

Effects of magnesium biotinate supplementation on serum insulin, glucose, and lipid parameters along with liver protein levels of intermediary metabolism in rats

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Abstract

Background: The objective of this study was to investigate the effects of a novel form of biotin (magnesium biotinate) on serum glucose, lipid profile, and hepatic lipid metabolism-related genes in rats.

Methods: Forty-two rats were divided into six groups and fed a standard diet-based egg white powdered diet supplemented with either d-biotin at 0.01, 1 or 100 mg/kg BW or magnesium biotinate at 0.01, 1, or 100 mg/kg BW.

Results: Serum total cholesterol and triglyceride decreased with biotin by both sources ($P < 0.05$). Concentrations were lower with magnesium biotinate when comparing the 1 mg/kg dose groups ($P < 0.05$). Serum, liver, and brain biotin, and liver cyclic guanosine monophosphate (cGMP) concentrations were greater when rats were treated with magnesium biotinate versus d-biotin, particularly when comparing the 1 mg/kg and 100 mg/kg dose groups ($P < 0.05$). Both forms of biotin decreased the liver SREBP-1c and FAS and increased AMPK- α 1, ACC-1, ACC-2, PCC, and MCC levels ($P < 0.05$). The magnitudes of responses were more emphasized with magnesium biotinate.

Conclusions: Magnesium biotinate, compared with a commercial d-biotin, is more effective in reducing serum lipid concentrations and in regulating gene levels of intermediary metabolism-related biomarkers.

Introduction

Biotin, also known as vitamin B7 or vitamin H, is an essential water-soluble vitamin. Biotin is a covalently bound coenzyme to five biotin-dependent carboxylases, which are involved in essential biological processes including fatty acid synthesis, gluconeogenesis, and amino acid metabolism [1]. Biotin is also functional in cell signaling, modulations of the expression of up to 2000 genes, immunity, and chromatin structure [2, 3]. Biotin administration has been shown to decrease serum concentrations of triglycerides [4, 5, 6] cholesterol [7], and free fatty acids [8, 9] in human and animal studies. The mechanism by which biotin is involved in the lipid homeostasis is mostly related to its presence in carboxylases. Biotin-dependent carboxylases include acetyl CoA carboxylase (ACC), propionyl CoA carboxylase (PCC), 3-methylcrotonyl-CoA carboxylase (MCC), geranyl-CoA carboxylase (GCC), and long-chain acyl-CoA carboxylase (LCC), as well as small organic compounds such as pyruvate carboxylase (PC) and urea carboxylase (UC) [10].

Acetyl CoA carboxylase is the key enzyme involved in the synthesis of fatty acids. Two isoforms of ACC are ACC-1 (also known as ACC α) and ACC-2 (also known as ACC β). ACC-1 is the key enzyme in the liver and adipose tissues of mammals catalyzing long-chain fatty acid biosynthesis, whereas ACC-2 is active mainly in the heart, muscles, and liver tissues [10, 11]. Pyruvate carboxylase catalyzes the conversion of pyruvate to oxaloacetate, and thus is crucial in intermediary metabolism, governing fuel partitioning toward gluconeogenesis, lipogenesis, and insulin secretion [12]. Propionyl-CoA carboxylase is an important enzyme that contributes to the catabolism of cholesterol and odd chain fatty acids as well as

certain amino acids (valine, methionine, isoleucine, and threonine) [10]. 3-Methylcrotonyl-CoA carboxylase is crucial for the catabolism of leucine and isovalerate [13].

Several factors are also involved in lipid metabolism and its regulation. Sterol regulatory element-binding proteins (SREBPs) adjust the synthesis and cellular uptake of cholesterol and fatty acids [14]. In addition, fatty acid synthase (FAS) catalyzes fatty acid synthesis (*de novo*), thus regulating energy homeostasis by transforming the excess food consumed into lipids (mainly palmitate) for storage. This process supplies energy when needed via beta-oxidation in animal and human tissues [15]. AMP-activated protein kinase (AMPK) acts as a key enzyme in skeletal muscle fat metabolism. AMPK regulates cellular energy homeostasis through mainly activating glucose and fatty acid uptake and oxidation when cellular energy is low [16]. AMPK protein complex consists of α , β , and γ subunits. Alpha (α) subunits have the catalytic domain of the AMPK.

Biotin is required for maintaining normal glucose metabolism through regulating the expression of insulin receptors [17] and improving beta cell functions in pancreatic islets [18, 19], increasing insulin secretion [20]. The beneficial effect of biotin in hyperglycemia has also been confirmed in both types 1 and 2 diabetes [21].

For adults, the U.S Recommended Dietary Allowance (RDA) for biotin is 30 $\mu\text{g}/\text{day}$. While biotin intake is 35–70 $\mu\text{g}/\text{day}$ with a typical Western diet [22], biotin supplementation has still been shown to provide various benefits. For instance, biotin supplementation at 6 mg/kg resulted in improvements in glucose metabolism, measured by increased IRS-1 liver expressions in rats [23]. Moreover, biotin supplementation increased serum glucose, cholesterol, triglycerides, creatinine, and MDA concentrations in diabetic rats supplemented with 300 μg biotin/kg BW [8]. High doses of biotin have also been studied in the treatment of multiple sclerosis [24]. However, the full mechanisms by which biotin exerts its effects on lipid, carbohydrate, and amino acid metabolism through gene expressions are still being elucidated, particularly with a new form of biotin, called magnesium biotinate (MB). Magnesium biotinate is a novel biotin complex of magnesium and biotin that is 40 times more soluble than commercial biotin, as well as having greater tissue and serum uptake [25]. Therefore, the objectives of the present work were; 1) to investigate the effects of magnesium biotinate as compared to commercial biotin at various levels, including pharmacological doses, on body weight, serum concentrations of glucose, insulin, cholesterol, and triglycerides, and serum and tissue biotin concentrations in rats and 2) to observe changes in the levels of intermediary metabolism-related genes such as SREBP-1c, FAS, AMPK-alpha1, ACC-1, ACC-2, PC, PCC and MCC in the rat liver.

