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Bioactivity of food peptides: biological response of rats to bovine milk whey peptides following acute exercise.

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ABSTRACT

The higher availability of bioactive peptides in whey protein hydrolysate (WPH) has been linked to a number of physiologically positive benefits.

Aims: The goal of this study was to examine the effects of WPH's four BCAA-containing dipeptides on immunological regulation, HSP expression activation, muscle protein synthesis, glycogen content, satiety signals, and the influence of these peptides on plasma free amino acid profiles.

WPH was used as a control group. The animals were placed into six groups: control, water, lle-Leu, lle, Leu, and Val-Val. WPH was also used as a control group. Except for the control, all animals were subjected to intense physical activity for a short period of time.

Results: Leu-Val increased HSP90 expression whereas lle-Leu promoted immunological response, hepatic and muscular glycogen, and HSP60 expression. We found that all three of the dipeptides lowered the levels of GLP-1 and GDF-1, but there was no change in leptin levels. NF-kB expression was suppressed by all peptides. Plasma amino acid time-course demonstrated peptide- and isomer-specific metabolic characteristics, including a rise in BCAA concentration.

According to the findings, lle-Leu was able to reduce the effects of exercise-induced immune suppression, increase glycogen levels, and activate an anti-stress impact (HSP). The expression of HSP90, p-4EBP1, p-mTOR, and p-AMPK was also elevated by Leu-Val. Peptides found in WPH may have a role in a variety of health benefits, according to the research.

Introduction

A number of biological responses have been shown to be improved by whey proteins (WPs), including cytoprotective effects mediated by increased heat shock protein (HSP) expression, increased glycogen content, increased protein synthesis in muscles, immune response modulation, and an increase in satiety [1–6]. An important aspect of the body's natural defence mechanism, the HSPs help cells withstand and tolerate various stresses while also repairing and preventing harm. During times of stress, HSPs preserve cell integrity and shape, hence boosting cell survival [7]. For example, hydrolyzed WPs produce bioactive peptides, particularly BCAA-containing dipeptides, which are capable of regulating physiologic activities at the cell and tissue level [9]. [8] Peptides from whey protein hydrolysate (WPH) may be responsible for the favourable health benefits of WP intake, notably the WPH's bioactive peptides [2]. Exercise and proper homeostasis are often related with the favourable benefits of WP consumption. Because regular physical activity has been shown to alter homeostasis, it is worth including into your routine.

To get a deeper understanding of how these proteins might help counterbalance some of the negative

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effects of exercise-induced changes in the body temperature, immunological suppression, muscle injury, or a rapid loss of glycogen. Given that the milk whey proteins include dipeptides L-leucyl-isoleucine, L-isoleucine, L-leucine, and L-valyl-leucine (Val-Leu), and since they are all dipeptides, the hypothesis is that the milk whey proteins contain the dipeptides L-leucyl-valine (Leu-Val).

We reasoned that any one of these dipeptides may explain some of the metabolic effects ascribed to hydrolyzed milk whey proteins since they were sufficiently stable to resist separation from a hydro lysate. So the goal of this research was to examine the effects of WPH's four BCAA-containing dipeptides on immunological modulation, HSP expression, muscle protein synthesis and glycogen content as well as the satiety signalling mechanisms. As essential nutrients and potential cell signalers, BCAAs and their peptides should be studied to see how plasma free amino-acid profiles change when these four dipeptides are consumed. As a consequence, the findings may reveal which peptides (components of whey) are accountable or implicated in the various effects associated with WPH ingestion.

Components and procedures

Approval based on moral principles

The University of Campinas (UNICAMP, BrazilEthics)'s Commission on Animal Use (CEUA-UNICAMP, protocol number 2845–1) authorised the comprehensive experimental methods for all animal manipulations.

