Haptoglobin polymorphism modulates cardiometabolic impacts of four consecutive weeks, dawn to sunset Ramadan intermittent fasting among subjects with overweight/obesity

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Research Article

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Abstract

Aims

Haptoglobin (Hp) is a multifaceted marker of inflammation, and mediates the interplay between obesity, inflammation, and cardiometabolic dysfunction. However, the role of the Hp phenotype in modulating intermittent fasting (IF)-induced changes in cardiometabolic markers remains to be elucidated.

Methods

Hp phenotype was determined for the study subjects. Cardiometabolic markers were assessed before and at the end of four consecutive weeks, dawn to sunset IF.

Results

A total of 114 subjects (75 males and 39 females, 38.7 ± 11.7 years, body mass index (BMI) of 30.41 ± 5.09 kg/m^2) were recruited. Hp2-2 (n = 55, 48.2%) and Hp2-1 (n = 53, 46.5%) were the predominant phenotypes. Significant reductions were observed in serum Hp, IL-6, TNF-α, triglycerides (TG), total cholesterol (TC), LDL, BMI, fat mass (FM), while the significant elevation was observed in serum CD163, HDL, and IL-10 at the end of the IF month for the whole population. Based on the Hp polymorphism, significant decreases in Hp, BMI, FM, TG, LDL, and TNF-α, with significant increases in HDL and CD163 levels were observed among subjects with Hp2-2 and Hp2-1 phenotypes. A more pronounced reduction in FM was reported in subjects with Hp2-2 in comparison with Hp2-1.

Conclusion

Hp gene polymorphism modulates IF-induced changes in cardiometabolic markers.

Introduction

A growing body of evidence supports the role of weight-reducing regimens, intermittent fasting (IF), and physical activity in reversing (or protecting against) the adverse metabolic derangements associated with obesity [1]. IF is a widely adopted dietary practice, used for both religious and non-religious (e.g., health improvement) purposes [2–4]. Recent evidence supports the health-improving, disease-preventing effects of IF, such as: maintaining healthy aging; reducing insulin resistance and improving insulin sensitivity; decreasing cancer risk by improving DNA repair and mitochondrial health, and triggering autophagy; reducing body weight; and improving cardiovascular and cerebrovascular health [2, 3, 5–7]. Among the different regimens of IF, religious form of IF such as Ramadan IF (RIF)[2] is one of the most extensively examined types of IF, with various impacts, have been reported in anthropometric [8, 9], dietary [10], inflammatory and oxidative stress [11–13], metabolic [14–16], hormonal [17] and lifestyle [18] aspects. Ramadan fasting month is the ninth month of the lunar calendar, through which adult Muslims are mandated to refrain from all food and drinks from dawn to sunset for 29–30 consecutive days. Fasting duration ranges from 12–20 hours depending on the geographical location and solar season of the fasting month [19].

Various single nucleotide polymorphisms (SNPs) are associated with the variable components of metabolic syndrome, and many of these SNPs have been shown to modify an individual's response to dietary and lifestyle interventions [20, 21]. Haptoglobin (Hp) is a plasma polymorphic glycoprotein that has an inflammatory potential and increases two- to five-fold in response to inflammatory stimuli [22]. The primary functions of Hp are to bind to the free hemoglobin (Hb) produced from red blood cell destruction, reduce the release of heme iron and the heme generation of reactive oxygen species (ROS), and consequently, inhibit oxidative tissue damage. Hp-Hb complex's uptake and release from circulation are regulated by the monocyte/macrophage scavenger receptor CD163 and hepatocytes [23, 24].

The three main Hp phenotypes in humans (Hp2-2, Hp2-1, and Hp1-1) are derived from two alleles: Hp1 and Hp2 [25]. Several studies have reported that the Hp phenotype has a crucial role in determining the antioxidant and anti-inflammatory responses, and hence the cardiometabolic risk [25, 26]. People with the Hp1-1 phenotype were found to be more resistant to oxidative stress (OS) than those with Hp2-2 and Hp2-1 phenotypes, by the better abilities to enhance the stability of the Hp-Hb complex, and prevent Hb oxidation and the formation of ROS. In contrast, Hp2-1 and Hp2-2 phenotypes have a lower Hp-Hb complex stability, which increases its association with higher OS and pro-inflammatory response, making Hp2-2 phenotype a risk factor for inflammatory disease [24, 26–28]. This is reinforced by the fact that individuals with the Hp2-2 phenotype have increased levels of oxidized LDL-cholesterol and decreased clearance rate of free Hb compared to Hp1-1 [26, 28–30]. These variations in the antioxidant activity and anti-inflammatory macrophage signaling between the three Hp phenotypes may explain the association between specific Hp phenotypes and increased obesity and metabolic disorder risk factors [31].

With the growing attention directed toward IF in reducing body weight and improving cardiometabolic health, along with the existence of variable Hp phenotypes; it becomes rationalized to examine how people with different Hp phenotypes respond to IF regimen. Further, the extent of how anthropometric, metabolic, inflammatory markers change in response to IF among people with obesity is worth to be examined. Thus, the primary objective of the current work is to elucidate how people with different Hp phenotypes respond to four-week, dawn to sunset Ramadan IF (RIF), and how Hp modulates the anthropometric, inflammatory, and metabolic changes among subjects with overweight/obesity. The study stems from the hypothesis that subjects with overweight/obesity with different Hp phenotypes will respond differently to the observed RIF, and that the anthropometric, metabolic, and inflammatory responses will vary according to the Hp phenotype.

Subjects And Methods

Study design

An observational, prospective cohort design was used to find out how RIF-induced changes of anthropometric, metabolic, and inflammatory markers will differ among subjects with overweight/obesity with three distinct Hp phenotypes.

This prospective study was conducted over two Ramadan fasting months over two consecutive years (during May-June 2017 and 2018). Data were collected at two-time intervals: baseline (T1, 2–7 days before commencement of RIF) and at the end of the fourth week of Ramadan month (T2, after completing 28–30 consecutive days of dawn-to-sunset RIF). During the fasting period (about 15 hours a day), individuals refrained from oral intake (including food and water) from dawn to sunset. Subjects received no particular dietary or physical activity regimens or recommendations during any stage of this study. According to Islamic laws of fasting, menstruating women are exempted from observing Ramadan fasting during their menstrual period; hence, the fasting period for participating women was less than that for men (23–25 days vs. 28–30 days).

Participant selection

A convenience sampling technique was followed. After announcing the research via social media, institutional emails, and personal communications, interested people who expressed their intention to observe Ramadan fasting, and visited the University Hospital Sharjah (UHS)/UAE for screening were recruited for this study. The study protocol was designed and conducted following the Declaration of Helsinki and approved by the UHS Research Ethics Committee (Reference no: REC-16-05-11-01). All enrolled subjects were provided with an information sheet describing the research plan, objectives, and requirements of participation. All subjects provided signed informed consent to participate in this study. Male and female subjects who were overweight/obese (Body Mass Index, BMI > 25 kg/m^2), willing to fast during Ramadan, and to participate in this study were considered eligible. Data were collected using a self-report questionnaire that covered the medical history and demographic information. The questionnaire was administered in individual face-to-face interviews. All interviews were conducted by trained research assistants. The exclusion criteria were: a history of metabolic syndrome, diabetes, cardiovascular disease; neuro-psychiatric patients taking regular medications; following a weight-reducing diet, a history of bariatric surgery within the last 9 months before commencing RIF; and being a pregnant or premenopausal woman.

