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Original Article

The Effects of Calorie Restriction and Time-Restricted Feeding on IGF1 Serum Level and Lipid Profile in Male Wister Rats with Previous Obesity

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Abstract

Background: Calorie restriction (CR) is known as a nutritional gold standard for life extension and different studies have shown that insulin-like growth factor (IGF1) reduction through CR may be involved in CR's anti-aging effects. Besides, time-restricted-feeding (TRF) is also highlighted due to more feasibility and positive health effects. We designed this study to compare the effects of CR and TRF on IGF1 and other metabolic parameters. **Methods:** Fifty-two male Wistar rats (3 weeks old) were subjected to either a control (CON, n = 11) diet or high-fat diet (HFD, n = 42) for 17 weeks. In the second phase of the study, the HFD group were divided into four groups (n = 9) 1) 30% CR, 2) Night Intermittent Fasting (NIF, active phase), 3) day intermittent fasting (DIF, rest phase), and 4) Ad-Libitum (AL) with a standard diet for 10 weeks. Blood samples were collected at the end of both phases. **Results:** HFD increased IGF1 and deteriorated lipid profiles, except for triglycerides (P: 0.018, 0.008.0.012, 0.032) but CR in these obese subjects could not lower the IGF1 level. HDL significantly decreased in DIF compared to CON and CR (P; 0.001). Meanwhile, HOMA-IR increased in DIF and was significant compared to CR (P: 0.002). Serum glucose levels decreased in CR compared to all groups except for CON (P: 0.001). **Conclusion:** Data indicates the role of previous obesity on the effect of CR on the IGF1 level and highlights the effect of inappropriate time of food intake on HDL and APOA1.

Keywords: *Apo A-I, calorie restriction, IGF-1, intermittent fasting*

Introduction

The incidence of obesity and obesity-related problems despite the considerable efforts of health care professionals is rising worldwide.[1] According to the World Health Organization's report, the number of obese people is doubling over the last 35 years and more than 50 million children at age 5 are obese.[2,3] The major explanation for the current trend is the overconsumption of high-calorie foods and disrupted circadian rhythm. Epidemiological studies in the USA, Canada, and China have indicated that obesity incidence increases by the increment of the average amount of fat in the diet.[4,5] Reduction of the calorie intake without malnutrition is the common strategy against obesity but beyond that, since 1935 different studies introduce, CR as a nutritional gold standard for life extension.[6] Several different pathways have been suggested to play a role in CR's mediated effects, including insulin growth factor-1 [IGF-1], Forkhead Box O (FOXO), and mammalian target of Rapamycin (mTOR), which eventually retards the damaged molecules accumulation.[7]

Previously, some different rodent studies have shown that CR can reduce IGF1 serum concentrations in rodents, and this multifaceted growth factor may be involved in CR's anti-aging and anti-cancer effects.[8] However, there is a controversy in human studies as a long term 25% calorie restriction (CR) did not lead to a IGF1 reduction while increased IGF binding1.[9] Several cellular and metabolic adaptations occur as a result of the IGF1 pathway suppression including upregulation of autophagic and apoptotic pathways, downregulation of growth pathways, increased resistance to multiple toxic agents, and increased genome stability.[9]

Moreover, due to the more feasibility and modulatory effects, intermittent fasting (IF) is known as one of the important CR's mimetics in weight loss as its primary outcome. It is noteworthy that, alternate day fasting is considered as if the protocol

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in most studies; while time-restricted feeding in dark/light cycle (similar to our protocol) is more practical and similar to human normal life.[8] For example, 8-week intermittent fasting specifically in the form of alternate-day fasting-induced body weight and fat mass lost specifically in obesity-prone ones.[10] Studies suggest that chronic diseases such as diabetes and cardiovascular disease are also prevented by intermittent fasting, even when there is little or no overall decrease in calorie intake.[9] Considering that light/dark cycle regulates central circadian clock which this, in turn, modifies the absorption of food, glucose or lipid transport and small peptides, depending on the moment of the day.[4] The comparison of the different effects of CR and IF in light (rest phase in rodent) and dark cycle (activity phase in rodent) against nutritional challenges that predispose an organism to obesity and related metabolic problems have yet to be investigated. Accordingly, the purpose of this study is to investigate the effect of CR and time-restricted feeding on IGF1 serum level and lipid profile in male Wister rats with previous obesity

