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Handgrip Strength and Vertical Jump and their Relationship with Body Fat in Hong Kong Chinese Children and Adolescents

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Abstract

Aim: To examine the associations of handgrip strength and vertical jump with gender, pubertal status and body composition, and establish normal reference values of handgrip strength and vertical jump of Hong Kong Chinese children and adolescents.

Methods: This study included 1154 children and adolescents aged between 8 and 17 years, who participated in a territory-wide cohort study. Data of anthropometry, pubertal status handgrip strength and vertical jump were collected. Percentile curves of handgrip strength and vertical jump were constructed using the LMS method. General linear model was used to evaluate the effects of age, sex, pubertal stage, body size, body fat and the possible 2-way interactions on handgrip strength and vertical jump.

Results: According to the international BMI cutoffs, the prevalence rate of overweight or obesity (20.7%) in our cohort of children was similar to that obtained from previous local report. General linear model revealed that handgrip strength and vertical jump increased with increasing age, and boys were significantly stronger than girls after aged 12 year or older. Among overweight/obese children, those with high body fat had significantly lower handgrip strength than those with low body fat. A full model including age, sex, BMI z score, body fat z score and age*sex interaction explained 67.8% and 60.1% of the variance of handgrip strength and vertical jump respectively. Handgrip strength and vertical jump was positively associated with age, male sex and BMI z score, but was negatively associated with body fat z score.

Conclusions: Classifying children's weight status by BMI cutoffs, additional information on children's body composition should also be considered. Reference values for handgrip strength and vertical jump are established for Hong Kong Chinese children and adolescents aged 8 to 17 years.

Keywords: bioelectrical impedance; normative fitness values; body fatness, muscular strength

Introduction

Childhood obesity is the most serious global public health challenges of the 21st century [1]. It is reaching alarming proportions in many countries, in just 40 years the number of schoolchildren with obesity has risen more than 10-fold, from 11 million to 124 million [2]. Hong Kong, as one of the most urbanized cities in China, cannot escape from this global epidemic and the overweight prevalence in Hong Kong children was 20.4% [3]. Childhood obesity undermines the physical, social and psychological well-being of children [4,5]. One of the most possible explanations for this global epidemic consists in the decline of fitness, produced primarily by decreases in physical fitness [6]. Obesity and physical fitness are two interrelated factors and changes in one may cause changes in the other [7]. A recent longitudinal study confirmed a strong reciprocal relationship between physical fitness and obesity in Hong Kong children [8].

Muscular strength, as an important component of physical fitness, has been increasingly recognized in the pathogenesis and prevention of disease [9,10]. Some evidence suggests that muscular strength is inversely and independently associated with cardiovascular and all-cause mortality events in both healthy adults and clinical populations [9,11]. Muscular strength is also inversely associated with age-related weight gain, risk of hypertension and prevalence of metabolic syndrome [9,12,13]. Similar associations have also been reported in children [14-16]. This phenomenon may be partly explained by the fact that muscle tissue is an important organ influencing metabolism and can directly affect risk of metabolic diseases [17]. However, muscular strength changes with growth, and therefore, age-specific values obtained in healthy children should serve as a reference for with acute and chronic conditions using muscle strength for diagnostic purposes, follow-up, or to assess the efficacy of therapy [18]. For population-based studies, it is essential that the techniques involved should be simple and quick, so that such studies do not have follow laboratory conditions strictly. Two tests which satisfy these conditions are the handgrip and the vertical jump.

The vertical jump, a measure of lower body power, and handgrip strength, a measure of upper-limbs muscular strength, have both been acknowledged as being strong measures of one's health, and recommended for potential use in school fitness testing which in line with recent recommendations [19]. Moreover, handgrip strength can be used as a tool to have a rapid indication of someone's general muscle strength [20]. Meanwhile, the vertical jump is a simple method to calculate peak leg power which is a component of test batteries used to assess physical ability [21]. Both measurements are inexpensive, easy and reliable method of muscular strength assessment [16,22,23].

