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Effect of urea application and gibberellic acid foliar spraying on growth characters and some physiological parameters of almond seedlings

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Abstract:

The present study was conducted in private farm, Homs province, Syria, during the season 2021-2022 to study the effect of urea application (0, 50, 100 g/seedling), GA_3 foliar spray (0, 0.5, 1 g/l) and their combinations on some morphological and physiological characters of almond seedlings cv. Zaghhori. The chemical analysis was carried out in laboratory of plant physiology, Horticultural science Department, Faculty of Agriculture, Damascus University. The combination treatment of urea (100 g/tree) and GA_3 (1g/l) significantly promoted the growth characters (203.55 cm, 119.44 cm, 54.57 cm, 5.18, 22.19, 5.96 cm for seedlings height, trunk height, structural expelled height, structural expelled number, branches number average, trunk diameter respectively), and improved the physiological parameters (9.81cm^2, 0.72 gm, 0.19 gm, 87.22 %, 3.50 mg/g fresh weight, 0.94 mg/ g fresh weight, 0.80 mg/ g fresh weight 1.92 %, 0.51 %, 1.92 % for leaf area, leaf fresh and dry weight, relative water content in the leaves, chlorophyll a, chlorophyll b, carotenoids, N, P and K respectively) comparing with all studied treatments and with control which resulted in the lowest values for all studied parameters.

KEY WORDS: Almond, Urea, Gibberellic Acid, Growth Characters, Physiological Parameters

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تأثير المعاملة باليوريا والرش الورقي بحمض الجبريليك في خصائص النمو وبعض المعايير الفيزيولوجية في غراس اللوز

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الملخص:

أجريت هذه الدراسة في حقل خاص في محافظة حمص، سوريا، خلال الموسم 2021 – 2022 بهدف دراسة تأثير المعاملة باليوريا (0، 50، 100 غ/غرسة) والرش الورقي بحمض الجبريليك (0، 0.5، 1 غ/ل) والتفاعل بينهما في خصائص النمو وبعض المعايير الفيزيولوجية لغراس اللوز صنف زخوري. تم إجراء التحليل الكيميائي في مخبر فسيولوجيا النبات، قسم علوم البستنة، كلية الزراعة، جامعة دمشق. أدت معاملة التفاعل بين اليوريا (100غ/شجرة) و حمض الجبريليك (1 غ/ل) إلى زيادة معنوية في جميع خصائص النمو (203.55 سم، 119.44 سم، 54.57 سم، 5.18، 22.19، 5.96 سم بالنسبة لارتفاع الغرسة، ارتفاع الجذع، طول و عدد الأفرع الهيكلية، متوسط عدد الطرود و الأفراخ /الفرع على التوالي) و المعايير الفيزيولوجية (9.81 سم2، 0.72 غ، 0.19 غ، 87.22 %، 3.5 مغ/ غ وزن رطب ، 0.94 مغ/ غ وزن رطب ، 0.80 مغ/ غ وزن رطب ، 1.02 %، 0.51 %، 1.92 % بالنسبة للمساحة الورقية، الوزن الرطب و الجاف للأوراق، محتوى الماء النسبي في الأوراق ، كلوروفيل أ، كلوروفيل ب، الكاروتينات، النتروجين، الفوسفور و البوتاسيوم على التوالي) و ذلك بالمقارنة مع باقي المعاملات المدروسة و الشاهد الذي أعطى أقل القيم بالنسبة لجميع المؤشرات المورفولوجية و الفيزيولوجية المدروسة.

الكلمات المفتاحية: اللوز، يوريا، حمض الجبريليك، خصائص النمو، معايير فيزيولوجية.