The use of animals in research and the ethics involved

A total of 56 male Wistar rats (21 days old, specific-pathogen-free), kept in separate cages with access to food and water, and maintained under controlled environmental conditions (reverse 12-hour light/dark cycle, 55 percent humidity, 22 1°C), were utilised in the first experiment. When the animals were 7 weeks old and weighed 278 g 14.33, they were divided into seven groups (n = 8) based on the type of supplement they received: control (no gavage), vehicle (water), L-isoleucyl lle lle, lle lle, lle lle, lle lle, L-leucyl lle lle, Lle lle, Lle lle, and whey protein hydrolysate (WPH). WPH has identified these BCAA-containing dipeptides. Previous studies have shown that some of these peptides have favourable effects [10]. Peptides with branched chain amino acids have also been shown to reach the circulation and tissues in their intact form, passing through the digestive system unaltered [11]. All

Except for the control, all participants performed a 60-minute exercise session on a treadmill at a speed of 18 m/min without any incline. WPH or water (vehicle) was given to animals immediately after the activity and they were killed three hours later. There was research done on the immunological system, expression of heat shock protein (HSP), satiety, and glycogenic reactions. It was utilised to create a time-course curve of 60 animals that had the same features as the first experiment (e.g., characteristics, food treatment, dosage, and exercise regimen) (0, 15, 30, 45, 60 min). After the activity, the animals were gavaged and tested for plasma amino acids throughout a 60-minute time span. The goal was to see how different dipeptides affected the plasma amino acid profiles. The animals were categorised into six groups: control, lle-Leu, Leu-lle, Val Leu, Leu-Val, and WPH.

Hydrolyzed whey protein peptides and peptides

By oral gavage, each animal received a single dosage of 3 mmol (0.75 g/kg) dissolved in water of the peptides or WPH (0.75 g/kg). BioBasic manufactured all dipeptides (purity > 98 percent) (Markham, Ontario, Canada). Hilmar Ingredients provided the WPH (Hilmar, CA, USA).

collection of blood and biochemical analysis

parameters Blood samples were taken three hours after peptides gavage and centrifuged at 3000 g (4°C, 15 minutes) to get the serum. We utilised Millipore's GIP, GLP-1, C-peptide, insulin levels, interleucine 1 and interleucine 10 (Recytmag-65K), and leptin assay kits according to the manufacturer's instructions and acquired them all from Millipore (Radpk 81K). An automated cell counter was used to measure haematological (immune system) characteristics in the blood drawn with EDTA anticoagulant (Ac.T5diff haematology analyzer, Beckman Coulter, High Wycombe, UK).

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Method of Western blotting

Animals had their soleus muscles excised and frozen in liquid nitrogen for further study. Antiprotease buffer was used to homogenise muscle samples as previously reported [2]. The Lowry technique [14] was used to determine the protein content of muscle homogenate. Samples were available for download.

transported to a Santa Cruz nitrocel lulose membrane by semi-dry electrophoresis, and then separated by SDS-PAGE (Bio-Rad, CA, USA). HSP90 (ADI-SPA 831), HSP60 (ADI-SPA 806), and GAPDH (ADI 905734) were used for the blots. The appropriate antibodies were used: Enzo Life Sciences – HSP90, HSP60 (ADI-SPA 831), GAPDH (ADI 905734); Abcam – OGT (ab59135), NF-kB p65 (ab7970), BCKDH (ab59747); Cell Signaling Technology – p-mTOR Ser 2448 (2971S), mTOR, MA 2972; Bethyl – 4EBP1 (TX A300501A); Upstate Biotechnology – PI A UVITEC Cambridge device was used to examine the membranes (model Alliance LD2). Blots were analysed digitally using Image J to quantify them.

Free amino acids in the plasma with time

After pep tides gavage, blood samples were drawn from the tail vein of each rat and kept at 20°C with heparin anticoagulant at intervals of 0, 15, 30, 45, and 60 minutes. Plasma was obtained by centrifuging the samples at 3000 g for 15 minutes at 4°C. Molecular exclusion filtration was used to extract the free amino acids from plasma (100 mg) using a solution of methanol (99.9%, HPLC grade) and 0.1 M hydrochloric acid (80%/20%, v/v) (Vivaspin 500 Sartorius). Vacuum cryogenic evaporation was used to evaporate the extract (40 L). A portion of the evaporated material was redissolved and homogenised in 20 L (methanol, triethylamine and ultrapure water) before being derivatized at room temperature using HPLC grade chemicals and then evaporated once more. A dibasic anhydrous sodium phosphate buffer (pH 7.4) was used to dissolve the derivatized materials, and a Luna C-18, 100 ; 3, 250 4.6 mm (00G-4251-E0) column (operating at 46°C and detecting at 254 nm) was used to chromatograph the mixtures [15].

