QUBAHAN ACADEMIC JOURNAL
VOL. 5, NO. 1, March 2025
https://doi.org/10.48161/qaj.v5n1a1290

Impact of HOT Instruction on Knowledge and Comprehension in Chemistry and Biology for Middle School Students

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ABSTRACT: Higher-order thinking (HOT) have proliferated in education. While traditional didactic methods may fail to engage students effectively, research suggests the potential pros of HOT-grounded learning in terms of educational outcomes. However, there is a dearth of studies investigating the impact of such approaches on biology and chemistry education at the middle school level. Hence, this study aimed to evaluate the effectiveness of an after-school program designed to foster biological/chemical literacy and metacognitive self-regulation through meta-learning activities. A total of 132 eighth-graders took part in structured educator-led activities over a 10-week period. Sessions centered upon multi-level understanding, connecting scientific concepts, and utilizing metacognitive prompts. Controls received traditional didactic instruction aligned with the standard curriculum. Results indicated significant improvements in biology comprehension alongside chemistry knowledge and comprehension among students who completed the experimental module as compared to untreated counterparts. Per chemistry, learners reporting higher pre-existing self-regulation tended to reach better knowledge acquisition at post-test, with interference control acting as a moderator. Baseline self-regulation predicted post-intervention biology understanding as well, and the effect was moderated by interference control. These findings advocate for the integration of HOT-based training into middle school curricula to bolster attainment across science disciplines.

Keywords: academic performance, interference control, metacognition, moderator analysis, self-regulation.

I. INTRODUCTION

Cultivating learners' higher-order thinking (HOT) has evolved to become a momentously pressing education goal in the 21st century [1 – 3] and is reflected in the latest competency models [4]. HOT embodies the cognitive prowess to surpass the given information for tasks such as categorization, inference and generalization within intricate scenarios [5]. Drawing upon the principles of constructivism [6], HOT encompasses cognitive processes that involve actively constructing meaning. In essence, HOT serves as a comprehensive descriptor for various forms of reflective thinking, including problem-solving, metacognition and critical thinking [7]. Metacognition, conceptioned in John Flavell's metacognitive theory as “knowledge and cognition about cognitive phenomena” [8], is a cornerstone HOT domain enabling individuals to monitor and regulate their cognitive processes [9].

Unfortunately, traditional didactic methods may be boring for the Z generation and may not fully engage students or encourage more profound understanding [10]. For instance, [11] conducted a study where secondary school students were requested to solve a task including pH calculation and prediction of a titration indicator color. They found 37% of the participants failed the problem-solving task since they could not design and implement an appropriate solution. This case sheds light on a critical point: despite the inherent problem-solving and analytical thinking skills that biology and chemistry require, students may struggle to apply these concepts in practical, real-world scenarios, particularly because of the abstract nature of the taught concepts [12]. Therefore, there is a pressing need to move beyond traditional, passive learning approaches in science education, particularly in fields like biology and chemistry, and explore innovative instructional strategies that foster deeper cognitive and metacognitive engagement. The application of HOT in science education involves encouraging students to engage in scientific inquiry, design investigations, analyze data, and construct evidence-based explanations, aligning with the practices of scientists. In the context of biology and chemistry, this means moving beyond simply memorizing facts to understanding the underlying mechanisms and applying scientific concepts to real-world problems. This accords with the upper tiers of Bloom's revised taxonomy, which outlines cognitive learning objectives beyond simple recall, specifically aiming for the cognitive processes of analyzing, evaluating, and creating [13]. For instance, students can be engaged in inquiry-based learning activities that require them to formulate hypotheses, design experiments, and interpret findings [14 – 17]. The transition from traditional didactic methods to HOT-driven instruction in biology and chemistry education represents a critical shift towards fostering a generation of scientifically literate individuals equipped to tackle the complex challenges of the 21st century [18].

While numerous researches concluded that HOT-driven solutions are profitable to students' educational performance [19 – 23], there remains a paucity of research specifically examining the impacts of such approaches on biology and chemistry education, particularly at the middle school level. Given the foundational nature of these subjects and their relevance to students' future academic and professional pursuits, investigating the effects of HOT-focused interventions in this domain is imperative. Moreover, while previous studies have demonstrated the positive effects of meta-learning models on various aspects of educational performance, further exploration into the underlying mechanisms driving these effects is necessary for advancing educational practice.