Introduction:

Almond (Prunus dulcis Mill.) is of the most economically important nut fruits (Ansari and Gharaghan, 2019, 137) and, almond seedlings are used as tolerant rootstocks for all stone fruit plants (Tagliavini *et al.,* 2000, 2007). In order to expand the almond cultivations, firstly, seedling production and growth of the grafted trees should be optimized in nurseries (Gutiérrez-Gordillo *et al.,* 2019, 38). Standard seedlings of almond have a height of 100-180 cm with three to five branches with 30-40 cm apart around the trunk (Ghasemi *et al.,* 2010). Soils in arid and semi-arid regions are mostly poor of nitrogen because of low organic matter content, Therefore, nitrogen application as organic and inorganic fertilizers is required to supply the plant needs (Chaker *et al.,* 2019, 23). Gibberellins are the longest phytohormones among the class of all the plant hormones. GA_3 is widely studied and used all around the world The main functions of gibberellins are to increase internodal length of the stem, enhancing seed germination, breaking seed dormancy and inducing flowering and fruit set in plants (Jong *et al.,* 2009), stimulate the elongation of stem and shoots (Kalra and Bhatla, 2018, 617; Hedden and Sponsel, 2015, 740).

Review of literature:

Nutrient losses in fertilization have often become a problem that causes the low efficiency. About 40–70% nitrogen, 80–90 % phosphorus, and 50–70 % potassium of the applied fertilizers is lost to the environment and cannot be absorbed by plants (Wu and Liu, 2008, 240). The losses of these nutrients from the soil can be caused by leaching (washing off) by the rainfall, irrigation water, and runoff. Besides causing economic losses, nutrient losses by leaching may lead to environmental problems (Himmah *et al.,* 2018, 104). Optimizing the plant nutrition in nursery is the most practical approach to achieve this considerable growth. Among the essential nutrients, nitrogen has the most important impact on plant growth and development (Marschner, 2012, 135). Almond trees need to receive considerable amounts of nitrogen for developing branches, flowering, and fruiting (Muhammad *et al.,* 2015, 52). Muhammad *et al* (2015, 52) obtained the highest almond yield by application of 466 g nitrogen at various times (20 % in late February, 30% in mid–April, 30% in late June and 20 % in September after harvest). Rahnamoun (2002) reported that application of 600 g nitrogen per tree in two parts in March (at the beginning of growing season) and 45 days later in May is required to obtain the highest yield and growth of almond ‘Azar’. Improving the nitrogen availability to almond trees, increases leaf chlorophyll concentration and promotes its photosynthesis capacity (Mohammadi, 2017, 33). According to its low price, readily availability and high nitrogen content, urea is the most dominant nitrogen fertilizer which is used for growing plant crops (Rui *et al.,* 2019, 4). Due to its high nitrogen content (46.7%), the transport, storage and application costs per nitrogen unit is lower than the other nitrogen fertilizers (Boncz *et al.,* 2012, 2453). Moreover, high acidification capability of this fertilizer may improve absorption of the other essential nutrients such as phosphorus, iron, zinc, copper and manganese in the alkaline calcareous soils of arid and semi-arid regions (Barker and Pilbeam, 2015, 21). However, urea fertilizers exposed to leaching due to its high solubility; therefore, farmers have to use more amounts of fertilizer to meet the plants' needs (Rui *et al.,* 2019, 4). The loss of fertilizer due to leaching not only increases production costs, but also contributes to soil hardening and pollution of groundwater resources. One way to improve the efficiency of nutrient use, is by using slow-release or controlled-release fertilizers, Slow-release fertilizers are made of materials complex structure and little solubility in water such as the urea products. Various synthetic organic products, matrix-based formulations, with the nutrients dispersed in the polymeric or inorganic matrices and polyphosphate-based micronutrient fertilizers so, release their nutrient contents gradually and coincide with the nutrient requirement of plant (Alshamaileh *et al.,* 2018, 11). Slow-release fertilizers are cost-saving, environment friendly and efficiently improve the utilization efficiency of fertilizers in agricultural production (Pang *et al.,* 2018, 2397). However, the time required for seedlings to reach a suitable size for transplanting may take 1 to 3 years. Mobli and Baninasab (2008, 363) tested the effects of foliar application of growth regulators on the vegetative growth and carbohydrate accumulation in shoots and roots of these species. Six-week-old seedlings were treated with gibberellic acid (GA_3) (100 mg/l) alone or with GA_3 followed by ethephon (100 and 200) mg/l), or chlormequat chloride (CCC) (500 and 1000 mg/l), or paclobutrazol (PBZ) (500 and 1000 mg/l). Most levels of plant growth regulators significantly enhanced seedling growth. However, GA_3 alone was most effective on stem height, leaf area, and shoot fresh and dry weights of both almond species. The thickest stems of P. amygdalus and P. webbii were obtained from the application of 100 mg/l GA_3 followed by application of 1000 and 500 mg/l of PBZ, respectively. Application of GA_3 alone on P. webbii and of GA_3 followed by 100 mg/l ethephon on P. amygdalus showed the highest root number, and root fresh and dry weights. High levels of soluble sugars and starch in the shoots and roots of both species were observed when GA_3 application was followed by PBZ. Application of plant growth regulators to the seedlings might be a useful way of enhancing growth of P. amygdalus and P. webbii and reducing the time and cost of seedling production. The involvement of nonstructural sugars in shoot growth of citrus seedlings was studied in 'Carrizo' citrange rootstocks by foliage applications of gibberellic acid (GA_3) and paclobutrazol (Mehouachi *et al.*, 1996, 747). PBZ inhibited length and dry weight of the stem, whereas GA_3 increased length (79 %) and stem dry weight (27 %). These observations may suggest that at this early stage of treatments, growing seedlings are acting as utilization rather than storage. Beneh (Pistacia mutica F. & M.) and Kolkhong (Pistacia khinjuk Stock) are used as rootstocks for pistachio cultivars. Poor germination and very low seedling vigor of these two species have been a major problem in using them as rootstocks for pistachio cultivars. Gibberellic acid (GA_3) at five concentrations (100, 250, 500, 750 and 1000 mg/l) was used during and after stratification to enhance seedling growth. The results showed that GA_3, application during and after stratification, significantly increased the length, trunk diameter, internode length, leaf area and fresh and dry weight of seedlings of both Beneh and Kolkhong species. However, application of GA_3 after stratification was more effective on seedling growth of Beneh. GA_3 application at higher concentrations (500 and 750 mg /l) increased the rate of growth. GA_3 at 250 mg/l enhanced seedlings growth of Kolkhong. It was concluded that application of GA_3 to the seedlings might be a useful method for promoting rapid shoot growth of Beneh and Kolkhong and reduce the expense of seedling production in glasshouse conditions (Rahemi and Baninasab, 2000, 336).