Methods

Animals and diets

Iran University of Medical Sciences Ethics Committee approved the study protocol ((ir.iums.rec. 0327-26581) and a veterinarian regularly monitored the health status of the rats throughout the experiment. In the first phase, 52 postweaning male Wistar rats (three weeks old) were obtained from Pasteur Institute of Iran and were individually housed in cages at controlled temperature (22 ± 2 C), and humidity (50%) in a designated room under a 12-h light-dark cycle. In the accommodation period, the animals had free access to food and water. Then, the animals were divided into two groups, including control diet group (n = 11) receiving standard chow (18.8 MJ/kg with 23.4% as protein, 4.5% as fat, and 72.1% as carbohydrate, Ralston-Purina and other necessary nutrients for the growth of rats) and high-fat diet (n = 41) offering 60% of calories from milk/butter for 17 weeks. A subsample from the HFD group was housed in metabolic cages to measure the rat food intake for the second phase of the study, and CRs evaluation. The experimental diets were freshly prepared every 3 days and were kept at 0–4°C to avoid any rancidity. After significantly increasing the HFD group weight (12.5% higher body weight compared with the control group), the first phase of the study was completed. After an overnight food deprivation, a subsample from both groups (n = 6) was anesthetized by ketamine and xylazine, and blood samples were collected from the aortic vein. Blood samples were centrifuged (1500 g, 15 min at 4°C), the serum collected and stored at −80°C for further analyses. In the second phase of the study, the HFD group was divided randomly into four groups (each of 9): 1) CR, 2) Night Intermittent Fasting (IFN), 3) day intermittent fasting (IFD), and 4) Ad-Libitum (AL). The second phase of the study continued with the control group and the four derivative groups from the high-fat diet for 10 weeks [Figure 1]. Under the time-restricted feeding, rats were allowed to access the food between ZT 13 (one hour after light off) and ZT1 (1 h after light on) or vise versa [Figure 1]. At the end of this stage, blood samples were taken in similar condition to the first phase.

Statistical analysis

The data are reported as mean ± SD. The statistical analyses were performed using SPSS software (2013- IBM SPSS Statistics for Windows, Version 22.0. Armonk NY: IBM Corp). The normality of the data distribution was checked through Kolmogorov–Smirnov test and Levene's test was used to assume the equal variance of each group. In the first phase of the study, data were analyzed through independent t-test or its nonparametric alternative, the Mann–Whitney test. In the second phase of the study and normal distribution of data, one-way analysis of variance (ANOVA), followed by Tukey posthoc tests, were used to compare the results. Otherwise, the Kruskal–Wallis and posthoc Bonferroni test were used. P values of <0.05 were considered significant.

Results

Day intermittent fasting leads to lower HDL and APOA1

The results of biochemical analyses are presented in Table 1. The increment of LDL/HDL ratio, cholesterol, and total cholesterol/HDL were observed over 17 weeks of a high-fat diet, and there was no significant change in the APOB100/APOA1 ratio. Pair-wise comparison in the second phase of the study showed that the HDL level was significantly lower in DIF (rest phase) compared to CON and CR (P: 0.0001), while there were no significant changes among other parameters. Moreover, APOA1 was lower in DIF compared to the others, which was significant

