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Research Article

Compliance and Tolerability of a 16-Hour Prolonged Nighttime Fasting Regimen in Healthy Males: a Preliminary Study

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Abstract

Background: Prolonged nighttime fasting is a type of intermittent fasting that may be a more maintainable method for improved health as compared to caloric restriction. This study aimed to determine the compliance and tolerability of a 4-week 16-hour nighttime fasting regimen.

Methods: Compliance and tolerability were assessed though a post-study survey and a compliance diary. Pre- and post-study health and longevity biomarkers were also assessed. Additionally, dietary intake was evaluated using the Automated Self-Administered 24-Hour Recall and physical activity using the International Physical Activity Questionnaire.

Results: All 11 subjects completed the prolonged fasting nighttime regimen. The mean fasting time was 16.5 hours, with an average of 2.4 days of noncompliance. Survey results confirmed tolerability, with no perceived changes in quality of life, sleep, physical activity, or daily tasks. The regimen did not significantly impact any health parameters or biomarkers assessed, except for a reduction of systolic blood pressure ($p=0.03$) and increased antioxidant capacity in the blood ($p=0.001$). Diastolic blood pressure tended to decrease, but did not reach statistical significance ($p=0.05$)

Conclusion: Prolonged nighttime fasting may be a maintainable variation of intermittent fasting, as the subjects in this study were able to comply with the regimen and believed it to be tolerable.

Keywords: intermittent fasting, caloric restriction, health, longevity, preventative medicine

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Introduction

Obesity and non-communicable chronic diseases continue to be public health concerns. In the United States (U.S.) the prevalence of obesity among adults is approximately 36% and among children approximately 17 [1]. The prevalence continues to rise. At least half of the adult population suffers from at least one chronic disease condition and at least 25% from multiple conditions, such as heart disease, hypertension, hyperlipidemia, stroke, diabetes, and cancer [2]. Thus, interventions to curb obesity and non-communicable chronic conditions are of necessity.

Often used for weight loss, caloric restriction is a behavioral intervention that has consistently shown to slow the intrinsic rate of aging and reduce morbidity in various species [3]. Traditionally, caloric restriction means restricting calories by ~30-40% per day on a continual basis, while maintaining adequate intake of essential micronutrients. Dedicated following of calorie restricted diets require meticulous calorie counting, timing of meals, and analysis of nutrients. This can make maintaining caloric restriction very difficult. There are also some discomforts and health risks associated with calorie restricted diets [3].

Due to the difficulty of adherence, scientists have studied alternative regimens of caloric restriction and fasting [4-8]. These alternatives may still offer benefits on health and longevity, but may make adherence easier. Intermittent fasting consists of extended periods of time (e.g., 16-48 hours) with little or no energy intake, followed by periods of ad libitum feeding. Preclinical trials and some human studies suggest the transient periods of fasting produce benefits on aging and health similar to "traditional" caloric restriction [4-8]. Many of the studied intermittent fasting protocols involve periods of 24 hours of little or no energy intake. Some believe that this regimen is still difficult to maintain long-term due to the lengthy 24 hour fasting periods [6].

Prolonged nighttime fasting is a regimen that may be more sustainable due to decreased awake-time fasting hours and absence of required calorie counting. Evidence exists from studies of Ramadan fasting that ~16 hours of daily fasting can confer health benefits [9-14]. Studies involving Ramadan fasting may not reflect the same benefits on health and longevity as prolonged nighttime fasting for two reasons. First, time of feeding has been shown to have differential effects on metabolism and biomarkers of health [15,16]. Secondly, Ramadan practice often includes overeating after sundown and disrupting normal sleep habits, which may negate some of the health benefits of fasting. Therefore, prolonged nighttime fasting may exert additive health benefits over Ramadan fasting or other regimens that allow feeding through nighttime hours.