This study sought to addresses the evidence gap by examining the effectiveness of an after-school program specifically designed to promote biological/chemical literacy and metacognitive self-regulation among eighth graders through HOT-based activities. The designed HOT module goes beyond traditional didactic methods by incorporating activities that encourage students to:

1) Recognize and utilize multiple levels of scientific understanding: The program explicitly trains students to identify and integrate different representational levels.
2) Connect scientific concepts across these levels: Students are not only exposed to scientific concepts but also actively engage in making connections between different representational levels, fostering a deeper understanding of the underlying principles.
3) Develop metacognitive self-regulation: The program incorporates prompts that encourage students to reflect on their learning process, monitor their comprehension, and independently regulate their learning strategies.

By comparing outcomes between students engaged in the experimental sessions and those following standard curriculum, this research can contribute to understanding effective methods for integrating HOT skills into science education and assessing their impact on learning outcomes. To note, researchers suggest that the advancement of students' learning success can be credited to the conflation of a deep grasp of the subject matter and adequate strategic approaches to problem solving [24]. In this regard, metacognitive self-regulation can make sense. Self-regulation emphasizes individuals' ability to manage their learning through metacognitive strategies like goal setting, planning, and monitoring their learning processes. Effective self-regulators are supposed to adeptly allocate their time and apply learning resources, which is in turn hypothesized to afford them successful learning experiences [25]. This provided the motivation for this investigation to be the first effort to explore the predictive power of students' pre-existing self-regulation levels on post-experimental knowledge and comprehension in biology and chemistry, and whether this relationship is moderated by interference control, which refers to the ability to suppress/ignore conflicting information or task-irrelevant stimuli [26]. Such insights are critical for educators and policymakers aiming to design effective curricula that not only impart knowledge but also develop the (meta)cognitive skills necessary for students to thrive in their academic and future professional endeavors. In light of these considerations, the present research addresses the following research questions (RQs):

1) RQ1. Will students engaged in HOT-based sessions have metacognitive self-regulation, biology/chemistry knowledge and comprehension significantly different compared to students who followed standard curriculum?
2) RQ2. Will pre-existing self-regulation level predict post-intervention biology/chemistry knowledge? And will this relationship be moderated by interference control level?

3) RQ3. Will pre-existing self-regulation level predict post-intervention biology/chemistry comprehension? And will this relationship be moderated by interference control level?

II. METHOD

1. PARTICIPANTS

Eligibility criteria were put forward for this research. To qualify for the final analysis, an individual had to: (a) be an eighth-grade student registered in the school, (b) regularly attend the school, (c) endorse willingness to participate (including parental consent), (d) complete both pre-test and post-test assessments, and (e) miss no more than one intervention session and complete all assignments. Eventually, this quasi-experimental study involved 132 eighth-grade students (74 males and 58 females; Mage = 15 years 4 months) attending two of the 21 [blinded for review] schools. Each of the schools has about one thousand students. To enroll, students must pass a competitive selection process, which results in the awarding of educational grants. Lessons are commonly followed by individual work in libraries, as well as educational and experimental laboratories. Teaching languages are [blinded for review] and English, depending on the subject.

Totally, four groups were set up: (a) busy-as-usual comparison group (school A, n = 34) who served as historical controls, (b) busy-as-usual comparison group (school B, n = 30) who served as historical controls, (c) experimental Biology group (school A, n = 37) who received a biology HOT module intervention, and (d) experimental Chemistry group (school B, n = 31) who received a chemistry HOT module intervention. Controls were recruited and pre-/post-tested a year before experimental to avoid any condition contamination. The research flow is detailed in Figure 1.