In recent studies, handgrip strength is reported to be differed significantly across ethnic groups, with lower handgrip strength associated with higher prevalence of type 2 diabetes mellitus [24,25]. This highlights the importance of ethnic-specific reference standards for screening and monitoring purposes. Normative data for handgrip strength and/or vertical jump have been developed for children in different countries [22,26-31]. Only one recent publication from China mainland reported the reference data of the muscular strength [32]. However, the association between muscular strength and the Anthropometric measurements was note addressed in this report. Among the published reports, few explored this association [22,26,33]. Furthermore, muscular strength is correlated with BMI and, particularly, muscle mass [34]. However, this could simply reflect the gender difference because of the effect of sex steroid hormones [35,36]. In fact, scientific evidence suggests that Asians have different associations between weight status, body composition and health risks than do European populations. For example, in some Asian populations a specific BMI reflects a higher percentage of body fat than in white or European populations [37]. The association of muscular strength, weight status, and body composition in Hong Kong Chinese children and adolescents is not known. In this study, we aimed to examine the associations of handgrip strength and vertical jump with gender, anthropometric variables and body composition. We also establish normal reference values for handgrip strength and vertical jump for Hong Kong Chinese children.

Materials & Methods

Design

This cross-sectional study measured grip strength in a cohort of healthy children and adolescents. The data were used to generate normative values for handgrip strength and vertical jump.

Subjects

This was a part of a territory-wide cohort study on 24-h ambulatory blood pressure of Chinese children and adolescents conducted in 2011 to 2012 [37]. A two-stage cluster sampling method was used. Data from the Education Bureau, the government of the Hong Kong Special Administrative Region, were used to compile a sampling frame of all schools in Hong Kong. In the first stage, one primary school and one secondary school were randomly selected from each of the 18 Districts in Hong Kong. In the second stage, students were selected randomly by computer generated numbers and were invited to join the study. Details were mentioned in our previous publication [37]. An information sheet explaining the purpose and procedure of the study was given to each child and his/her parents. All children completed a validated self-reported Pubertal Development Scale [38]. Informed assent was obtained from the children and consent from their parents before the measurements. This study was approved by the Joint Chinese University of Hong Kong and New Territories East Cluster Clinical Research Ethics Committee. (CRE-2009.540)

Procedures

Anthropometric Measurements

A team of three trained research staff visited each selected school on a pre-arranged date for data collection. Standing height without shoes was measured using a stadiometer (seca 217, UK) to the nearest 0.1 cm. Body weight and percentage body fat were measured with light clothing using foot-to-foot bio-electrical impedance by a validated electronic body composition analyzer (Model BF-522, Tanita, Japan) [39,40]. Children emptied their bladder before the measurement. They were asked to stand barefoot on the metal sole plates of the machine, and gender and height details were entered manually into the system. Body weight and percentage body fat, estimated using the standard built in prediction equations for children, were displayed on the machine and printed out. Body mass index was converted to z score using local normal reference [41]. Children were classified into underweight, normal weight, overweight or obese based on their body mass index (BMI) using the International Obesity Task Force cut-offs [42]. Percentage body fat was also converted to z score using local normal reference [40]. Children were categorized into high and low body fat groups using the 85th percentile of the local reference as the cutoff [40].

Handgrip Strength

Handgrip strength was done by an assessor with background of Sports Science and Physical Education. Each subject was given a brief demonstration and verbal instructions for the handgrip strength test using the Takei T.K.K.5001 GRIP-A handgrip dynamometer (Takei Scientific Instruments Co. Ltd, Tokyo, Japan). The dynamometer was adjusted according to the child's hand size. The test was done in the standing position, with the wrist in the neutral position and the elbow extended. Subjects were given verbal encouragement to 'squeeze as hard as possible' and apply maximal effort for at least 2 seconds. Two trials were allowed in the dominant arm and the highest score recorded as peak grip strength (kg) [43]. Limb dominance was determined by asking the children whether they are left-handed or right-handed.

Vertical Jump

Vertical jump skill was assessed by means of the process-oriented method proposed in the Western Australian Teachers Resources [Department of Education Western Australia (EDWA), 2013] done by two trained assessors. A demonstration of how to jump was provided to each subject and he/she was allowed to practice the jump until meeting the jump criteria, which usually takes two jumps. The jump was a countermovement jump with the use of arms. The jump began from a standing position, keeping the feet flat on the ground, with the preferred shoulder adjacent to a wall. Standing reach height was obtained by asking the subject to reach up with his/her hand as high as possible to touch the wall. After that, the child bent knees to about a 90 degree angle while moving their arms back in a winged position; then thrusted forward and upward and touched the wall at the highest point of the jump. The results of the jump was measured and recorded on a centimeter scale (cm).Vertical jump score was calculated as the difference in distance between the standing reach height and the jumping height. Two jumps using the correct technique were allowed for each subject and the best score was retained for analysis [30].