Materials And Methods

Animals and diets

Male Sprague-Dawley rats (n = 42, 8 weeks old) were provided from the Laboratory Animal Research Center, Firat University (Elazig, Turkey). The animals were kept in a room with standard conditions (22 \pm 2 °C temperature, 55 \pm 5% humidity, a 12-h light–12-h dark cycle). The rats were offered ad libitum water

and a standard diet with minor modifications formulated by the American Institute of Nutrition. The diet contained a protein source of only spray-dried egg white avidin to bind biotin to standardize biotin absorption across groups (egg white avidin protein binds about 1.44 mg biotin/kg of purified diet). The biotin concentrations of the present work were adequate for avidin binding.

The rats were randomly assigned to one of six treatment groups (n = 7 per group) and fed a standard egg white powdered diet supplemented with either commercial biotin (d-biotin) at 0.01, 1, or 100 mg/kg body weight (BW), or magnesium biotinate at 0.01, 1, or 100 mg/kg BW. The doses used at 0.01, 1 and 100 mg/kg BW from each source represented a standard dietary dose, high dietary dose, and pharmacologic dose, respectively. Magnesium biotinate contains 86% biotin. Therefore, any effects detected upon magnesium biotinate supplementation should be mainly attributed to biotin, not magnesium. The duration of the study was 35 days. Bodyweight changes as initial and final, as well as feed intake, were recorded weekly. At the end of the study, upon overnight fasting, blood samples were collected through the Cardiac Puncture Blood Collection method. The cervical dislocation was used for animal euthanasia, and liver and brain samples were obtained for analyses.

Ethics statement

The ethical permission of the experiment was obtained from the Animal Experimentation Ethics Committee of Firat University (2017/84–166) according to the relevant laws, guidelines, and restrictions.

Laboratory analyses

Sera samples out of the blood were yielded through centrifugation at $3.000 \times g$ for 10 min. Serum glucose, creatinine, urea, total cholesterol, and triglycerides levels, as well as enzyme activities of ALT and AST, were measured using an automated analyzer (Samsung LABGEOPT10, Samsung Electronics Co, Suwon, Korea). A rat insulin kit was used to analyze the insulin levels (Linco Research Inc, St. Charles, MO, USA) with an ELISA instrument (Elx-800, Bio-Tek Instruments Inc, Vermont, USA). The assay sensitivity was 0.35 ng/mL. Inter- and Intra- assay constants were 3.2% and 6.9%.

For determination of liver the cGMP, samples were diced into small pieces in cold PBS and homogenized in a cold 100 mM phosphate buffer at 16.000 rpm for 3 min (pH 7.5). The samples were re-homogenized with a glass homogenizer on ice and subjected to two freeze-thaw cycles accompanied by ultrasonication. The concentration of cGMP was detected using a commercially available assay kit (LSBio, Seattle, WA, USA). The inter- and intra- assay constants were CV < 10% and inter-assay < 12%, respectively.

For the assay of serum biotin concentration, samples were ultrafiltered as earlier described [26]. Tissue samples (300 mg) were processed in 5 volumes of ice-cold homogenization buffer (300 mmol/L mannitol, 10 mmol/L HEPES, pH 7.2, 1 mmol/L EDTA and protease inhibitor cocktail). The samples were then centrifuged at $15.000 \times g$ for 30 min at 4 °C. Before the biotin measurement, the supernatant was ultrafiltered. The pellet was brought to a concentration of 40 g/L in a homogenization buffer. The tissue samples were promptly frozen in a mixture of dry ice and isopropanol and stored at -80 °C until

analyzing. Serum, liver, and brain biotin levels were analyzed by HPLC (Shimadzu, Kyoto, Japan) as earlier defined with minor modifications [27, 28]. The C18-ODS-3 reversed-phase column (250 × 4.6 mm, 5 m) and biotin-containing chromatography fractions were used in HPLC. A stream of nitrogen was used to dry the samples before analyzing them.

