

Whey Protein Improves HDL/non-HDL Ratio and Body Weight Gain in Rats Subjected to the Resistance Exercise

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ABSTRACT

The aim of this study was to evaluate the effects of resistance exercise, such as weight-lifting (WL) on the biochemical parameters of lipid metabolism and cardiovascular disease risk in the rats fed casein (control) or whey protein (WP) diets. Thirty-two male Fisher rats were randomly assigned to sedentary or exercise-trained groups and were fed control or WP diets. The WL program consisted of inducing the animals to perform the sets of jumps with weights attached to the chest. After seven weeks, arteriovenous blood samples were collected for analysis. The WL or WP ingestion were able to improve the lipid profile, reducing the TC and non-HDL cholesterol concentrations, but only WP treatment significantly increased the serum HDL concentrations, thereby also affecting the TC/HDL and HDL/non-HDL ratios. However, WL plus WP was more effective in improving the HDL/non-HDL ratio than the exercise or WP ingestion alone and the body weight gain than exercise without WP ingestion.

Key words: dietary protein, lipid profile, cholesterol, cardiovascular disease risk

INTRODUCTION

Cardiovascular disease (CVD) remains a major cause of morbidity and mortality worldwide. The occurrence of CVD is strongly related to lifestyle and biological risk factors, such as dyslipidemia, physical inactivity and bad eating habits. Dyslipidemia, including elevation of total cholesterol (TC) and non-high-density lipoprotein (non-HDL) cholesterol fractions and a decrease in high-density lipoprotein (HDL) concentration, is an important risk factor for the CVD development (Glass and Witztum 2001). Epidemiological and clinical studies have confirmed the positive correlation between the CVD risk and plasma low-density lipoprotein (LDL) cholesterol

concentration, and an independent negative correlation with plasma HDL concentration (Kolovou et al. 2009). Lifestyle changes, including the practice of regular exercise and healthy eating habits, are essential factors to reduce the CVD risk (Miller et al. 1997; Fan et al. 2008). In this context, aerobic exercise has been considered the most preventive type of exercise (Tanasescu et al. 2002; Williams et al. 2007). Although resistance exercise (RE), such as weight-lifting (WL), may reduce multiple CVD risk factors (Hurley et al. 1998; Miller et al. 1994), its effects on lipid metabolism remain controversial in both animal and human studies (Williams et al. 2007; Frisch and Sumida 1999; Leite et al. 2009; Kelley and Kelley 2009; Sallinen

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et al. 2007). Frisch and Sumida (1999) observed that strength training for ten weeks did not affect the serum TC, LDL, or HDL in the rats. On the other hand, Leite et al. (2009) observed that a similar type of RE for 12 weeks significantly increased the serum concentration of HDL and reduced those of LDL and very low density lipoprotein cholesterol (VLDL). In a meta-analysis of randomized controlled human trials, Kelley and Kelley (2009) concluded that long-term RE resulted in decreased blood concentrations of TC, LDL, and triglycerides and increased concentrations of HDL. However, Williams et al. (2007) reported that in addition to lack of proper dietary controls, most human studies did not adequately control for normal variations in lipoproteins, nor determine certain relevant conditions, such as exercise in the subjects. After these factors are accounted for, there is usually no improvement in the lipid profile after RE (Williams et al. 2007). Sallinen et al. (2007) also observed no improvement in the blood lipid profile after 21 weeks of RE in elderly men. In addition to the effect of regular exercise, dietary factors can also affect lipid profile (Silva et al. 1999; Colla et al. 2008; Paula et al. 2009; Barbalho et al. 2009). Although low-saturated fat-containing diets have been reported to improve the lipid profiles (Aro et al. 1997; Sacks et al. 2002), biochemical parameters of lipid metabolism can also be influenced by dietary protein type (Beynen 1990). Whey protein (WP), a protein having a high biological value, has received attention because of reported benefits in the field of sports nutrition (Ha and Zemel 2003; Lands et al. 1999), including its effects on body composition and physical performance as well as its ability to preclude tissue lipid and protein oxidation after RE (Haraguchi et al. 2011). WP has also successfully been used in the treatment of different pathological conditions, including cystic fibrosis (Lands et al. 2010), human immunodeficiency virus infection (Sattler et al. 2008), liver diseases (Watanabe et al. 2000), cancer (Castro et al. 2009), and CVD (Nagaoka et al. 1992; Kawase et al. 2000). Regarding its effect on lipid metabolism, studies have described a hypocholesterolemic action (Nagaoka et al. 1992), although conflicting results have been reported in other studies (Sautier et al. 1983; Zhang and Beynen 1993; Haraguchi et al. 2009). Although many studies have reported the effects of exercise

or WP on lipid profile and CVD risk, the combined effects of RE and dietary WP on lipid profile and CVD risk have not been well studied. Therefore, the aim of the present study was to evaluate the effects of WL exercise, a form of RE, on the biochemical parameters of lipid metabolism and CVD risk in the rats. In addition, the ability of WP to enhance these RE-induced adaptations was also evaluated.

