

Working title: skin response to hyperbaric gaseous cryotherapy.

Hyperbaric gaseous cryotherapy: effects on skin temperature and systemic vasoconstriction.

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Abstract (255 words)

Objective: To compare skin surface cooling caused by application of an ice bag (15 min) and by projection of CO₂ microcrystals (2 min) under high pressure (75 bar) and low temperature (-78°C), a modality called **hyperbaric gaseous cryotherapy** (HGC).

Design: Randomized controlled trial with repeated measure.

Setting: Laboratory experiment.

Participants: 12 healthy male subjects (mean ± SD: 22.9 ± 1.8 years)

Interventions: Ice bag and hyperbaric gaseous cryotherapy were randomly applied on the skin of the non-dominant hand.

Main Outcome Measure: Skin temperature of the cooled (dorsal and palmar sides) and contra-lateral (dorsal side) hands were continuously measured with thermistor surface contact probes before, during and after (30 min) cooling.

Results: HGC projection induced a large decrease ($P < .05$) of the dorsal skin temperature of the cooled hand (from $32.5 \pm 0.5^\circ\text{C}$ to $7.3 \pm 0.8^\circ\text{C}$), and a significant decrease of the skin temperature of the palmar side and of the contra-lateral hand. The skin temperature of the dorsal side of the cooled hand was decreased with an ice bag (from $32.5 \pm 0.6^\circ\text{C}$ to $13.9 \pm 0.7^\circ\text{C}$; $P < .05$). However, the lowest temperature was significantly higher than during HGC, and no significant changes in the other skin temperatures were observed. Rewarming was equal after the two modalities, highlighting a more rapid increase of the skin temperature after HGC.

Conclusion: HGC projection decreased skin temperature of the cooled and contra-lateral hand suggesting a systemic skin vasoconstriction response. On the other hand the vascular responses triggered by ice pack cooling appeared limited and localized to the cooled area.

Key words: cold, cryotherapy, cutaneous temperature, ice bag, dry ice, sympathetic, autonomic nervous system

The term cryotherapy refers to the achievement of a therapeutic objective through the lowering of tissue temperature by the withdrawal of heat from the body. ¹ A critical level of tissue cooling is required for specific effects. ^{2, 3, 4, 5, 6} Ice massage, ⁷ mixture

of water and alcohol,⁸ or nitrogen cold air⁹ are efficient cooling techniques, but among the various cryotherapy modalities available for use both in clinical and on-field settings, ice or gel bag or pack are probably the most frequently used. A series of studies reported that critical temperatures are not typically achieved with these modalities, thus limiting the therapeutic effect.^{10,11}

Despite a general consensus regarding the physiologic effects of cryotherapy, there are no clear guidelines on the optimum application technique for acute cryotherapy. Thus, there is no strong evidence to justify the use of one cryotherapy modality over the others.¹² It is generally believed that greater cooling leads to more profound metabolic suppression, suggesting that cryotherapy modalities that produce lower temperatures are more efficacious.^{10, 11, 13,14, 15, 7} Similarly, it is generally assumed that cryotherapy techniques that provide more rapid cooling of tissues may offer some advantage over slower cooling techniques. With traditional ice and cold gel pack, achievement of temperatures low enough to produce local analgesia² by conduction are usually obtained after 5-10 minutes.^{8, 14}

With the intention to drastically shorten cooling time, the Cryonic® society developed a cryotherapy modality that lowers tissue temperature by convection. Reduced skin temperature is achieved through sublimation on the skin of CO₂ microcrystals under high pressure (75 bar) and low temperature (-78°C). The aim of the present study was to directly compare the skin surface temperature during and after the application of a commonly used latex ice bag and of hyperbaric gaseous cryotherapy (HGC) by carbon dioxide application.

