TITLE:
THE INFLUENCE OF MATURITY STATUS ON MUSCLE ARCHITECTURE IN
SCHOOL-AGED BOYS

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ABSTRACT

**Purpose:** To determine the differences in muscle architecture of the lower limb in pre-, circa- and post-peak height velocity (PHV) boys. **Methods:** Muscle architecture variables from both the gastrocnemius medialis (GM) and vastus lateralis (VL) were derived from ultrasonographic images in 126 school-boys. One-way ANOVA’s using Bonferroni post-hoc comparisons were employed to determine between-group differences and effect sizes were calculated to establish the magnitude of these differences. **Results:** All muscle architecture variables showed significant small to large increases from pre- to post-PHV, excluding GM fascicle length ($d = 0.59 – 1.39; p < 0.05$). More discrete between-group differences were evident as GM and VL muscle thickness and physiological thickness significantly increased between pre- and circa-PHV ($d > 0.57; p < 0.05$); however only the VL muscle thickness and physiological thickness increased from circa- to post-PHV ($d = 0.68; p < 0.05$). The post-PHV group also showed larger GM pennation angles than the circa-PHV group ($d = 0.59; p < 0.05$). **Conclusion:** The combined results show that maturation is associated with changes in muscle morphology. These data quantify the maturity-related changes in muscle architecture variables provide a reference to differentiate between training induced adaptations versus changes associated with normal growth and maturation.
INTRODUCTION

Notable disparities in anatomy and physiology exist between children and adults, and there are also clear differentiations between children and adolescents, mediated by growth and maturation (23, 35). Force-producing capacities are lower in children when compared to adolescents or adults, partly as a result of the architecture and size of the muscle (18, 20, 30) and specific muscle activation patterns (9). Throughout maturation, the neuromuscular system develops in a non-linear manner, resulting in large variations in physical performances of children of similar chronological age (26). It is known that maturity leads to increased body mass and fat free mass (23), however, the specific structural adaptations that occur naturally throughout growth and maturation are yet to be fully understood. Very few studies have specifically examined how muscle size changes throughout childhood and adolescence, and how the maturation process may result in specific architectural changes. Considering that muscle architecture is an important factor when it comes to determining a muscle’s function and influences both force production and contraction velocity (21), examining maturity-related developmental changes in muscle architecture is warranted.

Muscle architecture is typically characterised by parameters such as fascicle length, pennation angle and cross-sectional area (5). Despite fascicular arrangements significantly influencing a muscle function (7), particularly the fascicle’s force-length and force-velocity characteristics (36), age- and maturity-related comparisons of muscle architecture measures between adults, adolescents, and children are sparse. Research suggests that adults demonstrate greater muscle thickness than children (19, 29) while older adolescents exhibit greater muscle thickness relative to their younger peers (17, 18). Men have been shown to have longer fascicles than boys, and this remains the same for fascicle length when made relative to thigh length (20). Furthermore, 15-year-old adolescents have been shown to possess significantly longer muscle fascicles than children, but do not differ from adults (17), implying that fascicle length
reaches adult levels at or before the age of 15 years. Pennation angle of the knee extensor muscles seems to remain consistent from childhood through to adulthood (17), whereas the pennation angle of the gastrocnemius medialis has been reported to increase from birth before becoming stable following the adolescent growth spurt (3, 14). However, maturational status was not quantified in these studies, and the developmental effects on pennation angle throughout maturation is still unknown. The existing literature has focused on changes based on chronological age as opposed to grouping according to maturity status. Therefore, the manner in which muscle architecture changes throughout the non-linear process of maturational development remains unclear.

Literature has demonstrated that individuals of the same chronological age can differ markedly with respect to biological maturity (1). Furthermore, significant inter-individual variation exists for the level (magnitude of change), timing (onset of change), and tempo (rate of change) of maturation. Therefore, it is important to quantify muscle architecture in relation to maturity status as opposed to chronological age. Understanding how muscle architecture variables differ between maturity groups would better enable researchers to distinguish between changes caused by growth and maturation versus those mediated by physical training. Additionally, the majority of previous studies have compared a single age group during childhood versus adults and given that maturation is non-linear, the comparison of two discrete age groups does not highlight how muscle architecture changes throughout the fluctuating process of maturation (17, 20, 30). Finally, it should be noted that the majority of previous studies have not included children coincident with peak height velocity (PHV) which, due to the rapid gains in height and mass, is regarded as a unique stage of development within paediatric populations and can have significant effects on muscle architecture due to the differential lengthening of bone and muscle during this period.
Therefore, the aim of the current study was to investigate differences in muscle thickness, pennation angle, and fascicle length of vastus lateralis and gastrocnemius medialis of pre-, circa- and post-PHV boys. We hypothesised that muscle thickness, pennation angle, and fascicle length would increase across maturity groups, with the largest differences evident between the circa- and post-PHV groups.

