, and height were similar in cases and controls (Table 1).

Table 1. Characteristics of TGCC Cases and Controls in a Population-Based Case–Control study, Connecticut and Massachusetts, 2006–2010

Characteristics	Cases (%) ($n=356$)	Controls (%) ($n=513$)
Age (years, means)	35.42	38.54
Race		
Whites	338 (94.94)	459 (89.47)
Others	18 (5.06)	54 (10.53)
Years of education		
$\leqslant$12 years	101 (28.37)	137 (26.71)
>12 years	255 (71.63)	376 (73.29)
Tobacco smoking		
Never	224 (62.92)	309 (60.23)
Ever	132 (37.08)	204 (39.77)
Alcohol drinking		
Never	21 (5.90)	28 (5.46)
Ever	335 (94.10)	485 (94.54)
Height at reference date		
$\leqslant$68 inches	90 (25.28)	143 (27.88)
69–70 inches	98 (27.53)	125 (24.37)
71–72 inches	93 (26.12)	146 (28.46)
>72 inches	75 (21.07)	98 (19.10)
Missing	0 (0.00)	1 (0.19)
Undescended testes or cryptorchidism		
No	312 (87.64)	500 (97.47)
Yes	41 (11.52)	11 (2.14)
Missing	3 (0.84)	2 (0.39)
Family history of TGCC		
No	252 (70.79)	408 (79.53)
Yes	7 (1.97)	4 (0.78)
Missing	97 (27.25)	101 (19.69)
Injury to testes or groin[a]		
No	213 (59.83)	358 (69.79)
Yes	142 (39.89)	154 (30.02)
Missing	1 (0.28)	1 (0.19)
Vigorous exercise or sports activities[b]		
No exercise	19 (5.34)	25 (4.87)
$\leqslant$12 h per month	89 (25.00)	162 (31.58)
>12 h per month	119 (33.43)	161 (31.38)
Missing	129 (36.24)	165 (32.16)

Abbreviation: TGCC = testicular germ cell cancer.
[a]Injury to testes or groin that prevented normal activities for at least 5 min.
[b]Vigorous exercise or sports activities in the past 2 years.

Adjusted ORs for the association between use of MBS and risk of TGCC are presented in Table 2. Compared with men who never used MBS, the OR for ever use in relation to TGCC risk was 1.65 (95% CI: 1.11–2.46) (Table 2). Compared with men who did not use MBS, the strongest associations with risk of TGCC were observed in those who used MBS before the age of 25 years (OR = 2.21, 95% CI: 1.34–3.63), in men who ever used $\geqslant 2$ types of MBS (OR = 2.77, 95% CI: 1.30–5.91), and in those who used MBS for >36 months (OR = 2.56, 95% CI: 1.39–4.74) (Table 2).

Analyses by TGCC subtype suggested similar associations between use of MBS and the risk of seminoma and non-seminoma (Table 3) (all the *P*-values for hierarchical coefficients tests were >0.05). We further conducted exploratory stratified analyses examining associations with TGCC for the major types of MBS use reported by the study population and found that the use of MBS containing ingredients of both creatine and proteins increased the risk of TGCC significantly (OR = 2.55, 95% CI: 1.05–6.15).

Table 2. Association Between MBS Use and the Risk of TGCC, Connecticut and Massachusetts, 2006–2010

MBS use	No. of cases (%)	No. of controls (%)	OR (95% CI)	Adjusted OR (95% CI)[a]
MBS				
Never	289 (81.18)	451 (87.91)	1	1
Ever[b]	67 (18.82)	62 (12.09)	**1.69 (1.16–2.46)**	**1.65 (1.11–2.46)**
Age at first use (13–50 years)				
Never use	289 (81.18)	451 (87.91)	1	1
⩾25 years	17 (4.78)	30 (5.85)	0.88 (0.48-1.63)	1.00 (0.52–1.91)
<25 years	50 (14.04)	32 (6.24)	**2.44 (1.53–3.89)**	**2.21 (1.34–3.63)**
Number of types used				
Never use	289 (81.18)	451 (87.91)	1	1
1 type	42 (11.8)	51 (9.94)	1.29 (0.83-1.98)	1.38 (0.87–2.17)
⩾2 types	25 (7.02)	11 (2.14)	**3.55 (1.72–7.32)**	**2.77 (1.30–5.91)**
Duration of use				
Never use	289 (81.18)	451 (87.91)	1	1
⩽12 months	22 (6.18)	32 (6.24)	1.07 (0.61–1.88)	1.13 (0.63–2.05)
13–35 months	13 (3.65)	11 (2.14)	1.84 (0.82–4.17)	1.53 (0.64–3.65)
⩾36 months	32 (8.99)	19 (3.70)	**2.63 (1.46–4.73)**	**2.56 (1.39–4.74)**

Abbreviations: CI = confidence interval; MBS = muscle-building supplement; OR = odds ratio; TGCC = testicular germ cell cancer.
[a]Adjusted for age (continuous variable), race (Whites *vs* others), years of education (⩽12 *vs* >12 years), tobacco smoking (ever *vs* never), alcohol drinking (ever *vs* never), height (⩽68, 69–70, 71–72, >72 inches), undescended testes or cryptorchidism (no, yes, missing), injury to testes or groin (no, yes, missing), vigorous exercise or sports activities (no exercise, ⩽12 h per month, >12 h per month, missing), and family history of TGCC (no, yes, missing).
[b]At least once a week for 4 consecutive weeks.
The values in bold indicate statistically significant associations.