Anthropometric assessment

Anthropometric measurements were taken at two-time intervals, T1 and T2. Body weight (BW), body mass index (BMI), fat mass (FM), body fat percent (BFP), fat mass (FM), fat-free mass (FFM), and visceral fat surface area (VFSA) were measured before and at the end of the fasting month using direct segmental multi-frequency bioelectrical impedance analysis machine (DSM-BIA; TANITA, MC-980, Tokyo/Japan) following the manufacturer instructions. Before taking the BIA measurements, all accessories, metals, and/or jewelry were removed according to the manufacturer's instructions, and each subject was requested to purge surplus bodily fluids through urine. Because all people fasted for eight to ten hours before each of the two-time intervals, the impact of hydration and physical effort on BIA measurements was minimized, as was intra-individual variability. Furthermore, for T1 and T2, BIA and all other data were taken at the same time of day. The DSM-BIA machine measured the visceral fat rating (from 0 to 100); this value was converted into a VFSA by multiplying the obtained value by 10, consistent with the manufacturer's instructions. Height was measured using a fixed stadiometer to the nearest 0.1 cm. BMI was calculated as weight (kg) divided by height in m^2. Waist (WC) and hip circumference (WC) were measured to the nearest 0.01 m using a non-stretchable measuring tape (Seca, Hamburg/Germany), and their ratio was calculated accordingly.

Blood sampling

Alongside all anthropometric assessments, venous blood samples were collected from subjects after 8–10 hours of fasting at both time intervals (T1 and T2). A volume of 10 ml of blood was collected at the two-time intervals. At both time intervals, the samples were collected between 11 am and 1 pm to eliminate the effect of timing and dietary intake on the measured biochemical parameters and ensure consistency in the duration of fasting. Collected blood samples were divided into two aliquots. One aliquot was centrifuged at 2500 rpm for 15 minutes within 1 hour of the collection; the serum was aliquoted, coded, and stored at − 80°C until it was used for biochemical analyses. The second aliquot was used for RNA extraction, as explained below.

Biochemical assays and determination of Hp polymorphism

Hp phenotype was determined for the subjects using G-vertical polyacrylamide gel electrophoresis [32]. In this study, a fully automated clinical chemistry analyzer (Adaltis, Pchem1, Italy) was used to quantify fasting glucose (FBG), total cholesterol (TC), low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglycerides (TG) at the time points. Blood pressure was measured before blood sampling using a digital blood pressure monitor (GE, USA), with subjects in an erect, seated position after a 5-min resting period. Serum CD163 was measured using an ELISA kit (Elabscience, USA). The pro-and anti-inflammatory cytokines (IL-6 and TNF-α; and IL-10, respectively) were quantified using a multiplex assay (Luminex, Bio-Plex Pro™ Human Cytokine plex Assay).

Statistical analysis

Statistical analyses were performed using SPSS 26 (IBM, Armonk, NY, USA). The mean and standard deviation (SD) were calculated for continuous variables and percentage (%) for categorical variables. The normality distribution of the data was tested using Kolmogorov Smirnov. The change was calculated as [the endpoint value - baseline value], and the % change was calculated as [(endpoint value (T2) - baseline value (T1)) / baseline value] * 100%. The *P*-value for the trend was analyzed using a linear trend test. The Manne Whitney U-test was used to compare baseline data between two groups. The Wilcoxon signed-rank test for paired samples was used to compare changes within groups over the time course of the study. Changes in variables within groups over 4 weeks are presented as adjusted *P* values derived from a general linear model after adjusting for the baseline age, sex, waist circumference, and total caloric intake. The significance was considered at *P*-values < 0.05.

Results

Changes of anthropometric, lipid profile, and inflammatory markers of the study population after RIF

As presented in Table 1, subjects had significantly lost an average of 1.6% of their initial body weight (BW) and exhibited significant decreases in the BMI, BFP, FM, FFM, MM, VFSA, WC, HC, and WHR at the end of RIF. Moreover, significant reductions were observed in serum triglycerides (TG), total cholesterol (TC), LDL, while a significant elevation was observed in serum HDL at the end of the IF month for the whole population. Finally, a significant reduction in serum Hp, IL-6, and TNF was recorded at the end of the RIF month in comparison to the pre-fasting levels among the study subjects. The levels of serum CD163 and IL-10 of the study subjects, however, were significantly increased at the end of Ramadan.

Sex differences in the basic demographic, anthropometric, metabolic, and inflammatory indicators for the study subjects (females = 39, males = 75, total = 114) are presented in Supplementary Tables 1a,b,c, with most of the differences falling in the anthropometric measurements, with minimal differences in the metabolic and inflammatory parameters. Supplementary Table 2 shows that no differences were found in the prevalence of obesity classes among the three different haptoglobin phenotypes.

Hp phenotyping

The electrophoretic patterns of the three Hp phenotypes, presented in Table 2, revealed that the Hp2-2was the most abundant phenotype (n = 55, 48.2%) among the study subjects, followed by Hp2-1 (n = 53, 46.5%), and finally Hp1-1 (n = 6, 5.3%). Supplementary Fig. 1 shows the distribution of Hp polymorphism phenotypes among the study subjects using G-vertical polyacrylamide gel electrophoresis.

The mean age was 38.7 ± 11.7 years (Hp1-1: 29.50 ± 9.75 years, Hp2-1: 37.77 ± 11.85 years, and Hp2-2: 37.75 ± 11.91), and the mean BMI at baseline was 30.41 ± 5.09 kg/m^2 (Hp1-1: 33.02 ± 5.06, Hp2-1: 30.06 ± 4.48, and Hp2-2: 30.46 ± 5.63) (Table 2). No significant differences in any of the anthropometric or blood pressure measures among Hp phenotypes, except for BFP (Hp1-1: 35.63 ± 5.83, Hp2-1: 29.91 ± 7.28, Hp2-2: 29.45 ± 6.72%), with the Hp1-1 group having a significantly higher value than Hp2-2 and close to significantly higher than Hp2-1, despite being the youngest among the three groups. Regarding lipid profile, no significant differences were detected between the three phenotypes concerning FBG, TC, and HDL-C. Generally, Hp 2 − 1 had the highest values of FBG, TC, HDL-C, and TG. For instance, the Hp2-1 group reported significantly higher TG levels than Hp2-2, with a significant trend across the three phenotypes (P = 0.008). Yet regarding LDL-C, Hp2-2 reported numerically the highest LDL-C levels, which was nearly significant for subjects with Hp1-1. Concerning inflammatory markers, the Hp2-1 group exhibited numerically the highest values in the four tested parameters, with significantly higher levels of inflammatory markers IL-6 and IL-10 than Hp2-2, and close to significance for serum Hp. A clear significant trend (P = 0.003) was reported among the three phenotypes regarding serum levels of IL-10 (Table 2).

Changes in body weight, composition, and blood pressure after RIF based on Hp phenotype

The changes in anthropometric measurements in response to RIF based on the Hp phenotypes are shown in Table 3. The differences of the means at the baseline and end of Ramadan of all Hp phenotypes were calculated and analyzed to reveal the responses of each Hp phenotype individually to Ramadan and compare them to each other. The significance P-values were adjusted for the baseline age, sex, WC, and total caloric intakes (all adjusted P < 0.05).