Methods

Experimental design

We used a repeated-measures design to compare the skin surface temperature during the application of the two cryotherapy modalities. The independent variables were time and cryotherapy modalities while the dependent variable was skin surface temperature. Subjects were tested with 2 cryotherapy modalities: ice bag and hyperbaric gaseous cryotherapy on 2 occasions with at least a 24-hour interval between occasions. To control the order effects, subjects were randomly allocated. Subjects were also asked to refrain from consuming alcohol, caffeine, or food for 3 hours and from any vigorous activities for 24 hours prior to testing to help stabilize extremity blood flow. None of the subjects in this study reported negative reactions to the cold treatments.

Participants

Twelve male subjects (mean \pm SD: 22.9 \pm 1.8 years; 178.3 \pm 2.0 cm; 70.6 \pm 2.2 kg), from a university student population, voluntarily participated in this study. Their medical history and a medical examination were used to exclude subjects with history of smoking, cardiovascular or peripheral vascular disease, diabetes, neurologic pathologies, recent trauma or injury to the hand, presence of any sores or open wounds on the hand, local hot or cold insensitivity, and very fair skin. These criteria were chosen to avoid any harm or discomfort to the subjects, and were in keeping with previous studies.^{10, 11} The subjects were normotensive and none was taking medication. They were informed of the organization and details of the study, which was approved by our local ethics committee, and reminded of their right to withdraw

at any stage. Written informed consent was signed according to the Helsinki recommendation. None of the 12 prospects was excluded from the study.

Experimental procedures

A sole investigator made all data measurements to reduce measurement variability. Subjects were in supine position on a treatment table during the entire test. A control period was imposed for a minimum of 20 minutes before starting temperature measurements in an effort to allow body temperatures to stabilize and to control for temperature fluctuations resulting from any pre-experiment physical activity.

The dorsal face of the non dominant hand was cooled in each therapy. The ice bag was prepared from ice chips and had a diameter of 25 cm. Air was evacuated from the bag to allow the bag to better form to the hand surface. Care was taken to cool only the hand from the distal part of the wrist to the extremity of the fingers. With hyperbaric gaseous cryotherapy (HGC), cold was applied on the same area by sweeping motions. Due to the ability of HGC to rapidly withdraw heat, application time differed between modalities: cooling lasted 15 min with the ice bag^{7,10} and only 2 min with HGC (manufacturer recommendations).

Skin temperatures were recorded at several sites: 1) the centre point of the cooled area (middle of the third metacarpus), 2) the opposite side (palmar side) of the same hand and 3) the middle of the third metacarpus of the dorsal contra-lateral hand. Skin temperatures were recorded every 30 seconds during the first 2 minutes of application and then every two minutes until the end of application of the modality (ice bag only); every 30 seconds during the first 3 minutes of rewarming, then every minute from 3 to 6 minutes of rewarming and finally every 2 minutes during the remaining 30 minutes of rewarming.

Subjects were permitted to leave when sensation in the tested area returned to normal. No subjects experienced any adverse reactions to the experimental protocol.

Materials

Skin surface temperatures were measured by means of thermistor surface contact probes^a fixed on the skin with thin, air-permeable, adhesive surgical tape. By using uniform amounts of tape covering an equal area for each individual treatment, we controlled the potential insulating effect of the tape, and it was assumed that a uniform effect would be produced across all treatments. Therefore, it would not interfere with our ability to discriminate among treatments. Room temperature was maintained at a mean of $25 \pm 1^\circ\text{C}$ for all testing sessions.^b

Ice bag and hyperbaric gaseous cryotherapy by carbon dioxide were the modalities used in this study. The ice bag^c was a 25-cm diameter latex bag filled with chipped ice ($0-1^\circ\text{C}$). In this case, air was evacuated from the latex bag in an attempt to improve conforming of the bag to the body surface. The amount of ice was 400 g. For HGC, we used the **Cryo+** apparatus.^d This device throws up CO_2 microcrystals under low temperature (-78°C) and high pressure (50 bars at the outlet pipes, 2 bars on the skin). Medical CO_2 , in liquid form, is stored in bottles with a valve and a dip tube. Cold was applied on the dry skin with sweeping motions using a spray gun linked to the CO_2 bottle. The pipe was maintained at a distance of 7 to 10 cm of the skin surface. With this technique, it was possible to see the action of the cold by

observation of the microcrystals on the skin. Skin temperature reduction is controlled by an infrared thermometer incorporated into the gun and application time of the gas is displayed on a screen.