MATERIAL AND METHODS

Animals

Thirty-two male Fischer rats (60-day old; each weighing approximately 110 g) were used in the experiments. The animals were housed individually in galvanized wire cages in a room with controlled temperature ($23 \pm 1^\circ\text{C}$) and a 12-h light/12-h dark cycle. The animals were maintained and used in accordance with the guidelines of the Canadian Council on Animal Care (CCAC). The research was approved by the Ethical Committee of the Ouro Preto University, Protocol #036/2008.

Diets

The rats were randomly assigned to four groups: a control-sedentary (CS) and a control-exercised group (CE) fed with a semi-purified standard diet (AIN-93 M) (Reeves et al. 1993) containing casein as the protein source; a WP-sedentary (WS) and a WP-exercised group (WE) received the AIN-93 M modified diet containing WP instead of casein. The diet composition is presented in Table 1. Food and water were provided *ad libitum*. Body weight and food intake (corrected for spillage) were measured every week.

Exercise training program and experimental procedure

The RE program was developed as previously described (Haraguchi et al. 2011). In brief, the rats were induced to perform the sets of jumps in a circular plastic container at a water level corresponding to 150% of their body length. Weights were attached to the animal's chest to promote its submersion. When the rats touched the bottom of the container, they jumped to emerge and breathe. The water level in the container and the weight attached to the animal's chest generated the resistance for the exercise. The animals

performed four sets of ten jumps each day, five times per week for seven weeks. Each set of jumps was interrupted by a 1-min resting interval. The exercise intensity was increased weekly by increasing the attached weights relative to the animal's body weight (BW) (25% of BW in week 1, 30% in week 2, 35% in week 3, 40% in week 5, 50% in week 6, and 55% in week 7). The animals

in the CS and WS groups were kept in a 5-cm deep swimming pool for 15 min to generate the same amount of stress as experienced by the animals that performed the exercise. At the end of seven weeks of training, all the rats were fasted for 12 h, and arteriovenous blood samples were collected under anesthesia from the retro-orbital plexus of the eye for the analysis.

Table 1 – Composition (g/1000 g) of control (C) and whey protein (WP) diets.

Ingredients	C	WP
Casein ¹	140	-
Whey Protein ²	-	150
Mineral Mixture ³	35	35
Vitamin Mixture ⁴	10	10
Soybean oil	40	40
Choline	2.5	2.5
Cellulose	50	50
Sucrose	100	100
Cornstarch	622.5	612.5
Total	1,000	1,000

¹ Isofar[®] (Rio de Janeiro, Brazil), containing 85% protein as determined by Kjeldahl method (AOAC, 2000).

² Bio Protein ISOFORTE[®] (Vitafor, Brazil), containing 80% protein as determined by Kjeldahl method (AOAC, 2000).

³ Mineral mixture for the AIN-93M diet (Reeves *et al.* 1993)

⁴ Vitamin mixture for the AIN-93M diet (Reeves *et al.* 1993)

Biochemical Parameters

TC was measured by the end point colorimetric assay and glucose by quantitative, kinetic and end point assay. The HDL was measured in the supernatant after selective precipitation of LDL and VLDL. Serum albumin was determined by the colorimetric measurement and urea by the UV spectrophotometry using a two-point assay. Aspartate aminotransferase (AST) and alanine aminotransferase (ALA) were measured by the kinetic assay and creatinine by Jaffe's alkaline picrate reaction, all using the commercial kits (Labtest Diagnóstica, Lagoa Santa, Brazil). The following parameters were also assessed: Castelli's atherogenic ratio (TC/HDL) (Castelli, 1988), HDL/non-HDL ratio (Tuomilehto *et al.* 1990; Albers *et al.*, 1996), and the atherogenic index (AI = TC-HDL/HDL) (Rosenfeld, 1989).

Statistical Analysis

All the variables were tested for normal distribution using the Shapiro-Wilk test ($p > 0.05$).

Data were analyzed by two-way analysis of variance; the classification factors were diet (CS + CE x WS + WE) and exercise (CS + WS x CE + WE), as well as the interaction between the diet and exercise (CS x CE x WS x WE). Tukey's post-hoc test was used to determine the differences among the four groups when a statistically significant interaction between the diet and exercise was observed.

