

## Effect of L-carnitine and *Hoodia gordonii* Supplementation on Metabolic Markers and Physical Performance under Short Term Calorie Restriction in Rats

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### ABSTRACT

Calorie restriction can occur as a consequence of food shortage due to natural calamities, war like situations or voluntarily for health benefits. This state of negative energy balance leads to decrease in performance and increase in feeling of hunger. A normal individual can sustain himself on stored energy in form of body fat for a period of time. It was hypothesised that use of an appetite suppressant like *Hoodia gordonii* along with L-carnitine, which helps in fat oxidation can be used as strategy for coping adverse situation without compromising physical performance. The aim of the study was to evaluate the combined effect of *H. gordonii* and L-carnitine supplementation on metabolic changes and appetite regulatory peptides during calorie restriction. Male albino rats were divided into two groups (n=12 in each) i.e. control (without treatment) and treated (*H. gordonii* organic solvent extract and L-carnitine, orally for 5 days at a dose of 100 mg/kg under 25 per cent calorie restriction). Biochemical variables including regulatory peptides were estimated along with physical efficiency tests. Significant changes in ghrelin, leptin, corticosterone and thyroid hormones were observed in comparison to control. While blood glucose, AMP kinase decreased significantly in the treated group, an increase in CPT-1 activity was observed compared with controls. It is concluded that approach could be practically suitable and effective in emergency situations of combat or food shortage.

**Keywords:** Calorie restriction, *Hoodia gordonii*, L- carnitine, appetite regulatory peptides, forced swim test

### 1. INTRODUCTION

Calorie restriction (CR) is a dietary regimen where a reduction of 20-40 per cent in total energy intake in comparison to the basal food intake is followed. This is one of the most potent and broadly acting dietary interventions in obesity management<sup>1</sup> and may be useful during short term military missions<sup>2</sup>. Keys<sup>3</sup>, *et al.* stated that as the state of negative energy balance proceeds, performance is compromised and hunger is increased even under stressed conditions. Energy restriction results in a feeling of nagging hunger, which has been known to interfere with the ability to perform maximally<sup>4</sup>. Laessle<sup>5</sup>, *et al.* have described that during the periods of low calorie intake the subjects reported stronger hunger pangs, thought more about food, exhibited difficulties in concentrating and had greater fatigue even though there was minimal weight loss. The psychological preoccupation with food and feelings of hunger arising due to low energy intake are associated with afflicted performance<sup>6</sup>. Therefore, use of an effective therapeutic intervention could be an approach to lessen the negative impact of dietary restriction.

*Hoodia gordonii*, is a supplement of natural origin which has gained popularity and commercial interest due to its appetite suppressing properties. Belonging to the *Apocynaceae* family, the plant is a rich source of pregnane, oxypregnane and steroidal glycosides. The steroidal glycoside, P57, is the active ingredient in *H. gordonii* that stimulates satiety<sup>7</sup>. Studies have

reported a reduction in food intake and body weight of rats on *H. gordonii* administration<sup>8-10</sup>. Though limited human clinical trials have been done with *H. gordonii* so far, it has been in use by the indigenous Khoi-San tribes of Kalahari to curb hunger during their long hunting trips. *H. gordonii* administration in obese participants has shown a reduction in appetite and weight loss<sup>11</sup> along with a decrease in blood glucose and triglycerides<sup>12</sup>. Our previous studies have shown that supplementation with *H. gordonii* extract exerts changes in levels of appetite regulatory peptides in response to decreased food intake<sup>13</sup>. Even under CR condition, it demonstrates its anorectic activity through altered metabolic responses with a significant decline in ghrelin and increase in cholecystokinin (CCK)<sup>14</sup>.