Western blot analysis

The western blotting technique was used to detect specific proteins with procedures described earlier [29]. Liver samples were pooled and homogenized in 1 ml ice-cold hypotonic buffer A including 10 mM HEPES (pH 7.8), 10 mM KCl, 2 mM MgCl₂, 1 mM DTT, 0.1 mM EDTA, and 0.1 mM phenylmethylsulfonyl-fluoride (PMSF). Eighty µl of 10% Nonidet P-40 (NP-40) solution was added to the homogenates and the mixture then was implemented with centrifugation for 2 h at 14,000 *g*. Five-hundred µl of buffer A plus 40 µl of 10% NP-40 was used to wash the precipitates containing nuclei. The precipitates were then centrifuged and resuspended in 200 µl of buffer C [50 mM HEPES (pH 7.8), 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 20% glycerol], and centrifuged for 30 min at 14,800 *g*. The supernatant was removed to new tubes. Western blot analyses were run on the tissue homogenates for SREBP-1c, fatty acid synthase (FAS), (Thr172)-phosphorylated AMPK-α form, as well as acetyl-CoA carboxylase 1 (ACC 1), acetyl-CoA carboxylase 2 (ACC 2), pyruvate carboxylase (PC), propionyl-CoA carboxylase (PCC), and 3-methylcrotonyl-CoA carboxylase (MCC), and β-actin. Protein concentration was measured using the Lowry method. A pool of tissue samples was created with the same amounts of protein (50 µg) and the samples were electrophoresed (12% SDS-PAGE gels) followed by transfer to nitrocellulose membrane (Schleicher and Schuell Inc., Keene, NH, USA). The primary antibodies against SREBP-1c, FAS, (Thr172)-p-AMPK-α, ACC 1 and 2, PC, PCC, and MCC and β-actin were delivered (Abcam Inc., UK). The primary antibody was reduced in strength (1:1000) in the same buffer containing 0.05% Tween-20. The antibody with the nitrocellulose membrane was incubated overnight at 4 °C. After washing, the blots were incubated with goat anti-mouse IgG (horseradish peroxidase-conjugated secondary antibody) with a dilution of 1:5000 (Abcam, Cambridge, UK). To avoid data reproducibility, the blots were run several times. Protein bands were quantified via scanning densitometry using an image analysis system (Image J; National Institute of Health, Bethesda, USA). The protein bands were normalized by the corresponding β-actin band values and compared with the control group.

Statistical analysis

One-way ANOVA was used to analyze the data using SPSS for Windows version 21.0 (IBM Corp., Armonk, NY, USA). The Tukey post hoc test was also applied among treatment groups. *P* < 0.05 was the level of statistical significance. Data were reported as a mean and standard error of the mean.

Results And Discussion

Initial body weights, as intended, as well as final body weights and feed intake of rats, were similar among treatments (*P* > 0.05; Table 1). Similar to the results of the present work, unchanged final body weights of rats fed a diet supplemented with 6 mg biotin/kg of diet [23] and mice fed a diet supplemented with 97.70 mg of free biotin/kg diet [30, 31] has been reported.

Table 1
Effects of biotin supplementation on live weight changes and serum metabolites

Items	Treatments*						– P –
	B 0	B 1	B 100	MgB 0	MgB 1	MgB 100	
Initial Body Weight, g	248.43 ± 4.42	251.43 ± 4.83	246.71 ± 3.43	252.29 ± 2.29	249.86 ± 5.91	251.29 ± 4.51	0.946
Final Body Weight, g	295.43 ± 4.87	298.57 ± 4.70	294.00 ± 5.57	296.29 ± 5.73	297.43 ± 5.01	299.29 ± 4.27	0.977
Feed Intake, g	24.00 ± 0.65	24.86 ± 0.59	24.43 ± 1.07	24.71 ± 0.87	24.14 ± 0.55	24.43 ± 0.57	0.963
Glucose, mg/dL	89.00 ± 2.44	89.29 ± 2.74	91.71 ± 2.35	90.71 ± 3.11	83.57 ± 3.92	87.43 ± 3.18	0.480
Insulin, mIU/L	14.10 ± 0.92	16.13 ± 0.71	15.96 ± 1.10	14.77 ± 1.67	17.33 ± 1.04	15.67 ± 1.05	0.428
ALT, U/L	82.29 ± 1.60	81.14 ± 4.66	85.57 ± 2.44	83.43 ± 3.27	82.71 ± 2.95	81.43 ± 2.96	0.928
AST, U/L	126.29 ± 6.23	121.86 ± 5.80	123.57 ± 10.39	121.86 ± 11.92	122.00 ± 10.40	118.14 ± 6.05	0.993
Creatine, mg/dL	0.47 ± 0.06	0.41 ± 0.08	0.43 ± 0.06	0.44 ± 0.04	0.44 ± 0.11	0.40 ± 0.05	0.987
Urea, mg/dL	23.87 ± 2.04	22.44 ± 2.23	23.09 ± 0.99	22.80 ± 1.83	23.27 ± 2.22	24.16 ± 1.73	0.988
Total cholesterol, mg/dL	73.00 ± 1.81 ^a	64.57 ± 1.86 ^b	64.00 ± 1.98 ^b	74.00 ± 2.56 ^a	54.29 ± 1.66 ^c	60.86 ± 1.87 ^{bc}	0.0001
Triglyceride, mg/dL	68.00 ± 2.46 ^a	59.29 ± 4.86 ^{ab}	60.29 ± 1.54 ^{ab}	66.00 ± 3.18 ^a	45.14 ± 2.52 ^c	48.14 ± 1.91 ^{bc}	0.0001

* Dietary treatments contained biotin supplements as either commercial biotin (d-biotin) at 0.01 (B0), 1 (B1) and 100 (B100) mg/kg body weight or a new form of biotin (magnesium biotinate) at 0.01 (MgB0), 1 (MgB1), and 100 (MgB100) mg/kg body weight. The doses used at 0.01, 1, and 100 mg of biotin supplementations from each source represented standard dietary dose (control), high dietary dose, and pharmacologic dose, respectively. Statistical comparisons are indicated with different superscript (a-c) in the same row ($P < 0.05$; ANOVA and Tukey's post-hoc test). Mean values are demonstrated with ± standard deviations. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

In the present study, neither form of biotin influenced ($P > 0.05$) serum glucose or insulin concentrations. Although decreases in serum glucose concentrations in rats fed a diet supplemented with biotin have been reported [23], Lazo de la Vega-Monroy et al. [30] found increases in elevated glucose-stimulated serum insulin concentrations, but no changes in fasting glucose concentrations or insulin tolerance in

mice fed a diet supplemented with biotin. Enhanced fasting serum glucose levels have been observed with biotin supplementation in individuals with Type 2 diabetes who had low serum biotin concentrations before supplementation [21]. However, biotin administration (6.14 $\mu\text{mol/d}$) for 28 days to individuals with Type 2 diabetes did not change concentrations of glucose, insulin, triacylglycerol, or cholesterol [32]. Inconsistent results from the present work and the literature in experimental animals and individuals with Type 2 diabetes [21, 23, 30, 32] could have been due to differences in biotin doses, duration of supplementation, or the severity of diabetes, among others.