Results showed a significant decrease in BW, BMI, BFP, FM, FFM, MM, and WC for all of the three phenotypes (Hp1-1, Hp2-1, and Hp2-2) at the end of the fasting month in comparison to the pre-fasting levels. However, subjects with Hp1-1 showed a greater tendency of decreasing weight as compared to subjects with the other phenotypes, with an average of 2.5% decrease of their initial BW, which was significantly different from the BW change of both Hp 2 − 1 or Hp 2−2 phenotypes. Similarly, Hp1-1 experienced the largest decrease in BMI (-1.98 ± 2.58%), significantly different to Hp2-1 (-1.44 ± 3.10%); meanwhile, the BMI of Hp2-1 BMI was significantly decreased to a lesser extent than of Hp2-2 (-1.63 ± 1.83%). Despite so, Hp1-1 experienced the least decreases in BFP and FM (-0.66 ± 2.57 and − 3.32 ± 3.49% respectively), conversely to Hp2-2 phenotype that experienced the largest decreases in BFP and FM (-3.38 ± 8.51 and − 5.09 ± 8.96% respectively). Yet, only Hp2-1's changes of BFP and FM (-3.22 ± 6.97 and − 4.77 ± 8.33% respectively) were significantly different from Hp1-2. Moreover, Hp1-1 experienced the largest decreases of FFM and MM (-2.66 ± 0.73 and − 2.62 ± 0.81%, respectively), which was significantly different from Hp2-1 (-1.26 ± 7.32 and − 0.25 ± 2.54%, respectively). Contrarily, Hp1-1 solely experienced an increase in VFSA and WHR (+ 9.98 ± 22.74 and + 0.61 ± 6.25%, respectively), while the other two groups had their VFSA and WHR decreased (Hp2-1: -2.21 ± 15.23 and − 1.07 ± 4.66%; Hp2-2: -5.66 ± 16.73 and − 0.73 ± 4.36%, respectively). A significant trend (P = 0.044) was present regarding VFSA change across the three phenotypes, with subjects with phenotype Hp2-2 experiencing a significantly higher reduction in VFSA than those with the Hp2-1 phenotype (-6.26 ± 18.95 vs. -2.27 ± 9.71, respectively). Even so, Hp1-1 had the largest decrease in their WC (-4.23 ± 8.16%), which was significantly different from the WC decrease of Hp2-1 (-2.40 ± 5.11%).

Finally, all Hp phenotypes experienced significant changes in their SBP and DBP values over Ramadan, with decreases in SBP values of Hp2-1 and Hp2-2 phenotypes, and significant decreases in DBP values of Hp1-1 and Hp2-2 phenotypes. However, significant increases were reported in SBP and DBP for Hp1-1 and Hp2-1. No significant differences among the Hp phenotypes' blood pressure values, however, were present.

Changes in blood lipid and glucose profile after RIF based on Hp phenotype

Fasting blood glucose and serum lipid profile parameters were measured in the subjects according to their Hp polymorphism phenotype in response to RIF (Table 4). Serum levels of TC have significantly reduced in all types of Hp polymorphism at the end of RIF month compared to pre-fasting levels, according to the post-adjustment P-values. However, no significant difference was found between the three Hp phenotypes. Hp2-1 experienced the largest TC decrease among the three phenotypes. All three phenotypes experienced significant changes in their TG and LDL-C levels. In contrary to the other phenotypes, Hp1-1

experienced significant increases in both TG and LDL-C, while experiencing the highest numerical increase in HDL-C at the end of the Ramadan fasting month in comparison to the pre-fasting levels. Conversely, FBG and HDL-C serum levels were all significantly increased at the end of Ramadan for all of the three phenotypes, with Hp 2–2's HDL-C increase being significantly different from the other two phenotypes.

Changes in serum Hp, CD163, and inflammatory cytokines after RIF based on Hp phenotype

Inflammatory cytokines, CD163, and serum Hp were measured for all subjects based on their Hp phenotype in response to RIF (Table 5). At the end of RIF, all of the three Hp phenotypes experienced a significant decrease in IL-6 and TNF-α, with Hp1-1's decrease of IL-6 and TNF- being the smallest and significantly different than the other two phenotypes. In contrast, serum IL-10 and CD163 levels were significantly elevated for all Hp phenotypes after RIF, with the least increase in CD163 was reported for the Hp1-1 phenotype group. Serum Hp was significantly lowered for Hp2-1 and Hp2-2, while significantly increased for Hp1-1, with a clear trend for differences between the three phenotypes. The change of the Hp2-2 phenotype in serum Hp was significantly different from the other two phenotypes, with the highest reduction was reported Hp2-2 (Table 5).

Discussion

The religious form of IF (i.e.: Ramadan model) was shown to mitigate low-grade systemic inflammation, reduce pro-inflammatory cytokines and OS markers, and confer a short-term transient cardiometabolic protection [11, 15, 33].

While more than one study examined the effect of observing IF regimens on genetic expression (e.g., *FTO, Nrf2, TFAM, SOD2, SIRT1, SIRT3*, and *CLOCK*)[34–39], few studies have examined the impact of genetic variations on the metabolic response to IF regimen among overweight and obese subjects. In these, the IF was associated with higher weight reduction in comparison to low-calorie diet among overweight/obese people with GG genotype of the UCP2 gene, while no differences in weight loss were found between the two regimes among people with AA + GA genotype [40]. To our knowledge, this is the first study investigating the impact of Hp phenotypes (Hp1-1, Hp2-1, and Hp2-2) in obesogenic (including bodyweight reduction), metabolic and inflammatory markers in response to IF regimen. Our results demonstrated that the Hp phenotype might independently influence the outcomes of IF on abdominal obesity, lipid profile, inflammatory cytokines, serum Hp, and CD163 in overweight and obese individuals. Our results confirmed the momentous role of IF in reducing low-grade systemic inflammation and enhancing anti-inflammatory mechanisms, thereby mitigating health deteriorations accompanying people with obesity.

Impact of RIF on serum Hp, CD163, and other measured parameters

The effect of IF and caloric restriction on body weight change has been the subject of several studies; numerous reviews and original research have been published in attempts to elaborate on this perpetuate effect [3, 6, 41, 42]. Given that fasting during Ramadan represents a form of IF and TRF [43] that is globally observed by at least 1.5 billion Muslim people each year, the impact of RIF on body weight loss and the associated metabolic and inflammatory effects needs further elaboration.

The present study suggests that RIF is associated with reduced body weight, body fat percentage, and visceral fat area, with improvements in several cardiometabolic risk factors. These findings concerning body weight and composition are consistent with those of a previous study that reported significant reductions in body weight, fat mass, BMI, visceral fat area, and fat-free mass in healthy adults following Ramadan fasting during the summer [44, 45].

Additionally, IL-6 and TNF-α levels had significantly decreased, whereas IL-10 levels had significantly increased at the end of the Ramadan month. These significant reductions in TNF-α and IL-6 after observing RIF were consistent with significant reductions reported in a similar previous study on the Ramadan model of IF [13]. Furthermore and as discussed above, the secretion of pro-inflammatory cytokines IL-6, TNF-α, and anti-inflammatory cytokine IL-10 may be regulated by the Hp phenotype [46]. Besides, pro-and anti-inflammatory cytokines play crucial roles in the upregulation and downregulation of CD163 expression, respectively [47]. Hp expression depends on the Hp phenotype and the levels of the pro-inflammatory cytokines IL-6 and TNF-α, both of which have a crucial role in Hp concentration [48]. Therefore, Hp phenotype and Hp-Hb complex have leading roles in the regulation of serum Hp and CD163 levels. Several studies have shown that the pro-inflammation characteristics of serum Hp [30, 49], and the anti-inflammation activity of CD163 [50] altered in response to different Hp polymorphisms. These findings were consistent with our previous study that showed RIF had an ameliorating effect on pro-inflammatory cytokines and in enhancing the anti-inflammatory response [12, 45, 51]. The present results confirmed a significant increase in the anti-inflammatory serum CD163 and a significant decrease in the pro-inflammatory serum Hp following RIF when compared with pre-fasting levels. There may be, however, other unexamined factors that have an impact on these variables during the Ramadan month, such as changes in circadian rhythm that may influence IL-6 production, as reported in non-fasting research [52].

It is well established that the increased ratios of anti-inflammatory (IL-10) to pro-inflammatory (TNF-α and IL-6) cytokines represent a protective factor against atherogenesis [53]. In one study [54], the ratios of IL-10 to TNF-α and IL-10 to IL-6 showed preferred increments at the end of the fasting month. Their reported noticeable reduction in the IL-6:IL-10 ratio at the end of Ramadan suggests that RIF had a favorable protective effect against systemic inflammation and subsequent metabolic derangements [54].

Therefore, the lower level of IL-6 at the end of RIF in the present study could be explained by the decreased level of physical activity reported by most subjects during the month of Ramadan and in the literature [55]. A recent study showed that RIF is associated with reduced activity and sleeping time without changes to the resting metabolic rate or total energy expenditure [56]. This is consistent with the 0.5% reduction in muscle mass reported at the end of Ramadan.