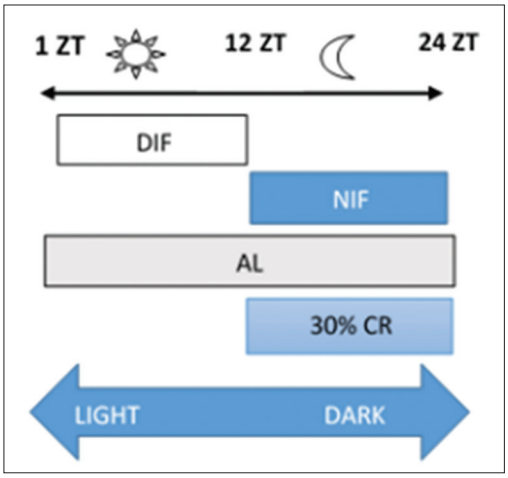

Figure 1: Second phase dietary pattern

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Table 1: Biochemical parameters in the first and second phase of the study

	1st phase				2nd phase			
	HFD	CON	*P*-value	CR	NIF	DIF	AL	CON	*P*-value
Glucose (mg/dl)	159.4±57.57	139.8±60.73	0.03	144.6±17.69abc	276.55±45.49a	256.11±51.46b	223.55±48.60c	132.66±32.61	0.0001
Insulin (µg/L)	0.16±0.04	0.20±0.06	0.37	0.06±0.02	0.21±0.07	0.22±0.07	0.13±0.04	0.10±0.04	0.39
HOMAIR	1.9±1.4	2.1±1.4	2.1	1.8±0.5a	3.36±1.64	4.01±2.41a	2.86±1.45	1.61±0.9	0.002
IGF1 (ng/ml)	178.06±18.49	145.32±16.38	0.018	170.65±9.95	163.33±13.62	173.12±24.54	158.62±21.95	166.25±18.21	0.46
Cholesterol (mg/dl)	137.8±16.39	93.2±23.15	0.008	94.11±13.04	97.22±20.79	75.66±12.78	95.44±17.52	95±16.23	0.051
Triglyceride (mg/dl)	69.4±33.29	73.24±33.24	0.32	55.88±8.46	76.66±32.01	63.88±18.23	88.77±24.79	62.66±21.91	0.051
HDL	44.6±7.19	53.2±14.13	0.27	67±9.06a	54±9.61	49.11±8.08ab	53.11±10.52	66.37±8.75b	0.0001
LDL/HDL	53.8±8.04	35±10.17	0.012	0.43±0.1	0.49±0.11	0.51±0.8	0.46±0.1	0.39±0.08	0.18
APOB100/APOA1	33.51±6.46	32.4±6.69	0.80	0.02±0.008	0.03±0.007	0.03±0.006	0.03±0.019	0.03±0.004	0.28
Total cholesterol/HDL	3.13±0.58	1.87±0.84	0.02	1.40±0.09	1.80±0.1	1.53±0.06	1.8±0.12	1.42±0.07	0.0001

Data presented as mean ± SD. Similar letters indicate a significant difference between groups. independent T-test or its non-parametric alternative, the Mann–Whitney test were used in the first phase and in the second phase, statistical differences between groups were assessed by, one-way (ANOVA), followed by Tukey posthoc tests, were used to or Kruskal–Wallis and posthoc Bonferroni. P-values of <0.05 were considered significant

among CR and DIF (*P*: 0.012). CR increased HDL and APOA1 levels in comparison to the first phase, which was not seen in other groups (*P*: 0.002, 0.045).

Glucose metabolism and IGF1

A high-fat diet leads to higher fasting blood glucose, though there was no significant change in HOMA- IR and serum insulin concentration in the first phase groups (*P*: 0.37). Between-group analyses in the second phase of the study showed a significantly lower fasting blood glucose in CR (except for CON), and higher HOMA-IR in DIF (*P*: 0.0001, *P*: 0.002), which was significant compared to IFN. HOMA-IR also increased in IFN and DIF compared to HFD (*P*: 0.04, 0.004). The serum concentration of IGF1 increased in response to a high-fat diet and weight gain, while there were no significant changes in the second phase groups. Nevertheless, IGF1 increased in response to CR, compared to AL, and control food intake (*P*: 0.46) [Table 1].