RESULTS

Body weight gain, food intake, and feeding efficiency

Table 2 summarizes the changes in food intake, gain in body weight, and feeding efficiency observed during the experiment. Throughout the period studied, the food intake was slightly lower in the rats undergoing exercise, relative to those which were sedentary, and it was not affected by

the WP inclusion in the diet. Body weight gain was similar in all the groups, except for the CE animals, which showed the lowest weight gain. The WE rats gained more body weight than those

fed casein, despite similar food intake. In addition, feeding efficiency was highest with the WP in the diet.

Table 2 - Initial body weight, food intake, body weight gain and feeding efficiency in the studied groups*.

Variables	Experimental Groups				Two-way ANOVA (P)		
	CS	CE	WS	WE	D	E	D x E
Initial body weight (g)	114 ± 16	113 ± 9	114 ± 8	113 ± 9	0.957	0.933	0.975
Food intake (g/day)	13.8 ± 1.19	11.9 ± 1.26	13.8 ± 1.07	13.1 ± 0.97	0.082	0.004	0.257
Body weight gain (g/day)	3.47 ± 0.24 ^a	2.75 ± 0.17 ^b	3.81 ± 0.36 ^a	3.57 ± 0.4 ^a	<0.001	<0.001	0.025
Feeding efficiency**	25.3 ± 1.8	24.3 ± 1.0	28.4 ± 1.9	25.7 ± 1.9	<0.001	0.011	0.542

*ANOVA, analysis of variance; CS, control sedentary group; CE, control exercised group; WS, whey protein-sedentary group and WE, whey protein-exercised group. Values were expressed as the mean ± SD. n=6-8 animals per group. D and E correspond to diet and exercise, respectively. D x E is the interaction between the corresponding parameters. Within a row, statistically different values are marked with different superscript letters when a significant interaction was observed (P < 0.05). **Feeding efficiency = (weight gain/daily intake) x 100.

Serum glucose, albumin, AST, ALA, creatinine, and urea levels

The effects of WL, with and without dietary WP, on serum glucose, albumin, AST, ALA, creatinine and urea levels are summarized in Table 3. Serum glucose was slightly lower in the rats undergoing

WL, whereas the rats of WP groups presented higher concentrations of serum glucose and albumin. Serum AST and ALA activities and creatinine and urea concentrations were similar among all the groups in this study.

Table 3 – Serum total cholesterol (TC), HDL cholesterol (HDL), non-HDL cholesterol (non-HDL), glucose, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and urea concentrations in the studied groups*.

Variables	Experimental Groups				Two-way ANOVA (P)		
	CS	CE	WS	WE	D	E	D x E
TC (mg/dL)	86.5 ± 8.4	72.4 ± 9.7	78.6 ± 5.5	63.1 ± 3.1	0.004	<0.001	0.818
HDL (mg/dL)	55.8 ± 14.1	45.1 ± 2.6	63.0 ± 3.4	57.3 ± 3.1	<0.001	0.002	0.310
Non-HDL (mg/dL)	33.8 ± 4.2	26.9 ± 8.6	15.6 ± 5.9	8.0 ± 4.1	<0.001	0.025	0.439
Glucose (mmol/L)	9.0 ± 2.4	7.7 ± 1.1	10.7 ± 2.4	9.3 ± 1.3	0.019	0.049	0.949
Albumin (g/L)	25.3 ± 0.7	25.0 ± 0.8	27.5 ± 1.2	26.9 ± 0.9	<0.001	0.190	0.593
AST (U/mL)	87.2 ± 12.7	91.9 ± 18.5	75.8 ± 11.9	87.3 ± 17.3	0.154	0.134	0.521
ALT (U/mL)	45.2 ± 8.9	37.1 ± 5.5	37.6 ± 8.6	37.0 ± 4.9	0.145	0.074	0.120
Creatinine (μmol/L)	61.0 ± 2.4	63.0 ± 4.8	62.0 ± 1.9	60.0 ± 3.0	0.179	0.636	0.097
Urea (mg/dL)	27.6 ± 3.2	26.8 ± 1.9	27.0 ± 1.9	26.9 ± 2.5	0.848	0.578	0.678

*ANOVA, analysis of variance; CS, control sedentary group; CE, control exercised group; WS, whey protein-sedentary group and WE, whey protein-exercised group. Values were expressed as the mean ± SD. n=6-8 animals per group. D and E correspond to diet and exercise, respectively. D x E is the interaction between the corresponding parameters.