Content of glycogen

There was a need to determine glycogen levels in various tissues. Glycogen was precipitated with ethanol after tissue samples were digested with potassium hydroxide until they were completely dissolved. Phosphor–sulphuric acid was used to react with the glycogen precipitate [16]. A spectrophotometer (Beckman-Coulter DU 640) read the absorbance at 490 nm. Analysis of data using statistical methods Analysis was performed using SPSS (Statistical Package for the Social Sciences, Chicago, USA–software version 17.0). The data were given as mean and standard error (mean SEM). p 0.05 was used as the cutoff point for statistical significance. In order to create the graphs, we used GraphPad Prism (CA, USA).

Results

Proteomic analysis of proteins

HSP90 and HSP60 expression were raised by Leu-Val and lle-Leu, respectively, among the WPH peptides (Figures 1(a, b)). Figure 1(c) shows that all peptides, except Val-Leu, showed a significant increase in the expression of OGT (O—acetylglucosaminyltransferase). Muscle nuclear factor kappa B (NF kB) expression was lowered in all pep tides. When compared to the vehicle, physical activity enhanced BCKDH expression and the expression of Val-Leu peptide (Figure 1e) was higher, but remained at control levels. The AMP-activated protein kinase (AMPK) phos phorylation was induced by Leu-lle and Leu-Val peptides (Figure 1f)). Figure 1(g) and Figure 1(h) show that Leu-Val promoted mTOR fosforilation and 4EBP1 expression, respectively. IDE levels were lowered in all peptides compared to the control group (Figure 1(i). Figures (j, k) show that there were no changes in the reactions of AKT and p85.

Glycogen levels and blood chemistry

Dipeptide lle-Leu better protected liver glycogen content than other dipeptides (Figure 2a) during exercise. When compared to the control, lle Leu increased the exercise-induced cardiac glycogen content, but there was no difference between lle Leu and the dipeptides When compared to the vehicle, Val-Leu, and Leu-Val dipeptides, lle-Leu caused a significant increase in muscle glycogen storage. However, compared to other peptides, the lle-leu dipeptide had the lowest amount of kidney glycogen, remaining at normal levels (Figure 2(d)). Compared to the control, all peptides lowered insulin levels. When compared to a control group, the C-peptide

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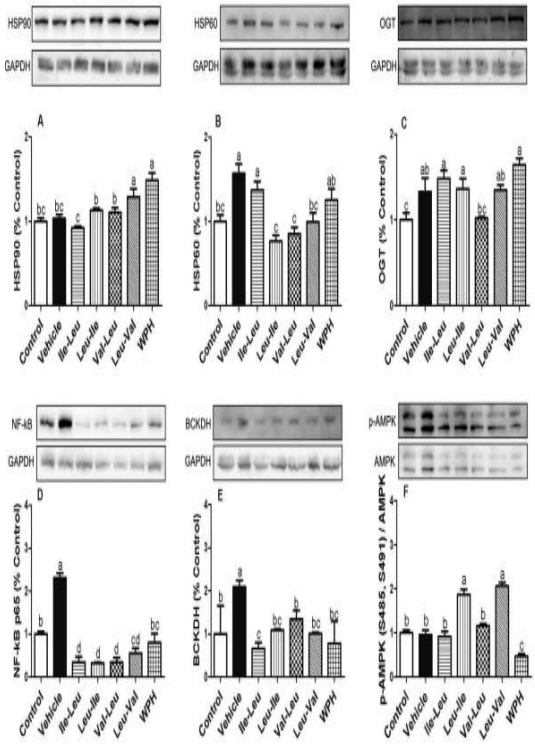
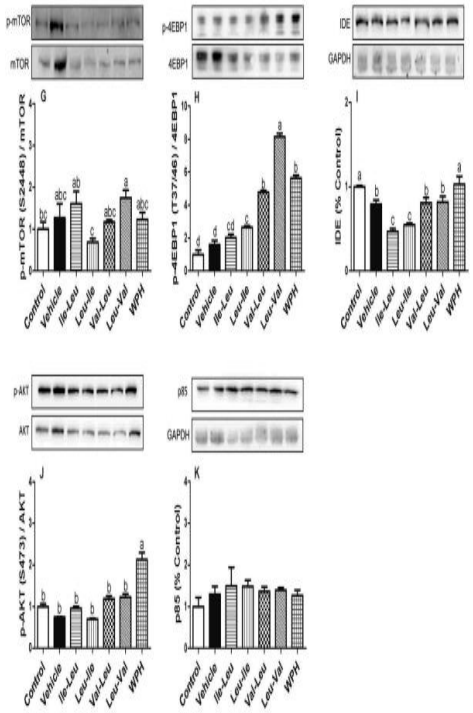
levels of all except the lle-Leu and WPH were lowered by exercise, as shown in Figure 2 (f). All peptides and WPH lowered glucose-dependent insulin sensitivity.