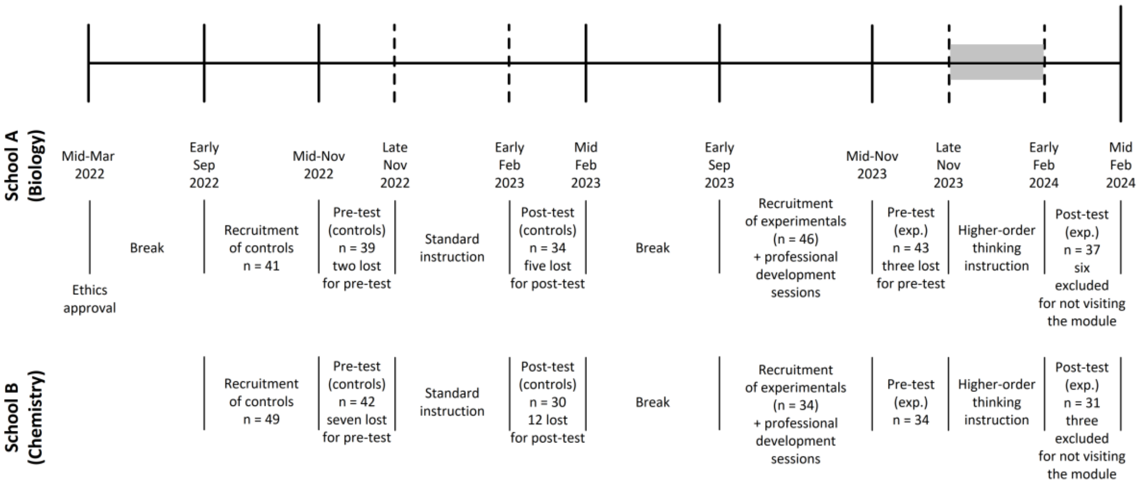

FIGURE 1. Algorithm of the research.

One teacher per school taught both an experimental and a control group. Both teachers had a teaching diploma. Prior to the research onset, they both received professional development sessions on delivering the HOT module.

2. ETHICS

The research protocol was approved by the ethics committee at the first author's institution. Consent to participate was obtained from each participant and their parent/legal guardian. Participants' anonymity was ensured. All assessments were administered by research assistants blinded to group assignments and students' identities, except unique digit and letter sequences to which scores were credited, so that individual baseline and post-experimental results could be connected at the data analysis stage.

3. INTERVENTION

This intervention comprised a series of structured educator-led activities delivered as an after-school program over a 10-week period, with sessions held twice a week for approximately one hour each, from late November 2023 to early February 2024. After regular school hours, the teacher gathered eighth-grade students from various classes into one classroom or laboratory. The module was aimed at promoting biological/chemical literacy and self-awareness among learners. The teacher organized an interactive learning environment where students actively engaged with scientific concepts and processes at multiple levels of understanding and were incited to connect theoretical knowledge with practical examples. For instance, in a lesson on the role of deoxyribonucleic acid (DNA) in the storage and transmission of hereditary information, the teacher directed students to a visual representation of DNA's structure and function. Students were asked to identify the components of DNA (e.g., sugar-phosphate backbone, nitrogenous bases) and relate them to its role in heredity (e.g., base pairing and complementary strand synthesis). The teacher then prompted the students to consider the molecular structure of DNA at the symbolic level, drawing the double helix and identifying the base pairs (A-T, G-C). In chemistry sessions exploring reactions involving hydrogen and oxygen, the teacher facilitated hands-on experiments to illustrate combustion reactions (e.g., burning a candle or igniting a hydrogen-filled balloon) and proposed students to draw the electron transfer process ($H2 + O2 \rightarrow H2O$) in their notebooks, reinforcing the concept at the symbolic level. Throughout the intervention, the teacher consistently encouraged students to articulate their learning process, fostering a deeper understanding of the subject matter. Specifically, following a brief introduction of a scientific concept, participants were presented with relevant materials (e.g., graphs or beakers with chemicals) and instructed to:

1) Identify the chemical/biological level. The module targeted various levels of understanding: macroscopic, microscopic, symbolic, and process (for chemistry) or organismal, cellular, molecular, and population (for biology). Teachers explicitly explained the significance of each understanding level and requested students to integrate as many understanding levels as possible when constructing their written and oral answers. Students were fostered to recognize the specific level of representation being used (e.g., macroscopic observations or symbolic formulas) when analyzing a concept. (Example: When discussing pollination, the teacher might ask, “What level are we using when we observe pollen grains landing on the stigma?”)
2) Connect levels. The educator used examples to demonstrate the process of analyzing scientific concepts using different understanding levels and facilitated student-led explanations connecting these levels. Learners were requested to explain the relationship between different representational levels and how they contribute to understanding the scientific concept. (Example: When exploring double fertilization, the teacher could prompt, “How does the structure of the pollen grain (symbolic level) relate to its function in delivering sperm cells (microscopic level)?”)
3) Utilize metacognitive prompts. Participants had to independently answer guided assignments that included prompts specifically designed to instigate students to reflect on their thought processes, monitor their understanding, and regulate their learning. Below are exemplary assignments:

Exemplary assignment (biology):

- Illustrate the stages of double fertilization, from pollination to the formation of seeds.
- Explain how double fertilization contributes to genetic diversity in plants.
- Analyze your thought process as you connected the concepts of genetic diversity in plants and double fertilization.

Exemplary assignment (chemistry):

- Compare the reactivity of oxygen with other oxidizers used in industrial processes.
- Reflect on the environmental impact of oxygen-based oxidation reactions.
- Describe any uncertainties or complexities you encountered and how you addressed them in your analysis.