METHOD

Participants

One hundred and eighty-three male secondary school children in the United Kingdom volunteered to participate in the study. Sample size was estimated a priori using statistical software (G*Power v 3.1) for the analyses of variance test, considering an effect size of 0.4, significance level of 0.05, and statistical power of 0.95, for the primary outcome variables of muscle thickness, pennation angle and fascicle length, a sample size of 102 was needed. All participants were free from lower extremity injury and were involved in regular sport and physical education-based activity programs, inclusive of strength and conditioning and games-based activities. Participants were asked to refrain from strenuous exercise at least 48 hours prior to testing. Written parental consent and participant’s assent were then obtained for participants under 18 years prior to involvement in the study, while ethical approval was granted by the Cardiff School of Sport Research Ethics Committee.

Procedures

Anthropometrics: Standing height (centimetres) and seated height (centimetres) were measured using a stadiometer (SECA 321, Vogel and Halke, Germany), and body mass (kilograms) was measured using a balance beam scale (SECA 770, Vogel and Halke, Germany). Biological maturity was then determined using years from PHV (28). Maturity offset values were
calculated using the sex-specific equation for boys: $-\left[9.236 + 0.0002708 \cdot \text{Leg Length and Sitting Height interaction}\right] - \left[0.001663 \cdot \text{Age and Leg Length interaction}\right] + \left[0.007216 \cdot \text{Age and Sitting Height interaction}\right] + \left[0.02292 \cdot \text{mass by Height ratio}\right]$. Due to the error in the prediction equation of approximately 6 months for boys (16), participants with a maturity offset of -1 to -0.5 years, and +0.5 to +1 years were removed from the study. Additionally, participants whose maturity offset was outside -3 or +3 years were also removed from the study to maximise accuracy. These selection criteria resulted in 57 participants being removed from the study. The final sample size was 126 subjects, which was still above the required number from the sample size analysis. This robust approach enabled the identification of three distinct maturity groups: pre-PHV group (maturity offset of < -1), circa-PHV (maturity offset between -0.5 – 0.5), and post-PHV group (maturity offset of >1). Descriptive statistics for each age group are presented in Table 1.

****Table 1 inserted here ******

Muscle architecture: Muscle structure of the gastrocnemius medialis (GM) and vastus lateralis (VL) were measured with real time B-Mode ultrasonography (Vivid E9, GE Healthcare, Chalfont St Giles, UK) with a 45-mm linear array probe. Water soluble gel was applied to both the ultrasound probe and the participants’ skin for acoustic coupling, to enhance the contrast of the images, and to avoid depression of the dermal layer. To measure the GM, participants laid prone on a massage couch with the hip angle at approximately 180° relative to the trunk, the knee set at approximately 180° relative to the thigh, and ankle positioned at approximately 90° relative to the shank. For the imaging of the VL, participants lay supine with the hip positioned at 90° relative to the trunk, and legs fully extended. For both muscles, the ultrasound probe was placed perpendicular to the skin, and the scanning surface was orientated until it was
positioned parallel to the muscle to collect sagittal plane images of each subject’s dominant leg. For the VL muscle, the image was taken at 50% of the distance between the greater trochanter and the lateral epicondyle of the femur (33) and the GM image was taken at 30% of the distance from the popliteal crease to the centre of the lateral malleolus (18). The subsequent analysis was carried out using open-source image analysis software (Image J, National Institute of Health, Bethesda, MD, USA) to calculate pennation angle, fascicle length and muscle thickness. Figure 1 depicts how the muscle architecture characteristics were calculated. Muscle thickness was measured as the distance from the superficial aponeuroses to the deep aponeuroses. The thickness of the proximal, distal and middle of the muscle belly was assessed in the image, and the average of these sites used for further analysis. Physiological muscle thickness was calculated by the following equation: (muscle thickness^2 + [tan x pennation angle x muscle thickness]^2)^0.5 (6). Pennation angle ($\theta_p$) was defined as the angle between the muscle fascicles and the deep aponeuroses. As the entire fascicle was not visible in the ultrasound image, it was calculated from the equation: fascicle length = muscle thickness (sin $\theta_p$)^{-1} (6). Each ultrasound image was assessed on three occasions, with the average value for each architectural variable used for analysis.