Only two studies have been published on prolonged nighttime fasting in humans. A retrospective study indicated that each 3-hour increase in nighttime fasting was associated with a 4% lower 2-hour glucose measurement and a 0.4-unit decrease in hemoglobin A1c in women who participated in the 2009-2010 U.S. National Health and Nutrition Examination Survey (NHANES) [17]. Furthermore, an increased nighttime fasting duration has been associated with a decrease in the risk of breast cancer reoccurrence [18]. Since the available data suggests that prolonged nighttime fasting may confer health benefits, we aimed to determine the tolerability and compliance of such a regimen. Biomarkers of health were also assessed.

Materials and Methods

Subjects: 11 healthy male faculty, staff, and/or students at Wingate University were recruited for the study. The eligibility requirements were as follows: 21-60 years of age; normal weight to overweight (BMI: 18.5-29.5); stable weight within the last three months; usual breakfast and dinner eaters who do not consistently overnight fast for more than 12 hours; sedentary to light exercisers; nonsmokers; no known history of metabolic disease, cardiovascular disease, cancer, or thyroid dysfunction; no documented hypoglycemia; and no chronic medication use. Participants received $100 at completion of the study. All subjects signed an informed consent form. All procedures were approved by the Institutional Research Review Board.

***Experimental Design*:** Subjects fasted 16 hours each night for a period of 4 weeks. Subjects were encouraged to fast between the hours of 6pm and 10am (consuming nothing but water) and to

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eat their normal diet during the non-fasting hours. Subjects were asked to keep a daily log of their fasting hours, listing any deviations from the protocol. Dietary intake before and during the fasting regimen was assessed using the online Automated Self-Administered 24-hour Recall (ASAS24) developed by the National Cancer Institute (www.asa24.nci.nih.gov) to determine daily caloric intake and daily nutrient intake. Subjects recorded their 24-hour dietary intake on specified days three times during a 2-week period before initiation of the fasting protocol and three times during the 4-week fasting period. Subjects were texted reminders to complete the ASA24 on designated days throughout the study period. Subjects also completed the long version of the validated International Physical Activity Questionnaire (IPAQ) before and on the last day of the fasting period, recording their physical activity within the previous 7 days [19,20]. Results were expressed as metabolic equivalent (MET)-minutes per week, which was calculated by multiplying the MET score of an activity by the minutes performed. Guidelines for data processing and analysis can be found at www.ipaz.ki.se. On the last day of the fasting period, subjects also completed an online survey to assess perceptions of compliance, tolerability, and feeding behavior relating to the fasting regimen.

Approximately two weeks before initiating the fasting protocol and on the last day of the 4-week fasting period, subjects underwent a clinical assessment at the Wingate University Health Clinic located on campus. Subjects reported to the clinic in the morning hours after fasting overnight. The assessment included measurements of height, weight, blood pressure, and a blood draw for chemical analysis. The blood sample was immediately sent to Carolinas Healthcare Systems Medical Group Laboratory for analysis of a standard lipid profile, glucose, insulin, fructosamine, insulin-like growth factor 1 (IGF-1), high sensitivity C reactive protein (hsCRP), and hydroxybutyrate. A second set of plasma samples were stored at -80C° for future analysis of markers of oxidative stress which encompassed total antioxidant capacity, DNA/RNA oxidative damage, and lipid peroxidation (thiobarbituric acid reactive substances [TBARS]). The total antioxidant assay did not measure any specific antioxidants. All oxidative stress assay kits were purchased from Caymen Chemicals (Ann Arbor, MI; Item numbers 709001, 589320, and 10009055) and directions from the manufacturer were followed.

Statistical Analysis

A student t-test was used to determine statistical significance pre and post completion of the fasting protocol. $p < 0.050$ was considered statistically significant. Data are represented as mean ± SEM.