(n = 8) Western blot analysis mean (SEM) values are shown in Figure 1. P-AKT p85 (k), p85 (l), p-AKT p90 (m), p-AMPK (f), p-mTOR (g), p-AMPK (h), IDE I p4EBP1 (h), HSP90 a, HSP60b (k). Significant differences (p 0.05) are denoted by different letters. loading control is the GAPDH. whey protein hydrolysate, L-isoleucyl-leucine, L-leucyl-isoleucine, and L-valyl leucine were all used as groups, as was a control (no gavage or treatment), vehicle (water), and the resting, untreated group (W)

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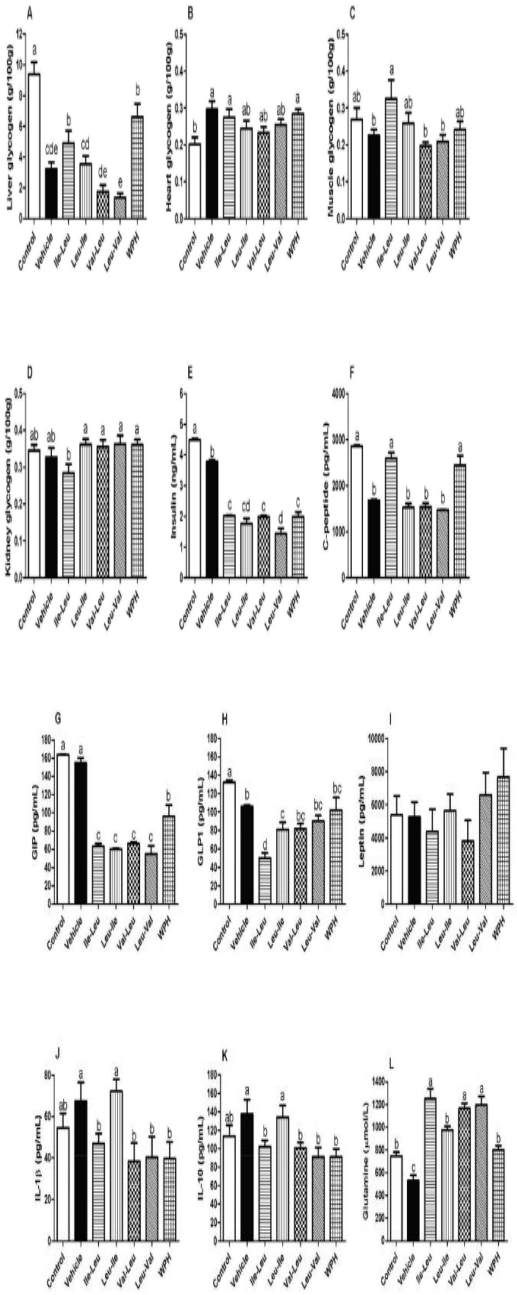

FIGURE 2: Glycogen content means (SEM) liver, heart, muscle, and kidney. Blood biochemical parameters (means SEM): There are a variety of hormones involved in weight loss: insulin, C-peptide, IL-1, GIP, GLP-1, leptin, GLP-10, GLP-1a, and glutamine free form (mol/L). Significant differences (p 0.05) are denoted by different letters. whey protein hydrolysate and whey protein hydrolysate were the only groups that received a placebo (WPH levels of GIP, but there was no difference between the dipeptides (Figure 2(g)). ” Figure 2(h) shows that the lle-Leu group had the lowest amount of the glucagon-like peptide-1 (GLP-1). Figure 2(i) shows no change in leptin levels. In comparison to other peptides, the levels of interleukin-1 and IL-10 increased with Leu-lle, but did not vary from control (Figures 2(j, k)). Plasma glutamine levels were increased by the lle-Leu, Val-Leu, and Leu-Val peptides (Figure 2(l)).