Figure 1. Ultrasound image of the gastrocnemius medialis, highlighting how the muscle architecture variables were calculated. MT: muscle thickness; PA: pennation angle; FL: fascicle length

Reliability
To establish intra-rater reliability of the ultrasonographic measurements, a sub-group of thirty-four school aged boys (14 = pre-PHV, 20 = post-PHV) attended a test session, where three images were taken for each muscle. Subsequently, two levels of reliability were quantified for each muscle architecture variable. Within-image reliability reflected the reliability of digitising an image and was assessed using three measurements of each variable in the final image taken during the test session. Between-image reliability determined the reliability of taking accurate images of the same muscle site, and was assessed by calculating an average measure from the three trials of each muscle architecture variable from each of the three images within the test session.

**Statistical Analysis**

Within-image and between-image reliability for each variable was determined using mean coefficients of variation (CV%), intraclass correlation coefficients (ICC) and the change in mean. Magnitudes of ICC were classified according to the following thresholds: Poor < 0.49; Moderate 0.5 – 0.74; Good 0.75 – 0.89; Excellent > 0.9 [18]. All reliability statistics were calculated using an online spreadsheet run through Microsoft Excel for Mac version 16.3 (11).

Descriptive statistics (means ± standard deviations) were calculated for all muscle architecture variables for each maturity group. Differences in muscle architecture variables between maturity groups and across both muscles were assessed using a 3 x 2 (maturity group x muscle) mixed model ANOVA. Homogeneity of variance was assessed via Levene’s statistic and where violated, Welch’s adjustment was used to correct the F-ratio. Post-Hoc analysis was used to identify the groups that were significantly different to one another using either Bonferroni or Games-Howell post-hoc analysis, where equal variances were and were not assumed, respectively. Cohen’s $d$ effect sizes were also calculated, using pooled standard deviations to establish the magnitude of any between-group differences using the following
classifications: trivial < 0.19; small 0.2 – 0.59; moderate 0.6 – 1.19; large 1.2 – 1.9; very large 2.0 – 3.9; > extremely large > 4.0 (12). The strength of relationships between muscle architecture variables in the whole sample, and when sub-divided by maturity group (pre-PHV vs circa-PHV vs post-PHV) were assessed using Pearson’s correlation coefficient, and interpreted as: < 0.2 no relationship; 0.2 – 0.45 weak; 0.45 – 0.7 moderate; > 0.7 strong based on previous recommendations (31). All significance values were accepted at \( p \leq 0.05 \) and all statistical procedures were conducted using SPSS v.23 for Macintosh.

RESULTS

Reliability of ultrasonography

Data for all architectural variables in both muscle sites showed excellent ICC and acceptable CV% for within-image reliability in both maturity groups (pre-PHV: ICC = 0.87 – 1.00; CV = 0.9 – 7.1%, post-PHV: ICC = 0.92 – 1.00; 0.6 – 3.1%). Apart from VL fascicle length in the pre-PHV cohort, all architectural variables ICC’s for between-images indicated moderate to excellent reliability and acceptable CV % for both muscle sites in the pre-PHV (ICC = 0.59 – 0.97; CV = 3.3 – 11.1%) and post-PHV groups (ICC = 0.75 – 0.95; CV= 2.9 – 5.9%).

