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# Effects of Cervical Rotatory Manipulation on Internal Carotid Artery in Hemodynamics Using an Animal Model of Carotid Atherosclerosis: A Safety Study

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

ABCE 1,2 **Taiyuan Guan\***  
BC 3 **Yan Zeng\***  
DE 4 **Ji Qi\***  
DE 1,2 **Bo Qin\***  
FG 1,2 **Shijie Fu\***  
EF 1,2 **Guoyou Wang\***  
AFG 1,2 **Lei Zhang**

1 Department of Orthopedics, Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou, Sichuan, P.R. China  
2 Academician Workstation in Luzhou, Luzhou, Sichuan, P.R. China  
3 Department of Nephrology, Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou, Sichuan, P.R. China  
4 School of Traditional Chinese Medicine, Southern Medical University, Guangzhou, Guangdong, P.R. China

\* These authors contributed equally to this work

**Corresponding Author:**

Lei Zhang, e-mail: 307501597@qq.com

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**Background:** Spinal manipulation, particularly in cervical rotatory manipulation (CRM), has become increasingly popular in physical therapies, with satisfying effect. However, it is still unclear whether CRM affects internal carotid arteries (ICA) with mild carotid atherosclerosis (CAS), especially in hemodynamics.

**Material/Methods:** Nine cynomolgus monkeys were randomly divided into 3 groups: the CAS-CRM, the CAS, and the blank control groups. CAS models were developed in the left ICA in the CAS-CRM and the CAS groups. The monkeys in the CAS-CRM group underwent CRM intervention for 3 weeks. Histology and hemodynamics were measured, including peak systolic velocity (PSV), end-diastolic velocity (EDV), time average velocity (TAV), resistance index (RI), and pulsatility index (PI). Measurements were made separately at 3 different rotation angles (0°, 45°, and 90°).

**Results:** In the 3 groups, with the increase of rotation angle, the decreasing tendency of PSV, EDV, and TAV and the increasing tendency of RI and PI were statistically significant. At each angle, the monkeys in the CAS-CRM and the CAS groups had lower levels of PSV, EDV, and TAV and higher levels of RI and PI compared with the blank control group. No significant difference in hemodynamics was found between the CAS-CRM and the CAS groups.

**Conclusions:** Both the rotational angle and the atherosclerotic disease can affect the blood flow of the ICA. However, CRM does not cause adverse effects on hemodynamics in cynomolgus monkeys with mild CAS, and appears to be a relatively safe technique.

**MeSH Keywords:** **Atherosclerosis • Carotid Artery Diseases • Carotid Artery, Internal • Manipulation, Spinal**

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## Background

Spinal manipulation has become increasingly popular in physical therapies, with satisfying clinical effects [1–3]. In 1995, Spitzer et al. [4] demonstrated that a regimen of manipulation or mobilization can be used to treat patients with neck pain after whiplash injuries, which was published in *Spine*. Therefore, the use of cervical spinal manipulation (CSM) in treating cervical diseases is becoming more and more popular [5,6]. However, adverse effects or complications following massage manipulation were occasionally reported [7–9]. The safety of cervical manipulation needs further study, and some researchers even expressed that CSM should not be used [10,11].

Artery dissection was considered as one of the most severe complications, possibly leading to severe events, such as cerebrovascular diseases [12–14]. Although related research proposed that the neurovascular injury caused by spinal manipulative therapy is rare, with reported occurrence ranging from 1 in 100 000 to 1 in several million [15,16], others held the opposite opinion that many problems are unreported due to delayed adverse effects [17].

Indeed, it has been reported that cervical rotatory manipulation (CRM) and other high-velocity thrust (HVT) cervical techniques may be associated with serious vertebral artery trauma and carotid artery dissection [18]. Previous studies mainly focused on the effects of CSM on the vertebral artery, and it is unclear whether cervical manipulative techniques have adverse effects on the carotid artery (CA), particular in carotid atherosclerosis (CAS) patients [16,19,20].

As one of the major arteries, the CA supplies blood to the brain and face, and it is vulnerable due to its special anatomic position relative to the neck. It is also a common site for atherosclerosis. CAS has been considered as one of the potential risk factors leading to ischemic stroke (IS), possibly due to carotid stenosis, unstable plaque, and even ruptures [21–23]. This raises the question of whether CAS should be a potential contraindication of CRM due to its high prevalence in epidemiology studies and high risks involving cerebrovascular diseases.