Results

Eleven subjects participated in the study. The average age was 28.09 ± 1.89 years with a range of 21-39. All subjects completed the 4-week prolonged nighttime fasting regimen. According to the fasting compliance diaries, the mean fasting time each day was 16.5 ± 0.17 hours. Each subject had an average fasting time ≥ 16 hours/day and the mean number of days with a fasting time ≤ 15.5 hours (considered noncompliant) was 2.43 ± 0.97. The majority of the subjects (71.4%) had at least one day where they did not fast for at least 15.5 hours. The number of noncompliant days ranged between 1-6 days.

A survey confirmed tolerability, with the majority of subjects stating the fasting regimen did not alter quality of life, nor did it impact their ability to sleep, daily physical activity, or ability to complete usual daily tasks. The majority of subjects (54.6%) believed that the regimen did not negatively impact their energy level. Additionally, the majority of subjects (63.6%) believed that prolonged nighttime fasting reduced their daily food intake and felt that the regimen affected the types of food that they ate and craved. 36.4% believed they increased their intake of healthier foods, while 18.2% believed they decreased intake of healthy foods. 27.3% and 18.2% believed they increased or decreased intake of unhealthy foods, respectively. The majority (54.5%) felt that their cravings for unhealthy foods increased. The majority (72.8%) stated that their friends and family were supportive of their adherence to the fasting protocol and 81.8% would consider or maybe consider adopting this fasting regimen as a part of a long-term dietary plan. See Table 1 for complete survey questions and responses.

The fasting protocol did not affect the subjects' daily caloric

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Table 1: Survey questions and responses (percent of subjects).

	Greatly Improved or Greatly Increased	Improved or Increased	Neutral or No Change	Decreased	Greatly Decreased
How did the fasting protocol affect your quality of life?	0%	9.1%	81.8%	9.1%	0%
How did the fasting protocol impact your ability to sleep?	9.1%	9.1%	54.5%	27.3%	0%
During the study, how did your daily food intake (quantity) change?	0%	18.2%	18.2%	63.6%	0%
How did the fasting protocol impact your normal daily routine involving physical activity?	0%	27.3%	63.6%	9.1%	0%
How did the fasting protocol affect your energy level?	0%	9.1%	45.5%	45.5%	0%
How did the fasting protocol affect your ability to complete usual daily tasks?	0%	18.2%	63.6%	18.2%	0%
	Increased Intake or Cravings of Healthier Foods	**Decreased Intake or Cravings of Healthier Foods**	**Did Not Impact the Types of Foods That I Ate or My Cravings**	**Increased My Intake or Cravings of Unhealthy Foods**	**Decreased My Intake or Cravings of Unhealthy Foods**
How did the fasting protocol affect the types of foods that you ate? (Answer all that apply)	36.4%	18.2%	18.2%	27.3%	18.2%
How did the fasting protocol affect your food cravings? (Answer all that apply)	18.2%	9.1%	27.3%	54.5%	0%
	Very Supportive	**Supportive**	**Neutral**	**Unsupportive**	**Very Unsupportive**
How supportive were your friends and family regarding adherence to the fasting protocol?	36.4%	36.4%	9.1%	9.1%	9.1%
	Yes	**No**	**Maybe**		
Would you consider adopting a fasting protocol as a part of a long-term dietary plan?	54.5%	18.2%	27.3%		

intake which was assessed before and during implementation of the protocol using the ASA24 (2079 ± 186 vs 2129 ± 136 KCAL, respectively; p = 0.85). The subjects also did not appear to alter the types of food that they ate regarding percentage of protein (23.1 ± 2.27 vs 23.0 ± 2.42%, p=0.94), fat (22.7± 2.06 vs 21.3 ± 1.97%, p=0.29), carbohydrates (54.2 ± 4.04 vs 55.8 ± 3.88%, p=0.61), sugar (21.4 ± 3.18 vs 20.9 ± 3.14%, p=0.88), and fiber (5.3 ±0.63 vs 5.9 ± 0.76%, p=0.30).