In contrast, control groups followed traditional didactic methods without extracurricular higher-order thinking sessions. However, the curriculum for the experimental activities aligned with the regular class syllabus. Specifically, the key biological concepts completed during the investigation were: pollination, double fertilization in flowering plants, the role of DNA in the storage and transmission of hereditary information, genital system in vertebrates, the number and arrangement of chromosomes in different organisms, artificial selection, centers of origin of cultivated plants, variability and its types, and the role of heredity and variability in evolution. The chemistry topics that were taught during the intervention period included hydrogen as a reductant, oxygen as an oxidizer, hydrogen and oxygen reactions, calculations in chemistry, the heat effect of a chemical reaction, energy change in chemical reactions, the benefits and harms of combustibles, and comparisons of energy change in combustion, respiration, and corrosion reactions.

4. MEASUREMENT

Pre- and post-questionnaires were used before and after the students had studied the module. Both the pre- and the post-questionnaires included four parts: the first part inquired respondents' demographics, the second one related to biological/chemical conceptual knowledge, while the third part assessed biological/chemical understanding, and the fourth part gauged metacognitive self-regulation.

Demographic information entailed two dummy variables, namely gender (male vs. female) and socioeconomic background (medium vs. high). The latter was categorized based on participant's self-identified social class (poor / working class / middle class / upper middle class or wealthy) and the education level of their most highly educated parent or primary guardian (high school or less / college degree / professional degree) using the approach reported in [27]. None of the subjects included in the final analysis selected options aggregating to the low socioeconomic status, which is why the variable was condensed to medium and high levels.

1.1 Subject Knowledge

Chemistry and biology knowledge inventories designed by the researchers comprised ten items each (see a sample item below). The items in pre-test and post-test evaluations were different to prevent memorization. Piloting proved that time 1 and time 2 versions of the test were equally difficult to complete. Performance on the test was calculated as number of correct answers. Exemplary item of the multiple-choice knowledge test:

- Which of the following beakers containing solutions of HCl and Na2S2O3 placed in a water bath at the same temperature will show the fastest initial rate of SO2 evolution?
- 100 mL of 0.5 M Na2S2O3 solution with large Na2S2O3 crystals, (b) 50 mL of 1.0 M Na2S2O3 solution with finely powdered Na2S2O3, (c) 100 mL of 1.0 M HCl solution with large Na2S2O3 crystals, (d) 100 mL of 0.5 M HCl solution with finely powdered Na2S2O3, (e) I do not know.

1.2 Subject Comprehension

Investigator-developed tests were administered to assess how well learners understand biology/chemistry concepts. Each test presented three open-response items. The respondent received one point for every level of chemistry understanding reflected in their answer. The final score for a participant was derived by summing the scores for the three items. Below is a sample item.

Exemplary item of the comprehension test: Here are biology-related concepts: pollination, DNA, and artificial selection. Please choose one concept and explain it using as much understanding levels as you can.

1.3 Metacognitive Self-regulation

This variable was gauged using the Metacognitive Activities Inventory (MCAI) [28], the tool specifically crafted to evaluate students' capacity to regulate their cognitive processes when solving chemistry tasks. This questionnaire comprises 27 statements (eight of which are reverse-coded) like “I try to determine the form in which the answer or product will be expressed” rated according to a five-point Likert scale (1 = strongly disagree, 5 = strongly agree). Responses on the negative items were inverted upon the survey completion. The MCAI score per participant was the mean of scores reported on all 27 items. A higher score indicated a better self-regulator.

1.4 Interference Control

This executive inhibitory ability was examined at pre-test using the Eriksen flanker task, which was modified by the researchers drawing on a blend of approaches employed in [29] and [30]. The participant performed the task sitting in front of a desktop with a keyboard. The test was run through the E-Prime software (Psychology Software Tools, Pittsburgh, PA) and included 100 trials distributed between two 50-trial blocks. Each trial lasted 1,800 ms, commencing with the display of a horizontal array of five arrows in the middle of the screen for 800 ms, after which the screen was blank for 1,000 ms. The central arrow was encircled by four distracting arrows (flankers) that were either aligned in the same direction (congruent) or the contrary direction (incongruent). During the period from the onset of the array and the end of the blank screen, students had to press the key corresponding to the direction indicated by the central arrow as quickly and accurately as they could. There was an interstimulus interval of 1,700 ms between trials, and a pause of 30 s between the two blocks, with a ten-second-long warning before resumption. Equal numbers of congruent and incongruent trials were administered in a randomized sequence. Prior to starting the actual task, students went through instructions and performed ten practice trials each for congruent and incongruent ones, receiving feedback on response accuracy. Each participant's proportion of correct responses (%) and the mean reaction time (ms) were registered by the software. A summary measure of interference control was the inverse efficiency score (IES), which is the mean reaction time divided by the accuracy percent. Lower IES implies an individual with greater control over their attention, allowing them to effectively filter out unimportant details.