The body's defence system

One bout of acute exercise lowered lym phocytes, monocytes, platelets and erythrocytes while boosting MCHC, leukocytes and neutrophils (comparison of control (at rest) with a vehi cle), according to the results in Table 1. (exercised). While Val-Leu only enhanced neutrophils, lle-Leu stimulated both leukocytes and neutrophils. To counteract the decrease in lymphocytes brought on by exercise, no other peptide except for lle-Leu was successful; lle-Leu was the only peptide that successfully maintained lymphocyte levels while failing to bring them back to control levels. Increases in monocytes and platelets were seen with Leu-lle. Eosinophils, basophils, and hematocrit did not change (Hct). Lymphocyte and haemoglobin counts were increased with Leu-Val.

Time-course of plasma free amino acids

Free amino acid profiles in plasma may be shown to react to all peptides in general (Figure 3). Since all of the peptides examined included solely BCAAs, the concentrations of leucine, isoleucine, and valine should be the most important findings. It is clear from Figures 3(a, b) that all of the peptides increased leucine levels, although Val-Leu was by far the least abundant. Peptides lle-Leu and Leu-lle both raised isoleucine levels, whereas Leu-Val increased valine levels, but Val-Leu did not. Each peptide had a distinct effect on the other amino acids, as seen by the findings that glutamic, serine, cysteine and taurine were all elevated in the same manner, but in various ways. Lysine and tryptophan were also elevated, but in different ways.

Discussion

The study's goal was to see whether four dipeptides that are anticipated to be generated during digestion

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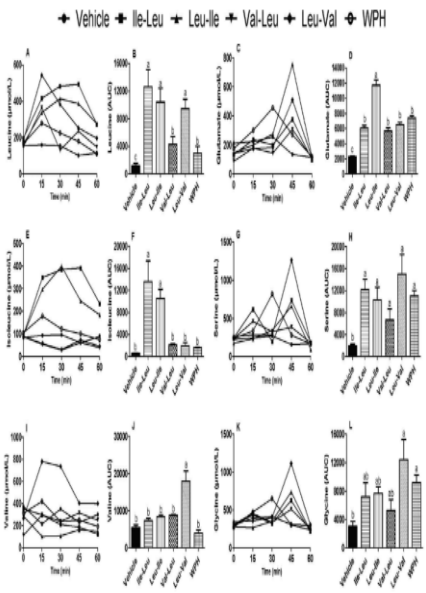
of the milk WPs were responsible for some of the

Table 1. Immune system.