Zhang et al. found that the uniaxial tensile properties of rabbit arteriosclerotic ICAs decreased after CRM intervention [24]. The effect of the manipulation on hemodynamic properties of the CA is still unclear during CRM, and this information would help predict the adverse effects on the CA. Hence, we presented a hypothesis that the effects on mechanical properties of CA existed and the hemodynamics of the CA indicate an abnormal trend during the procedure of CRM.

It is difficult to conduct a study about this problem in the human body. Therefore, the animal model of CAS has been widely

used to imitate atherosclerosis in the human body [25]. Taking this into consideration, this study conducted a CRM intervention on the prepared atherosclerotic ICA model of cynomolgus monkeys and observed the trend of blood flow in the CA to explore the effect of CRM on hemodynamics.

## Material and Methods

### Ethics statement

Ethics approval was given by the Ethics Inspection Committee on Animal Experiments of Yunnan Yinmore Biological Technology Co. (reference number: YBT1602). The welfare of animals was guaranteed by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), and animal cares conformed to the standard of “Guide for the Care and Use of Laboratory Animals” (Office of Science and Health Reports CPRR/NIH 1996).

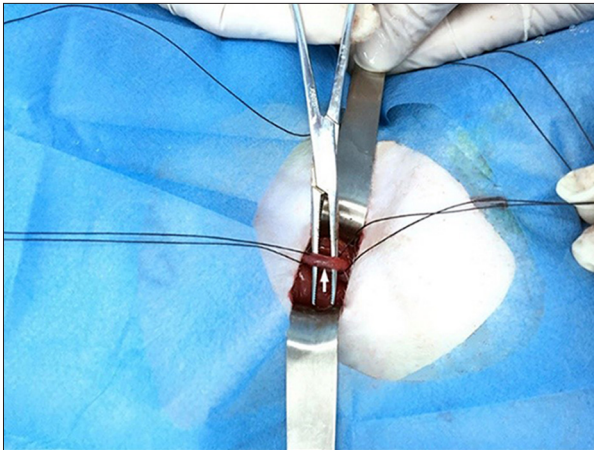
### Animals and grouping

Nine male specific pathogen-free (SPF) cynomolgus monkeys, weighing 6.0 to 7.0 kg, were selected and provided by the Yunnan Yinmore Biological Technology Co. Animal feeding and experiments were conducted at the Laboratory Animals Breeding Center of Yunnan Yinmore Biological Technology Co. The monkeys were housed in cages (1.5 m (H)×2 m (W)×1.5 m (D)), where the monkeys could sleep, eat, and rest. In addition, space was ensured to be adequate. There was a 12: 12 h light: dark cycle, with a temperature of 22–24°C and relative humidity of 45–65%, and all monkeys had sufficient sleep and sunshine. Checked water supply equipment regularly to ensure adequate cleaning water. All animals were moved out of cages to another spacious activity room, approximately measuring 4 m (H)×12.5 m (W)×8 m (D), for 6 to 8 h of free time per day. We dynamically observed the monkeys' behavior and played video and music irregularly to relax them.

All cynomolgus monkeys were randomly divided into 3 groups: the CRM-CAS group (n=3), the CAS group (n=3), and the blank control group (n=3).

### CAS modeling

In the CRM-CAS and the CAS groups, the CAS models were developed on the left side by puncturing and scratching the carotid artery intima. Monkeys were fasted before surgery, then anesthetized by Zoletil 50 (Virbac, France, 5 mg/kg, IM). The monkeys were fixed in dorsal position with shaved skin on the neck, and the skin was disinfected in the region of the neck surgery. At 1 cm on the outside of prominentia laryngea, the operators made a longitudinal incision, about 4 cm, followed



**Figure 1.** Surgical procedure of carotid atherosclerosis modeling. We isolated the carotid artery of the cynomolgus monkey and fixed it with a silk thread. A needle was inserted into the artery and we scratched the inner wall of the artery repeatedly and gently.

by step-by-step separation of the skin and subcutaneous tissue. In clearance between the sternocleidomastoid and prominentia laryngea, we isolated the common carotid artery, and made it fixed with surgical suture. We repeatedly scratched artery walls with a 5-ml syringe needle, piercing into the artery but not piercing through the artery (Figure 1). Finally, the needle was pulled out and gauze was pressed on the needle point. After there was no obvious oozing of blood, the wound was washed and sutured.