Biotin supplementation has been shown to increase glucose-stimulated insulin secretion in rats [33] and mice [30] via affecting morphology and the number of cells in the pancreas. Tixi-Verdugo et al. [34] found that mice fed a diet supplemented with 100 mg of biotin/kg diet had greater beta-cell proportions (%) and an elevated number of islets per pancreatic area. Biotin is known to improve glycemic control through stimulating pancreatic and hepatic glucokinases while inhibiting the hepatic gluconeogenic enzyme phosphoenolpyruvate carboxykinase [35]. Glucose is considered the major lipogenic substrate for most tumor cells, which have greater lipid synthesis and requirement for amino acids [36]. Therefore, supplementing biotin can be indirectly involved in the prevention and therapy of cancer, diabetes, obesity, and other diseases.

Serum ALT and AST enzyme activities, as well as concentrations of creatine and urea, remained similar among treatments ($P > 0.05$). Similarly, [31] found no changes in the ALT activity of urea concentrations but greater AST enzyme activities in biotin-supplemented mice (97.70 mg of free biotin/kg diet). Although greater AST enzyme activity was found in the biotin-supplemented group, the values were within the normal range (55.0-352 U/L). Results from the present work and the literature [31] indicate that neither pharmacological doses of commercial d-biotin or magnesium biotinate influence indicators of liver damage.

Serum total cholesterol and triglyceride concentrations in the rats decreased with biotin supplementation from both sources ($P < 0.05$). However, supplementing with magnesium biotinate provided greater decreases in blood lipid concentrations, particularly with the 1 mg/kg dose ($P < 0.05$), compared to commercial biotin. In accordance with the results of the present work, Turgut et al. [23] also reported decreases in serum concentrations of cholesterol and triglyceride in rats fed a diet supplemented with 6 mg/kg biotin. Larrieta et al. [5] also found reduced serum triglyceride concentrations in mice fed a diet supplemented with 97.7 mg of free biotin/kg diet. Similarly, plasma concentrations of triacylglycerol and VLDL-cholesterol were reported to decrease in biotin-supplemented (61.4 $\mu\text{mol/day}$) individuals with Type 2 diabetes [4].

As expected, biotin supplementation with both forms resulted in increases in serum, liver, and brain biotin concentrations ($P < 0.05$, Table 2). However, biotin concentrations of the blood and the organs were greater with magnesium biotinate compared with the same doses of commercial d-biotin, particularly in the 1 and 100 mg/kg BW groups ($P < 0.05$). Similarly, elevated serum biotin concentrations were also reported in a previous study where mice were fed a diet supplemented with biotin [30].

Table 2
Effects of biotin supplementation on biotin and liver cGMP contents

Items	Treatments*						– P –
	B 0	B 1	B 100	MgB 0	MgB 1	MgB 100	
Serum biotin, nmol/L	23.65 ± 2.60 ^c	136.67 ± 2.73 ^c	3517.14 ± 87.93 ^b	23.41 ± 2.31 ^c	171.13 ± 3.02 ^c	5161.43 ± 250.96 ^a	0.0001
Liver biotin, nmol/g	0.02 ± 0.01 ^e	0.56 ± 0.04 ^d	1.38 ± 0.02 ^b	0.03 ± 0.01 ^e	0.71 ± 0.03 ^c	1.62 ± 0.02 ^a	0.0001
Brain biotin, nmol/g	0.14 ± 0.01 ^e	0.42 ± 0.05 ^d	1.37 ± 0.04 ^b	0.14 ± 0.01 ^e	0.66 ± 0.04 ^c	1.65 ± 0.03 ^a	0.0001
Liver cGMP, pmol/mg protein	8.46 ± 0.26 ^d	12.01 ± 0.26 ^c	14.68 ± 0.32 ^b	8.60 ± 0.28 ^d	13.04 ± 0.98 ^{bc}	17.07 ± 0.28 ^a	0.0001
*Dietary treatments contained biotin supplements as either commercial biotin (d-biotin) at 0.01 (B0), 1 (B1) and 100 (B100) mg/kg body weight or a new form of biotin (magnesium biotinate) at 0.01 (MgB0), 1 (MgB1), and 100 (MgB100) mg/kg body weight. The doses used at 0.01, 1, and 100 mg of biotin supplementations from each source represented standard dietary dose (control), high dietary dose, and pharmacologic dose, respectively. Statistical comparisons are indicated with different superscript (a-e) in the same row ($P < 0.05$; ANOVA and Tukey's post-hoc test). Mean values are demonstrated with ± standard deviations.							

Cyclic guanosine monophosphate (cGMP) functions as anti-apoptotic and anti-inflammatory in cells and regulates multiple physiologic processes in the cardiovascular system [37]. Biotin supplementation to the diet of mice has been shown to decrease blood triglyceride concentrations through increased cGMP content [6, 9]. Comparable to the results reported by other researchers [6, 9], liver cGMP contents of animals in the present work also increased with biotin supplementation. However, liver cGMP contents were higher in rats supplemented with magnesium biotinate compared with the same doses of commercial d-biotin, particularly when comparing the 100 mg/kg BW groups ($P < 0.05$). Cyclic guanosine monophosphate has also been proposed to have a substantial effect on beta-cell functions [38]. In the present study, although cGMP contents of the liver increased with biotin supplementation, neither serum glucose nor insulin concentrations were significantly altered.