Another bioactive compound is L-carnitine, which is a popular nutraceutical because of its role in the fat oxidation to provide energy. It is recognised that supplementation with L-carnitine might enhance the oxidation of fatty acids during exercise, sparing the use of muscle glycogen, thereby delaying the onset of fatigue and culminating in enhanced physical performance. There are several studies indicating L-CAR supplementation is beneficial for exercise performance<sup>15,16</sup>. On contrary many studies have also shown that carnitine supplementation has no beneficial effect on exercise performance<sup>17</sup>. Although inconsistency in results exists, yet, carnitine has been reported to exhibit therapeutic function during altered metabolic states as in pathological and stress

conditions<sup>17,18</sup>. Likewise, we have also observed that L-carnitine supplementation has performance enhancing properties even under short term calorie restriction<sup>9</sup>.

Based on authors previous work<sup>13,14</sup> and available information, authors hypothesize that supplementation with L-carnitine and *H. gordonii* together might have a beneficial effect in overcoming the hunger pangs and improve physical performance during low calorie intake. This study examined the combined effect of these two supplements under short term calorie restriction. The effect of combination of supplements on appetite regulatory peptides, biochemical changes, and physical performance under short term calorie restriction one evaluated.

## 2. MATERIALS AND METHODS

### 2.1 Supplements/ Drugs

#### 2.1.1 L-carnitine

Carnitor-500 tablets, manufactured by Modi-Mundipharma Pvt. Ltd under license from Sigma-Tau India Pvt. Ltd. were purchased and used in the study. The tablets were crushed and then dissolved in deionised water and given at a dose of 100 mg/kg body weight orally to the rats.

#### 2.1.2 Plant Material

Authenticated dry powder of (cultivated) *H. gordonii* was a gift from M/S Farm Vredelus PTY LTD, Farm Douglas, Namibia. Certificate of Analysis along with the liquid chromatography mass spectrometry (LC/MS) chromatogram for *H. gordonii* were also provided by them (LC/MS analysis was done at University of Stellenbosch, South Africa). This was further validated by HPTLC in our laboratory<sup>14</sup>.

#### 2.1.3 Preparation of Plant Extract for use in Animal Study

Organic solvent extract was prepared using a mixture of dichloromethane– methanol (1:1)<sup>20</sup>. Powdered crude drug was weighed (20 g) and mixed with a 100 ml solvent mixture and kept overnight for extraction. The organic layer was separated from raw plant material by centrifugation at 3000 ×g for 15 min at room temperature. Extraction was repeated twice under identical conditions; supernatants were combined and dried at 40 °C. The yield of the crude extract was 8.0 per cent of the dry powder supplied by the manufacturer.

### 2.2 Experimental Animals and Treatment

Male albino rats (*Sprague Dawley*) weighing 200–250 g, bred and reared in the experimental animal facility of the institute, were used in the study. Animals were housed in cages (46 × 24 × 20 cm) in a temperature (22° ± 1 °C), humidity (55–60 per cent) and light-controlled room (lights on at 6:30 h, lights off at 18:30 h). Rats were provided commercial rodent diet M/s Golden Feed Pvt. Ltd., Delhi. Restricted day-time feeding regimen was employed in the study for monitoring of food intake, where in the food was provided only for 6 h during the light phase of the day. Rats were habituated to this regimen two weeks prior to the experimentation. Food left-over after the 6 h duration was weighed on each day. In case of treated rats all the food was consumed as 25 per cent

less food in comparison to basal intake was given. This ensures calorie restriction. Average food intake was used to determine amount of feed to be given during 25% calorie restriction. All procedures and protocols used in the present study were approved by the animal care and use committee of the Institute and followed the guide lines documented in the National Institute of Health's Guide for the Care and Use of Laboratory Animals. The animals were randomly divided into two groups (n = 12 in each) i.e. Control group, and treated group. Treated group was subjected to 25 per cent calorie restriction along with supplementation with *H. gordonii* and L-Carnitine. The rats were administered the doses orally using a rodent feeding needle (Brand name- Swent). The crude extract of *H. gordonii* was administered at a dose of 100 mg/kg body weight (minimum dose effective for causing a reduction in food intake was selected after a dose response study)<sup>13</sup>. L-carnitine was administered 2 h after *H. gordonii* was given to the rats at a dose of 100 mg/kg body weight<sup>19</sup>. The control rats were given equal amount of water using feeding needle along with food and water that was available *ad libitum* for 6 hours and left over food was weighed. In case of treated group all food was consumed by rats. Supplementation was done in the morning for 5 consecutive days at the time of providing food and water to the test animals. Changes in body weight were monitored daily post treatment.