Statistical Analysis

Statistical analyses were performed using PASW Statistics 21.0 (IBM SPSS Inc., New York, USA). Percentile curves were constructed using LMS method [44]. The LMS method estimates the measurement centiles in terms of three age-sex-specific cubic spline curves: the L curve (Box-Cox power to transform the data that follow a Normal distribution), M curve (median) and S curve (coefficient of variation). In brief, if Y(t) denotes an independent positive data (e.g. handgrip) at age t, the distribution of Y(t) can be summarized by a normally distributed SD score (Z) as follows:

$$Z = \frac{\left[Y(t)/M(t) \right]^{L(t)} - 1}{L(t)S(t)}$$

Once the L(t), M(t), and S(t) have been estimated for each parameter at age t, the 100α th centile at t age could be derived from

C100α(t) = M(t) [1 + L(t)S(t)Zα]1/L(t)

where Zα is the α centile of the Normal distribution (for example for the 95th centile, α = 0.95 and Zα = 1.65). The LMS program (version 12.43, Institute of Child Health, London, UK) was employed to fit the data.

The Q-Q test was used to assess the normality of the anthropometric, handgrip and vertical jump variables ($p > 0.05$). Estimated marginal means for handgrip strength and vertical jump were generated and age and gender interactions were determined using two-way analysis of covariance (ANCOVA) with mass and stature as covariates. General linear model was used to evaluate the effects of age, sex, pubertal stage, body size, body fat and the possible 2-way interactions on handgrip strength and vertical jump. Significance level was set at $p < 0.05$.

Sample Size Calculation

Assuming both handgrip strength and vertical jump are normally distributed among each age and gender, sample size was calculated in terms of the standard deviation of the

$100\alpha^{th}$ centile ($SD_{c100\alpha}$) and the age- and gender-specific SD described by Healy [45] as:

$$SD(c100\alpha) = \sqrt{\left[\left(1+k^2/2\right)/n\right]} * SD$$

$$i.e. \qquad n = \frac{\left(1+k^2/2\right) * SD^2}{SD(c100\alpha)^2}$$

where k is the appropriate value from the standard normal distribution. For 97th centile, k = 1.88.

To find out the age and gender-specific means and SDs for sample size calculation, pilot data were collected from 200 healthy children aged 8-17 years. The required sample sizes for each gender and age group to obtain an extreme centile, i.e. the 97th centile, with an error of ±4% were listed in supplementary table. The estimated total number of subjects required for handgrip strength and vertical jump are 944 and 794, respectively (S1 Table).

S1 Table: Handgrip strength and vertical jump data collected from 200 healthy children aged between 8-17 years.

	Age, y	N	Mean	SD	N required to obtain the 97th centile with 4% error
Handgrip strength, kg					
Girls	8 to 9	20	10.7	2.0	34
	10 to 11	20	12.3	3.4	58
	12 to 13	20	17.3	4.3	49
	14 to 15	20	19.5	3.4	30
	16 to 17	20	21.2	4.9	45
Boys	8 to 9	20	10.8	3.5	69
	10 to 11	20	14.0	3.4	47
	12 to 13	20	18.1	4.1	44
	14 to 15	20	25.2	5.1	37
	16 to 17	20	32.1	9.0	59
				Total	(Sum of the above x 2 =) 944
Vertical jump, cm					
Girls	8 to 9	20	22.2	5.4	48
	10 to 11	20	24.0	4.9	38
	12 to 13	20	27.4	4.6	28
	14 to 15	20	27.5	4.1	24
	16 to 17	20	30.2	7.8	52
Boys	8 to 9	20	23.4	4.1	30
	10 to 11	20	25.2	6.1	48
	12 to 13	20	34.2	7.0	38
	14 to 15	20	45.9	10.6	45
	16 to 17	20	48.3	11.3	46
				Total	(Sum of the above x 2 =) 794

Results

Subject Characteristics

A total of 1175 subjects aged 8-17 years from 32 schools (14 primary and 18 secondary schools) participated in the study. Twenty-one students were excluded due to incomplete data. The remaining 1154 subjects (49.3%, 569 boys) were included in the final analysis. Sex- and age-specific characteristics are shown in (Table 1). No subjects had any previous history of metabolic disease, and no participants were taking any type of medication. The mean ± SD age for boys and girls were 12.6y ± 2.7 (range: 8.2–17.9y) and 12.7y ± 2.8 (range: 8.1–17.9y) respectively.