Statistics

Standard statistical methods were used for the calculation of mean \pm SD. Statistical comparisons of skin surface temperature were made by utilizing a repeated-measures two-way ANOVA. A Student-Newman-Keuls test was employed post hoc when interactions were significant. The statistical significance was established at the $P < .05$ level. All analyses were performed using SigmaStat®. ^e

Results

Mean skin surface temperature for the two modalities at the end of the baseline periods were $32.5 \pm 0.6^\circ\text{C}$ (ice bag) and $32.5 \pm 0.5^\circ\text{C}$ (HGC) for the dorsal cooled hand, $33.7 \pm 0.7^\circ\text{C}$ (ice bag) and $33.5 \pm 0.6^\circ\text{C}$ (HGC) for the palmar cooled hand and $31.0 \pm 0.7^\circ\text{C}$ (ice bag) and $30.8 \pm 0.6^\circ\text{C}$ (HGC) for the dorsal contra-lateral hand (Figure 1). At baseline, no significant differences were observed between ice bag and HGC.

Ice bag and HGC decreased the temperature of the dorsal aspect of the cooled hand significantly throughout the cold application (Figure 1). However, the rate of skin temperature decrease and the drop in skin temperature was dependent of the technique used. With the ice bag, skin temperature decreased to $26.5 \pm 0.7^\circ\text{C}$ (-6°C) in the first 30 seconds ($P < .05$ compared to baseline) and then more slowly to reach $21.8 \pm 0.6^\circ\text{C}$ at 2 min (-11°C ; range $-5.0^\circ\text{C} / -8.1^\circ\text{C}$) and finally $13.9 \pm 0.7^\circ\text{C}$ (-19°C ; range $-15.6^\circ\text{C} / -20.8^\circ\text{C}$) at the end of the 15 min application. With HGC, skin temperature decreased to $12.8 \pm 1.2^\circ\text{C}$ (-20°C ; range $-14.4^\circ\text{C} / -25.8^\circ\text{C}$) within 30 seconds ($P < .05$ compared to baseline and ice bag), and then more progressively to reach $7.3 \pm 0.8^\circ\text{C}$ (-25°C ; range $-18.4^\circ\text{C} / -30.0^\circ\text{C}$) at the end of the 2 min application. The overall reduction in skin temperature was larger with HGC compared to ice bag ($P < .05$). After removal of the application, rewarming was faster after HGC, particularly at the very beginning of recovery. Thirty seconds after removal of ice bag, the skin temperature was $16.9 \pm 0.8^\circ\text{C}$ ($+3^\circ\text{C}$; range $+1.9^\circ\text{C} / +4.0^\circ\text{C}$) while it was $13.5 \pm 0.6^\circ\text{C}$ ($+6^\circ\text{C}$; range $+3.8^\circ\text{C} / +9.1^\circ\text{C}$) for HGC ($P < .05$ compared to ice bag). After one minute of rewarming, no significant difference in skin temperature was observed between ice bag and HGC. After 20 min of recovery, neither cryotherapy modalities was significantly different than baseline values.

Like the temperature reductions on the dorsal side of the cooled hand, the palmar skin temperature changes were also dependent of the cooling technique used (Figure 1). A trend for an increase in the skin temperature was observed during the first 6 minutes of ice bag application (up to $34.0 \pm 0.7^\circ\text{C}$; $+0.3^\circ\text{C}$; range $-0.4^\circ\text{C} / +1.2^\circ\text{C}$; NS) before decreasing to reach $33.2 \pm 0.9^\circ\text{C}$ at the end of the cooling period. At this time, no significant difference with the baseline skin temperature was observed. During HGC, the palmar skin temperature decreased ($P < .05$) to reach $19.2 \pm 1.2^\circ\text{C}$ (-14°C ; range $-6.5^\circ\text{C} / -21.5^\circ\text{C}$) at the end of the 2 min application.