According to the results of the IPAQ, prolonged nighttime fasting did not significantly impact the subjects' self-reported quantity of physical activity pre- vs post-initiation of the regimen, which was 4420 ± 1605 vs 2595 ± 773 MET-minutes/week, respectively (p=0.12).

The fasting regimen did not negatively impact any health parameters that were assessed including BMI, fasting glucose, insulin, fructosamine, hsCRP, and lipid profile. Systolic blood

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pressure (SBP) improved after 4-weeks of fasting (124 ± 3.0 vs 118 ± 2.3 mmHg, respectively; p=0.03). Although it did not quite reach statistical significance, diastolic blood pressure (DBP) also improved (79 ± 2.5 vs 75 ± 2.1 mmHg, respectively; p=0.05) (Table 2).

Lastly, 4-weeks of prolonged nighttime fasting increased antioxidant capacity of the blood (2.27 ± 0.12 vs 2.92 ± 0.14 mM, pre- and post-fasting, respectively; p=0.001), with no significant impact on DNA/RNA oxidative damage or lipid peroxidation. See Table 2.

Discussion

For a preventative intervention to be effective, individuals must be able to comply long-term. A prolonged nighttime fasting regimen is a type of intermittent fasting that may be more manageable to adhere to as a lifestyle. The average number of days of noncompliance was low, indicating a high level of compliance. Furthermore, the fasting regimen did not appear to interfere with activities of daily life or negatively affect quality of life. In general, the fasting regimen appears to be tolerable and maintainable.

Despite a perceived change in caloric intake and food cravings from the survey, the fasting regimen did not alter the actual intake of foods consumed as far as caloric or macronutrient content according to ASA24 results. One concern was that individuals may gorge, resulting in increased daily caloric intake. This may be due to feelings of hunger after a period of fasting or the tendency to

Table 2: Biomarkers of health pre-and post-implementation of prolonged nighttime fasting. Results expressed as mean ± SEM. *Statistically significant.

	PRE	POST	
BMI (Kg/m^2)	25.2 ± 0.99	24.9 ± 1.0	p=0.14
SBP (mmHg)	124.0 ± 3.0	118.0 ± 2.3	p=0.03*
DBP (mmHg)	79.0 ± 2.5	75.0 ± 2.1	p=0.05
Glucose (mg/dL)	90.8 ± 2.4	92.5 ± 5.2	p=0.65
Insulin (mIU/mL)	9.2 ± 1.1	9.2 ± 1.9	p=0.99
Fructosamine (umol/L)	259.9 ± 3.7	259.8 ± 6.8	p=0.98
hsCRP (mg/L)	0.8 ± 0.2	0.6 ± 0.1	p=0.14
IGF1 (ng/mL)	10.1 ± 2.6	11.3 ± 2.8	p=0.45
TC (mg/dL)	186.3 ± 10.8	186.3 ± 10.5	p=1.00
TG (mg/dL)	98.6 ± 16.7	96.1 ± 16.3	p=0.80
HDL (mg/dL)	56.6 ± 5.1	55.8 ± 5.7	p=0.70
LDLc (mg/dL)	110.0 ± 9.1	111.2 ± 8.5	p=0.80
nonHDL (mg/dL)	129.7 ± 9.5	130.5 ± 10.4	p=0.88
Hydroxybutyrate (mg/dL)	1.70 ± 0.6	1.66 ± 0.4	p=0.94
Antioxidant Capacity (mM)	2.27 ± 0.12	2.92 ± 0.14	p=0.001*
Nucleic Acid Oxidative Damage (pg/mL)	515 ± 22.42	549 ± 24.20	p=0.68
Lipid Peroxidation (TBARS) (uM)	2.15 ± 0.30	2.79 ± 0.23	p=0.169

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overeat to prevent anticipated feelings of hunger during the fasting hours. However, the data suggests that the daily caloric intake did not change. This is in agreement with several other studies of intermittent fasting where the daily caloric intake remained the same or tended to decrease, rather than increase [14,21-23].