Table 1: Subject characteristics of 1154 children by age and sex.

	Age	n	Height, cm	Weight, kg	BMI, kgm^{-2}	Body fat, %	Handgrip, kg	Vertical jump, cm	Pubertal stage
Male	8	51	133 ± 6	32.2 ± 9.5	17.8 ± 3.9	21.1 ± 8.0	11.0 ± 2.4	22.4 ± 4.4	2 (2 to 2)
	9	78	136 ± 6	33.5 ± 8.1	18.0 ± 3.4	20.1 ± 6.9	12.0 ± 2.9	24.7 ± 5.0	2 (1 to 2)
	10	64	140 ± 6	37.3 ± 9.6	18.8 ± 3.8	21.1 ± 7.9	13.4 ± 3.2	23.8 ± 5.8	2 (2 to 2)
	11	51	147 ± 8	42.3 ± 12.8	19.2 ± 4.2	20.8 ± 7.4	15.2 ± 4.0	26.8 ± 6.6	2 (2 to 2)
	12	70	154 ± 9	46.6 ± 10.6	19.4 ± 3.5	18.4 ± 6.4	18.4 ± 5.3	33.2 ± 7.7	2 (2 to 3)
	13	69	161 ± 8	52.3 ± 13.3	20.0 ± 4.0	18.5 ± 7.1	21.8 ± 6.1	37.3 ± 9.2	3 (3 to 4)
	14	51	165 ± 8	53.8 ± 10.9	19.5 ± 2.9	16.4 ± 5.1	24.5 ± 6.0	40.7 ± 7.9	4 (3 to 4)
	15	55	170 ± 6	60.3 ± 13.9	20.8 ± 4.3	19.6 ± 6.3	26.5 ± 5.7	43.4 ± 8.7	4 (3 to 4)
	16	46	173 ± 6	65.3 ± 16.6	21.8 ± 4.8	20.5 ± 7.0	30.5 ± 7.9	45.5 ± 10.0	4 (4 to 4)
	17	34	171 ± 6	61.0 ± 10.4	20.9 ± 3.2	20.4 ± 6.0	32.8 ± 6.7	49.1 ± 7.8	4 (4 to 5)
Female	8	55	130 ± 6	29.0 ± 7.1	17.0 ± 3.5	18.0 ± 8.2	10.1 ± 2.0	21.2 ± 5.7	1 (1 to 2)
	9	86	135 ± 7	30.9 ± 6.9	16.7 ± 2.7	17.5 ± 6.6	11.5 ± 2.4	23.1 ± 5.5	1 (1 to 2)
	10	60	143 ± 7	37.4 ± 10.3	18.0 ± 3.5	19.0 ± 7.1	13.7 ± 3.4	24.4 ± 4.4	2 (1 to 2)
	11	62	150 ± 8	43.1 ± 10.7	18.9 ± 3.8	21.3 ± 7.4	15.4 ± 3.7	26.0 ± 5.9	2 (2 to 3)
	12	56	154 ± 6	45.9 ± 10.5	19.2 ± 3.7	22.3 ± 7.8	17.7 ± 3.3	27.7 ± 5.5	3 (2 to 4)
	13	51	155 ± 6	47.5 ± 9.1	19.6 ± 3.2	23.9 ± 6.9	19.1 ± 4.6	27.5 ± 5.2	3 (3 to 4)
	14	56	158 ± 5	50.5 ± 7.9	20.2 ± 2.9	25.0 ± 6.0	19.8 ± 4.2	27.2 ± 5.5	4 (4 to 4)
	15	49	160 ± 5	52.3 ± 8.9	20.5 ± 3.1	26.3 ± 6.9	20.8 ± 4.5	29.2 ± 6.3	4 (4 to 4)
	16	75	160 ± 5	51.2 ± 7.4	20.1 ± 2.5	25.6 ± 5.8	20.9 ± 4.0	30.1 ± 6.5	4 (4 to 4)
	17	35	158 ± 4	49.9 ± 6.4	20.0 ± 3.0	25.1 ± 6.4	22.0 ± 3.9	30.1 ± 5.8	4 (4 to 4)

According to the BMI cutoffs from the International Obesity Task Force (IOTF) [42], 13.9% (160/1154), 65.4% (755/1154), 15.1% (174/1154) and 5.6% (65/1154) of subjects were classified as underweight, normal weight, overweight and obese, respectively. The prevalence rate of overweight or obesity (20.7%) in our cohort of children was similar to that (20.4%) obtained from the previous Hong Kong Student Health Service Survey in 2008/2009 [3]. A total of 235 (20.4%) subjects were classified as having high percentage body fat, of whom 184 were overweight/obese and 51 were normal weight by IOTF definitions.