### 2.3 Sample Collection and Preparation

Upon completion of the five day treatment, rats were fasted overnight, anaesthetised and sacrificed. Blood was collected from heart in heparinised tubes; blood plasma was recovered by centrifugation at 1000 ×g for 10 min at 4 °C. The weight of the organs - liver, spleen, kidney, brain was recorded along with gastrocnemius muscle and epididymal fat tissue. For glycogen estimation, the weighed portions of liver were dissolved in 30 per cent KOH immediately after removal, precipitated with 95 per cent ethanol in the presence of sodium sulphate. Ten per cent liver homogenates (w/v) were prepared in 150 m MKCl using Polytron homogeniser and were centrifuged at 3000 ×g for 15 min at 4 °C. The supernatants were divided into aliquots and frozen at -80 °C until assayed. Liver mitochondria were separated by differential centrifugation at 12,000 ×g for 30 min at 4 °C and stored at -80 °C for Carnitine Palmitoyl Transferase-1 (CPT-1) activity analysis.

### 2.4 Analysis of Biochemical Parameters

Blood glucose was measured using glucose oxidase–peroxidase method. Tissue glycogen was estimated using the method of Montgomery<sup>21</sup>. CPT-1 activity was assayed in liver mitochondria by the method of Halperin and Pande<sup>22</sup>. Mitochondrial protein content was measured according to the method by Lowry<sup>23</sup>, *et al.* Radio immune assay (RIA) kits from Phoenix Pharmaceuticals, Burlingame, CA were used with a detection range of 10–1280 pg/ml for the hormones- ghrelin, NPY and CCK in blood plasma. Leptin concentrations were measured with a commercially available ELISA kit for rats from Ray Biotech, Inc., USA. The intra and inter assay coefficients of variance (CV) were 10 per cent and 12 per cent, respectively and sensitivity of 30 pg/ml. Rat plasma insulin

concentrations were determined using a direct ELISA kit bought from Mercodia, Uppsala, Sweden with interand intra assay CV of 5 per cent and 10 per cent resp. The detection limit was 0.12 µg/l. Ethyl ether extraction was performed on plasma for corticosterone according to the manufacturer's instructions using an ELISA kit (Neogen Corporation, USA). Adiponectin enzyme Immunoassay (EIA) kit from Ray Biotech, GA was used for detecting adiponectin. Plasma levels of insulin-like growth factor 1 (IGF-1) were analysed with an EIA kit from Mediagnost, Germany. Rat tri-iodothyronine (T3) ELISA kit, thyroxine (T4) ELISA kit and serotonin/5-hydroxytryptamine (5-HT) EIA kit used to measure the plasma concentrations were products of Cusabio biotech Co. Ltd. AMP kinase (AMPK) was measured using the phosphorylated adenosine monophosphate activated protein kinase ELISA kit.

## 2.5 Physical Performance Tests

### 2.5.1 Forced Swim Test

Swimming, the exhaustive type of exercise developed by Porsolt<sup>24</sup>, *et al.* has been selected in the present study as a model of physical exercise so that muscle trauma caused by other types of exercises like prolonged running on treadmill and exercise stimulated electric shock could be avoided<sup>25</sup>. In this test, the animal is released in a tank of water from which there is no escape and allowed to swim till the animal is unable to surface. The period of time that the animals swim till they are unable to rise to the surface within 10s is recorded as swimming time. The temperature of the water is maintained at room temperature ( $30 \pm 2$  °C). The animals were removed and allowed to recover for 5 min. before putting them back to their cages<sup>26</sup>. This test was conducted on a separate batch of rats. The animals were divided into groups of 8 animals each; with a similar mean weight of each group. The swimming test was carried out before any treatment was given on Day 0 (basal data) and on Day 5 after 2 h of drug supplementation.