Biotin is thought to reduce levels of blood lipids (hypotriglyceridemia) and glucose (hypoglycemia) through regulation of the mRNA abundance of lipogenic enzymes and transcription factors such as SREBP-1c, FAS, ACC, and pyruvate kinase, among others [39]. One of the main objectives of the present work was to detail the effects of biotin on such factors to determine the mechanism by which biotin, particularly the new form of biotin, works. Liver SREBP-1c and FAS levels decreased while the level of AMPK-alpha increased with both biotin forms ($P < 0.05$; Fig. 1). The magnitudes of responses were more emphasized with magnesium biotinate, particularly when comparing the 1 mg/kg dose groups for SREBP-1c and the 100 mg/kg dose groups for FAS and AMPK-alpha ($P < 0.05$). Liver ACC-1, ACC-2, PCC, and MCC levels increased ($P < 0.05$; Fig. 2) with both biotin forms. This effect was more apparent with

magnesium biotinate when compared to similar doses of commercial d-biotin ($P < 0.05$). The liver PC level increased with biotin supplementation, with no differences found from dose or biotin form ($P > 0.05$).

The functions of SREBP-1c involve activating several enzymes including FAS and ACC in catalyzing various steps in fatty acid and TG synthesis pathways [40]. Therefore, decreases in both SREBP-1c and FAS were consistent with reduced serum lipid concentrations of total cholesterol and triglycerides seen in the present work. Over-nutrition or intake of energy-dense molecules (sugar and saturated fatty acids) results in an increase in SREBP-1c expression and consequently lipogenesis in the liver [41]. Through regulation of energy metabolism, biotin supplementation, particularly magnesium biotinate, can reduce SREBP-1c levels and consequently reduce serum lipid concentrations.

Low cellular energy causes activation of AMPK which inactivates both ACC isoforms, ACC-1, and ACC-2, resulting in reduced *de novo* lipogenesis and increased fatty acid oxidation [42]. Similarly, biotin supplementation in mice and rats has been reported to increase the active form of AMPK, which phosphorylates ACC-1 and ACC-2, resulting in decreases in the rate of lipid synthesis and increases in fatty acid oxidation rates [43, 44]. Biotin supplementation in mice has also been shown to increase the level of the active form of AMPK and decrease FAS and SREBP-1c levels [6, 44]. Moreno-Méndez et al. [44] found that ACC-1 and FAS reduced the acetate incorporations into total lipid fractions in response to biotin supplementation, resulting in lower fatty acid synthesis in mice adipose tissues.

While ACC is related to fatty acid metabolism via generating malonyl-CoA for fatty acid synthesis, MCC is involved in leucine catabolism, and PC and PCC are anaplerotic, meaning they form intermediates of a metabolic pathway such as the TCA cycle [45]. Therefore, changing activities of these enzymes influence not only lipid but also carbohydrate and protein metabolism. However, in the present study, only lipid parameters were influenced by altered levels of carboxylases, namely ACC, PC, PCC, and MCC.

In the present study, the magnitude of the responses was more emphasized (greater) with magnesium biotinate compared with commercial d-biotin. This effect could be attributed to the fact that magnesium biotinate is a bioavailable form of biotin and is 40 times more soluble than d-Biotin and is more significantly absorbed into the blood and tissues in rats [46]. This idea is supported by evidence from a clinical study that showed that healthy female subjects orally supplemented with 10, 40, or 100 mg of magnesium biotinate had dose-dependent increases of biotin levels in the blood with no adverse effects [46].

Conclusions

Results of the present work indicated that a new form of biotin, magnesium biotinate, is more effective in reducing serum lipid concentrations and in regulating gene levels of lipid metabolism-related biomarkers compared to commercial d-biotin.

Abbreviations

ACC: acetyl CoA carboxylase; PCC: propionyl CoA carboxylase; MCC: 3-methylcrotonyl-CoA carboxylase; GCC: geranyl-CoA carboxylase; LCC: long-chain acyl-CoA carboxylase; PC: pyruvate carboxylase; UC: urea carboxylase; SREBPs: Sterol regulatory element-binding proteins; FAS: fatty acid synthase; AMPK: AMP-activated protein kinase; RDA: Recommended Dietary Allowance; MB; magnesium biotinate; BW: body weight.

Declarations

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Authors' contributions

KS designed the experiments. CO, FE, MT and NS carried out the experiments. CO, FE, MT and NS analyzed the experimental results. CO and OK conducted the statistical analysis. JK, and SPO wrote the draft manuscript; KS and OK wrote the manuscript. All the authors read and approved the final manuscript.

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Declaration of competing interest

JK and SPO are employees of Nutrition 21 LLC, NY, USA. The remaining authors state no conflict of interest.

Compliance with ethics requirements

The Animal Experimentation Ethics Committee of Firat University (2017/84-166) approved this study.