Handgrip Strength

The smoothed age-specific centile curves for boys and girls are shown in (Figure 1). General linear model revealed that handgrip strength was positively associated with age (F=1763, p <0.001), male sex (F=96.8, p <0.001) and the age*sex interaction (F=159, p <0.001). The age- and sex-specific error bar chart demonstrated that the sex difference was significant for subjects aged 13 years or older. (Figure 2) The pubertal stage*sex interaction was also significant (F=22.5, p <0.001). Significant sex differences were observed in subjects of pubertal stage III or later. (Figure 3)

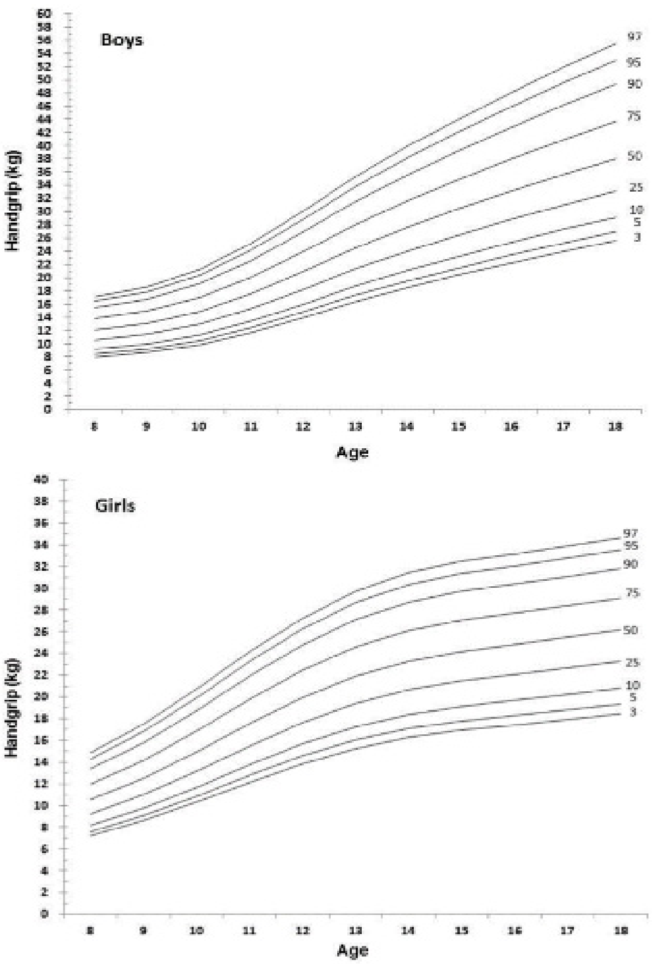

Figure 1: Smoothed centiles curves of handgrip strength for Hong Kong Chinese Children aged 8 to 17 years.

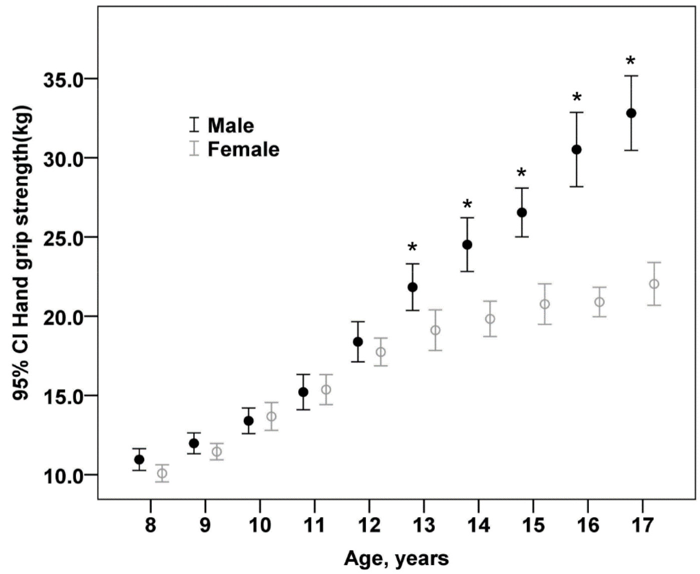

*Figure 2: Error bar charts of handgrip strength by age and sex *indicates significant sex difference, p <0.05.*