Aim of research:

The objective of this research was to explore the efficiency of slow-release compound fertilizer (granular urea 46) and treatment with GA_3 on reducing the time of seedling production by improving of nutritional status and accelerating growth.

Materials and methods:

– A field experiment was conducted during summer season of 2021-2022, on sandy, loamy soil of private farm at Homs province, Syria, to study the effect of urea and gibberellic acid on optimizing growth of almond specie cv. Zaghhori. This variety is fast and vigorous. The tree is medium sized, drought tolerant and high production.

– Grafted Zaghhori seedlings (on prunus amyglalas rootstock) were planted in terraces with a 5 * 6 m distances between lines and plants. Chemical analysis was performed at the Laboratory of plant physiology, Faculty of Agriculture, Damascus University, Syria. Organic fertilizer (bovine manure) was added (1 kg/ seedling) two times (at leaf emerge and after one month of leaf emerge).

– The soil samples were taken (before culture) and analyzed to be described physically and chemically as shown in table (1).

Table (1): The physical and chemical characters of the culture soil

K_2O available	P_2O_5 available	N total	Organic matter	EC Extract 5:1	pH suspended	mechanical analysis of soil (%)		
mg/kg				$ds.m^{-1}$	(2.5:1)	Clay	silt	Sand
245	0.17	0.2	2.5	0.46	7	48.62	25.18	26.2

−Urea was applied with irrigation water at three concentrations (0, 50, 100 g/tree) for three times: with leaves emerge, after 20 and 40 days of leaves emerge.

−Gibberellic acid was foliar sprayed at three concentrations (0, 0.5, 1 g/l) for three times: with leaves emerge, after 20 and 40 days of leaves emerge.

- The experiment treatments were as follows:

- Un-Treated control.
- Treatment with urea at a concentration of 50 g/tree
- Treatment with urea at a concentration of 100 g/tree.
- Treatment with GA_3 at a concentration of 0.5 g/l.
- Treatment with GA_3 at a concentration of 1 g/l.
- Treatment with urea at a concentration of 50 g/tree + GA_3 at a concentration of 0.5 g/l.
- Treatment with urea at a concentration of 50 g/tree + GA_3 at a concentration of 1g/l.
- Treatment with urea at a concentration of 100 g/tree + GA_3 at a concentration of 0.5 g/l.
- Treatment with urea at a concentration of 100 g/tree + GA_3 at a concentration of 1g/l.