### 2.5.2 Forelimb Grip Strength

A Grip Strength Meter (dual/single channel, dunnett firmware version 2.3) from Linton Instrumentation, UK was used to measure forelimb grip strength. It is a determinant of muscular strength and an indicator of neuromuscular function. Method used has been described previously by Ta<sup>27</sup>, *et al.*. The grip strength meter was positioned horizontally and rats held by the tail were allowed to grasp the smooth, metal pull bar (forelimbs only). They were then pulled backward in the horizontal plane. The force applied to the bar at the moment the grasp was released was recorded as the peak tension (g). The test commenced 2 h after the dose administration. It was repeated 3 consecutive times within the same session and the highest value recorded as the grip strength of that animal. Normalised values are expressed as g/kg body weight. Rats were trained prior to testing and each rat was tested (3 trials equal one test session). The test was carried out before any treatment was given on Day 0 (basal data) and on Day 5 after 2 h of drug supplementation during the study period.

## 2.6 Statistical Analysis

All the data in the text, tables and figures are presented

as mean  $\pm$ SD. Statistical analysis was performed using unpaired student 't' test for comparison between control and treated groups. Analysis of variance followed by Newman-Keuls post hoc test was used for data of swim test and grip strength. The *p* value < 0.05 was considered a significant change.

## 3. RESULT AND DISCUSSION

### 3.1 Food Intake, Body Weight and Organ Weights

The mean food intake of control rats was 16 g/rat. No food was left in the cage after 6 h duration in case of experimental rats. The gain in weight in control rats was 5.3 per cent over a period of 5 days. Body weight regulation and energy homeostasis is controlled by numerous metabolic pathway intermediates and neuroendocrine control systems<sup>28</sup>. Calorie restriction, typically leads to a decrease in body weight, as also seen in the treated group (3.14 per cent). However, it was observed that the reduction in body weight was much lesser than on a CR regime alone for 5 days (6.8 per cent) and with *H. gordonii* (6.7 per cent)<sup>14</sup>. This could also be due to the carbohydrate sparing effect, arising from enhanced fatty acid oxidation, leading to efficient energy utilisation. Studies by Jatoi<sup>29</sup>, *et al.*; Leij-Halfwerk<sup>30</sup>, *et al.* have shown that ATP prevents the deterioration in body weight. The hepatic ATP content is reduced following a hypocaloric diet<sup>31</sup>, but *H. gordonii*<sup>7</sup> and L-carnitine<sup>32</sup> both are important factors in ATP production. Hence, might be a contributing factor towards lesser mean body weight loss of the treated rats. However, no differences in the organ weights were observed (Table 1).

**Table 1. Effect of L-carnitine (100 mg/kg body weight) and *H. gordonii* (100 mg/kg body weight) supplementation under calorie restriction for five days, on biochemical parameters**

Variables	Control	Treated
Liver (g)	6.55 $\pm$ 1.50	6.32 $\pm$ 0.74
Heart (g)	0.58 $\pm$ 0.11	0.84 $\pm$ 0.09
Kidney(g)	1.38 $\pm$ 0.19	1.44 $\pm$ 0.42
Gastrocnemius muscle(g)	2.50 $\pm$ 0.62	3.35 $\pm$ 0.33
Spleen (g)	0.53 $\pm$ 0.11	0.89 $\pm$ 0.07
Brain (g)	1.80 $\pm$ 0.13	1.86 $\pm$ 0.10
Adipose tissue (g)	1.38 $\pm$ 0.37	0.98 $\pm$ 0.20