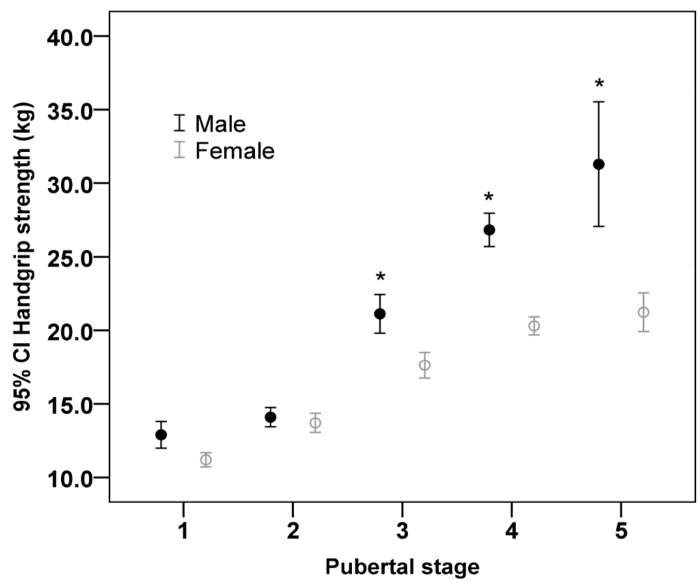

*Figure 3: Error bar charts of handgrip strength by pubertal stage and sex *indicates significant sex difference, p <0.05.*

BMI z score was positively associated with handgrip strength (F=12.9, p <0.001). The interaction between BMI z score and body fat z score was also significant (F=12.9, p <0.001). Further analysis revealed that among overweight/obese children, those with high body fat had significantly lower handgrip strength than those with low body fat [estimated marginal mean (SE): 18.0kg (0.5) c.f. 20.3kg (1.0), p = 0.039].

A full model including age, sex, BMI z score, body fat z score and age*sex interaction explained 67.8% of the variance of handgrip strength. The model demonstrated

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that handgrip strength was positively associated with age, male sex and BMI z score, but was negatively associated with body fat z score. (Table 2) The BMI z score*body fat z score interaction became insignificant (p = 0.83) in the fully adjusted model.

Table 2. Significant correlates of handgrip strength and vertical jump

	Handgrip strength				Vertical jump		
	β (SE)	p	Partial Eta squared (effect size)		β (SE)	p	Partial Eta squared (effect size)
Age, y	1.5 (0.1)	<0.001	0.65		1.1 (0.1)	<0.001	0.47
Sex (1 = male, 2 = female)	-11.5 (1.2)	<0.001	0.08		-19.5 (1.9)	<0.001	0.10
Age*Sex	1.1 (0.1)	<0.001	0.12		2.1 (0.1)	<0.001	0.18
BMI z score	2.4 (0.2)	<0.001	0.10		2.3 (0.4)	<0.001	0.04
Body fat z score	-1.3 (0.2)	<0.001	0.03		-3.4 (0.4)	<0.001	0.09
R squared	0.678				0.601		

Vertical Jump

The smoothed age-specific centile curves for boys and girls are shown in (Figure 4). General linear model revealed that vertical jump was positively associated with age (F=800, p <0.001), male sex (F=116, p <0.001) and the age*sex interaction (F=229, p <0.001). The age- and sex-specific error bar chart demonstrated that the sex difference was significant for subjects aged 12 years or older. (Figure 5) The pubertal stage*sex interaction was also significant (F=36.0, p <0.001). Significant sex differences were observed in subjects of pubertal stage II or later. (Figure 6) Vertical jump was positively associated with BMI z score (F=9.5, p = 0.002) but negatively associated with body fat z score (F=16.7, p <0.001). The interaction between BMI z score and body fat z score was not significant (F=2.1, p = 0.15).