No significant change in the dorsal skin temperature of the contra-lateral hand was observed during ice bag application (Figure 1). However, a significant decrease was observed with HGC: the temperature dropped to $30.5 \pm 0.6^{\circ}\text{C}$ (-0.3°C ; range $-0.1^{\circ}\text{C} / -0.7^{\circ}\text{C}$) at the end of the cooling period. The skin temperature remained significantly lower than that of baseline during the 2 first minutes after the end of cold application.

Discussion

This study investigated the effect of application of a traditional latex ice bag and of a hyperbaric gaseous cryotherapy (HGC) device on the surface skin temperature of the human hand. The results suggest that greater reductions in skin temperatures and greater rate of heat removal are achieved with HGC compared to ice bag.

Cold modalities work by absorbing heat from their immediate environment, particularly from the tissues being treated. The transfer of heat and the capacity of the cold modality to absorb this heat determines the modality's effectiveness and depend on several factors, such as the relative masses of the bodies, the size of the contact area, the difference in starting temperatures and the heat capacity or specific heat of each material.¹⁶ In the present study, the only difference between treatments was due to differences in the specific heat of the modalities used and their ability to absorb heat. Hyperbaric gaseous cryotherapy is based on a source of liquid carbon dioxide which converts, when applied on the skin, to a white solid phase (dry ice) through a process called deposition. Then, at atmospheric pressure, dry ice gradually sublimates to carbon dioxide gas (sublimation). The amount of heat absorption through this process is greater compared to the heat absorption operated through convection with the ice bag, explaining the greater and more rapid decrease in skin temperature. Also, condensation occurred at the surface of the ice bag during the 15 min application and thus a small amount of cold water was in contact with the skin of the subject's hand. On the other hand, convection during HGC involved dry gas. One potential advantage of such a difference is that pain is lessened during a dry cold than during a wet cold stimulation.¹⁷ However, pain sensation was not evaluated in the present study.

Comparing the present results with other research findings is difficult because of the different protocol used (time and site of cold application,¹²). However, the relative clinical merits of each modality can be established by reviewing the tissue temperatures achieved in relation to target tissue temperatures. Based on previous research, skin temperature below 13.6°C is required to induced localized analgesia,² reduction of nerve conduction velocity is observed at a temperature of 12.5°C ^{3, 4, 5} and tissue temperatures between 10°C and 11°C reduced metabolic enzyme activity.^{3,}
⁶ It is of note that large variation exists in individual responses to cold. Clinically, this variation is a concern, given the importance of the actual skin temperature that must be achieved to induce the different physiologic effects. It is therefore worth questioning whether generic application protocols and times will always ensure clinically effective tissue cooling. The ice bag did not achieve a mean skin temperature below 13.9°C . This suggests that the ice bag would not be suitable to achieve these particular clinically relevant tissue temperatures when applied to the hand in this manner. In the present study, skin temperature on the cooled surface was lower than 13.6°C in 6 subjects and lower than 12.5 and 11°C in only 2 over 12

subjects after 15 min of ice bag application. The inter-individual variation was large in this case, as skin temperature ranged from 9.7 to 17.7°C. Since there was a continual decrease in the mean surface temperature for the duration of ice pack application, clinically relevant temperatures may have been reached with longer application time. However, prolonged application at low temperatures should be avoided, as this may cause serious side effects, such as frostbite and nerve injuries.¹⁸ ¹⁹ On the other hand, HGC lead to a skin temperature below 11°C in every subject at the end of the 2 min application. This suggests that HGC has adequate heat abstraction capabilities to justify its use for achieving localized analgesia, reduced nerve conduction velocity and cell metabolism. It is not known if very rapid cooling could also have side effects but since the HGC has a great capacity to absorb heat, this modality may also caused injuries if mismanaged.