5. STATISTICAL ANALYSIS

To answer RQ1, three separate analyses of covariance (ANCOVAs) were conducted per knowledge, comprehension and self-regulation within each discipline. ANCOVA was chosen to compare post-test scores between groups while controlling for pre-existing differences that could influence the outcomes, thereby increasing the precision of the comparisons. Post-test scores were input as the response variable, with pre-test scores, gender, and socioeconomic status as covariates to account for potential biases in the sample, as past research has shown that gender and socioeconomic status can influence academic performance [31, 32]. Group (control vs. experimental) was the independent variable. A Bonferroni-corrected significance threshold of .017 (.05/3) was set to preclude the family-wise error rate. The Levene's test proved equality of variances.

To answer RQ2 and RQ3, two separate moderated multiple regression analyses were performed with either subject knowledge or comprehension as an output variable. Pre-intervention metacognitive self-regulation scores (predictor) and interference control values (moderator) alongside their interaction term were centered in order to eliminate multicollinearity. The alpha level was Bonferroni-adjusted to .025 (.05/2). Variance inflation factor and tolerance values affirmed the lack of multicollinearity. The Durbin-Watson test results excluded autocorrelation. Effect size was judged by η2p, with 0.01, 0.06, and 0.14 inferred as small, medium and large effects.

III. RESULTS

1. EFFECTS OF THE INTERVENTION (RQ1)

The following were the descriptives of the investigated variables (Table 1). The ANCOVA results revealed that within the biology arm, there was no significant difference at the post-test between the groups for biology knowledge (F(1, 66) = .83, p = .367, η2p = .012) as well as for metacognitive self-regulation (F(1, 66) = .51, p = .477, η2p = .008) while adjusting for gender, SES, and baseline scores. However, the students who attended the experimental module had significantly higher biology comprehension scores compared to the untreated individuals, with a large effect size (F(1, 66) = 35.06, p <0.001, η2p = .347).

Table 1. Mean scores for knowledge, comprehension & metacognition (biology: n=71, chemistry: n=61).

Variable		**Metacognitive group**		**Control group**	
		Pre-test	Post-test	Pre-test	Post-test
Biology					
	Knowledge	1.14 (.82)	8.41 (1.12)	1.26 (.75)	8.12 (1.49)
	Comprehension	3.86 (.82)	5.59 (1.66)	3.97 (.87)	4.21 (.88)
	Self-regulation	3.18 (.78)	3.27 (.55)	3.09 (.71)	3.15 (.70)
Chemistry					
	Knowledge	.65 (.49)	8.84 (1.07)	.80 (.55)	8.23 (1.14)
	Comprehension	4.52 (1.03)	5.90 (1.54)	4.20 (1.0)	4.47 (1.22)
	Self-regulation	2.93 (.91)	2.81 (.75)	3.31 (.81)	3.03 (.80)

Regarding the chemistry cohort, the HOT group upon the research conclusion significantly outperformed controls in terms of chemistry knowledge (F(1, 56) = 6.87, p = .012, η2p = .108) and chemistry comprehension (F(1, 56) = 17.61, p <.001, η2p = .239). The groups did not differ significantly in metacognitive self-regulation scores though (F(1, 56) = .79, p = .379, η2p = .014).

2. REGRESSION ANALYSIS ON KNOWLEDGE (RQ2)

In the biology trial, the moderated multiple regression analysis showed that neither prior self-regulation nor interference control levels were significant predictors of post-experimental biology knowledge scores, along with their interaction term (Table 2). Moreover, when the interaction term was included in the model, the addition was insignificant, $\Delta R^2 = .06$, $\Delta F\ (1, 67) = 4.55$, $p = .037$. Given the Bonferroni correction, this result evinces the lack of moderation. The total model explained 15% ($R^2 = .15$) of the variance in the regressand.

Table 2. Results of the moderated multiple regression analysis. Dependent variable: post-test biology knowledge scores.