A full model that included the same list of factors as those correlated with handgrip strength, i.e. age, sex, BMI z score, body fat z score and age*sex interaction, explained 60.1% of the variance of vertical jump. (Table 2) The model demonstrated that vertical jump was positively associated with age, male sex and BMI z score, but was negatively associated with body fat z score. (Table 2)

Discussion

We established age and gender specific normative values of handgrip strength and vertical jump in Hong Kong Chinese children. Although another report has provided normative data previously [32], the subgroups according to age and gender only mean and standard deviation were

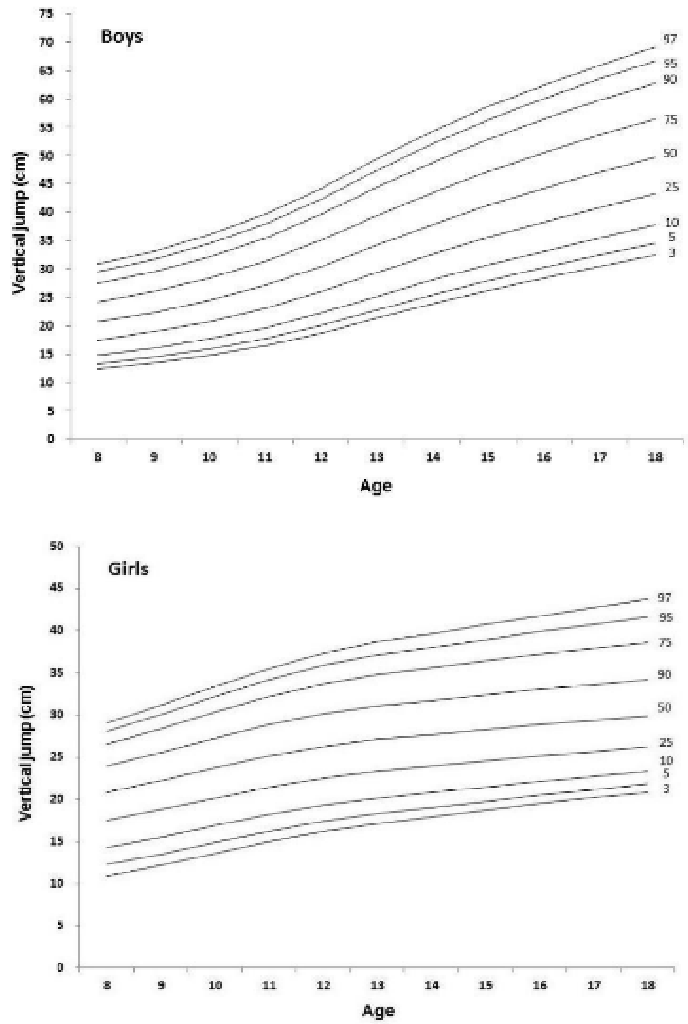

Figure 4: Smoothed centiles curves of vertical jump for Hong Kong Chinese Children aged 8 to 17 years.

shown in most studies [46]. Handgrip strength and vertical jump increase with age in both genders, with boys stronger than girls particularly after the age of 12 years.

Similar to previous investigations, maximal handgrip strength was measured in ACFIES [43], EUROFIT [47] and CHMS [48], while the maximal jump height was reported in a sample of English school children [30]. Our results are close to the Britain children in both handgrip strength [43] and vertical jump [30]. As expected, our result was very different from those of CHMS, performed in Canadian children with handgrip strength between 24 and 89 kg in boys and between 21 and 56 kg in girls aged 8–19 years old [48]. Our finding indicates the importance of having a reference value for different populations.

In regard of the gender difference, body composition is largely due to the action of sex steroid hormones [35], probably leading to a difference in muscular strength.

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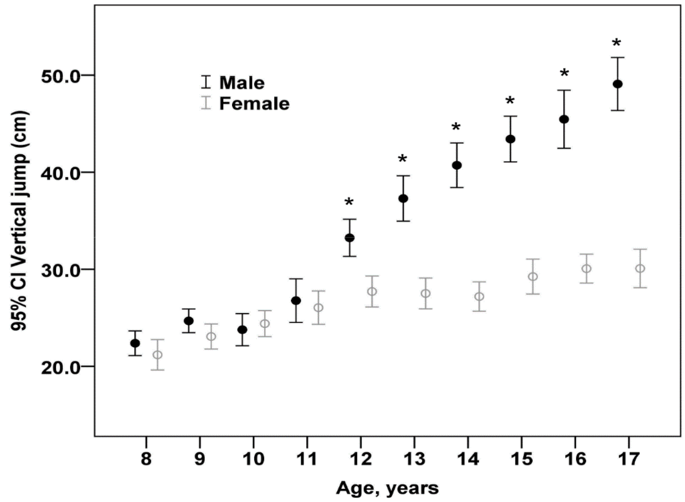