Between-group differences in muscle architecture

Muscle architecture variables for pre-, circa- and post-PHV groups are displayed in figure 2a-e. For GM muscle thickness and physiological thickness, there was a moderate, significant difference between the pre- and post-PHV groups (\( d = 1.19 \) and 1.12; \( p < 0.05 \), respectively); and also small to moderate, significant differences between the pre- and circa-PHV cohorts (\( d = 0.96 \) and 0.57; \( p < 0.05 \), respectively). There were moderate, significant differences for GM pennation angle between the post-PHV and both the pre- and circa-PHV groups (\( d = 0.94 \) and 0.59, respectively; \( p < 0.05 \)). However, there was no significant difference in GM fascicle
length between any groups. For VL muscle thickness and physiological thickness there were moderate to large, significant differences between the pre- and post-PHV groups, pre- and circa-PHV, and circa- to post-PHV cohorts ($d = 0.68 - 1.40$, respectively). For both VL pennation angle and fascicle length, there were significant differences between the pre-PHV and the post-PHV group ($d = 0.59$ and $0.66$, respectively; $p < 0.05$), but no differences between any other groups. When made relative to leg length, there was no difference between groups for fascicle length for either GM or VL muscles.

With regards to differences in architectural variables between muscles, both VL muscle thickness and fascicle length were significantly greater than GM muscle thickness and fascicle length ($p < 0.001$) in the pre-, circa- and post-PHV groups. Contrastingly, for all maturity groups, GM pennation angle was significantly greater than VL pennation angle ($p < 0.001$).

*****Figure 2 a-e inserted here *****

Figure 2. Differences between pre-, circa-, and post-PHV groups in a) physiological muscle thickness, b) muscle thickness, c) pennation angle, d) fascicle length, and e) relative fascicle length

* significant difference between groups ($p < 0.05$). $d =$ Cohen’s effect size.

For both the GM and VL muscle, data showed significant, weak correlations between thickness and pennation angle for the whole group, and the pre- and post-PHV group ($r = 0.28 - 0.43$; $p < 0.05$). Additionally, the relationship between both GM and VL muscle thickness and fascicle length showed weak to strong, significant relationships across the whole group, and the three maturity groups ($r = 0.37 - 0.76$; $p < 0.05$). Finally, there were significant,
moderate to strong, negative correlations between GM and VL pennation angle and fascicle length in all the groups ($r = -0.51$ to $-0.79$; $p < 0.05$).

DISCUSSION

The initial hypothesis can be accepted, as the results showed an increase in GM and VL muscle thickness, GM and VL pennation angle, and VL fascicle length from pre- to post-PHV. However, the larger differences in muscle architecture variables were seen between the pre- and circa-PHV groups. In light of existing literature, this appears to be the first study to compare muscle architecture variables across all maturity stages in a sample of male youth. While previous literature has compared circa- and post-PHV groups with adults, this is the first study to examine a pre-PHV cohort. The main findings of the current study showed that all muscle architecture variables showed significant small to large increases from pre- to post-PHV with the exception of GM fascicle length. More discrete between-group differences were evident as GM and VL muscle thickness and physiological thickness significantly increased between pre- and circa-PHV; however only the VL muscle thickness and physiological thickness increased from circa- to post-PHV. The post-PHV group also showed larger GM pennation angles than the circa-PHV group. Finally, for both the VL pennation angle and fascicle length, there were small to moderate, significant differences between the pre-PHV and post-PHV groups, but no differences between any other groups.

The current study showed that GM muscle thickness increases from pre-PHV to both circa-PHV and post-PHV. For both maturity groups, these changes in muscle thickness were larger than the typical error (CV%), and are therefore considered as meaningful changes likely due to maturation as opposed to merely reflecting noise in the measurement. The non-significant change between circa- and post-PHV groups would indicate that natural developments in GM muscle thickness may have started to stabilise around the time of PHV.
Growth of the muscle throughout maturation may occur due to increases in body mass potentially through increases in skeletal mass, and internal organs intensifying the mechanical load on the skeletal system during everyday tasks (5). Additionally, changes in stature will enhance muscle growth as the load or stretch applied to muscles during bone growth may act as a stimulus for increases in muscle size (37). Previous studies, albeit examining age-related differences, have shown significant increases in GM muscle thickness between elementary school children, junior school children, and adults (18). The differences between adults and junior school children suggest that there may be further development of muscle size during ageing, beyond that of adolescence. This could be due to large changes in activity profile and body mass during adulthood driving increases in mechanical loading that underpin the mechanism of muscle growth (5). Data from Binzoni and colleagues (3) showed approximate increases in GM muscle thickness of 7% per year, which was then confirmed in an empirical study with children between the ages of 5-12 years (2). Expressing results from the current study by chronological age as opposed to maturity, shows an approximate 9% increase in GM muscle thickness per year between 12 years to 14 years, but only a 5% increase per year between 14 to 16 years. Cumulatively, these results support the notion of a slower rate of growth in the GM muscle following PHV, which may infer that further maturity-related increases in lower limb strength (27) may be due to growth of different muscles or ongoing changes in neural properties (9). For example, as children mature they have a greater potential to produce force, as there is a reduced agonist–antagonist co-contraction, a greater ability to recruit high-threshold type II motor units, and greater muscle activation rates (10).