The model establishment was completed by the same researchers. At 3 days after surgery, levofloxacin hydrochloride and sodium chloride injection (Heng Ao, China, 8 mg/kg, 1 time/12 h, IV) was used to prevent infection. We closely observed the condition of the wound. Tramadol hydrochloride injection (QiMaiTe, China, 2 mg/kg, 1 time/day, IM) was used to relieve pain.

After the operation, monkeys in the CRM-CAS and the CAS groups were fed a high-fat diet (containing 2% cholesterol, 10% lard, and 88% regular granulated feed), and monkeys in blank control group were fed with a regular diet, lasting for 8 weeks. During this period, we closely observed the condition of the wound, animal swallowing, and eating.

### CRM intervention

At the end of modeling, monkeys in the CRM-CAS group received CRM intervention lasting for 3 weeks, 2 days per time. According to previous research [18,24] on CRM intervention, we followed these steps: (1) Fixed the monkeys at a sitting position, then 2 doctors fixed the monkeys' mandible and occiput. (2) Pressed a gentle but stable torsion force to cervical



**Figure 2.** Intervention of cervical rotatory manipulation. After successful modeling, we fixed the monkeys of the CRS-CAM group in a sitting position and pressed a gentle but stable torsion force to cervical vertebrae with the rotation angles 0°, 45°, and 90°.

vertebrae, which was similar to any types of traditional spinal manipulation, but more gently and delicately. (3) Applied 2 rotation movements to each side and within the range of motion (ROM) to the cervical spine. (4) The doctor pushed against the spinous process of the involved segment with his thumb steadily and firmly, similar to high-velocity and low-amplitude (HVLA) manipulation, until the end of the ROM during the process (Figure 2).

### Color Doppler ultrasound

After the intervention, color Doppler ultrasound examinations were performed on all monkeys by use of a Philips iU22 ultrasound machine (Koninklijke Philips Electronics N.V., Eindhoven, Netherlands) with a mini probe (Philips, L15-7io). The procedure was as follows: (1) Marked for the probe on the skin so that we could collect all the data in the same place during the experiment. (2) Anesthetized with 3% pentobarbital sodium (120 mg/kg, IV) and fixed at dorsal position with shaved skin in neck. (3) Observed whether there were typical atherosclerotic plaques in the monkeys' ICAs. (4) Measured the basic situation of the ICAs' lumen, including the ICA's stenosis rate (ICASR) and cross-sectional area of vascular lumen (CAVL). (5) Chose 3 positions on each left ICA in the experimental group randomly, and measured the atherosclerotic stenosis rate, and the maximum stenosis rate as the final stenosis rate of the ICA. (6) Refer to the CAVL, measured the area of the left ICA in the experimental group where the maximum stenosis rate was recorded, while for the right ICA in the control group and the left ICA in the blank control group, they were measured from the ICA at 1 cm above the carotid bulb.

Then, the hemodynamic parameters of all monkeys' ICAs were measured. (1) The monkeys rested in supine position for 15 min to stabilize hemodynamics. (2) Fixed the neck in a neutral position (Pre0°) to measure the blood flow of ICAs bilaterally by using a Philips iU22 ultrasound machine with a broadband (9-3 MHz) linear array transducer. The neutral position was maintained by the principal investigator, while both sides of ICAs were scanned and examined for pathology. (3) Set the sample volume at about one-third of the total diameter and placed it in the center of the vessel to avoid the natural turbulence at the edge of the lumen. The Doppler angle was maintained at 56° to avoid overestimation of the velocity. All measurements of peak systolic velocity (PSV), end-diastolic velocity (EDV), time average velocity (TAV), resistance index (RI), and pulsatility index (PI) were taken for both sides of ICAs in the neutral position.

After measuring the blood flow on the left and right ICA in the neutral position, the necks were rotated slowly to the right and the blood flow of the left ICAs was measured. The rotation angles were 45° and 90°. Each rotation angle was maintained for at least 10 s before scanning. If necessary, a period of fixing time added to measure all measurements.

Again, all parameters were taken at each angle. These parameters were taken for blood flow in the ICA contralateral to the direction of rotation. Testing was discontinued on the provocation of any abnormal signs or symptoms. The monkey's neck was then returned to a neutral position for a 60-s resting period while an assessment was made of any latent signs or symptoms.