Although 45.5% of participants felt the fasting protocol negatively affected their energy level, only 9.1% of the participants felt that the protocol negatively impacted their level of physical activity. The results of the IPAQ confirmed that their level physical activity was not significantly impacted.

The fasting regimen did not negatively impact any biomarkers of health or oxidative stress that were assessed, with improvements noted in blood pressure and total antioxidant capacity of the blood. Reductions in blood pressure are associated with reduced risk of heart disease, heart attack, heart failure, stroke, kidney disease, and sexual dysfunction [24]. With a high global prevalence, this regimen has the potential to improve health outcomes of over a billion individuals [25]. Furthermore, higher total antioxidant capacity has been associated with lower cardiovascular disease risk [26].

The results are in both agreement and disagreement with several of the studies involving 3-4 weeks of daily intermittent fasting in healthy subjects observing Ramadan, which are currently the only human studies published encompassing daily intermittent fasting periods. The results are in agreement with Faris et al. and Sungar et al. in that daily intermittent fasting decreased the SBP and DBP [9] and did not alter the lipid profile [12] in healthy subjects. Ibrahim et al., reported no change in the levels of oxidized macromolecules in the blood, which is consistent with our current findings [11]. A review on the health impacts of religious fasting reported that the BMI of subjects remained unchanged or decreased during the period of Ramadan fasting [14]. None of the studies included in the review reported an increase in BMI. These results are consistent with our study in that there was no statistically significant change in BMI, but there was a trend towards a decrease.

The results from our study are in disagreement with other studies in that 3-4 weeks of Ramadan fasting were shown to improve various biomarkers of health such as HDL, glucose, and/or insulin levels in healthy subjects [10,27], all of which were not affected in the current study. CRP was also found to significantly decrease in other studies; while it remained unchanged in our study, it did trend towards a decrease [12].

The lack of a significant effect on most biomarkers assessed may be due to several factors, including: the small sample size, the healthy nature of the subjects, and/or the relatively short length of the study period. The inclusion criteria required subjects to be healthy, taking no medications, and with no chronic conditions or diseases. Although the inclusion criteria allowed subjects to be in the range of normal weight to overweight (BMI = 18.5-29.5 kg/m^2), the BMI of most subjects were in the healthy range, which is 18.5-24.9 kg/m^2. The BMI of the subjects before initiating the fasting regimen was 26.0 ± 1.5 versus 24.9 ± 1.0 kg/m^2 at the end of the 4-week regimen. The healthy nature of the subjects may have narrowed the range for significant improvement in the assessed biomarkers. It is also possible that prolonged nighttime fasting, without a reduction in daily caloric intake, does not impact some biomarkers of health.

The fasting regimen seems tolerable and individuals are able to comply. Although this study did not show that the regimen improved many health parameters that we monitored, it did reduce blood pressure and improved antioxidant capacity, without any negative effects on the others. Therefore, the fasting regimen is worth pursuing by individuals wanting to reduce blood pressure, which is a large proportion of the global population, or wanting to improve antioxidant capacity. Furthermore, the results suggest that the fasting regimen is worth further research to determine additional benefits utilizing a larger cohort, for a longer period of time, and/or in less healthy individuals with more room for improvement.

Conclusion

Various modes of intermittent fasting have been shown to impact morbidity and mortality. This is the first non-retrospective human study to evaluate prolonged nighttime fasting as a feasible variation. Prolonged nighttime fasting may be a variation that is sustainable as a long-term lifestyle change, as the subjects in this study were able to comply with the regimen and believed it to be tolerable.

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The 16-hour daily fasting regimen improved blood pressure and increased antioxidant capacity, with no negative impact on all other health parameters assessed. More comprehensive studies, with a larger sample size from a variety of populations, will be required to determine the impact on biomarkers of health and longevity, and ultimately the impact on morbidity and mortality.

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