	Erythrogram					Leukogram						
	Erythrocytes ($\mu L \cdot 10^3$)	Hct (%)	Hemoglobin (g/dL)	MCV (fL)	MCHC (g/dL)	Leukocytes (µL)	Neutrophils (µL)	Lymphocytes (µL)	Monocytes (µL)	Eosinophils (µL)	Basophils (µL)	Platelet ($\mu L \cdot 10^3$)
Control	7945^{c} (5)	40 (1)	16.05^{c} (0.05)	50.3^{a} (0.01)	40.1^{b} (0.1)	5850^{c} (50)	819^{a} (7)	4914^{a} (42)	58.5^{ab} (2.5)	0 (0)	0 (0)	1.023
Vehicle	7585^{d} (15)	37 (1)	15.95^{c} (0.04)	48.7^{b} (0.15)	43.05^{a} (0.20)	6200^{b} (100)	2480^{e} (40)	3720^{d} (60)	0^{b} (0)	0 (0)	0 (0)	967^{e} (8)
Ile-Leu	7905^{c} (25)	39 (1)	16.45^{b} (0.05)	49.25^{b} (0.10)	42.15^{a} (0.15)	6700^{a} (100)	2412^{e} (36)	4221^{b} (63)	67^{ab} (1)	0 (0)	0 (0)	1016^{b} (10.5)
Leu-Ile	7879^{c} (5)	38 (1)	15.95^{c} (0.07)	48.15^{b} (0.05)	41.95^{a} (0.12)	5550^{d} (50)	1498^{d} (13)	3885^{c} (35)	111^{a} (1)	0 (0)	0 (0)	1062^{b} (8.5)
Val-Leu	7885^{c} (5)	38 (1)	16.15^{c} (0.04)	48.15^{b} (0.05)	42.45^{a} (0.1)	5350^{d} (50)	2461^{a} (23)	2782^{e} (26)	53.5^{ab} (3.0)	0 (0)	0 (0)	938^{e} (2)
Leu-Val	8565^{b} (25)	39 (1)	17.05^{a} (0.05)	45.5^{c} (0.1)	43.65^{a} (0.25)	4300^{e} (10)	1720^{e} (3)	2537^{f} (10)	43^{c} (0.3)	0 (0)	0 (0)	983^{e} (6)
WPH	8415^{b} (25)	39 (1)	16.8^{a} (0.03)	46.3^{c} (0.13)	43^{a} (0.1)	6050^{bc} (50)	2299^{b} (19)	3690^{d} (30)	60^{b} (0.5)	0 (0)	0 (0)	985^{e} (9)

Mean and (SEM). Different letters in the same column represent significance differences ($p < 0.05$). Hct: hematocrit; MCV: mean cell volume; MCHC: mean corpuscular hemoglobin concentration.



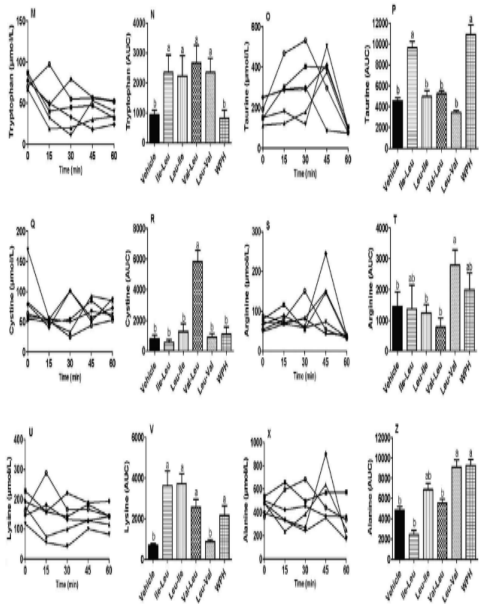


Figure 3 shows the temporal history of the plasma free amino acids profile (mol/L): leucine, glutamate and isoleucine concentrations, as well as the area under the curve for leucine (AUC). Plasma serine, (f) Isoleucine AUC, (g) The plasma AUC for serine (h) is shown in the figure below. The AUCs for plasma valine I and glycine (j) can be found in the table below. The AUCs for plasma tryptophan (m) and tryptophan (n) can be found in the table below. The AUCs for plasma taurine (o) and taurine (p) can be found in the table below (z). Significant differences (p 0.05) are denoted by different letters. Whey protein hydrolysate and whey protein hydrolysate are two of the six groups that make up the whey protein hydrolysate (WPH). During the 60-minute time-course, amino acid levels were found to have restored to their pre-workout levels. In this study, the water group was employed as a control. Animals with typically functioning bodies have HSPs, which work as part of an endogenous defence mechanism to repair damage produced by stressors [7,17]. HSPs play an important cytoprotective role in this system.

Adaptive mechanisms, such as increased production of HSPs, are designed to protect tissues against sudden changes in homeostasis (anti-stress effect) as happens during exercise. HSP90, a protein that protects the skeletal muscle of horses exercising on a treadmill, increased in expression when lipoic acid was supplemented [18]. It has been shown to be effective in rats [1,2,19] to stimulate HSP response