The whole procedure was then repeated in each rotation angle on the opposite side.

## Histology

At the 11<sup>th</sup> week, after euthanasia, the damaged vessels of cynomolgus monkeys were harvested, rinsed by isotonic saline, then soaked for 24 h in 4% paraformaldehyde, then routine alcohol dehydration was performed. After embedding in paraffin, 5- $\mu$ m sections were prepared using a rotary microtome (Leica, CM3050S, Germany). Then, hematoxylin-eosin (HE) staining was used to stain the tissue sections, observing typical atherosclerosis histology.

## Statistical analysis

A two-factor, group (CAS-CRM group, CAS group and blank control group)  $\times$  rotation angle (0°, 45°, 90°), and mixed-model repeated measures ANOVA analysis was conducted to compare PSV, EDV, TAV, RI, and PI. Whenever the sphericity assumption was violated, the degrees of freedom were adjusted and

reported using the Greenhouse-Geisser epsilon correction. The LSD post hoc test was used to determine where the significant differences occurred among the 3 rotation angles in each group. At the same rotation angle, the values of hemodynamic parameters among the 3 groups were compared with one-way ANOVA and LSD pos hoc test.

## Results

### CAS modeling

CAS modeling was successfully developed in 6 monkeys of the CAS-CRM and the CAS groups. In terms of ultrasound, the lumen area of ICA decreased significantly in comparison with the blank control group ( $P < 0.05$ ) (Table 1, Figure 3). No significant difference was found in plaque location, the size of plaque, and the stenosis rate of ICA. The histology confirmed the existence of atherosclerotic plaques (Figure 4). In terms of the plaque location (distance above the carotid bulb), plaque size and the stenosis rate of lumen, no significant difference was found between the CAS-CRM group and the CAS groups ( $P > 0.05$ ) (Table 1).

### Hemodynamics

Among the 3 groups, the effects of rotation angle were significant in PSV, EDV, TAV, RI, and PI ( $P < 0.05$ ). With the increase of rotation angle, the decreased tendency of PSV, EDV, and TAV and the increased tendency of RI and PI were significant ( $P < 0.05$ ) (Tables 2, 3, Figure 5).

At each rotation angle, there was a significant difference in the above index among the 3 groups ( $P < 0.05$ ). The monkeys in the CAS-CRM and the CAS groups had significantly lower levels of PSV, EDV, and TAV and a higher level of RI and PI in comparison with the blank control group ( $P < 0.05$ ). No significant differences in hemodynamics were found between the CAS-CRM group and the CAS group ( $P > 0.05$ ) (Tables 2, 3, Figure 5).