		95% Confidence Intervals			
Predictor	**B**	**Lower**	**Upper**	**β**	**p**
Prior self-regulation	.220	-.265	.704	.125	.368
Interference control	-.025	-.048	-.001	-.290	.038
Prior self-regulation * Interference control	-.024	-.047	-.002	-.213	.037

As for the chemistry subpopulation, pre-test self-regulation and interference inhibition ability exerted a significant effect on post-intervention chemistry knowledge scores (Table 3). The interaction of baseline self-regulation and interference control provided a significant addition to the model, $\Delta R^2 = .05$, $\Delta F\ (1, 57) = 5.81$, $p = .019$, confirming there was moderating influence. The total model explained 50% ($R^2 = .50$) of the variance in the output variable.

Table 3. Results of the moderated multiple regression analysis. dependent variable: post-test chemistry knowledge scores.

		95% Confidence Intervals			
Predictor	**B**	**Lower**	**Upper**	**β**	**p**
Prior self-regulation	.338	-.088	.587	.261	.009
Interference control	-.044	-.061	-.027	-.521	<.001
Prior self-regulation * Interference control	-.023	-.042	-.004	-.237	.019

Simple slopes analysis was performed to further look into the moderating impact of interference control on the link between participants' pre-test metacognitive self-regulation and post-intervention chemistry knowledge scores. The results suggest that the students reporting higher ability to self-regulate were more likely to better grasp chemistry concepts and processes. Specifically, when the interference control value was one standard deviation (SD) below its mean, the simple slope was significant, B = .645, p <.001, 95% CI [.280, 1.010]. At the mean of the interference control scores, the slope remained significant, B = .338, p = .009, 95% CI [.088, .587]. However, when the variable was one SD above the mean, the simple slope was insignificant, B = .031, p = .860, 95% CI [-.317, .379]. Figure 2 delivers a visual representation of this moderated correlation.

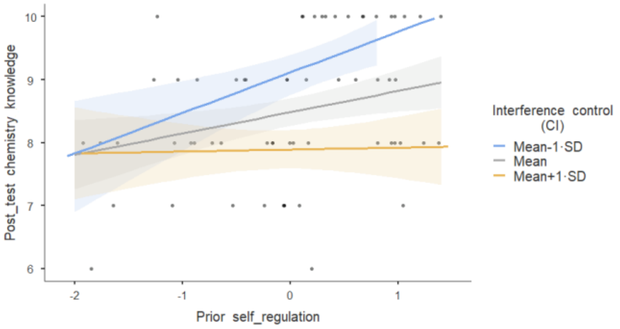

FIGURE 2. Simple slopes for the relationship between baseline self-regulation and post-test chemistry knowledge as moderated by interference control.

3. REGRESSION ANALYSIS ON COMPREHENSION (RQ3)

Within the biology arm, the moderated multiple regression analysis unearthed that both prior self-regulation scores and distraction inhibition ability were significant antecedents of post-test biology comprehension level (Table 4). The interaction of these centered variables yielded a significant addition to the model, $\Delta R^2 = .07$, ΔF (1, 67) = 6.08, p = .016, which supports the presence of moderation. The total model explained 29% ($R^2 = .29$) of the variance in the outcome variable.

Table 4. Results of the moderated multiple regression analysis. dependent variable: post-test biology comprehension scores.

		95% Confidence Intervals			
Predictor	**B**	**Lower**	**Upper**	**β**	**p**
Prior self-regulation	.629	.256	1.141	.311	.017
Interference control	-.030	-.054	-.005	-.304	.019
Prior self-regulation ✻ Interference control	-.030	-.054	-.006	-.226	.016

Simple slopes analysis proved that the association between pre-experimental metacognitive self-regulation and post-test biology comprehension was positive and tended to increase with decreasing index of interference control. The simple slope was not significant when the inhibition index was at one SD above its mean, B = .172, p = .558, 95% CI [-.411, .754], while being significant when the index was at its mean, B = .629, p = .017, 95% CI [.118, 1.141], and one SD below its mean, B = 1.087, p = .002, 95% CI [.409, 1.764]. These interactions are depicted in Figure 3.

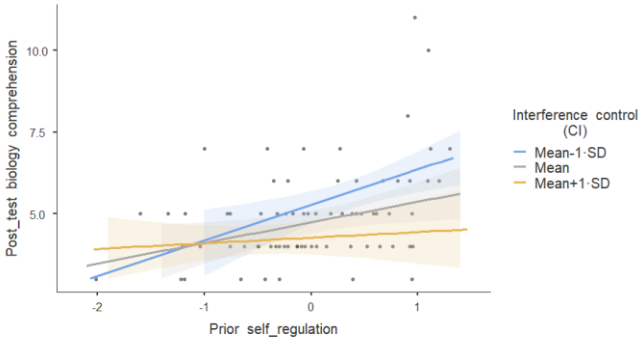

FIGURE 3. Simple slopes for the relationship between baseline self-regulation and post-test biology comprehension as moderated by interference control.