HDL level was significantly increased at the end of RIF in this study. This finding was consistent with a previous study involving 81 fasting subjects, in which HDL decreased significantly at the end of RIF [57]. This result was inconsistent with the findings of the majority of previous studies on RIF, where HDL

increased or remained unchanged during the Ramadan month [58]. A systematic review and meta-analysis of 57 studies investigating RIF among healthy subjects (N = 2771) showed a significant, but small, pooled reduction in HDL at the end of Ramadan fasting [33].

This discrepancy in the effect of RIF on lipid profile and HDL may be attributable to differences in cultural foods and dietary practices among different populations, especially in the types and amounts of dietary fats consumed. Further, the genetic makeup of study subjects affects the way their bodies respond to various changes during the Ramadan month, which in turn affects their lipid profile at the end of the fasting month.

It has been proposed that the fasting state (i.e.: Ramadan IF) induces an elevation in free fatty acids and ketone bodies, such as beta-hydroxybutyrate, which in turn may impose damage on the metabolically active mitochondria in the neurons of the fasting organism. This damage, however, can be corrected by several mechanisms, including upregulated antioxidant defense genes and enhanced mtDNA repair [59].

The interplay between Hp polymorphism and IF in relation to CD163 and other measured parameters

The present study found that obese individuals with the Hp1-1 phenotype experienced a higher decrease in BW and BMI after RIF than those with the Hp2-1 or Hp2-2 phenotypes, with the differences in the change of BW being significantly different among the three Hp phenotypes. Overall, each Hp polymorphism underwent a significant decrease in BW and BMI due to RIF. Although expected, this result was interesting as there were significant changes in body weight and BMI in response to RIF in general. In addition, these results support the hypothesis of a previous study that suggested that the phenotype of Hp has a crucial role in modulating the oxidative-antioxidative status in both obesity and diabetes [60]. Furthermore, our results showed a significant reduction in body fat percentage, fat mass, fat-free mass, and muscle mass for all of the three Hp phenotypes. Phenotype Hp1-1 had a tendency also to lose the greatest muscle/fat-free mass in comparison to the other two phenotypes, with the difference being significantly different, particularly against the Hp2-1 phenotype.

Previous studies showed that BMI is an indicator of total body fat, whereas waist circumference reflected visceral fat. Visceral fat deposition is known to have more metabolically adverse effects than subcutaneous fat [61]. Therefore, waist circumference measurement is a better indicator of metabolic and inflammatory disorders related to obesity. Both waist circumference and BMI showed the strongest positive correlations with Hp among the various obesity measures [62, 63]. In a recent study, waist circumference and visceral fat area were found to significantly decrease in Hp2-1 after RIF compared with before RIF, whereas Hp1-1 and Hp2-1 showed non-significant reductions.

Interestingly, the WC reduction experienced by obese individuals withHp1-1 phenotype following RIF compared was significantly different from the ones of Hp2-1 and Hp2-2. Similarly, a recently published paper that investigated hypo-caloric dietary programs among obese women showed these dietary programs had a stronger positive influence on abdominal obesity (waist circumference, total body fat, and fat mass) of the Hp1-1 phenotype compared with the Hp2-1 and Hp2-2 phenotypes [64]. That study supported our finding that individuals with an Hp1-1 phenotype had higher expression of anti-inflammatory cytokines and were more resistant to OS than Hp2-1 and Hp2-2 individuals. Incidentally, Hp2-1 and Hp2-2 individuals have been reported to have a higher incidence of risk factors for obesity, diabetes, and cardiovascular diseases [27].

Due to its association with pro-inflammatory activity, higher levels of Hp have been associated with an increased risk for obesity, type 2 diabetes, and cardiovascular diseases [65–67]. Many studies have suggested that Hp polymorphism not only affects serum Hp but also influences lipid profile levels. In other words, there is a positive correlation between serum Hp and TC, non-HDL-C, HDL-C, and TG concentrations in obese individuals [68]. Our results are consistent with this finding. Moreover, several studies [67, 69], including the present study, demonstrated a higher concentration and positive association of LDL-C in obese Hp2-2 individuals compared with obese Hp1-1 individuals. Furthermore, the present study showed a significant decrease in TG and LDL in both Hp2-1 and Hp2-2 subjects after RIF. Additionally, HDL-C levels were significantly higher following RIF in all Hp polymorphisms. Conversely, TC was significantly reduced in all of the Hp phenotypes. The current results suggest that Hp2-2 individuals with obesity have more anti-inflammatory characteristics and will have a greater response to RIF and obtain optimum benefits from IF than those with Hp2-1 and Hp2-2 phenotypes.

The present study investigated the role of the environmental factors in modulating anti-inflammatory markers (IL-10) and pro-inflammatory cytokines (IL-6 and TNF-α) in response to IF in people with different Hp polymorphism phenotypes. It also explored the expression of an essential anti-inflammatory factor, CD163, in both RIF and Hp polymorphism. With the observed interesting effect of RIF on significantly reducing levels of IL-6 and TNF-a in all Hp phenotypes, this confirms previous findings demonstrating the positive impact of the Ramadan model of IF on the health of obese individuals [70]. In contrast, IL-10 showed a significant increase in all of the Hp phenotypes after observing RIF.

As previously noted, serum Hp and CD163 levels are associated with and regulated by the pro-and anti-inflammatory cytokines (IL-6, TNF-α, and IL-10) alongside Hp polymorphism [50]. There was a significant increase in serum of the anti-inflammatory CD163 and a considerable decrease of pro-inflammatory serum Hp in response to RIF. Additionally, serum CD163 levels were significantly increased in all of the Hp phenotypes after RIF, particularly Hp2-2 whose increase was significantly different from the Hp2-1 and Hp2-1 groups. Moreover, the serum CD163 mean difference values between Hp phenotypes revealed a trend in increment through Hp1-1, Hp2-1, and Hp2-2 respectively, but the changes were not significant between Hp1-1 and Hp2-2. In contrast, serum Hp showed significantly lower expression in the Hp2-1 and Hp2-2 phenotypes, yet an increase was experienced by the Hp1-1 phenotype after RIF.

Understandably, the Hp2-2 phenotype is mainly associated with increased risks for obesity, diabetes, and cardiovascular diseases, in addition to enhancing pro-inflammatory metabolites. RIF also has a positive impact on modulating and strengthening inflammatory cytokines. Interestingly, our findings revealed that Hp2-2 had a better response in moderating and enhancing anti-inflammatory markers than Hp1-1, which highlighted the influence of RIF on health outcomes for people with obesity, suggesting metabolites may offer a pathway to a healthier positive response.

Our results confirmed the decisive role of IF in reducing low-grade systemic inflammation and OS and enhancing anti-inflammatory mechanisms, thereby improving the health conditions of individuals with obesity. Moreover, this provided an example of the epigenetic factors of the different Hp phenotypes in

response to RIF and confirmed that Hp1-1 individuals had a higher anti-inflammatory response than Hp2-1 and Hp2-2 individuals. Because of the positive impact of IF on the health of individuals with obesity in developing and enhancing adipocytokine pathways, Hp2-2 expressed a higher anti-inflammatory response to RIF compared with Hp1-1. Therefore, we propose that IF contributes to health benefits for individuals with obesity in moderating and refining their oxidative and inflammatory mechanisms.

In conclusion, four consecutive weeks of dawn-to-sunset IF was found to significantly and variably affect the anthropometric, metabolic, and inflammatory markers in relation to Hp polymorphisms. Our results confirmed the decisive role of IF in reducing low-grade systemic inflammation and OS and enhancing anti-inflammatory mechanisms, thereby improving the health conditions of individuals with obesity. Moreover, this provides an example of the epigenetic factors of the different Hp phenotypes in response to IF and confirmed that Hp1-1 individuals had a higher anti-inflammatory response than Hp2-1 and Hp2-2 individuals.