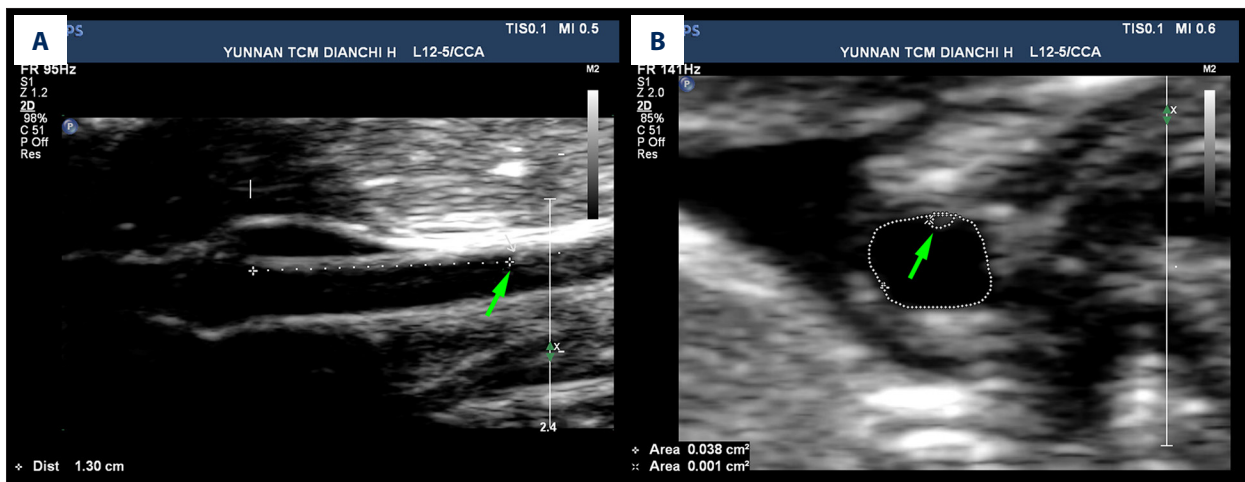
## Discussion

In this study, cynomolgus monkeys were selected as the subjects, as they are more anatomically and physically similar to humans than are other animals. On the other hand, they are sensitive to a high-fat diet and are easy to imitate the formation process of CAS plaques in hyperlipidemia environment to achieve the desired effect. To simplify the procedure of CAS modeling and to increase the success rate, we created a modified method to develop the CAS model. Finally, the ultrasound and histology outcomes confirmed the success of the model.

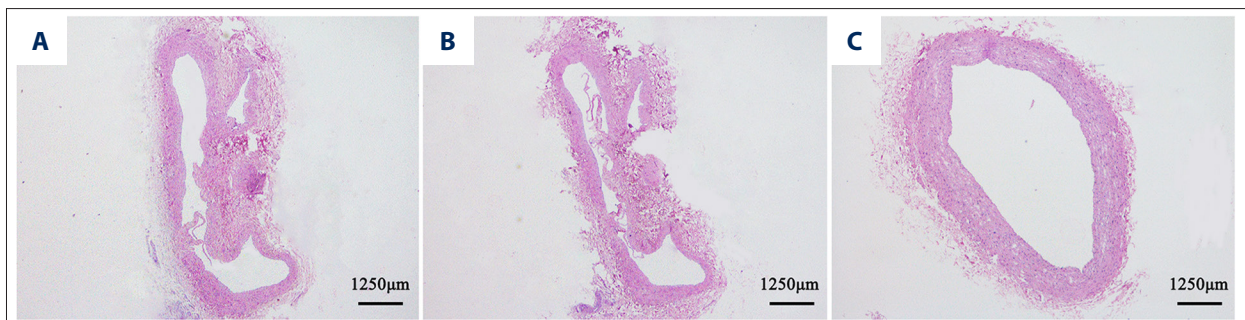
**Table 1.** Measurement of atherosclerotic plaque in the CAS-CRM and CAS groups (mean ±SD).

	CAS-CRM group	CAS group	Blank control group	F/T value	P value
Area of lumen (cm <sup>2</sup> )	0.05±0.00*	0.07±0.00*	0.57±0.01	7.000	0.027
Plaque Location (cm)	1.37±0.12	1.37±0.12	N/A		
Diameter of lumen (cm)	2.23±0.06	2.27±0.06	2.23±0.06	0.333	0.729
Area of plaque (cm <sup>2</sup> )	0.01±0.00	0.01±0.00	N/A		
Stenosis rate (%)	18.89±1.92	18.89±1.92	N/A		

CAS – carotid atherosclerosis; CRM – cervical rotatory manipulation. \* P<0.05 vs. blank control group.



**Figure 3.** Atherosclerotic plaque (arrows) and lumen stenosis in carotid artery were observed under carotid ultrasonography. (A) Longitudinal image. (B) Cross image. Under anesthesia, the carotid artery of cynomolgus monkeys was measured using carotid ultrasonography. There was formation of atherosclerotic plaques, and the green arrow points to plaques in longitudinal and cross images, respectively.



**Figure 4.** Histology of carotid artery with typical atherosclerosis characteristics. Histology of carotid artery in the 3 groups (HE staining, ×40). Carotid atherosclerotic plaque and lumen stenosis were observed in the CRM-CAS group (A) and the CAS group (B). Normal carotid artery was observed in the blank control group (C), with regular cell arrangement in arterial wall.

We used color Doppler ultrasound to examine blood flow in the carotid arteries in different neck positions and compare the measurements of blood flow with that in the neutral position to identify if the positions were potentially more hazardous than others. The results demonstrated that both the rotation angle and the atherosclerotic disease could affect the blood flow of the CA. Significant differences were observed under

color Doppler ultrasound. Measurements of PSV, EDV, TAV, RI, and PI are commonly applied in diagnosis and evaluation of arterial diseases, such as atherosclerosis and stroke [26–28].