With regard to the chemistry trial, the regression analysis (Table 5) found that the levels of self-regulation pre-existing in participants and their ability to ignore distractors failed to influence the chemistry comprehension level at time two. Furthermore, the analysis indicated that the ability to control interference did not act as a moderating factor in the link between the students' self-regulation and their comprehension of chemistry content. This was clear from the non-significant change in the regression model upon including the interaction term, with no change in explained variance ($\Delta R^2 = 0$) and an insignificant F-change value, $\Delta F (1, 57) = .04$, $p = .836$. Hence, the simple slopes analysis was not computed. The overall model explained 11% ($R^2 = .11$) of the variance in the response variable.

Table 5. Results of the moderated multiple regression analysis. Dependent variable: post-test chemistry comprehension scores.

		95% Confidence Intervals			
Predictor	**B**	**Lower**	**Upper**	**β**	**p**
Prior self-regulation	.573	.116	1.029	.322	.015
Interference control	-.001	-.032	.029	-.011	.935
Prior self-regulation ✻ Interference control	-.004	-.039	.031	-.027	.836

IV. DISCUSSION

1. IMPACT OF THE INTERVENTION ON KNOWLEDGE AND COMPREHENSION

This ten-week-long investigation was intended to explore if extra-curricular HOT practice could be beneficial for middle schoolers' metacognition and attainment in chemistry and biology. Earlier research is almost unequivocal that training focused on (meta)cognitive strategies is instrumental for learning gains. Actually, our literature review retrieved only one example of ineffective HOT-driven experiment, namely a quasi-experiment [33] in which university students received an online metacognitive learning strategy intervention that ultimately "did not have significant contribution to their development of programming skills". Conversely, in a recent randomized controlled trial [34], learners enrolled in a hybrid chemistry basic concepts course went through ChatGPT-assisted learning guiding students with hints instead of outright solutions over 10 weeks, and this metacognitive strategy resulted in their comprehension of chemistry substantially surpassing those who used ChatGPT in a standard manner. As reported in an experimental study [35], skill training promoting metacognitive and regulatory strategies within a biology course was effective on students' exam scores in biology. A mixed-methods study among 11th graders [36] showed that inquiry-based chemistry instruction in the course of 12 weeks prompted a modest but significant improvement in chemistry attainment relative to a conventional teaching condition. Hubers [37] undertook a course redesign towards enhancement of higher order thinking skills, which brought about students' superior performance on their final paper as compared to the previous years.

We here augment those findings with additional evidence that when the student can analyze scientific phenomena at higher-order levels, while being able to recognize and manage their problem-solving patterns and come up with suitable solutions, it favors educational achievement. Convergent with the majority of studies in the field, the current study states that the proposed HOT-grounded intervention boosted learners' knowledge and understanding of chemical phenomena. Yet eighth graders engaged in the module that homed in on biology considerably outdid controls only on understanding level. These incongruous outcomes between biology and chemistry groups could ensue from various factors, including the instructional nuances inherent to the discipline itself [38]. The interactive and multi-level engagement strategies employed in the HOT program might have been particularly conducive to understanding chemistry concepts, where hands-on experimentation and symbolic representation are crucial for comprehension. Biology, being more conceptually diverse [39], might have required additional time or different instructional approaches to yield significant knowledge gains. Moreover, the lack of significant between-group difference at the post-test biology knowledge assessment can be attributed to the alignment between the content covered in the school curriculum and the scientific concepts introduced within the experimental sessions. Therefore, any substantial differences in learning outcomes would not likely manifest at the level of knowledge acquisition. Overall, a combination of these factors likely contributed to the lack of significant results in biology knowledge. Nonetheless, this absence of effect does not necessarily negate the effectiveness of the intervention. Rather, the potential for differentiation between the intervention participants and untouched controls lies at levels of subject matter comprehension. While both groups may have acquired similar factual knowledge from the standard curriculum, the intervention group likely had opportunities to engage with the material at a deeper level, synthesizing information, making connections, and applying their understanding in novel contexts. This deeper engagement with the subject matter could have facilitated enhanced comprehension among the intervention participants regardless of discipline. The findings align with cognitive load theory, which suggests that when learners are engaged in activities that require them to manage and optimize their cognitive resources, they are more likely to achieve deeper understanding and better comprehension. We believe that the use of historical controls helped to adjust for any secular trends or external factors that might influence the outcomes.