Twenty minutes after the removal of the cryotherapy modalities, the mean skin surface temperature was not significantly different from baseline. It is interesting to note that the 2 cryotherapy modalities achieved a similar level of mean skin surface temperature during the rewarming despite the fact that the mean skin surface temperatures before removal differed. Because heat always transfers from the warmer environment into the cooler environment^{14, 20} the similar level of mean skin surface temperature at the end of the rewarming may imply that the cooling effects of the topical cryotherapy application in this study are short-lived. The important changes in skin temperature observed with HGC may be of interest, based on the assumption that immediate cryotherapy application will be more beneficial than delayed application because the sooner the metabolic rate is reduced after injury, the less the secondary damage.¹⁴

In the present study, we chose to cool the hand instead of the thigh, as usually performed in studies comparing different cryotherapy modalities (e.g.,^{8, 14}). This was done because the subjects' skinfold thickness is thin at this site and the vascular bed is highly developed, therefore magnifying the thermal alterations. Also, changes in hand skin temperature could be used as an indicator of the sympathetic activity,^{21, 22, 23} giving information on the vascular autonomic control during cooling. In the present study, a significant decrease in the skin temperature of the palmar face of the cooled hand and of the contra-lateral hand was found with HGC and not with ice bag cooling, suggesting that the cutaneous vasoconstriction triggered by HGC was systemic while that triggered by the ice bag was localized only to the cooled area. This should be noted since evidence has shown that sympathetic activity modulates inflammation and the release of cytokines²⁴ and that involvement of the autonomic nervous system is importance in the treatment of inflammation.²⁵

Limitations

Several methodological restrictions limit the generalization of our findings. The subjects of the present study were a selected group of young healthy men, which enhances internal validity and data purity. However they are not representative of the population as a whole^{26, 27, 28}, restricting the external validity of this study. Likewise, injured subjects may not respond in the same fashion because of their inflammatory status and higher temperature at the wounded site. During cold application, there is a direct relationship between adipose thickness,²⁹ vascularity of the tissue, local blood flow³⁰ and both the required cooling time and the temperature change, suggesting adjustments to cryotherapy duration may be necessary to produce similar temperature changes at different body segments. HGC was compared to ice pack because it is

likely the most frequently used modality in clinics and on the field. However, more rapid and deeper tissue cooling compared to ice bag could be achieved with other cryotherapy modalities.^{7,8,9} It is necessary to compare the efficacy of HGC to these alternative modalities. Finally, in lieu of more direct measures, the clinical efficacy of cryotherapy is often assessed through skin surface temperature measurements. It is assumed that skin surface temperature declines in a fashion similar to that of intramuscular temperature during the course of cryotherapy application;^{14, 7, 20} however, this may be incorrect.³¹ Also, the duration of the intramuscular temperature reduction is dependent of the cooling modalities.^{15, 32}

Conclusion

Hyperbaric gaseous cryotherapy was superior to the ice bag in reducing skin temperature. This modality decreased the mean skin surface temperature to levels required for therapeutic effects in all the subjects studied, while ice bag did not. Also, and unlike the ice bag, hyperbaric gaseous cryotherapy triggered a systemic vasoconstriction. Confirmation of these results with a larger population is necessary and the clinical benefits of hyperbaric gaseous cryotherapy need to be evaluated.

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Suppliers:

^a Thermistor surface contact probes [series 400, type 409B, Yellow Springs Instrument (YSI); accuracy $\pm 0.1^{\circ}\text{C}$.

^b [DiGi-Sense Thermister Thermometer](#), Eutech Instruments Europe B.V., Wallerstraat, The Netherlands.