and change homeostasis. WPH improves the muscle expression of exercise-induced HSP90, but not HSP60, in rats [3]. This is consistent with our findings. Our new findings suggest that the WPH capacity to increase HSP90 expression may entail the dipeptide Leu-Val, while merely lle-Leu may boost exercise-induced HSP60 expression. It is thought that OGT, a protein in the hexosamine pathway, is involved in the glutamine-mediated increase of HSP [20]. This is contrary to recent findings with WPH, which showed a correlation between HSP increase and OGT expression after the ingestion of BCAA-containing dipeptides. Glutamine may be the sole amino acid that has a correlation. Exercising has been shown to have a short-lived effect on the immune system, producing immuno-suppression for the first few hours, but recovering to normal levels after 15 hours. People may be more vulnerable to illnesses during this time period, known as the "open window" phenomenon [21]. These new findings (Table 1) corroborate previous studies showing that a single bout of exercise increases leukocytes [6,22], lowers lymphocyte counts [23], and has no effect on basophils or eosianophils [22]. In addition, we found that platelets decreased after exercise, which is consistent with the findings of previous research [6]. Fewer than a handful of research have examined the role of peptides in the immunological response of whey protein [5,24–27]. [9] The immunological modulatory potential of milk protein Peptides Tyr-Gy Gy and Tyr-Gy was discovered, however their method of action remains a mystery [9]. BCAAs may potentially impact immunological indicators, according to some research [23]. Leukocytes and neutrophils were raised, whereas lymphocytes were retained, suggesting that lle-Leu dipeptide might lessen the immunological suppression brought on by exercise. It has been hypothesised that WP's immunomodulatory properties may be linked to its high glutamate concentration [6,27]. WPH has been shown to increase the production of glutamine synthetase, an enzyme that may use the nitro gen provided by BCAAs to produce glutamine. [1] As a consequence, we measured the plasma free glutamine concentration and found that the lle-Leu peptide, which affects a variety of immunological indicators, also causes an increase in the plasma free glutamine concentration. It is also possible that the production of HSPs might elicit an immunological response [28], although the mechanisms are yet unknown.

Few approaches exist to detect HSPs in extracellular settings or on membranes, where HSPs are thought to impact the immune response [29]. Depending on the HSP family and the kind of immune cell with which the HSP interacts, HSPs may either promote or inhibit inflammation. Because our method did not discriminate between intracellular and extracellular HSPs, we are unable to relate our findings to this new kind of activation at this time. Whey protein and bioactive peptides have been shown to have a positive impact on interleukin levels during intensive exercise. Kerasioti et al. (31) found that supplementing with whey protein after strenuous exercise increased levels of the anti-inflammatory IL-10. Pro- and anti-inflammatory interleukins were raised simultaneously, although NF-kB expression was not. This mismatch may be due to the fact that interleukins and NF-kB were measured in distinct tissues. According to our findings, all of the tested anti-inflammatories lowered the NF-kB expression. Glycogen levels in several tissues are known to be elevated and/or preserved when WP is consumed, even after intense activity (3,2). An experiment conducted recently found no increase in liver, muscle, or heart glycogen levels after consuming WPH's lle-Leu and Leu-lle components (10). Our findings, on the other hand, suggest that lle-Leu may have a role in the preservation of glycogen in whey (Figure 2). The discrepancy in results between the two experiments was most likely caused by the fact that the peptides were consumed at different times. The recovery time after gavage in Morato et al. (10) was just 30 minutes, but in the current research it was 3 hours. During instances of restricted oxygen supply, renal glycogen is thought to be critical for cell survival (32). Our findings are consistent with the idea that exercise may cause a minor change in kidney glycogen, which may be seen 24 hours after the activity (33). AMPK activation has been suggested as a possible mechanism through which exercise-induced muscle glucose uptake increases (34). AMPK phosphorylation was induced by Leu-lle, but the glycogen level remained unchanged, which is consistent with our findings. AMPK phosphorylation is also stimulated by the Leu-Val dipep tide, which has no impact on glycogen. Consumption of whey protein leads to greater feelings of fullness than other protein sources or glucose (4,35–37). GLP-1 and GIP are hormones involved in appetite control and satiety promotion, and previous research shows that whey protein stimulates their production (36–39). In addition,

peptides in whey have been hypothesised to enhance satiety signals, although no identification of such peptides or the underlying processes involved have been advanced (40). Our findings show that none of the BCAA-containing dipeptides contribute to the reported satiety effect of whey if GIP and GLP-1 are taken into account.