2. IMPACT OF THE INTERVENTION ON METACOGNITIVE SELF-REGULATION

In contrast to the multitude of previously published papers consistently claiming efficacy of meta-learning initiatives for fostering self-regulation abilities [40 – 42], the schoolers in this study did not report improved metacognitive self-regulation across both subjects. The likeliest reason behind this discrepancy with the past literature seems to be the fact that while objectives instruments were employed in the here reported research to examine knowledge, comprehension, and interference inhibition, metacognitive self-regulation was gauged via a self-reported scale as a sole option, which entails the risk of mono-method bias [43]. Self-reported measures, while convenient, may not fully capture the complexities of metacognitive processes, which are inherently introspective and can be challenging to assess accurately through self-report alone. Additionally, students may overestimate or underestimate their self-regulatory abilities due to social desirability bias or a lack of metacognitive awareness. Therefore, the absence of significant improvements in self-regulation could be attributed to the limitations of the measurement tool rather than the ineffectiveness of the intervention itself. Future studies might benefit from incorporating multiple methods of assessment, such as behavioral observations, think-aloud protocols, or ecological momentary assessments, to provide a more comprehensive understanding of students' metacognitive self-regulation. Additionally, the specific content area and the nature of the intervention may play a role. Our study focused on biology and chemistry, which are complex subjects with unique cognitive demands. It is possible that the higher-order thinking skills required in these disciplines may not directly translate to improvements in self-regulation as measured by our instrument.

3. SELF-REGULATION AS A PREDICTOR OF KNOWLEDGE AND COMPREHENSION

Literature tends to declare that students with higher levels of self-regulation are more likely to achieve better learning outcomes as they tend to use more effective learning strategies and have a better grasp of the subject matter [44]. However, there is not much empirical backing of the assumption in the science education literature. In particular, a two-terms-long study [45] confirmed that students who exhibited better self-regulation skills gained higher points in business mathematics. Likewise, a cross-sectional study among undergraduate students [46] disclosed that participants' self-regulation was positively related to their educational attainment. Nonetheless, a qualitative study involving middle school students [47] spotted the lack of interrelation between self-regulation and learning success.

In the present research, the participants of the chemistry trial with greater initial self-regulation eventually scored higher in both comprehension and knowledge. However, the interference control value moderated only the predictive effect on knowledge. Per biology, prior self-regulation scores were positively related to biology comprehension success solely, and interference control moderated this association. Taken together, these findings imply that adequate self-regulative skills combined with the ability to focus in distracting environments might be crucial for middle school students' learning outcomes.

4. LIMITATIONS AND FUTURE DIRECTIONS

There are limitations to this study that revolve around a sample size of 132 individuals and a focus on specific schools in a country, potentially limiting the generalizability of the results to broader contexts. Furthermore, the duration of this study is confined to a period of 10 weeks. A longer instructional timeframe may yield different outcomes. Considering both the findings and limitations of this study, some directions for future research can emerge. First, given that the effects of the experimental module varied by subject, it would be promising to carry out similar studies in the future across a wider range of scientific disciplines. This could provide a more categoric picture of how HOT-based learning influences school students. Secondly, future investigations could delve deeper into the underlying mechanisms and factors not covered in this study, such as learning styles that mediate or moderate the impacts of HOT training on students' learning. This exploration could shed light on the reasons and conditions for the efficacy of the proposed approach and offer insights for its optimization.

V. CONCLUSION AND IMPLICATIONS

Despite the lack of statistically discernable impact on metacognition, this study demonstrates the potential of additional HOT instructional practices in enhancing students' comprehension and knowledge acquisition in science, particularly in chemistry and especially for students with strong interference control. The findings reported here highlight the need for learning schemes that explicitly target the development of HOT skills within the science education. Perhaps such redeveloped instruction could empower learners to not only perform well in science courses but also become more effective learners across disciplines due to a deeper understanding of scientific concepts and processes. Additionally, the implication is that the proposed approach could be integrated into the middle school curriculum. The obtained evidence implies that meta-learning practices may have a subject-specific impact, which is a crucial consideration for educational interventions. Drawing from these contributions, the research reported here may offer valuable references for researchers and educators seeking to design suitable teaching models, especially within the context of school science curriculum design.

Funding Statement

The authors wish to acknowledge that no specific funding or support was provided for this study.

Author Contribution

All authors made an equal contribution to the development and planning of the study. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available from the authors upon request.

Acknowledgments

Not applicable.

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