^c Laboratoires Clement-Terkan – Produits Burnet, Montrouge, France

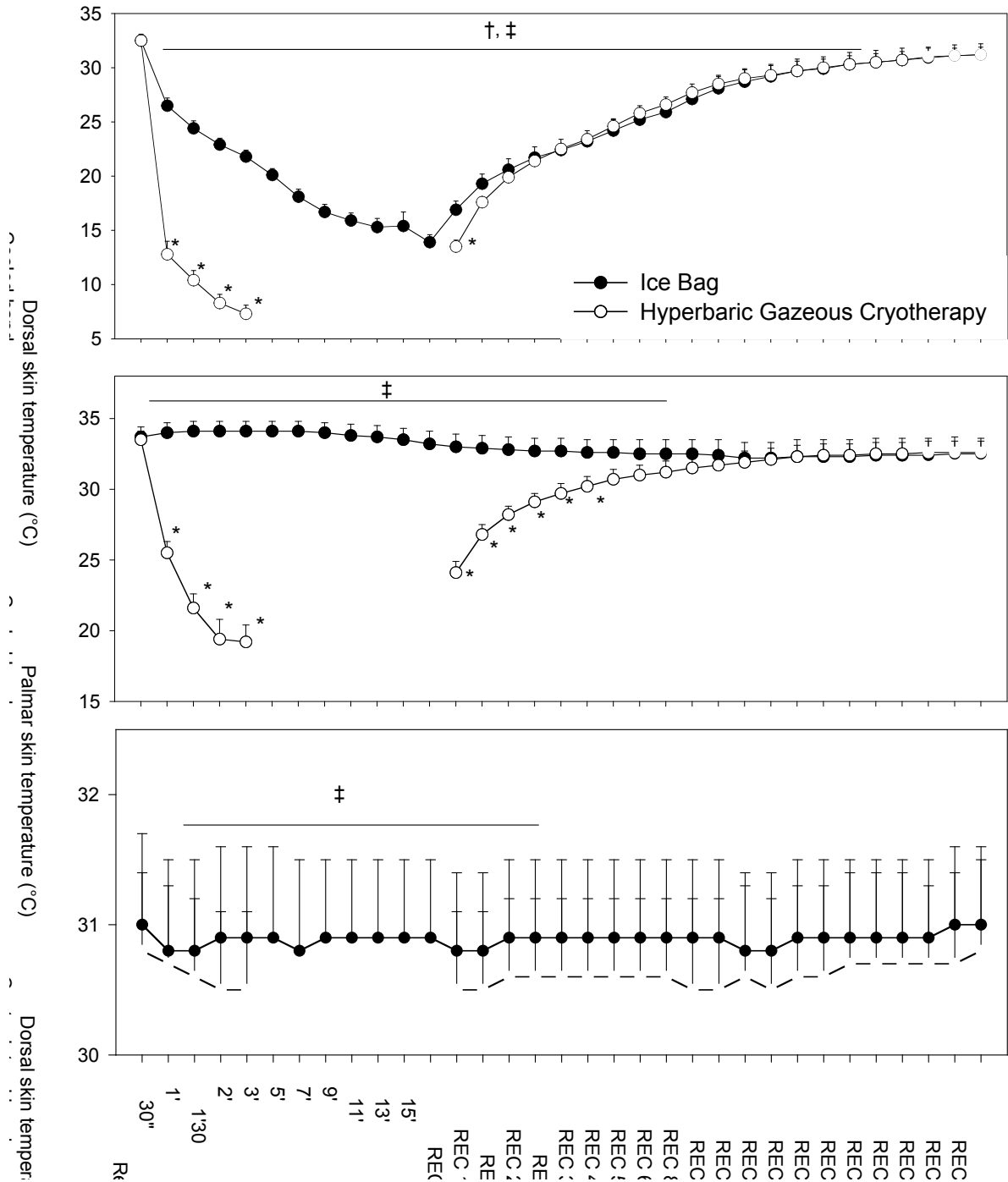
^d Cryonic Medical S.A., Le Martinet du Haut, Salins les Bains, France

^e SPSS Inc, Chicago, USA.

Legend:

Figure 1. Skin surface temperatures of the cooled and of the contralateral hand during and after 15 min of ice bag application and 2 min of hyperbaric gaseous cryotherapy (HGC).

Figure 1.



* significant difference between ice bag and HGC, † significant difference from the reference values (ice bag), ‡ significant difference from the reference values (HGC) at the $P < .05$ level.

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