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Figures

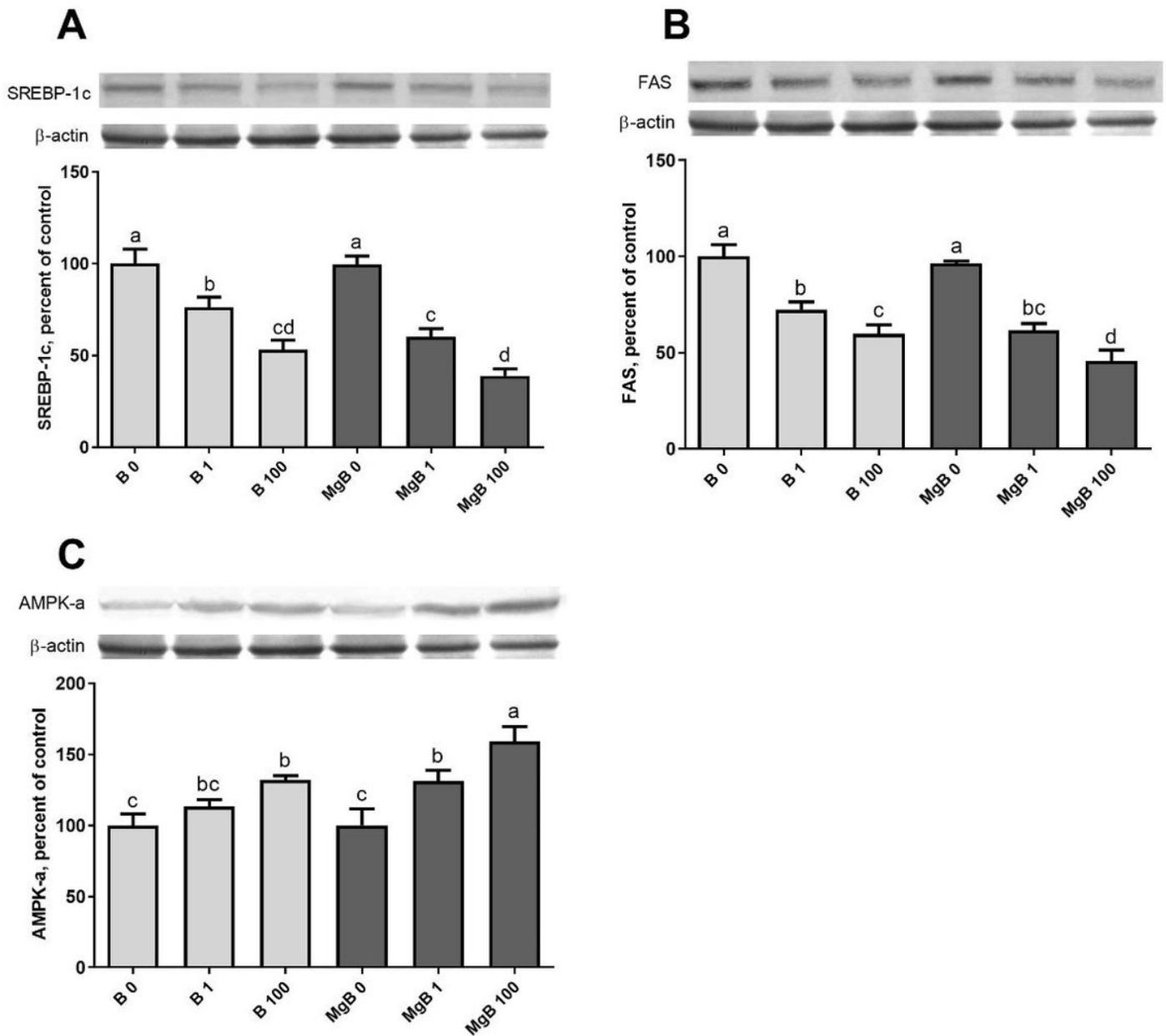


Figure 1

Effects of biotin supplementation on liver levels of sterol regulatory element-binding proteins (SREBP-1c) (A), Fatty acid synthase (FAS) (B), and AMP-activated protein kinase (AMPK-alpha1) (C). Dietary treatments contained biotin supplements as either commercial biotin (d-biotin) at 0.01 (B0), 1 (B1) and 100 (B100) mg/kg body weight or a new form of biotin (magnesium biotinate) at 0.01 (MgB0), 1 (MgB1), and 100 (MgB100) mg/kg body weight. The doses used at 0.01, 1, and 100 mg of biotin supplementations from each source represented standard dietary dose (control), high dietary dose, and pharmacologic dose, respectively. Statistical comparisons are indicated with different superscript (a-c) in the same row ($P < 0.05$; *ANOVA and Tukey's post-hoc test).