This research also used samples collected 3 hours after dipeptide gavage, although most satiety studies use longer periods of time for sample collection and measurement of GIP and GLP-1 parameters. Temporary and short-term hormonal modifications have been shown to reduce hunger sensations (41–43) during or immediately after exercise (41–43). Figures 2(g, h) show a fall in GIP and GLP-1 levels after the exercise, which seems to be in line with previous findings. At this point, the activity has exhausted all of the animal's reserves of energy and is more likely to induce a catabolic condition than a resting animal (the "control"). Insulin and C-peptide are both secreted and stored in the blood at equimolar amounts (44). We may have detected differing amounts of C-peptide and insulin in our experiment because the C-peptide has a half-life roughly 10 times longer than insulin (45). Type-1 diabetes patients given C-peptide saw an increase in skeletal muscle blood flow when it was administered, proving that it had biological activity (44). In line with our findings, which revealed that all peptides except for lle-Leu had lower insulin and C-peptide levels when compared to C-peptide levels, a recent study found that ingestion of whey increases glycemia reduction via insulin-independent pathways (38). It seems that little is known about the variables that control the expression of IDE, particularly whether whey protein-derived peptides have any impact. Insulin degradation has been linked to IDE. An increase in insulin clearance rather than alterations in insulin liberation may be more responsible for the decrease in insulin levels seen after four weeks of swimming according to Kim et al. (46) who discovered overexpression of the IDE gene. There was no evidence of IDE overexpression after a single exercise session in our investigation (Figure 1(i)). WP intake is known to stimulate the mTOR and 4EBP1 indicators of muscle protein synthesis, particularly in conjunction with exercising. Supplementation with leucine, regardless of the protein source (whey or casein), was shown by Lollo et al. (47) to activate the mTOR pathway. Leu-Val peptide may have contributed to the overall impact of whey since it boosted both mTOR and 4EBP1 while not reaching the stage of any muscle mass growth (data not provided) in a single workout session, as evidenced by our findings In response to certain of the peptides, plasma amino acids reacted quite specifically, indicating that metabolic modifications had taken place, either because of the peptides' cell-signaling capabilities or because the component amino acids have been liberated from hydrolysis. Amino acid imbalances may be the cause of this, or the peptide's distant action may be to blame for the rise in amino acids other than BCAAs, which is an intriguing aspect. For example, peptides lle-Leu and Val-Leu enhanced taurine and cysteine, respectively (Figure 3).

Increases in cysteine and taurine in this instance may be linked to improved muscular performance (48). After hydrolysis, neither lle-Leu nor Leu-lle produced any isomer-specific changes in the amino acids they released as constituents. Even though there were large differences between the isomers Val-Leu and Leu-Val when synthesising arginine in two steps (Figure 3(r) and 3(t)), this suggests that the two isomers work in concert to produce cysteine. Another interesting feature was the late release (peaking at 45 minutes) of Glu, Ser, Cys, Tau, Arg and Ala, which suggested that their syntheses needed some time; the trend of WPH contributing significantly to Glu Ser Cys Tau Arg and Ala production, which suggested the presence of other peptide sequences with additional functions; 3) in spite of variations in free amino acid profiles (time-c) Despite the fact that the four peptides were found in whey protein sequences, their blood levels were not tested in this investigation. The lle-Pro-Pro milk dripeptide sequence has already been proven to be absorbed into the blood in humans in a complete form (11). However, based on our findings, it can be concluded that if these peptides are present in the blood, they modify the free amino acid profile significantly, which suggests that they persist and degrade over time.

Conclusion

It has been shown that WPH contains four BCAA-containing peptides that may affect crucial physiologic parameters during acute exercise. We found that the WPH-related activities of lle-Leu and Leu-Val dipeptides stood out in our study. It is possible that the lle-Leu dipeptide might boost

glycogen, offer an anti-stress effect (HSP), and reduce exercise-induced immunosuppression. Finally, the Leu-Val dipeptide increased the expression of HSP90, mTOR, and 4EBP1. These findings imply that a succession of directives given by a variety of peptide combinations might play a role in helping the body cope with stress, boost its immune system, simplify protein production, and provide tissue protection.

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