Discussion

CR is known as one of the most effective interventions against chronic diseases and a life-extension protocol from yeast to primates.[11] IGF1 as a growth factor is highlighted in CR's mediated benefits and plays an important role in cell differentiation, growth, and development. In some circumstances, this survival factor leads to higher cell proliferation, which may cause cancer and obesity, while in others acts as a cell protective agent and preserves myocytes and cardiomyocytes.[12,13] However, the exact mechanism of CR has not been recognized yet. Data from experimental and genetic studies have indicated that a higher level of IGF1 is correlated with reduced lifespan and CR can decrease the IGF1 level.[14,15] The first-phase results indicated that the HFD group had a higher level of IGF1. Data on IGF1 and obesity are controversial.[7,9,16] It seems that they have a bilateral relationship, since higher food intake increases IGF1 in those with a low level of serum concentration, while on the other hand, higher levels of IGF1 increases SREBP, cholesterol, and adipocyte differentiation.[15,17,18] In contrast, it seems that CR can effectively reduce IGF1 and protect the organism against cancer and other age-related diseases.[19,20] In the second phase of the study, no reduction was observed in the serum concentration of IGF1 following CR, while surprisingly a mild increment was observed in this group compared to AL (not significant). Previously, Fontana *et al*. reported that long-term CR in a normal-weight person did not lead to IGF1 reduction, due to a lack of significant changes in protein intake followed by mild CR.[7] Hence, it seems that a higher level of energy restriction can decrease the IGF1 level and the involved mechanism in this kind of dietary approach differs in normal weight and obese subjects due to the disturbed insulin signaling tailored by obesity.[21] This is seen early after CR, as shown by decreased FBS and insulin resistance (compared to DIF).

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A high-fat diet in the first phase of the study increased FBS significantly in the HFD group; however, the insulin concentration did not change significantly between groups. Fiorino reported that HFD started post-weaning did not change serum insulin concentration. Besides, a conjugated linoleic acid-rich diet (higher butter consumption) may activate PPARγ, which can increase free fatty acids transfer to adipose tissue, which is accompanied by higher fat pad weight and lower insulin serum concentration.[22-24] While in the CR period, higher Sirtuin may down-regulate PPARγ to improve fat hydrolysis and positively regulate PPAR-coactivator 1 (PGC1α) to coordinate the shift from glucose to fat oxidation.[25] Moreover, SIRT can enhance glucose-induced insulin secretion by inhibiting uncoupling protein 2 (UCP2).[26] In the same way, Dastbarhagh *et al*. reported that 8 weeks CR with or without carbohydrate limitation can enhance GLUT4 gene expression in male Wistar rats.[27] TRF did not influence FBS and insulin sensitivity favorably. It seems that TFR lasting for more than 21 h can have a positive effect on insulin resistance through an increment of beta-hydroxybutyrate, pyruvate dehydrogenase bypass, and acetyl-CoA increases.[28] Unlike the observations around insulin and FBS, DIF significantly decreased HDL and APOA1 serum concentrations compared to CON and CR, which points to the effect of inappropriate food intake time on HDL. This is noteworthy, due to the impact of HDL (rather than LDL) on cardiovascular disease.[29]

In summary, in contrast to previous studies, no changes in IGF following CR or TRF indicates the role of previous obesity in addition to calorie intake on CR-induced changes in IGF. However, the changes in this factor are studied and compared, different amount of CR or longer period of fasting may have different effects which were not studied on the other hand, the comparison between protein restriction which may have a sting role on IGF1 level with TFR (of course is not in the form of alternate-day fasting) is suggested. Moreover, this study reports an adverse effect of inappropriate food intake time on HDL and APOA1, which may be a risk factor for future cardiovascular disease and further studies in this regard may help to lead to a clear conclusion.

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Conflicts of interest

There are no conflicts of interest.

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