−The study included 9 treatments, each treatment was repeated for three time, where each replicate contains 10 plants. A Simple Random Design (SRD) was used. Results were analyzed using the statistical analysis program (XL-STATE, 2016).

−The averages were compared according to fisher's test and calculated the least significant differences (LSD) at the level 95 % of significance.

- The Studied parameters were as follow:

- Growth characters:

−At the end the season (in 15 Feb), seedling height (cm), trunk height (cm), branches height (cm), branches number, number of twigs/ branches, trunk diameter (cm), leaf fresh weight (g), leaf dry weight (g),

−Leaf area (cm^2): Five leaves were taken randomly from each plant, then scanned on A4 paper and measured using image j program.

−Relative Water Content % was estimated in the leaves using the method of Barrs and Weatherley (1962) according to the following equation:

$$RWC (\%) = (WF-WD)/(WS-WD) \times 100$$

Where's: (WF): Leaves fresh weight. (WD): Leaves dry weight. (WS): Saturated leaves fresh weight.

Physiological parameters:

At the end of the experiment the following physiological parameters were tested:

−**N concentration %:**

Leaves were digested in according to Jackson (1985) and then, N concentration was estimated using the method of Kjeldahl.

−**P concentration %:**

P concentration was estimated spectrophotometry in the leaves using the method of Jones *et al* (1991).

−**K concentration %:**

K concentration was estimated in the leaves using the Atomic flame method (Tendon, 1993).

- Chlorophyll a, b and carotenoids (mg/ g fresh weight):

were calculated according to Beerh and Siddappa (1959).

Results and discussion:

1- Effect of urea application and GA_3 foliar spraying on the growth characters:

Data presented in Table (2) summarize the effect of urea application and GA_3 foliar spraying on growth characters of almond trees. All studied treatments significantly improved growth characters comparing with control non- treated trees. Using high concentration of urea and GA_3 either alone or in combinations significantly promote the growth characters comparing with low concentrations. The combination treatment of urea (100 g/tree) and GA_3 (1 g/l), however, resulted in the best values (203.55 cm, 119.44 cm, 54.57 cm, 5.18, 22.19, 5.96 for tree height, trunk height, branches height, branches number, number of twigs/ branch, trunk diameter respectively), meanwhile, the lowest values (78.37 cm, 42.96 cm, 14.99 cm, 3.93, 15.16, 3.10 for tree height, trunk height, branches height, branches number, number of twigs/ branch, trunk diameter respectively) were observed in control.

Similar results were obtained by Baha *et al* (2009, 524) who pointed that urea application increased leaf number, leaf area, stem height, leaf fresh and dry weight in Prunus amygdalus seedlings. In addition, Mobli and Baninasab (2008) who mentioned that application of GA_3 alone was most effective on stem height, leaf area, and shoot fresh and dry weights of almond tasted species comparing with adding GA_3 with other plant growth regulators. They also proved that application of plant growth regulators to almond seedlings might be a useful way of enhancing growth and reducing the time and cost of seedling production. This might be due to the direct effect of GA_3 in increase stem elongation by increment the spaces between internodes (Hartman *et al.*, 2002, 880). It was concluded that application of GA_3 to the seedlings might be a useful method for promoting rapid shoot growth of beneh and kolkhong and reduce the expense of seedling production in glasshouse conditions (Rahemi and Baninasab, 2000, 336).

Table (2): effect of urea application and GA_3 foliar spray on tree height (cm), trunk height (cm), brunches height (cm), branches number, number of twigs/ branches, trunk diameter (cm).