*Figure 5: Error bar charts of vertical jump by age and sex *indicates significant sex difference, p <0.05.*

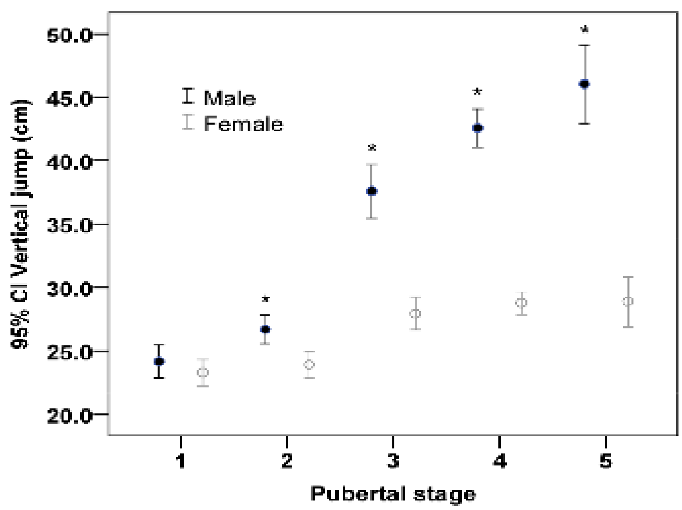

*Figure 6: Error bar charts of vertical jump by pubertal stage and sex *indicates significant sex difference, p <0.05.*

Nevertheless in boys, growth hormone and testosterone have more effects on muscular strength than in girls [49]. In our study, age, gender, BMI, and body fat were important predictors of handgrip strength and vertical jump, which were in line with previous findings from other countries [22,26-31]. There were only a few reports on the associations between handgrip strength and weight status [22,26]. Our finding was similar to these studies [22,26] that handgrip strength increased with weight status, as reflected by BMI. Importantly, our further analysis showed that overweight and obese children with high body fat had significantly lower handgrip strength compared to their overweight and obese peers with low body fat. Our study also found that vertical jump was positively associated with BMI but negatively associated with body fat. Children who were heavier, or being classified into overweight and obese categories, may have increased or no increased lean muscle mass in addition to fat [50], that the increased lean muscle mass may contributes to the better performance of handgrip strength and vertical jump.

BMI, as a measure of weight adjusted for height, correlates with body fat and with cardiovascular risk factors in children and adolescents, and a high value also predicts future adiposity, morbidity and death [51], Although BMI is the most widely used surrogate measure for screening for obesity, it cannot distinguish between fat mass and lean muscle mass. Thus, individuals with increased muscle mass may have increased BMI and although classifying as overweight their body fat level may still within normal range and they have low risk for cardiovascular risk factors. In our sample, about 20% of the children we tested fell into this category. It highlights the importance that when classifying children's weight status by BMI cutoffs, additional information on children's body composition such as percentage body fat, or fat-free mass should also be considered.

This study has some limitations. Our findings should be interpreted with caution as it is a cross-sectional study, it cannot demonstrate cause-and-effect. A longitudinal study is required to assess the longer-term health outcomes which may be associated with handgrip strength and vertical jump. Second, we utilized bioelectrical impedance as a measure of percentage of body fat and this technique is not without its limitations in children. Poor validity and measurement error have been reported [52], although, previous work in the same population has shown it is an adequate surrogate for percentage of body fat when compared to dual x-ray absorptiometry [53].

Advantages of this study include – huge sample size and pretty representative of the territory. It is noteworthy considering handgrip strength and vertical jump as a physical fitness test battery for the schoolchildren because it is more likely to be implemented in normal physical education settings.

Conclusion

The reported data enables health professionals to identify children and adolescents with poor strength according to age, gender and body composition, and to evaluate the effects of therapeutic interventions. Reference values for handgrip strength and vertical jump are provided for Hong Kong Chinese children and adolescents aged 8 to 17 years.

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Authors' Contributions

CCWY led the study conception and designed the study, participated in the coordination and execution of the study, and drafting, writing, and revising of the manuscript; HKS participated in coordination and execution of the study and drafting, and revising of the manuscript; CTA contributed to the acquisition of data analysis and interpretation of data; AMM, AML and RYTS participated in conceptualizing and designing the study, and revising of the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing Interests

The authors declare that they have no competing interests.

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