Declarations

Clinical Trial Registration number: ISRCTN18205186; https://trialsearch.who.int/?TrialID=ISRCTN18205186

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Tables

Table 1
Changes in anthropometric, metabolic, and inflammatory markers between pre-fasting and the end of Ramadan fasting month.

Variable	Before Ramadan (T1)	End of Ramadan (T2)	(P-value)
Anthropometric			
Weight (kg)	88.99 ± 15.51	87.57 ± 15.08	**
Body mass index (BMI, kg/m^2)	30.4 ± 5.09	29.91 ± 4.99	**
Fat mass (FM, kg)	26.9 ± 9.46	25.69 ± 9.25	**
Body fat percent (BFP, %)	29.98 ± 7.02	29.09 ± 7.18	**
Fat-free mass (FFM, kg)	61.41 ± 10.52	60.75 ± 10.73	*
Muscle mass (MM, kg)	58.34 ± 10.02	57.97 ± 9.78	**
Viseral fat surface area (VFSA, cm^2)	97.85 ± 48.0	94.3 ± 46.22	**
Waist circumference (WC, cm)	96.84 ± 13.48	94.6 ± 12.41	**
Hip circumference (HC, cm)	108.0 ± 10.14	106.6 ± 9.78	**
Waist to hip ratio (WHR)	0.895 ± 0.081	0.886 ± 0.074	*
Systolic blood pressure (SBP, mmHg)	123.4 ± 11.65	122.3 ± 11.67	NS
Diastolic blood pressure (DBP, mmHg)	71.73 ± 9.57	71.32 ± 9.93	NS
Glucoregulatory markers and lipids profile			
Fasting blood glucose (FBG, mg/dl)	97.97 ± 20.93	101.85 ± 19.97	NS
Total cholesterol (TC, mg/dl)	180.64 ± 37.63	175.0 ± 33.5	*
Triglycerides (TG, mg/dl)	102.39 ± 60.42	87.15 ± 38.7	**
Low-density lipoprotein cholesterol (LDL-C, mg/dl)	115.1 ± 31.7	107.9 ± 32.2	**
High-density lipoprotein cholesterol (HDL-C, mg/dl)	45.1 ± 7.73	49.73 ± 12.9	***
Haptoglobin and inflammatory markers			
Serum haptoglobin (Hp, mg/dl)	149.9 ± 46.08	127.74 ± 46.0	**
Serum CD163 protein (µg/ml)	177.54 ± 127.5	225.49 ± 103.7	**
Interleukin-6 (IL-6, pg/ml)	49.36 ± 30.70	24.86 ± 30.62	**
Tumor necrosis factor-α (TNF-α, pg/ml)	25.53 ± 18.32	17.05 ± 10.3	**
Interleukin − 10 (IL-10, pg/ml)	17.14 ± 2.31	18.55 ± 2.2	**

Table 2
Baseline sociodemographic, anthropometric, biochemical, and inflammatory characteristics of subjects with overweight/obesity stratified by haptoglobin phenotypes (n = 114) before the commencement of Ramadan intermittent fasting.

Variable		Haptoglobin phenotype (Hp)			*P* for trend [a]	*P*-value (1–1 vs. 2 – 1)[b]	*P*-value (1–1 vs. 2–2)[b]	*P*-value (2 – 1 vs. 2–2)[b]
		Hp1-1 (n = 6)	Hp2-1 (n = 53)	Hp2-2 (n = 55)				
Sociodemographic data								
Age (years)		29.50 ± 9.75	37.77 ± 11.85	37.75 ± 11.91	0.253	0.106	0.107	0.990
Sex	Males, n (%)	2 (1.8%)	38 (33.3%)	35 (30.7%)				
	Females, n (%)	4 (3.5%)	15 (13.2%)	20 (17.5%)				
Anthropometric measurements								
Body weight (BW, kg)		94.60 ± 16.86	88.91 ± 14.03	88.46 ± 16.86	0.658	0.399	0.362	0.883
Body mass index (BMI, kg/m^2)		33.02 ± 5.06	30.06 ± 4.48	30.46 ± 5.63	0.404	0.181	0.245	0.686
Body fat percent (BFP, %)		35.63 ± 5.83	29.91 ± 7.28	29.45 ± 6.72	0.122	0.058	**0.041**	0.734
Fat mass (FM, kg)		32.97 ± 5.83	26.91 ± 9.18	26.33 ± 9.71	0.266	0.139	0.104	0.747
Fat-free mass (FFM, kg)		59.67 ± 14.92	61.96 ± 9.53	61.33 ± 11.10	0.904	0.659	0.715	0.863
Muscle mass (MM, kg)		56.67 ± 14.24	58.61 ± 9.09	58.28 ± 10.57	0.903	0.657	0.711	0.867
Viseral fat surface area (VFSA, cm^2)		85.00 ± 40.37	100.00 ± 41.32	97.19 ± 54.83	0.764	0.473	0.558	0.764
Waist circumference (WC, cm)		100.75 ± 16.77	97.59 ± 12.39	95.71 ± 14.25	0.595	0.589	0.389	0.473
Hip circumferwance (HC, cm)		113.75 ± 11.06	108.04 ± 9.41	107.46 ± 10.72	0.356	0.194	0.152	0.766
Waist to hip ratio (WHR)		0.88 ± 0.08	0.90 ± 0.08	0.89 ± 0.09	0.433	0.268	0.199	0.692
Systolic blood pressure (SBP, mmHg)		127.00 ± 8.29	124.60 ± 10.72	121.91 ± 12.73	0.366	0.633	0.312	0.234
Diastolic blood pressure (DBP,mmHg)		76.83 ± 8.98	72.13 ± 9.31	70.79 ± 9.85	0.315	0.256	0.144	0.467
Glucose and lipid profile								
Fasting blood glucose (FBG, mg/dl)		85.83 ± 9.28	99.54 ± 23.23	97.79 ± 19.26	0.316	0.131	0.186	0.664
Total cholesterol (TC, mg/dl)		162.63 ± 19.97	183.75 ± 37.72	179.62 ± 38.87	0.415	0.196	0.297	0.570
High-density lipoprotein cholesterol (HDL-C, mg/dl)		42.62 ± 8.29	45.80 ± 8.13	44.73 ± 7.36	0.558	0.344	0.529	0.473
Triglycerides (TG, mg/dl)		85.08 ± 32.18	121.11 ± 73.18	86.25 ± 41.79	**0.008**	0.155	0.963	**0.002**
Low-density lipoprotein cholesterol (LDL-C, mg/dl)		77.73 ± 44.28	103.31 ± 36.85	108.38 ± 35.60	0.149	0.108	0.054	0.474
Pro-Inflammatory, anti-inflammatory markers								
Interleukin-6 (IL-6, pg/ml)		21.08 ± 8.99	64.63 ± 90.39	37.74 ± 46.15	0.085	0.150	0.580	**0.048**
Tumor necrosis factor-α (TNF-α, pg/ml)		23.34 ± 8.27	28.53 ± 19.54	22.89 ± 17.66	0.269	0.511	0.955	0.112
Interleukin − 10 (IL-10, pg/ml)		16.88 ± 1.87	17.92 ± 2.40	16.42 ± 2.08	**0.003**	0.283	0.630	**0.001**

Continuous data are presented as mean ± SD, categorical data are presented as frequencies (percentages). Bold denotes to statistically significant difference at $P < 0.05$.

[a] *P*-value for trend was analyzed by a linear trend test.

[b] *P*-value: the difference between haptoglobin phenotypes was analyzed by the Manne-Whitney U-test.