Table 3. Association Between MBS Use and the Risk of TGCC, by Histological Type, Connecticut and Massachusetts, 2006–2010

		Seminoma			Non-seminoma		
MBS use	No. of controls (%)	No. (%)	OR (95%CI)	Adjusted OR (95% CI)[a]	No. (%)	OR (95% CI)	Adjusted OR (95% CI)[a]
MBS use							
Never	451 (87.91)	154 (81.48)	1	1	119 (80.41)	1	1
Ever	62 (12.09)	35 (18.52)	**1.65 (1.05–2.60)**	**1.90 (1.17–3.08)**	29 (19.59)	**1.77 (1.09–2.88)**	1.58 (0.94–2.64)
Age at first use							
Never	451 (87.91)	154 (81.48)	1	1	119 (80.41)	1	1
⩾25 years	30 (5.85)	10 (5.29)	0.98 (0.47–2.04)	1.12 (0.51–2.45)	6 (4.05)	0.76 (0.31–1.86)	0.87 (0.34–2.22)
<25 years	32 (6.24)	25 (13.23)	**2.29 (1.31–3.98)**	**2.63 (1.45–4.76)**	23 (15.54)	**2.72 (1.54–4.83)**	**2.13 (1.16–3.93)**
Number of types used							
Never	451 (87.91)	154 (81.48)	1	1	119 (80.41)	1	1
1 type	51 (9.94)	22 (11.64)	1.26 (0.74–2.15)	1.58 (0.90–2.76)	18 (12.16)	1.34 (0.75–2.37)	1.33 (0.73–2.42)
⩾2 types	11 (2.14)	13 (6.88)	**3.46 (1.52–7.89)**	**3.23 (1.35–7.75)**	11 (7.43)	**3.79 (1.60–8.95)**	**2.57 (1.04–6.33)**
Duration of use							
Never	451 (87.91)	154 (81.48)	1	1	119 (80.41)	1	1
⩽12 months	32 (6.24)	9 (4.76)	0.82 (0.38–1.76)	1.03 (0.47–2.28)	12 (8.11)	1.42 (0.71–2.84)	1.33 (0.64–2.75)
13–35 months	11 (2.14)	7 (3.70)	1.86 (0.71–4.89)	1.85 (0.67–5.15)	5 (3.38)	1.72 (0.59–5.05)	1.40 (0.45–4.37)
⩾36 months	19 (3.70)	19 (10.05)	**2.93 (1.51–5.68)**	**3.33 (1.64–6.74)**	12 (8.11)	**2.39 (1.13–5.07)**	2.09 (0.95–4.60)

Abbreviations: CI = confidence interval; MBS = muscle-building supplement; OR = odds ratio; TGCC = testicular germ cell cancer.
[a]Adjusted for age (continuous variable), race (Whites *vs* others), years of education (⩽12 *vs* >12 years), tobacco smoking (ever *vs* never), alcohol drinking (ever *vs* never), height (⩽68, 69–70, 71–72, >72 inches), undescended testes or cryptorchidism (no, yes, missing), injury to testes or groin (no, yes, missing), vigorous exercise or sports activities (no exercise, ⩽12 h per month, >12 h per month, missing), and family history of TGCC (no, yes, missing).
The values in bold indicate statistically significant associations.

DISCUSSION

MBS use was found to be associated with an increased risk of TGCC. The associations were stronger among early users, men using ⩾2 types of MBS, and longer use of MBS. To our knowledge, this is the first analytical epidemiological study to explore the association between MBS use and the risk of TGCC.

Little is known about the aetiology of TGCC, particularly factors that would explain the rapid incidence increases in this disease. The increasing trends for seminoma and non-seminoma are similar, suggesting that they may share some important causal factors (Richiardi *et al*, 2004), which was also suggested in our present study as the risk associated with MBS use was similar by subtype. In addition to the ingredients in MBS that are known, there are also so-called natural components that may act as artificial hormones and other impurities that may vary by product. It has been documented that some commercially available supplement products contain less active ingredients than indicated on the product label or 'hidden' ingredients that are not listed on the label (Green *et al*, 2001). An international study found that ~15% of commercially available non-hormonal supplements contained undeclared anabolic androgenic steroids, including prohormones of nandrolone, which have been associated with development of testicular cancer in rats (Geyer *et al*, 2004; Chimento *et al*, 2012). Whether those ingredients have a role in the risk of TGCC in humans is currently unclear. Therefore, our preliminary findings suggest that the long-term

effects of MBS use, such as increased cancer risk, and its mechanisms, need to be further investigated. Of particular interest would be further evaluation of the potential effects of the combined use of multiple types of MBS at the same time.

In our study, nearly 20% of cases with TGCC had used MBS, which was similar to the previous case series study (Chang *et al*, 2005). Despite the fact that self-reported questionnaire data was used in our study, differential recall of MBS use by the cases and controls is unlikely as an association with MBS has not previously been reported in an epidemiological study and therefore this exposure would likely not be a suspected risk factor for TGCC among study subjects at the time of the interview. We also note that the associations with MBS use for TGCC remained significantly elevated after adjustment of the models for major potential confounders, with risks of over two-fold persisting in the adjusted models for the earliest and longest users of MBS. Strengths of our population-based study include the use of standardised in-person interviews conducted by trained interviewers that included detailed questions on lifetime MBS use and inclusion of only histologically confirmed incident TGCC cases in our study, which minimised the possibility of disease misclassification.

In conclusion, our study suggests that MBS use might contribute to the risk of TGCC, both seminoma and non-seminoma. Considering the magnitude of the association and the observed dose–response trends, MBS use may be an important and potentially modifiable exposure that could have important scientific and clinical importance for preventing TGCC development if this association is confirmed by future studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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