Treatments	Seedling height (cm)	Trunk height (cm)	Structural expelled height (cm)	Structural expelled number	Branches number average	Trunk diameter (cm)
Control	78.37 [g]	42.96 [h]	14.99 [h]	3.93 [c]	15.16 [e]	3.10 [d]
Urea= 50 g/ tree	108.39 [f]	64.06 [g]	18.83 [g]	4.50 [bc]	16.31 [d]	3.83 [c]
Urea= 100 g/ tree	157.63 [d]	92.08 [d]	37.16 [d]	5.05 [ab]	16.68 [cd]	4.48 [bc]
GA_3= 0.5 g/ l	118.68 [e]	79.29 [f]	23.36 [f]	4.84 [ab]	17.32 [bc]	3.95 [c]
GA_3= 1 g/ l	161.71 [d]	97.79 [c]	44.87 [c]	5.10 [ab]	17.71 [b]	4.54 [bc]
Urea= 50 g/ tree + GA_3= 0.5 g/ l	160.80 [d]	88.97 [e]	33.38 [e]	4.88 [ab]	17.31 [bc]	4.02 [c]
Urea= 50 g/ tree + GA_3= 1 g/ l	195.17 [b]	108.94 [b]	45.01 [c]	5.11 [ab]	21.55 [a]	4.86 [b]
Urea= 100 g/ tree + GA_3= 0.5 g/ l	179.53 [c]	110.16 [b]	50.18 [b]	5.13 [a]	21.35 [a]	5.63 [a]
Urea= 100 g/ tree + GA_3= 1 g/ l	203.55 [a]	119.44 [a]	54.57 [a]	5.18 [a]	22.19 [a]	5.96 [a]
LSD 5 %	5.65	2.96	2.3	0.62	0.93	0.71

The same letters at the level of columns indicate no significant differences at the 0.05 significance level.

2- Effect of urea application and GA_3 foliar spraying on the physiological parameters:

All studied treatments significantly increased leaf area (cm²) comparing with non-treated control (Table, 3). The highest leaf area (9.81 cm²), however, was observed in the combination treatment of urea (100 g/ seedling) and GA_3 (1 g/l). Meanwhile the lowest value (7.10 cm²) was recorded in the control. The lowest leaf fresh weight (0.26 gm) and dry weight (0.02 gm) were observed in control plant meanwhile, the highest values (0.72 gm and o.19 gm for leaf fresh and dry weight respectively) were observed in the combination treatment of urea (100 g/ seedling) and GA_3 (1 g/l). Concerning relative water content (%), the interaction treatments of urea and GA_3 increased the relative water content in the leaves (%) comparing with using of urea alone or GA_3 alone or comparing with control which resulted in the lowest value (78.37 %), meanwhile the highest value (87.22 %) was recorded in the combination treatment of urea (100 gm/ seedling) and GA_3 (1 g/ l).

Table (3): effect of urea application and GA_3 foliar spray on leaf area, leaf fresh and dry weight (g), relative water content in the leaves (%).

Treatments	Leaf area (cm^2)	Leaf fresh weight (gm)	Leaf dry weight (gm)	Relative water content in the leaves (%)
Control	7.10 e	0.26 e	0.02 c	78.37 e
Urea= 50 g/ tree	7.70 d	0.32 de	0.06 abc	79.35 de
Urea= 100 g/ tree	8.30 c	0.48 bcde	0.12 abc	80.71 cde
GA_3= 0.5 g/ l	8.66 bc	0.36 cde	0.02 bc	82.94 bcd
GA_3= 1 g/ l	9.03 b	0.49 bcd	0.13 abc	84.21 abc
Urea= 50 g/ tree + GA_3= 0.5 g/ l	8.20 cd	0.40 bcde	0.10 abc	81.63 cde
Urea= 50 g/ tree + GA_3= 1 g/ l	9.08 b	0.60 ab	0.17 ab	86.65 a
Urea= 100 g/ tree + GA_3= 0.5 g/ l	9.67 a	0.56 abc	0.15 abc	85.47 ab
Urea= 100 g/ tree + GA_3= 1 g/ l	9.81 a	0.72 a	0.19 a	87.22 a
LSD 5%	0.56	0.22	0.14	3.64

The same letters at the level of columns indicate no significant differences at the 0.05 significance level.