Pennation angle in the GM showed a moderate, significant increase between pre- and post-PHV, and a small, significant increase between circa- to post-PHV. The circa- to post-PHV changes were larger than the typical error (CV%), and can be considered as meaningful changes; however, the change from pre- to circa-PHV was only greater than the smallest-
worthwhile change (SWC) and not larger than the typical error, so should be viewed with caution. These changes suggest that larger changes in pennation angle occur following PHV, which may be explained by the nature of muscle architecture adaptation. The current study found significant, small relationships between GM muscle thickness and pennation angle in the whole cohort, as well as in the pre- and post-PHV groups. A greater muscle thickness would necessitate a larger pennation angle, and therefore pennation angle increases may happen in response to a muscle increasing in size (7). This may explain the significant increases in GM muscle thickness between pre- to circa-PHV and then the subsequent increase in GM pennation angle between circa- and post-PHV, where changes in muscle size manifest prior to alterations in pennation angle. Comparable results between younger children have been reported in previous literature, where pennation angle has been shown to be similar between children aged between 5-12 years (2). The current study found significantly greater GM pennation angle in the post-PHV than in either the pre- or circa-PHV groups. The lack of difference between the pre- and circa-PHV group suggest that GM pennation angle occurs later in maturation, potentially following the preceding growth in muscle thickness. The larger pennation angle as a result of maturation would likely improve the force-generating capabilities of a muscle (21), through an increase in PCSA and muscle gearing (36), which would ultimately increase explosive-type exercise commonly reported throughout maturation (22, 25, 35).

Muscle thickness in the VL significantly increased from pre- to circa-PHV and circa-to post-PHV, with between-group differences exceeding the typical error (CV%). This continuous increase in VL muscle thickness throughout maturation coincides with the significant increase in body mass reported between the three maturity groups, again suggesting the importance of mechanical loading on muscle growth. Interestingly, the increases in muscle thickness of the VL across the different maturity groups differ to the growth pattern of the GM, where changes between circa- to post-PHV were not significant. More growth occurs in the
femur compared to the tibia during childhood and adolescence (13), where the tibia may slow
in growth around the time of PHV, and the femur continues to grow further into adolescence.
Considering that growth in skeletal structures provides a stimulus for adaptation of muscle
tissue (37), the extended growth typically seen in the femur may explain the increases in VL
muscle thickness between circa- and post-PHV that are not seen in the GM. Similar increases
in muscle size for the VL have been reported between children and adults (20, 30), but these
findings do not offer insights into the complex and fluctuating process of maturation.

Pennation angle and fascicle length of the VL increased significantly between pre- to
post-PHV, and while all were above the SWC, only the change in VL fascicle length was above
the typical error. Similar findings related to fascicle length have been reported in previous
literature, whereby men were shown to have longer VL fascicles than boys (30). However, in
terms of VL pennation angle, findings from the current study differ to previous research, which
reported that pennation angle of the VL remained relatively stable between boys aged 11 years,
to 14 years and into adulthood (17), and that boys of approximately 9 years of age have similar
VL fascicle lengths to adults (30). The discrepancy in results between the current study and
previous research may be due to smaller sample sizes in the previous research. Intuitively,
greater increases in muscle thickness compared to pennation angle would require an increase
in fascicle length. This concept is supported by the current study, where a large negative
relationship was observed between pennation angle and fascicle length in both muscles.
Therefore, it can be assumed from this study that the increases in VL thickness are greater than
pennation angle, resulting in increased VL fascicle length. However, it can then be assumed
that the increase in thickness and pennation angle are similar in the GM, resulting in no
difference in fascicle length between the three groups.