Ultrasound studies have shown certain neck positions can alter craniocervical arterial blood flow velocities [26,27,29,30]. The CSM procedure is conducted by moving the head and

**Table 2.** Differences in velocity of carotid artery among 3 groups at different angles (mean ±SD).

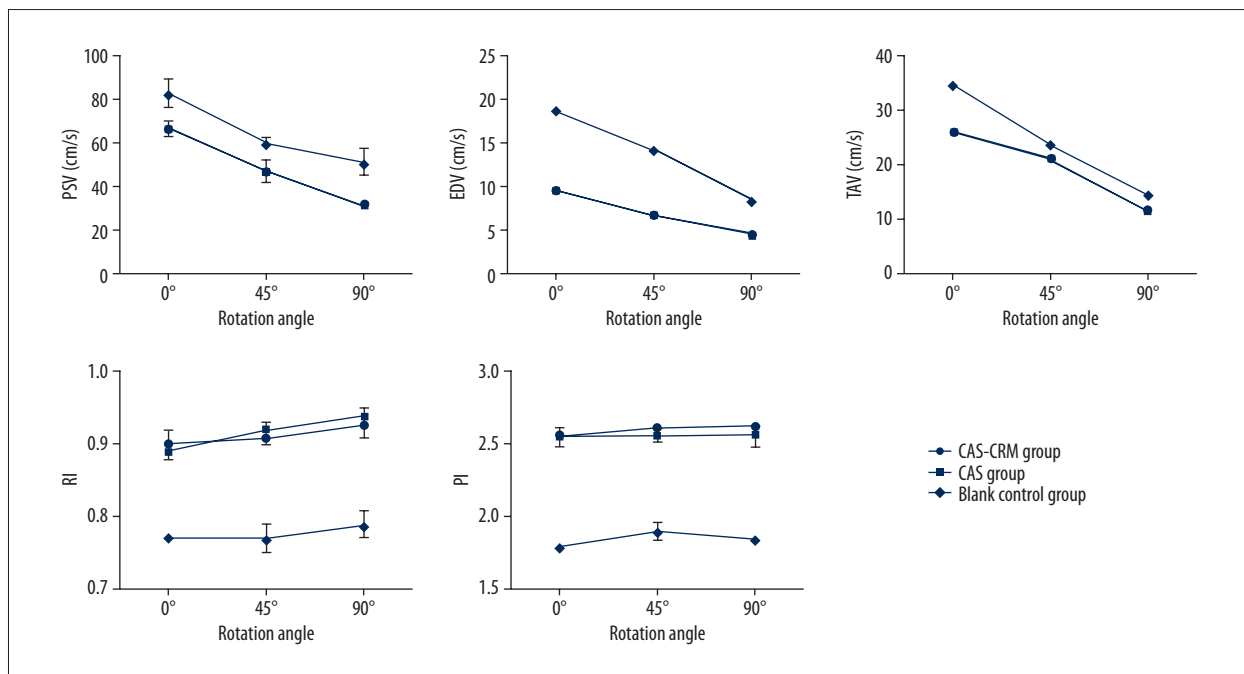
Group	PSV (cm/s)			EDV (cm/s)			TAV (cm/s)		
	0°	45°	90°	0°	45°	90°	0°	45°	90°
CAS-CRM	66.90± 1.21*	47.20± 2.09*	31.23± 0.78*	9.70± 0.26*	6.73± 0.21*	4.43± 0.21*	25.57± 0.58*	20.70± 0.40*	10.93± 0.15*
CAS	66.77± 1.31*	47.40± 1.66*	30.57± 0.45*	9.87± 0.21*	6.60± 0.20*	4.47± 0.31*	25.50± 0.20*	20.86± 0.67*	10.50± 0.52*
Blank-control	83.20± 2.51	60.33± 0.90	51.33± 2.34	18.87± 0.25	14.27± 0.21	8.33± 0.32	34.27± 0.40	23.10± 0.30	13.90± 0.20

PSV – peak systolic velocity; EDV – end-diastolic velocity; TAV – time average velocity. \* P<0.05 vs. blank-control group.

**Table 3.** Differences in RI and PI of carotid artery among 3 groups at different angles (mean ±SD).

Group	RI			PI		
	0°	45°	90°	0°	45°	90°
CAS-CRM	0.90±0.02*	0.91±0.01*	0.93±0.02*	2.55±0.01*	2.61±0.03*	2.62±0.03*
CAS	0.89±0.01*	0.92±0.01*	0.94±0.01*	2.55±0.06*	2.56±0.04*	2.56±0.07*
Blank-control	0.77±0.00	0.77±0.02	0.79±0.02	1.79±0.00	1.90±0.06	1.84±0.02

RI – resistance index; PI – pulsatility index; CAS – carotid atherosclerosis; CRM – cervical rotatory manipulation. \* P<0.05 vs. blank-control group.