Data presented in table (4) clearly exhibit the effect of urea application and GA_3 foliar spraying on photosynthetic pigments (chlorophyll a, b and carotenoids). No significant differences were observed in the combination treatment of urea and GA_3 at all studied concentrations with the superiority of treatment with urea (100 gm/ seedling) and GA_3 (0.5gm/l) which resulted in the highest values for chlorophyll a (3.52 mg/gm fresh weight) and carotenoids (0.81 mg/ gm fresh weight), the highest value for chlorophyll b (0.94 mg/gm fresh weight) was observed in the combination treatment of urea (100 gm/ seedling) and GA_3 (1gm/l). On the other hand, the lowest values (2.12 mg/gm fresh weight, 0.56 mg/gm fresh weight, 0.44 mg/gm fresh weight for chlorophyll a, b and carotenoids respectively) were recorded in the control.

Concerning NPK % concentrations, the lowest N (1.32 %), P (0.28 %) and K (1.32 %) were recorded in the control. Table (4) also shows that all studied treatment increased the concentrations of N, P and K comparing with control and the highest values (1.92 %, 1.51 % and 1.92 % for N, P and K respectively) were observed when urea (100 gm/ seedling) was added in combination with GA_3 (1 gm/l). Similar results were obtained by Al-Aaragi (2010, 76) when GA_3 was foliar sprayed on Beach seedlings cv. *Dexy Red*. Nitrogen supply to almond trees, increases leaf chlorophyll concentration and promotes its photosynthesis capacity (Mohammadi, 2017). Due to its high nitrogen content (46.7 %) in urea (Boncz *et al.*, 2012, 2453), high acidification capability of this fertilizer may improve absorption of the other essential nutrients such as phosphorus, iron, zinc, copper and manganese (Barker and Pilbeam, 2015, 21). Similar results were obtained by Al-Aaragi (2010, 76) who proved that urea application increased N % concentration in the leaves of peach seedling. This might be due to that the availability of nitrogen in the culture soil, increase the absorption by the plant and increase its concentration in the leaves (Al-Aaragi, 2010, 76).

Table (4): effect of urea application and GA_3 foliar spray on photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids (mg/ g fresh weight), and NPK % concentration:

Treatments	Chlorophyll a mg/g fresh weight	Chlorophyll b mg/g fresh weight	Carotenoids mg/g fresh weight	N %	P %	K %
Control	2.12 ^{d}	0.56 ^{b}	0.44 ^{d}	1.32 ^{f}	0.28 ^{g}	1.32 ^{f}
Urea= 50 g/ tree	2.26 ^{d}	0.72 ^{ab}	0.55 ^{cd}	1.35 ^{f}	0.30 ^{fg}	1.35 ^{f}
Urea= 100 g/ tree	2.81 ^{bc}	0.75 ^{ab}	0.70 ^{abc}	1.55 ^{cd}	0.37 ^{e}	1.55 ^{cd}
GA_3= 0.5 g/ l	2.51 ^{cd}	0.79 ^{ab}	0.59 ^{bcd}	1.42 ^{ef}	0.33 ^{ef}	1.42 ^{ef}
GA_3= 1 g/ l	3.05 ^{ab}	0.85 ^{ab}	0.72 ^{abc}	1.61 ^{c}	0.45 ^{cd}	1.61 ^{c}
Urea= 50 g/ tree + GA_3= 0.5 g/ l	3.13 ^{ab}	0.87 ^{ab}	0.73 ^{abc}	1.48 ^{de}	0.43 ^{d}	1.48 ^{de}
Urea= 50 g/ tree + GA_3= 1 g/ l	3.33 ^{a}	0.92 ^{a}	0.78 ^{ab}	1.82 ^{b}	0.50 ^{ab}	1.82 ^{b}
Urea= 100 g/ tree + GA_3= 0.5 g/ l	3.52 ^{a}	0.93 ^{a}	0.81 ^{a}	1.79 ^{b}	0.47 ^{bc}	1.79 ^{b}
Urea= 100 g/ tree + GA_3= 1 g/ l	3.50 ^{a}	0.94 ^{a}	0.80 ^{a}	1.92 ^{a}	0.51 ^{a}	1.92 ^{a}
LSD 5 %	0.48	0.32	0.2	0.1	0.04	0.1

The same letters at the level of columns indicate no significant differences at the 0.05 significance level.

Conclusion:

Treatment with urea and foliar application with GA_3 improve growth of almond seedlings. The combination treatment of urea (100 g/tree) and GA_3 (1g/l) significantly promote the growth characters, and physiological parameters.

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