The cumulative findings of the current study indicate that maturation may trigger
architectural adaptations within the GM and VL muscles that are likely to facilitate greater
force production and rate-of-force-development. Considering that muscle size is a major predictor of maximum strength and power in children (34), the increases in muscle size with maturation could be a major factor contributing to the improved capacity to produce force as children transition through adolescence. Furthermore, considering that the VL muscle plays a major role in sprinting and jumping tasks (8), the increase in the thickness of this muscle may be a key factor in the improvement in the physical performance measures reported during childhood and adolescence (22, 25, 35). The larger pennation angle demonstrated by the more mature participants would allow the muscle to produce greater force, as a larger pennation angle will increase the physiological cross-sectional area, resulting in a greater number of contractile elements attaching to the aponeurosis or tendon for a greater force transfer (15). The increase in pennation would also allow a muscle to take advantage of the “gearing” effect during movement (36), which permits that muscle fascicles do not need to shorten to the same extent as the overall muscle, permitting slower fascicle velocities in relation to whole muscle velocities (36). This enables the fascicles to operate on a more optimal region of the force–velocity curve and work at a favourable region of the force–length curve over a longer period, maximising the force that the muscle can develop, without impacting on the capacity for rapid movement production. Finally, the increased fascicle length due to the maturation process would result in a greater muscle shortening velocity (21). The longer fascicles seen in the more mature subjects would have a greater ability to produce force at higher velocities and over larger length ranges, as more sarcomeres in series results in greater cumulative length change of a fascicle within a given time. The increased number of sarcomeres in series may also result in each individual sarcomere operating at a lower relative velocity, meaning greater force can be produced by each fascicle (7).

To our knowledge, this is also the first report on the between-image and within-image reliability of GM and VL architectural properties attained from ultrasound images recorded at
rest in pre- and post-PHV groups. Data indicated that the muscle architecture variables could be reliably digitised at both within-image and between-image levels, with large to nearly perfect ICC and CV values below the acceptance threshold (<10%). While reliability data on ultrasonography-derived measures of muscle architecture remain scarce in youth populations, when compared to results from a cohort of strength trained males (24), data from the current study showed that the reliability of the digitising process both within- and between-images was more varied in the pre-PHV cohort, and similar in the post-PHV cohort. The increased variability in the pre-PHV cohort compared with older populations may be due to a larger fat mass seen in this population (23). Adipose tissue has less degrees of reflection than muscle due to different acoustic impedance (32). The greater fat mass seen in the younger children may weaken the generated reflection and reduce the quality of the ultrasound image (32), causing more variance in the digitisation and determination of muscle architectural variables.

The current study has made a novel contribution to the developmental literature surrounding paediatric muscle architecture. However, it is important to consider certain limitations within the study. Considering the dimensions of the linear probe being used, it was necessary to estimate fascicle length from measures of muscle thickness and pennation angle using a previously published equation (6). The model assumes that fascicles are straight and does not account for fascicule curvature, however this equation has been used in previous studies (6) and has been shown to result in an error of only approximately 3% for relaxed muscles with short fascicles (4). Additionally, fascicle length of the GM and VL was made relative to leg length in the current study. However, for more accurate relative measures, the GM fascicle length would need to be made relative to the shank length, and VL fascicle length made relative to thigh length. The current study used a cross-sectional research design; however, future research is now required to validate these findings by longitudinally measuring changes in muscle architecture characteristics in youth of varying maturity levels. Furthermore,
while this study has gone someway into highlighting the differences in muscle architecture during maturity, the influence that this has on physical performance in tasks such as sprinting and jumping remains unknown.

CONCLUSION

The current study showed that all muscle architecture variables showed significant small to large increases from pre- to post-PHV, with the exception of GM fascicle length. These findings indicate that maturation underlies changes in muscle morphology, and that maturation may trigger architectural adaptations within the GM and VL muscles that are likely to facilitate greater force production and rate-of-force-development. This study establishes maturity-related changes in muscle architecture variables that provides researchers and practitioners with reference data that can be used to differentiate between training induced adaptations versus those realised as a consequence of growth and maturation. For example, the data showed that an increase in 0.3 cm was observed in VL muscle thickness between pre- to circa-PHV; therefore, any adaptations would need to exceed this value to confirm changes were not attributable solely to the maturational process.

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