Variable	Haptoglobin phenotype (Hp)			*P* for trend [a]	*P*-value (1–1 vs. 2 – 1)[b]	*P*-value (1–1 vs. 2–2)[b]	*P*-value (2 – 1 vs. 2–2)[b]
	Hp1-1 (n = 6)	Hp2-1 (n = 53)	Hp2-2 (n = 55)				
CD163 protein (μg/ml)	182.8 ± 52.1	194.1 ± 140.3	166.6 ± 120.5	0.629	0.891	0.770	0.338
Serum haptoglobin (Hp) (mg/dl)	155.47 ± 29.52	158.48 ± 46.75	141.10 ± 45.87	0.140	0.879	0.466	0.050

Continuous data are presented as mean ± SD, categorical data are presented as frequencies (percentages). Bold denotes to statistically significant difference at $P < 0.05$.

[a] *P*-value for trend was analyzed by a linear trend test.

[b] *P*-value: the difference between haptoglobin phenotypes was analyzed by the Manne-Whitney U-test.

Table 3
Changes in blood pressure and anthropometric measurements among subjects with overweight/obesity stratified by haptoglobin phenotypes before and after Ramadan intermittent fasting (n = 114).

Variable	Haptoglobin phenotype (Hp)			*P* for trend [c]	*P*-value [a]	*P*-value [a]	*P*-value [a]
	Hp1-1 (n = 6)	Hp2-1 (n = 53)	Hp2-2 (n = 55)		(1–1 vs. 2 – 1) adjusted	(1-1 vs. 2–2,) adjusted	(2 – 1 vs. 2–2) adjusted
Body weight (BW, kg)							
Baseline (pre)	94.60 ± 16.86	88.91 ± 14.03	88.46 ± 16.86	0.658			
Week 4 (post)	92.15 ± 15.94	87.70 ± 13.56	86.96 ± 16.52	0.727			
P-value [b]	**0.028**	**< 0.001**	**< 0.001**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-2.45 ± 1.69	-1.21 ± 1.68	-1.50 ± 1.64	0.200	**0.016**	**0.001**	**0.001**
Change % [d]	-2.49 ± 1.63	-1.29 ± 1.80	-1.65 ± 1.79	0.239	**0.013**	**0.001**	**0.001**
Body mass index (BMI, kg/m^2)							
Baseline (pre)	33.02 ± 5.06	30.06 ± 4.48	30.46 ± 5.63	0.404			
Week 4 (post)	32.37 ± 5.12	29.59 ± 4.25	29.97 ± 5.63	0.436			
P-value [b]	0.093	**< 0.001**	**< 0.001**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-0.65 ± 0.90	-0.47 ± 1.11	-0.49 ± 0.54	0.893	0.139	**0.003**	**0.001**
Change % [d]	-1.98 ± 2.58	-1.44 ± 3.10	-1.63 ± 1.83	0.847	0.118	**0.001**	**0.001**
Body fat percent (BFP, %)							
Baseline (pre)	35.63 ± 5.83	29.91 ± 7.28	29.45 ± 6.72	0.122			
Week 4 (post)	35.48 ± 6.46	28.87 ± 6.97	28.62 ± 7.25	0.080			
P-value [b]	0.833	**< 0.001**	**0.001**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-0.15 ± 0.85	-1.04 ± 2.60	-0.83 ± 1.86	0.621	0.682	**0.005**	**0.002**
Change % [d]	-0.66 ± 2.57	-3.22 ± 6.97	-3.38 ± 8.51	0.709	0.556	**0.001**	**0.005**
Fat mass (FM, kg)							
Baseline (pre)	32.97 ± 5.83	26.91 ± 9.18	26.33 ± 9.71	0.266			
Week 4 (post)	32.00 ± 9.42	25.50 ± 8.51	25.19 ± 9.82	0.227			
P-value [b]	0.058	**< 0.001**	**< 0.001**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-0.97 ± 1.09	-1.42 ± 3.25	-1.14 ± 1.75	0.817	0.083	**0.003**	**0.001**

Continuous data are presented as the mean ± SD; categorical data are presented as frequencies (percentages). Bold denotes to statistically significant difference at $P < 0.05$.

[a] The *P*-value was analyzed by a general linear model after adjusting for baseline age, sex, waist circumference, and total caloric intake.

[b] *P*-value: The difference between baseline and 4 weeks was analyzed by Wilcoxon signed-rank test.

[c] *P*-value for trend was analyzed by a linear trend test.

[d] Change (%) was calculated as [(end point-baseline value)/baseline value] * 100.

Variable	Haptoglobin phenotype (Hp)			*P* for trend [c]	*P*-value [a] (1–1 vs. 2 – 1) adjusted	*P*-value [a] (1-1 vs. 2–2,) adjusted	*P*-value [a] (2 – 1 vs. 2–2) adjusted
	Hp1-1 (n = 6)	Hp2-1 (n = 53)	Hp2-2 (n = 55)				
Change % [d]	-3.32 ± 3.49	-4.77 ± 8.33	-5.09 ± 8.96	0.885	0.067	**0.001**	**0.001**
Fat-free mass (FFM, kg)							
Baseline (pre)	59.67 ± 14.92	61.96 ± 9.53	61.33 ± 11.10	0.904			
Week 4 (post)	58.02 ± 14.26	60.92 ± 10.37	60.89 ± 10.86	0.817			
P-value [b]	**0.028**	0.127	**0.017**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-1.65 ± 0.74	-0.77 ± 4.10	-0.45 ± 1.58	0.614	**0.003**	0.179	**0.040**
Change % [d]	-2.66 ± 0.73	-1.26 ± 7.32	-0.67 ± 2.52	0.633	**0.001**	0.214	**0.054**
Muscle mass (MM, kg)							
Baseline (pre)	56.67 ± 14.24	58.61 ± 9.09	58.28 ± 10.57	0.903			
Week 4 (post)	55.12 ± 13.57	58.42 ± 8.84	57.86 ± 10.34	0.734			
P-value [b]	**0.028**	0.263	**0.029**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-1.55 ± 0.74	-0.19 ± 1.43	-0.42 ± 1.52	0.094	**0.004**	0.334	0.044
Change % [d]	-2.62 ± 0.81	-0.25 ± 2.54	-0.66 ± 2.55	0.086	**0.001**	0.482	0.060
Visceral fat surface area (VFCA, cm^2)							
Baseline (pre)	85.00 ± 40.37	100.00 ± 41.32	97.19 ± 54.83	0.764			
Week 4 (post)	95.00 ± 51.28	97.73 ± 40.22	90.93 ± 51.46	0.750			
P-value [b]	0.414	**0.009**	**< 0.001**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	10.00 ± 25.30	-2.27 ± 9.71	-6.26 ± 18.95	**0.044**	0.377	0.095	**0.018**
Change % [d]	9.98 ± 22.74	-2.21 ± 15.23	-5.66 ± 16.73	0.074	0.331	0.296	**0.015**
Waist circumference (WC, cm)							
Baseline (pre)	100.75 ± 16.77	97.59 ± 12.39	95.71 ± 14.25	0.595			
Week 4 (post)	95.91 ± 14.31	95.03 ± 11.26	94.04 ± 13.44	0.888			
P-value [b]	0.207	**< 0.001**	**0.039**				

Continuous data are presented as the mean ± SD; categorical data are presented as frequencies (percentages). Bold denotes to statistically significant difference at $P < 0.05$.

[a] The *P*-value was analyzed by a general linear model after adjusting for baseline age, sex, waist circumference, and total caloric intake.

[b] *P*-value: The difference between baseline and 4 weeks was analyzed by Wilcoxon signed-rank test.

[c] *P*-value for trend was analyzed by a linear trend test.

[d] Change (%) was calculated as [(end point-baseline value)/baseline value] * 100.