### 3.2 Blood Glucose, Liver and Muscle Glycogen

Numerous studies have shown that weight loss improves glycemic control and since calorie restriction leads to weight loss consequentially, the effect on glycemic control becomes evident<sup>33</sup>. In the present study, experimental rats had significantly lower blood glucose levels in comparison to the control rats (*p*<0.05) (Table 2). The role of L-carnitine in glucose oxidation remains controversial. However, our previous studies show that both L-carnitine<sup>19</sup> and *H. gordonii*<sup>14</sup> lower the blood glucose levels when supplemented during CR. Besides, experimental evidence suggests that there are specific neuronal cell types in the arcuate nucleus that sense the availability of peripheral nutrients, fatty acids and in turn suppress endogenous glucose production<sup>34,35</sup>. All these aspects together might have resulted

in the lowered blood glucose levels of the CR+CAR+HG rats. Despite the glycogen lowering effect of caloric restriction<sup>36</sup>, an increase in liver glycogen content observed in the experimental group ( $p < 0.05$ ) might be an effect exerted by *Hoodia* as it is known to increase the sugar storage in liver<sup>37</sup> and L-carnitine that leads to 'glycogen sparing' in liver<sup>38</sup>. Muscle glycogen however, remained unaffected (Table 2).

### 3.3 Enzyme Activities, Plasma Protein and Muscle Protein

Carnitine palmitoyl transferase-1 is known to be the rate limiting enzyme for fatty acid oxidation. Previous studies from our laboratory have already shown that supplementation with L-carnitine and *H. gordonii* increases CPT-1 activity under CR<sup>14,19</sup>. The activity of CPT-1 in treated rats was significantly ( $p < 0.05$ ) higher than that of control rats (Table 2), thus emphasizing the role of L-carnitine and dietary energy restriction in fatty acid oxidation. Another energy sensing enzyme that acts as a fuel gauge is AMP kinase. It has been found that fatty acid oxidation and AMPK activation are linked, particularly in rodents but there is inconsistency<sup>36</sup>. It is activated by cellular stresses resulting in ATP depletion and is considered as a master switch regulating glucose and lipid metabolism<sup>39</sup>. Research has shown that L-carnitine and *Hoodia gordonii* facilitates ATP production<sup>7,32</sup>. Association of AMPK with glycogen has been established with some studies saying that it may be inhibited by glycogen<sup>32,33</sup>. However, there has been conflicting data on this<sup>42</sup>. The significantly reduced AMPK activity of the treated group vs control ( $p < 0.05$ ) could be due to these reasons. Calorie restriction is known to increase the levels of plasma proteins<sup>43</sup>, an effect which was not observed here<sup>14,19</sup> (Table 2).

**Table 2. Effect of L-carnitine (100 mg/kg body weight) and *H. gordonii* (100 mg/kg body weight) supplementation under caloric restriction for five days, on biochemical parameters**

Variables	Control	Treated
Blood glucose (mg/dl)	60.18 ± 8.32	40.4 ± 2.10*
Liver glycogen (mg/g wet tissue)	0.469 ± 0.75	0.76 ± 0.50*
Muscle glycogen (mg/g wet tissue)	0.14 ± 0.02	0.16 ± 0.033
Plasma protein (g/dl)	5.40 ± 1.20	6.5 ± 1.36
Muscle protein (mg/ml)	18.97 ± 4.09	18.33 ± 2.99
CPT-1 activity (umol/min/mg protein)	10.81 ± 4.5	29.06 ± 6.5*
AMP kinase (pg/mg protein)	314 ± 54	123.34 ± 28.84*

Expressed as mean ± SD (n=12). \* indicates control vs treated group. Significance was set at  $p < 0.05$ .