**Figure 5.** The effect of the rotation angle and grouping on the hemodynamic parameters (PSV, EDV, TAV, RI, and PI) in the carotid artery. Means for hemodynamic parameters at 0° (neutral), 45°, and 90° positions are given. Data expressed as mean ±SD.

neck into various positions, usually from the neutral position to the end range [18,24].

Some researchers reported that the positions of neck rotation close to the end of the physiological range might contribute to adverse neurovascular events following the manual treatment of the neck, as it could cause temporarily compromised blood flow [31]. In this experiment, the PSV, EDV, and TAV of ICAs in the 3 groups at 0° were significantly higher than those at 45° and 90°. Moreover, those velocities at 45° were statistically higher than at 90°. On the contrary, the RI and PI of ICAs in 3 groups at 90° were significantly higher than those at 45° and 0°; and those indexes at 90° were higher than at 45°. These results demonstrate that rotation of the head and neck from the neutral position can decrease the blood velocity of ICA, increase the pulse, and resist the blood flow at the same time. The effect of rotation on the blood flow of the ICA was also significant over angles, which may be because the rotation procedure exerts a distortion load on the vessel and decreases the relative area of the lumen in comparison with the neutral position.

On the other hand, at each rotation angle, ICAs of the CAS-CRM and the CAS groups had significantly lower levels of PSV, EDV, and TAV and significantly higher levels of RI and PI, compared with the blank control group. Since typical atherosclerotic diseases cause obvious stenosis of an artery, the findings of this study might be attributed to atherosclerosis and lumen stenosis. The results under ultrasound also confirmed the above characteristics. The findings were similar to those of related studies [32–35].

Our study also explored the potential adverse effect of CRM on the arteries. In the comparison between the CAS-CRM group and the CAS group, no significant difference was found in hemodynamics at each rotation position. The evidence did not prove the previous hypothesis. Recently, Zhang et al. [24] found that CRM decreases the uniaxial biomechanics of ICA in rabbits with CAS. Our findings were slightly different. CRM appears to be safe in terms of blood flow of atherosclerotic carotid arteries. The possible reasons are a difference in modeling animals, and the blood flows of CA are connected and balanced via the circle of Willis (CoW). The CoW plays an important role in cerebral collateral circulation. The hemodynamic changes in the CoW are usually associated with ICA stenosis, but whether CRM affects it remains unknown. Lin et al. [18] found that CRM does not have an obvious influence on the

blood flow in the CoW when the stenosis of ICA is less than 90%. In contrast, the stenosis of ICA in the experiment was less than 50%. As a result, mild atherosclerosis and stenosis might be one of the factors leading to a lack of significant effect of CRM on the blood flow of the atherosclerotic carotid arteries.

Although this kind of manipulation seems to be a safe technique in clinical applications with mild CAS, manipulation injuries are usually considered to be caused by embolization from dissection of the arteries, not just the plaque displacement. The justification may be difficult to infer from the blood flow changes only.

Our study existed has some limitations. First, although the sample size of subjects allowed for sufficient statistical power, it was still relatively limited due to animal size, characteristics, and restriction of funds and animal ethics. Secondly, the lesion degree of CAS is limited. This experiment mainly aimed at the mild CAS of cynomolgus monkeys, making it hard to evaluate whether the CRM has an adverse effect on intermediate and advanced CAS. Thirdly, Doppler ultrasound has advantages in terms of comfort, non-invasiveness, and relative time of performance in comparison to angiography; however, there are a number of potential problems, such as high operator dependence, poor reliability, and difficulties with localization of the target vessel. Future studies need to address these problems.

## Conclusions

In conclusion, this study supports the evidence that both the rotational angle and the atherosclerotic disease affect the blood flow of the ICA. The ICA blood flow increased with the increase of the rotational angle. In addition, cervical rotatory manipulation does not have a significant effect on the blood flow in the mild atherosclerotic carotid artery. Therefore, from the perspective of hemodynamics, this kind of manipulation seems to be a relatively safe technique in clinical application.

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## Conflict of interests

None.

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