Variable	Haptoglobin phenotype (Hp)			*P* for trend [c]	*P*-value [a] (1–1 vs. 2 – 1) adjusted	*P*-value [a] (1-1 vs. 2–2,) adjusted	*P*-value [a] (2 – 1 vs. 2–2) adjusted
	Hp1-1 (n = 6)	Hp2-1 (n = 53)	Hp2-2 (n = 55)				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-4.84 ± 9.10	-2.55 ± 5.50	-1.67 ± 6.89	0.462	0.250	**0.001**	0.078
Change % [d]	-4.23 ± 8.16	-2.40 ± 5.11	-1.45 ± 6.35	0.459	0.259	**0.001**	0.095
Hip circumference (HC, cm)							
Baseline (pre)	113.75 ± 11.06	108.04 ± 9.41	107.46 ± 10.72	0.356			
Week 4 (post)	108.03 ± 9.89	106.48 ± 8.27	106.60 ± 11.18	0.935			
P-value [b]	0.077	**0.005**	0.117				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-5.72 ± 6.30	-1.56 ± 3.90	-0.86 ± 4.00	**0.024**	**0.020**	**0.007**	0.374
Change % [d]	− 4.85 ± 5.17	-1.32 ± 3.44	-0.79 ± 3.53	**0.033**	**0.024**	**0.009**	0.441
Waist to hip ratio (WHR)							
Baseline (pre)	0.88 ± 0.08	0.90 ± 0.08	0.89 ± 0.09	0.433			
Week 4 (post)	0.88 ± 0.07	0.89 ± 0.07	0.88 ± 0.08	0.624			
P-value [b]	0.144	**0.022**	0.939				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	0.004 ± 0.06	-0.01 ± 0.04	-0.01 ± 0.04	0.777	0.887	0.219	0.606
Change % [d]	0.61 ± 6.25	-1.07 ± 4.66	-0.73 ± 4.36	0.779	0.961	0.265	0.746
Systolic blood pressure (SBP, mmHg)							
Baseline (pre)	127.00 ± 8.29	124.60 ± 10.72	121.91 ± 12.73	0.366			
Week 4 (post)	127.17 ± 14.85	123.40 ± 10.16	120.77 ± 12.61	0.295			
P-value [b]	0.753	0.532	0.524				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	0.17 ± 9.99	-1.20 ± 9.30	-1.15 ± 11.09	0.952	0.969	0.351	0.447
Change % [d]	0.01 ± 7.59	-0.66 ± 7.27	-0.52 ± 8.96	0.981	0.999	0.509	0.670
Diastolic blood pressure (DBP, mmHg)							
Baseline (pre)	76.83 ± 8.98	72.13 ± 9.31	70.79 ± 9.85	0.315			
Week 4 (post)	76.17 ± 11.79	72.72 ± 9.60	69.46 ± 9.84	0.111			

Continuous data are presented as the mean ± SD; categorical data are presented as frequencies (percentages). Bold denotes to statistically significant difference at $P < 0.05$.

[a] The *P*-value was analyzed by a general linear model after adjusting for baseline age, sex, waist circumference, and total caloric intake.

[b] *P*-value: The difference between baseline and 4 weeks was analyzed by Wilcoxon signed-rank test.

[c] *P*-value for trend was analyzed by a linear trend test.

[d] Change (%) was calculated as [(end point-baseline value)/baseline value] * 100.

Variable	Haptoglobin phenotype (Hp)			*P* for trend [c]	*P*-value [a] (1–1 vs. 2 – 1) adjusted	*P*-value [a] (1-1 vs. 2–2,) adjusted	*P*-value [a] (2 – 1 vs. 2–2) adjusted
	Hp1-1 (n = 6)	Hp2-1 (n = 53)	Hp2-2 (n = 55)				
P-value [b]	0.750	0.598	0.397				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-0.67 ± 7.23	0.58 ± 9.19	-1.33 ± 8.67	0.534	0.830	0.645	0.261
Change % [d]	-0.94 ± 9.70	1.58 ± 12.96	-1.15 ± 12.00	0.508	0.822	0.379	0.479

Continuous data are presented as the mean ± SD; categorical data are presented as frequencies (percentages). Bold denotes to statistically significant difference at $P < 0.05$.

[a] The *P*-value was analyzed by a general linear model after adjusting for baseline age, sex, waist circumference, and total caloric intake.

[b] *P*-value: The difference between baseline and 4 weeks was analyzed by Wilcoxon signed-rank test.

[c] *P*-value for trend was analyzed by a linear trend test.

[d] Change (%) was calculated as [(end point-baseline value)/baseline value] * 100.

Table 4
Changes in blood glucose and lipid profile among subjects with overweight/obesity stratified by haptoglobin phenotypes before and after Ramadan intermittent fasting (n = 114).

Variable	Haptoglobin phenotype (Hp)			*P* for trend [c]	*P*-value [a]	*P*-value [a]	*P*-value [a]
	Hp1-1 (n = 6)	Hp2-1 (n = 53)	Hp2-2 (n = 55)		(1–1 vs. 2 – 1) adjusted	(1–1 vs. 2–2) adjusted	(2 – 1 vs. 2–2) adjusted
Fasting blood glucose (FBG, mg/dl)							
Baseline (pre)	85.83 ± 9.28	99.54 ± 23.23	97.79 ± 19.26	0.316			
Week 4 (post)	99.98 ± 16.51	100.24 ± 20.83	103.62 ± 19.64	0.665			
P-value [b]	0.116	0.595	**0.008**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	14.15 ± 17.84	0.70 ± 27.46	5.83 ± 23.74	0.345	0.110	0.854	0.074
Change % [d]	17.39 ± 23.25	4.01 ± 26.01	8.29 ± 21.44	0.347	0.126	0.267	**0.006**
Total cholesterol (TC, mg/dl)							
Baseline (pre)	162.63 ± 19.97	183.75 ± 37.72	179.62 ± 38.87	0.415			
Week 4 (post)	158.55 ± 22.33	176.47 ± 34.37	175.44 ± 33.81	0.464			
P-value [b]	0.600	0.106	0.602				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-4.08 ± 22.74	-7.28 ± 28.30	-4.18 ± 30.80	0.853	0.678	0.067	0.318
Change % [d]	-1.90 ± 14.64	-2.13 ± 17.00	-0.64 ± 15.92	0.892	0.764	0.365	0.766
High-density lipoprotein cholesterol (HDL-C, mg/dl)							
Baseline (pre)	42.62 ± 8.29	45.80 ± 8.13	44.73 ± 7.36	0.558			
Week 4 (post)	50.53 ± 12.94	49.85 ± 13.45	49.51 ± 12.76	0.979			
P-value [b]	0.141	0.143	**0.043**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	7.92 ± 10.95	4.05 ± 14.11	4.78 ± 14.14	0.808	0.137	**0.041**	**0.015**
Change % [d]	19.33 ± 25.26	11.04 ± 31.91	13.26 ± 32.97	0.815	0.120	**0.015**	**0.004**
Triglycerides (TG, mg/dl)							
Baseline (pre)	85.08 ± 32.18	121.11 ± 73.18	86.25 ± 41.79	**0.008**			
Week 4 (post)	95.33 ± 28.95	97.24 ± 46.74	76.52 ± 27.22	**0.017**			
P-value [b]	0.345	**0.018**	0.080				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				

Continuous data are presented as the mean ± SD; categorical data are presented as frequencies (percentages). Bold denotes to statistically significant difference at $P < 0.05$.

[a] The *P*-value was analyzed by a general linear model after adjusting for baseline age, sex, waist circumference, and total caloric intake.

[b] *P*-value: The difference between baseline and 4 weeks was analyzed by Wilcoxon signed-rank test.

[c] *P-value* for trend was analyzed by a linear trend test.

[d] Change (%) was calculated as [(end point-baseline value)/baseline value] * 100.