Lipid profile and CVD risk

The effects of dietary WP in an RE rat model, summarizing the lipid profiles and CVD risk are presented in Table 3 and Figs. 1-2. The exercise, as well as WP treatment, significantly reduced the TC and non-HDL concentrations in the serum. However, only WP treatment significantly increased the serum HDL concentrations, thereby also affecting the derived TC/HDL and HDL/non-HDL ratios (Fig.1), and the AI (Fig. 2). These are

important clinical parameters used to determine the risk of developing atherosclerosis (Castelli 1988; Tuomilehto et al. 1990; Albers et al. 1996; Rosenfeld 1989). RE reduced the HDL concentration, although this reduction did not affect the AI nor TC/HDL or HDL/non-HDL ratios. The animals of the WE group showed significantly higher ratios of HDL/non-HDL than the rats in the other three groups.

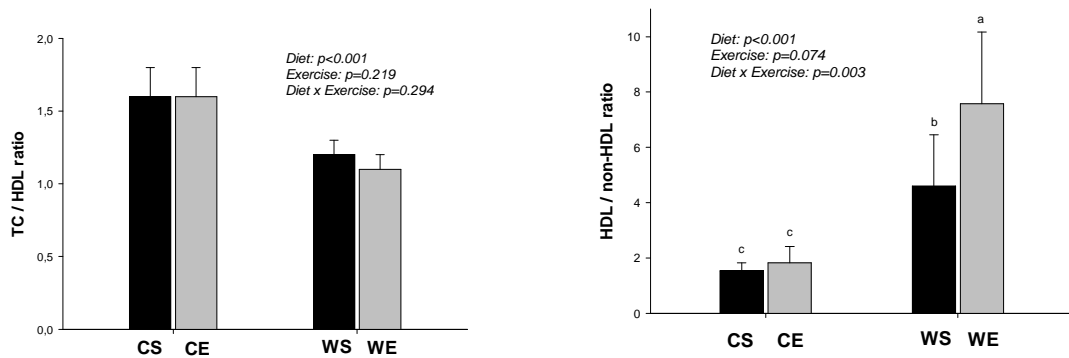


Figure 1- Total cholesterol (TC)/HDL cholesterol and HDL/non-HDL cholesterol ratios of sedentary (black bars) or resistance-trained (grey bars) rats fed control (CS and CE) or WP (WS and WE) diets. Results are expressed as means \pm SD; n = 8 per group; statistically different values are marked with superscript letters when a significant interaction was observed.

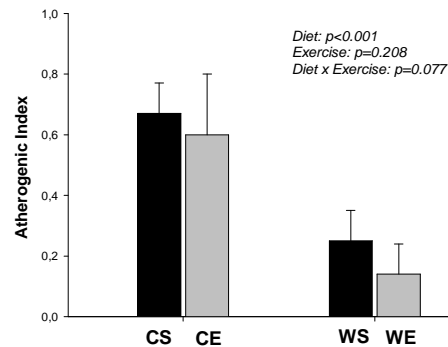


Figure 2- Atherogenic index (AI) [AI = (TC-HDL)/HDL] of sedentary (black bars) or resistance-trained (grey bars) rats fed control (CS and CE) or WP (WS and WE) diets. Results are expressed as means \pm SD; n = 8 per group.

DISCUSSION

The aim of this study was to evaluate the effects of WL exercise on the biochemical parameters of lipid metabolism and CVD risk factors in the rats. In addition, the ability of WP to enhance adaptations induced by the RE was also evaluated. Both the WL exercise and WP ingestion were able to significantly reduce the serum TC and non-HDL concentrations. However, only WP treatment was able to significantly increase the HDL concentration independently of RE, resulting in a lower TC/HDL ratio and AI, and a higher HDL/non-HDL ratio. On the other hand, RE resulted in lower serum HDL concentration. A literature review indicates that the effects of RE on serum lipid and CVD risk have resulted in

frequently contradictory findings (Williams et al. 2007; Frisch and Sumida, 1999; Leite et al. 2009; Kelley and Kelley, 2009; Sallinen et al. 2007). In the present study, although exercise resulted in lower HDL concentration, it clearly appeared to be as a consequence of lowered TC; the RE effect on HDL seen here would not represent an increase in the CVD risk factors, because CT/HDL and HDL/non-HDL ratios and AI were not significantly affected. In fact, data from the present study suggested a positive effect of WL, as a type of RE, to improve the lipid profile by reducing the serum TC and non-HDL concentrations. Although some authors have reported that RE could increase the HDL concentration (Leite et al. 2009; Leeds et al. 1986), the outcome in those reports, as well as in the

present study, was an improvement of lipid and lipoprotein status, suggesting that WL, as a type of RE could reduce the risk of CVD.