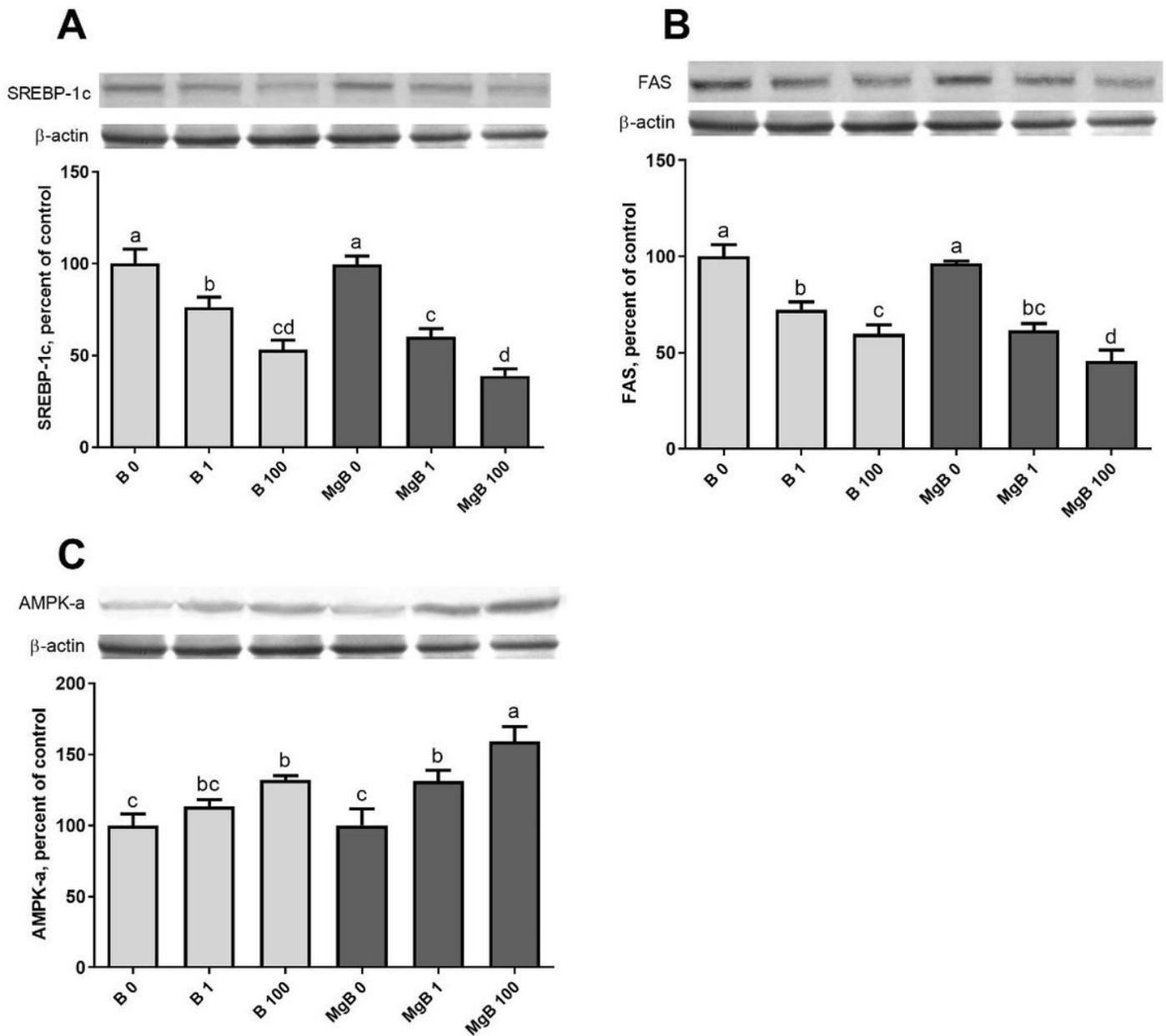


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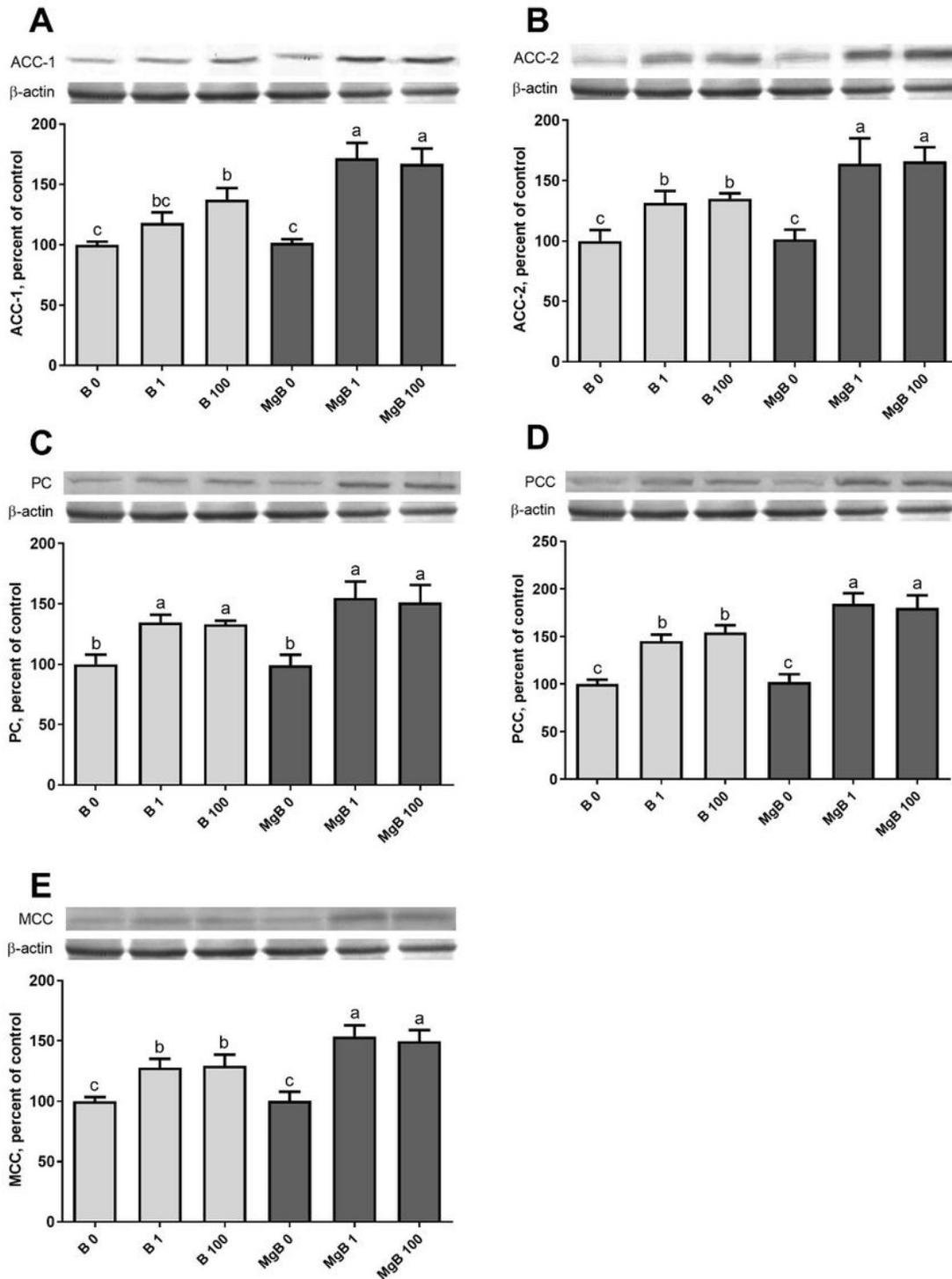