Variable	Haptoglobin phenotype (Hp)			*P* for trend [c]	*P*-value [a] (1–1 vs. 2 – 1) adjusted	*P*-value [a] (1–1 vs. 2–2) adjusted	*P*-value [a] (2 – 1 vs. 2–2) adjusted
	Hp1-1 (n = 6)	Hp2-1 (n = 53)	Hp2-2 (n = 55)				
Change [d]	10.25 ± 22.65	-23.87 ± 57.65	-9.73 ± 32.26	0.107	0.318	**0.004**	**0.029**
Change % [d]	12.04 ± 41.71	-19.70 ± 32.60	-0.72 ± 37.53	0.108	0.247	0.062	0.887
Low-density lipoprotein cholesterol (LDL-C, mg/dl)							
Baseline (pre)	77.73 ± 44.28	103.31 ± 36.85	108.38 ± 35.60	0.149			
Week 4 (post)	78.62 ± 34.37	96.95 ± 39.44	103.20 ± 40.94	0.319			
P-value [b]	0.753	0.117	0.163				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	0.88 ± 32.24	-6.36 ± 31.70	-5.18 ± 24.97	0.839	0.949	0.150	0.130
Change % [d]	18.61 ± 63.85	-1.47 ± 54.05	-4.58 ± 25.76	0.416	0.507	0.844	0.193

Continuous data are presented as the mean ± SD; categorical data are presented as frequencies (percentages). Bold denotes to statistically significant difference at $P < 0.05$.

[a] The *P*-value was analyzed by a general linear model after adjusting for baseline age, sex, waist circumference, and total caloric intake.

[b] *P*-value: The difference between baseline and 4 weeks was analyzed by Wilcoxon signed-rank test.

[c] *P-value* for trend was analyzed by a linear trend test.

[d] Change (%) was calculated as [(end point-baseline value)/baseline value] * 100.

Table 5
Changes in inflammatory markers among subjects with overweight/obesity stratified by haptoglobin phenotypes before and after Ramadan intermittent fasting (n = 114).

Variable	Haptoglobin phenotype (Hp)			*P* for trend [c]	*P*-value [a]	*P*-value [a]	*P*-value [a]
	Hp1-1 (n = 6)	Hp2-1 (n = 53)	Hp2-2 (n = 55)		(1–1 vs. 2 – 1) adjusted	(1–1 vs. 2–2,) adjusted	(2 – 1 vs. 2–2) adjusted
Interleukin-6 (IL-6, pg/ml)							
Baseline (pre)	21.08 ± 8.99	64.63 ± 90.39	37.74 ± 46.15	0.085			
Week 4 (post)	18.09 ± 7.99	22.07 ± 21.55	28.30 ± 38.53	0.494			
P-value [b]	0.173	**< 0.001**	**0.010**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-2.99 ± 5.46	-42.57 ± 94.29	-9.44 ± 63.95	0.076	0.237	**0.002**	0.278
Change % [d]	-5.99 ± 30.25	-26.56 ± 348.56	– 138.90 ± 521.38	0.363	0.648	0.581	**0.053**
Tumor necrosis factor- α (TNF-α, pg/ml)							
Baseline (pre)	23.34 ± 8.27	28.53 ± 19.54	22.89 ± 17.66	0.269			
Week 4 (post)	18.60 ± 3.54	18.19 ± 12.51	15.79 ± 8.22	0.452			
P-value [b]	0.173	**< 0.001**	**< 0.001**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	-4.74 ± 6.69	-10.33 ± 20.18	-7.10 ± 17.16	0.578	0.143	**0.001**	**0.003**
Change % [d]	-6.69 ± 50.89	-7.51 ± 150.55	– 29.76 ± 185.31	0.733	0.760	0.718	0.239
Interleukin – 10 (IL-10, pg/ml)							
Baseline (pre)	16.88 ± 1.87	17.92 ± 2.40	16.42 ± 2.08	**0.003**			
Week 4 (post)	18.84 ± 2.64	18.64 ± 2.10	18.43 ± 2.36	0.844			
P-value [b]	0.078	**0.025**	**< 0.001**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	1.96 ± 3.57	0.72 ± 2.84	2.01 ± 2.74	0.059	0.237	0.069	**0.001**
Change % [d]	13.32 ± 26.10	5.48 ± 15.87	13.67 ± 19.64	0.065	0.266	**0.015**	**0.001**
CD163 protein (μg/ml)							
Baseline (pre)	182.81 ± 52.1	194.1 ± 140.3	166.6 ± 120.5	0.629			
Week 4 (post)	194.9 ± 64.4	232.2 ± 117.1	222.2 ± 108.0	0.705			
P-value [b]	0.600	**0.005**	**0.001**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	12.0 ± 4.2	41.8 ± 138.6	57.9 ± 118.7	0.625	0.521	**0.032**	**0.001**

Continuous data are presented as the mean ± SD; categorical data are presented as frequencies (percentages). Bold denotes to statistically significant difference at $P < 0.05$.

[a] The *P*-value was analyzed by a general linear model after adjusting for baseline age, sex, waist circumference, and total caloric intake.

[b] *P*-value: The difference between baseline and 4 weeks was analyzed by Wilcoxon signed-rank test.

[c] *P*-value for trend was analyzed by a linear trend test.

[d] Change (%) was calculated as [(end point-baseline value)/baseline value] * 100.

Variable	Haptoglobin phenotype (Hp)			*P* for trend [c]	*P*-value [a] (1–1 vs. 2 – 1) adjusted	*P*-value [a] (1–1 vs. 2–2,) adjusted	*P*-value [a] (2 – 1 vs. 2–2) adjusted
	Hp1-1 (n = 6)	Hp2-1 (n = 53)	Hp2-2 (n = 55)				
Change % [d]	8.15 ± 22.93	127.23 ± 372.68	335.70 ± 810.24	0.155	0.424	**0.016**	**0.004**
Serum haptoglobin (Hp, mg/dl)							
Baseline (pre)	155.47 ± 29.52	158.48 ± 46.75	141.10 ± 45.87	0.140			
Week 4 (post)	161.04 ± 35.33	139.65 ± 46.09	112.63 ± 42.12	**0.001**			
P-value [b]	0.600	**0.003**	**< 0.001**				
P-value [a] (Adjusted)	**< 0.001**	**< 0.001**	**< 0.001**				
Change [d]	5.57 ± 57.01	-18.83 ± 46.81	-28.46 ± 33.48	0.121	0.820	**0.005**	**0.001**
Change % [d]	8.13 ± 35.52	-8.91 ± 31.64	-18.73 ± 23.00	**0.036**	0.599	**0.045**	**0.001**

Continuous data are presented as the mean ± SD; categorical data are presented as frequencies (percentages). Bold denotes to statistically significant difference at $P < 0.05$.

[a] The *P*-value was analyzed by a general linear model after adjusting for baseline age, sex, waist circumference, and total caloric intake.

[b] *P*-value: The difference between baseline and 4 weeks was analyzed by Wilcoxon signed-rank test.

[c] *P*-value for trend was analyzed by a linear trend test.

[d] Change (%) was calculated as [(end point-baseline value)/baseline value] * 100.

Supplementary Materials

Supplementary Figure 1. Distribution of haptoglobin polymorphism phenotypes among the study subjects using G-vertical polyacrylamide gel electrophoresis.

https://www.dropbox.com/scl/fi/1wod2ovmnmccsmo6zn2rr/Supplementary-Figure-1-Haptoglobin-polymorphuism.docx?dl=0&rlkey=ojzc2akvirdola97a7vcgg3td

Supplementary Tables 1 for sex differences in the basic demographic, anthropometric, metabolic, and inflammatory indicators for the study subjects (males=75, females=39, total=114).
https://www.dropbox.com/s/6siay34nmljidb1/Supplementary%20Tabl%201%20sex%20differences%20Haptoglobib%20polymorphism%20Updated%2019220dl=0

Supplementary Tables 2 for sex differences in the prevalence of obesity classes among the three different haptoglobin phenotypes.

https://www.dropbox.com/scl/fi/9u95hg12h0g959ghg8p93/Suplemnetary-Table-2-BMI-classes-Hp-phenotype.docx?dl=0&rlkey=jb5r39j9xc1667zkurnosm1jl