In the literature, WP ingestion has been reported to improve the lipid profiles in certain animal models (Lovati et al. 1990), but in the rat, its effect has remained controversial (Nagaoka et al. 1992; Sautier et al. 1983; Zhang and Beynen 1993; Haraguchi et al. 2009). For example, Sautier et al. (1983) observed that dietary WP exerted a hypocholesterolemic effect in the rats receiving the diets without cholesterol addition, although this effect was associated with reduction in serum HDL concentration. On the other hand, Choi et al. (1989) observed that the dietary WP did not exert hypocholesterolemia without cholesterol addition to the diet, but a reduction in the TC concentration was observed in the rats receiving the diets that contained added cholesterol (0.5%). Recently, Haraguchi et al. (2009) reported that WP was unable to significantly reduce the TC and non-HDL concentrations in hypercholesterolemic rats, although a tendency toward the reduction was observed. However, diets used in the studies of Haraguchi et al. (2009) were hypercholesterolemic (1% added cholesterol) and hyperlipidic (25% added soy oil). Zhang and Beynen (1993) observed that the diets with 30% WP reduced TC concentration in the rats also fed with 1% added dietary cholesterol, but this effect was not observed in the diets with 15% protein. Further, studies have reported an age-dependent effect of dietary WP on lipid metabolism in the rats (Choi et al. 1989; Minehira et al. 2000). These reports indicate that WP can cause a reduction in TC in adults (8–9 months old) but not in young animals (3–4 weeks old). Thus, the data of the present study, along with the results previously described by others, suggested that the effect of WP on lipid profiles resulted from the complex interactions with different factors, such as dietary cholesterol and protein, and the age of the animals studied. Regarding the combined effects of dietary WP and RE, although no interaction was indicated with respect to TC, HDL, or non-HDL concentrations, WE rats showed a higher ratio of HDL/non-HDL than other animals, suggesting an important interaction between the RE and WP treatment that resulted in reduced CVD risk factors.

The WP treatment resulted in highest serum albumin and glucose concentrations in the rats after overnight fasting. WP contains amino acids with gluconeogenic potential, i.e., relatively high

levels of branched-chain amino acids (BCAA). In addition to supporting protein synthesis, the BCAA also can be used as an energy source in muscle tissue (Blomstrand et al. 2006). Nitrogen removal from the BCAA in muscle stimulates alanine and glutamine synthesis, which are important in glycemic homeostasis, in a pathway known as the alanine-glucose cycle. The alanine-glucose cycle is an important pathway for glucose replenishment during prolonged exercise and after overnight fasting (Layman 2003). Thus, consistent with the previous findings (Haraguchi et al. 2010), data from the present study indicated that the WP represented an effective substrate source for glycemic homeostasis during overnight fasting, as indicated by elevated serum glucose concentrations. Moreover, WP fulfills more biological requirements than casein, showing higher digestibility, protein efficiency ratio, and net protein ratio (Haraguchi et al. 2010). The higher biological quality of WP, associated with the bioactive peptides present in the protein, may well contribute positively to a better hepatic production of albumin, resulting in elevated serum albumin concentrations.

The results of the present study showed differences between the body weight gain in CE and WE groups. The rats in the CE group showed less body weight gain than CS rats, as was typically observed in the resistance-trained animals (Morifuji et al. 2005; Barauna et al. 2005). Interestingly, WP counteracted the effects promoted by RE, resulting in similar gain in body weight to that observed in the CS and WS rats, but higher than that observed in the CE rats. Differences in amino acid composition of casein and WP, biological requirements, digestion/absorption kinetics (Boirie et al. 1997; Dangin et al. 2003), and even antioxidant properties (Haraguchi et al. 2011) could partially explain the differences in body weight gain between the rats fed with casein or WP. The activities of ALT and AST and serum creatinine concentrations were similar among all the groups, suggesting that hepatic failure or renal failure did not occur.

In conclusion, the present study demonstrated that the WL exercise for seven weeks improved the lipid profiles in the rats. Although the exercise resulted in reduced HDL, it was not correlated with increased CVD risk factors, because the TC/HDL and HDL/non-HDL ratios and AI were not affected by the RE. The rats treated with

combined RE and dietary WP showed higher ratios of HDL/non-HDL and higher body weight than the CE rats. This suggested that the combination of WL and dietary WP was more effective in increasing the body weight gain than the exercise without dietary WP and the combination could reduce the CVD risk factors more than RE or WP ingestion alone.

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