Figure 2

Effects of biotin on liver levels of biotin-dependent carboxylases including; A: acetyl CoA carboxylase-1 (ACC-1), B: acetyl CoA carboxylase-2 (ACC-2), C: pyruvate carboxylase (PC), D: propionyl CoA carboxylase (PCC), and E: 3-methylcrotonyl-CoA carboxylase (MCC). Dietary treatments contained biotin supplements as either commercial biotin (d-biotin) at 0.01 (B0), 1 (B1) and 100 (B100) mg/kg body weight or a new form of biotin (magnesium biotinate) at 0.01 (MgB0), 1 (MgB1), and 100 (MgB100) mg/kg body weight.

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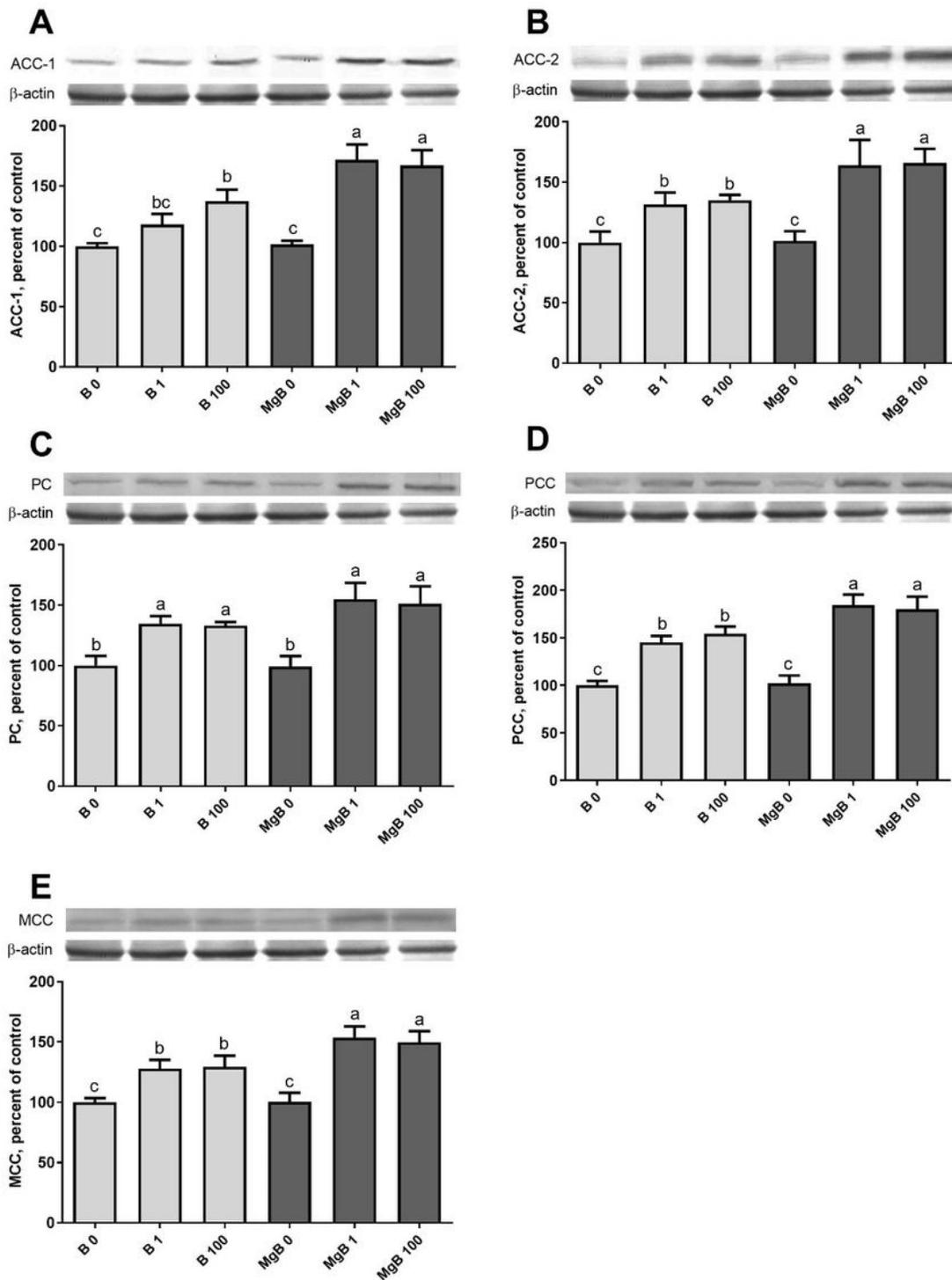


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