### 3.4 Appetite Regulatory Hormones

Leptin acts as a mediator of long-term regulation of energy balance acting through suppressing food intake. The significantly higher leptin levels of the experimental group than the control ( $p < 0.05$ ) (Table 1), might be suggestive of the role of L-carnitine here, as plasma leptin values were still very high than exerted by *H. gordonii* under CR as reported earlier<sup>14</sup>. Wolkowicz<sup>44</sup>, *et al.* reported that CPT-1 linked

fatty acid oxidation is a key modulator of leptin expression. Few studies have shown increased leptin concentrations on L-carnitine supplementation in healthy ponies<sup>45</sup> and gestating sows<sup>46</sup>. The list of nutritional factors that affect the circulating leptin concentrations is small and there are no studies so far reporting the effect of L-carnitine and *H. gordonii* on leptin concentrations. Interestingly, ghrelin was also found to be increased. It is still not clear whether an elevated level of circulating leptin causes a reduction in ghrelin levels. Conversely, it appears that leptin does not have a direct influence on ghrelin. Likewise, ghrelin is not a direct regulator of leptin and insulin<sup>47</sup>. The insulin levels remained unchanged in the present study. CR is accompanied by decreased circulating leptin levels, increased ghrelin levels and increased appetite<sup>48</sup>. This was distinctly observed here with increase in ghrelin levels vs *ad libitum* fed. Nonetheless, these ghrelin levels were significantly lower than those of CR+HG rats as reported earlier<sup>14</sup>. Since ghrelin is known to mediate its effects through lipid metabolism<sup>49</sup>, we speculate the action of L-carnitine through modulation of fat oxidation. The expression of adipokines like leptin and adiponectin may be influenced by fatty acids directly by interaction with transcription factors, or indirectly via unknown mechanisms possibly linked to fatty acid oxidation, synthesis or storage<sup>50</sup>. The slight rise in adiponectin could also be linked with enhanced fatty acid oxidation through CPT-1 activity<sup>51</sup> (Table 1). Since feeding requires integration, it is unclear how the expression of the neuropeptides is regulated; by which specific signals and through what efferent systems they accurately match the bioenergetic needs of the organism<sup>52</sup>. Although the rats were on a CR diet, but since fatty acids act as a stimulator for CCK release, an increase was observed (Table 1). Research has proposed that *H. gordonii* influences appetite control also through secretion of CCK<sup>13,53</sup>. There are no studies reporting the direct effects of L-carnitine supplementation on CCK. Nutritional energy balance and macronutrient composition of the diet also appear to be major determinants of IGF-1 bioactivity<sup>54</sup>. It is speculated that the rise of IGF-1 levels, though statistically not significant (Table 1) in the treated rats is attributed to L-carnitine supplementation since no change in insulin was observed. Studies have confirmed that carnitine supplementation causes an increase in IGF-1 levels in rats, pigs, chickens and humans<sup>55</sup>. Since IGF-1 also has hypoglycemic effects, the decrease in blood glucose levels of CR+CAR+HG could be related to the increase in plasma IGF-1. Thyroid hormone action is also modulated by L-carnitine. A significant increase in T3 and T4 levels of the treated rats ( $p < 0.05$ ) (Table 2) observed in the present study could be an effect of L-carnitine supplementation. It has been previously reported that L-carnitine supplementation elevates plasma tri-iodothyronine (T3) levels<sup>56,57</sup>. In addition, Benvenega<sup>58</sup>, *et al.* reported that L-carnitine is a peripheral antagonist of thyroid hormone action and inhibits both T3 and T4 entry into the cell, thereby leading to their accumulation in the peripheral blood. Thyroid hormones are also known to interact profoundly with fatty acid oxidation, in particular through activation of carnitine dependent fatty acid import<sup>59</sup>. An increased CPT-1 activity in the present study might also be associated with the thyroid hormone action.

**Table 3. Effect of L-carnitine (100 mg/kg body weight) and *H. gordonii* (100 mg/kg body weight) supplementation under calorie restriction for five days on different hormones in plasma**

Appetite Regulatory Hormones	Control	Treated
Leptin (pg/ml)	835.32 ± 390.18	1447.75 ± 574.77*
Ghrelin (pg/ml)	1208.45 ± 143	2045 ± 134.42*
Insulin (ng/ml)	0.36 ± 0.05	0.31 ± 0.06
Adiponectin (ng/ml)	84.11 ± 54.48	118.68 ± 33.32
CCK (pg/ml)	283.27 ± 16.63	388.83 ± 22.09
IGF-1 (ng/ml)	475.08 ± 136.86	511.65 ± 125.66
Tri-iodothyronine (ng/ml)	0.97 ± 0.15	1.53 ± 0.25*
Thyroxine (ng/ml)	36.64 ± 2.46	47.77 ± 4.03*
Corticosterone (ng/ml)	67.55 ± 11.77	42.28 ± 7.98*
Brain serotonin (ng/ml)	0.96 ± 0.36	0.89 ± 0.66

Expressed as mean ± SD (n=12). \* indicates control vs treated group. Significance was set at p<0.05.

Accumulating evidence from animal and human studies indicate that pharmacologic doses of L-carnitine have immunomodulatory effects resembling those of glucocorticoids as it is a ‘nutritional modulator’ of glucoreceptors<sup>60</sup>. The significant decrease in plasma corticosterone (p<0.05) (Table 1) in the present study is thus, an effect of L-carnitine supplementation. Similar results have also been reported by Elgazzar<sup>57</sup>, *et al.* L-carnitine supplementation has an influence on dopamine out flow. A study by Juliet<sup>61</sup>, *et al.* reported increased levels of dopamine, epinephrine, and serotonin in brain on carnitine supplementation. Though, the present study demonstrates no change in the brain serotonin of the treated rats in comparison to control (Table 2).

### 3.5 Forced Swim Test and Forelimb Grip Strength

In the present study, the treated (78 per cent) rats had longer swim time on day 5 than on day 0 (p<0.05) (Table 4). Increased glycogen stores in liver and skeletal muscle, and increased fatty acid oxidation are effective mechanisms to improve endurance exercise<sup>62</sup>. Previous studies have shown that calorie restriction does not deteriorate work efficiency<sup>63-65</sup> and modest energy restriction (upto 20 per cent of intake) for a period of 2 weeks has shown not to impair performance<sup>66</sup>. L-carnitine under short term CR improves swim time of rats<sup>19</sup>. Tulp<sup>9</sup>, *et al.* has reported the use of *H. gordonii* in improving endurance by the San people of South Africa. The increase in the swim time to exhaustion in the treated rats could be due to

**Table 4. Effect of L-carnitine (100 mg/kg body weight) and *H. gordonii* (100 mg/kg body weight) supplementation under calorie restriction for five days, on swim time (min.) of rats**

Experimental groups	Day 0	Day 5
Control	54.50± 10.27	58.60± 11.03
Treated	73.58± 11.46	131.37± 29.33 **

Expressed as mean ± SD (n=12). \*p<0.05, treated group day 5 vs control group day 0; \*\*p<0.05, treated group day 5 vs control group day 5.

all of these factors together. The insignificant increase in the swim time exhibited by the control rats depicts the learning behavioral adaptation within the rats for the task (Table 4). However, no change in the forelimb grip strength was observed with the treatment (Table 5). No change in the muscle protein might be a factor contributing to this.

**Table 5. Effect of L-carnitine (100 mg/kg body weight) and *H. gordonii* (100 mg/kg body weight) supplementation under calorie restriction for five days, on forelimb grip strength (g/kg) of rats**

Experimental groups	Day 0	Day 5
Control	1.21 ± 0.27	1.36 ± 0.21
Treated	1.40 ± 0.32	1.33 ± 0.27

Expressed as mean ± SD (n=12).

In summary, the purpose of this study was to investigate the supplemental effects of *H. gordonii* and L-carnitine together under CR on regulatory peptides, biochemical variables and physical performance. Our results support the hypothesis, that the combined treatment positively had an impact on physical performance and modulated the levels of appetite regulatory peptides. It is concluded, that although the combined supplementation is beneficial for improving endurance capacity, it is not that potent in curbing hunger as is *H. gordonii* alone and/or under CR, as shown in our previous studies. Nonetheless, this line of approach could be practically suitable and effective in emergency situations of combat or food shortage.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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