Serum adiponectin and leptin in young healthy men: Preliminary data from a small pilot cohort

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Abstract. Adiponectin and leptin are adipokines involved in metabolic regulation and inflammatory signaling. Despite the high burden of metabolic disease, locally generated data remain limited. The present descriptive pilot feasibility study was conducted to generate preliminary assay-specific serum adiponectin and leptin data in young men and to inform the design of larger standardized studies. In the present study, serum adiponectin and leptin levels were measured in 10 healthy young men. The study participants had a mean age of 28.4±10.1 years, a median age of 23.5 years, an age range of 20-45 years, and a mean body mass index of 23.1±5.4 kg/m^2. Serum adiponectin and leptin levels were measured using commercial Quantikine ELISA kits, and the data were analyzed descriptively. Preliminary descriptive analysis revealed a mean serum adiponectin level of 31.2 ng/ml (0.0312 μg/ml); range, 16.1-46.4 ng/ml (0.0161-0.0464 μg/ml) and a mean serum leptin level of 681.6 pg/ml (0.6816 ng/ml); range, 353.7-1045.8 pg/ml (0.3537-1.0458 ng/ml). On the whole, the present pilot feasibility study provides preliminary assay-specific serum adiponectin and leptin data in a small cohort of apparently healthy men. Larger, methodologically standardized studies are warranted to establish robust population-specific reference data.

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Introduction

Adiponectin and leptin are adipokines that link adipose tissue biology to metabolic regulation and inflammatory signaling. Adiponectin has generally been associated with insulin sensitization and anti-inflammatory effects, whereas leptin is more widely recognized for its role in appetite regulation, energy balance and broader immunometabolic activity (1-3). As both molecules are influenced by adiposity, metabolic state and other host factors, circulating concentrations have been investigated as potential biomarkers in obesity, insulin resistance and cardiometabolic disease (3-5). At the same time, the interpretation of adiponectin and leptin concentrations across studies remains challenging. Reported levels are affected not only by sex, age and body composition, but also by sampling conditions, cohort characteristics and assay methodology (4,5). As a result, values reported in one population may not be directly transferable to another, particularly when studies differ in participant selection or analytical platform. Establishing population-appropriate reference data therefore requires well-characterized cohorts and careful attention to pre-analytical and analytical standardization. This is of particular importance when comparing adipokine values across ethnic or population groups, where both biological variation and assay-related differences may influence the reported concentrations. This issue is particularly relevant in Saudi Arabia, where locally generated adipokine data remain limited and published values show substantial variability. Previous reports have described adiponectin concentrations

in Saudi cohorts that differ markedly from one another, and broad reference ranges from external clinical sources may not be directly applicable in the local setting (6-8). Such variability may reflect differences in study design, participant characteristics and assay approach. These considerations highlight the need to establish population-specific reference ranges for both adiponectin and leptin using standardized methods in Saudi cohorts.

Therefore, the present pilot study was conducted to quantify circulating serum adiponectin and leptin in a small cohort of apparently healthy Saudi men using standardized commercial enzyme-linked immunosorbent assay (ELISA). The aim of the present study was to generate preliminary assay-specific data that could help inform the design of larger, methodologically standardized studies and support the development of more robust population-specific reference ranges for adiponectin and leptin in Saudi cohorts. The primary objective of the present study was feasibility and preliminary distribution estimation rather than hypothesis testing.

Subjects and methods

Subjects. Fresh blood was collected from Saudi male donors recruited between February and June, 2021 from the College of Medicine (COM) and the College of Science and Health Professions (COSHP) at King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), Riyadh, Saudi Arabia. Participants were recruited as a small convenience sample of apparently healthy adult Saudi men. Participants had a mean age of 28.4±10.1 years, a median age of 23.5 years, an age range of 20-45 years, and a mean body mass index (BMI) of 23.1±5.4 kg/m^2. The inclusion criteria were Saudi male adults who were willing to provide a blood sample and written informed consent and who reported no known diagnosed medical conditions at the time of consent. Exclusion criteria included refusal to provide consent and incomplete sample collection or participant data required for analysis. Based on self-report at the time of consent, all participants were non-smokers and reported a moderate level of physical activity. Blood samples were collected in the non-fasting state between 8:00 and 11:00 a.m. Age and BMI were recorded at the time of enrollment. The phlebotomy procedure was performed by a certified phlebotomist, and written informed consent was obtained from all participants prior to blood collection. The institutional review board (IRB) at KSAU-HS approved the study (RC18/004/R).

Assays. Serum samples were collected and stored at -80˚C for ELISA. Human total adiponectin and leptin levels were measured using the Quantikine ELISA kit (cat. nos. DRP300 and DLP00, respectively; R&D Systems, Inc.) according to the manufacturer's protocol. Generally, serum samples were processed and stored at -80˚C within 1 h of collection. Each sample underwent one freeze thaw cycle prior to analysis, and all samples were assayed in duplicate. According to the manufacturer, the Human Total Adiponectin/Acrp30 Quantikine ELISA (DRP300) has a serum/plasma intra-assay CV of 2.5-4.7% and an inter-assay CV of 5.8-6.9%, while the Human Leptin Quantikine ELISA (DLP00) has an intra-assay CV of 3.0-3.3% and an inter-assay CV of 3.5-5.4%.

For adiponectin assay, briefly serum adiponectin levels were measured using a quantitative sandwich enzyme immunoassay. In this method, a microplate pre-coated with a monoclonal antibody specific to the human adiponectin globular domain captures the target protein. Serum samples were diluted 100-fold by adding 10 μl of the sample to 990 μl Calibrator Diluent RD6-39 to ensure optimal detection within the dynamic range of the assay. A standard curve was established by reconstituting the human adiponectin standard with the same calibrator diluent to create a stock solution of 250 ng/ml, followed by serial dilutions. For the assay, 100 μl Assay Diluent RD1W were added to each of the 20 wells, and 50 μl of either the standard, control, or diluted sample was then introduced. The plate was covered and incubated at room temperature for 2 h to allow binding. Post-incubation, the wells were aspirated and washed four times with wash buffer (included with the kit) to remove unbound substances. Subsequently, 200 μl human adiponectin conjugate (included with the kit) was added to each well, and the plate was incubated for a further 2 h at room temperature. Following a second series of washes, 200 μl substrate solution (included with the kit) were added to each well to initiate the colorimetric reaction. The reaction was terminated by the addition of 50 μl Stop Solution to each well, causing a color change from blue to yellow. The optical density of each well was measured within 30 min using SpectraMax Plus 384 microplate reader (Molecular Devices, LLC) set to 450 nm.

For the leptin assay, the same principle was used, with different reagents. For the assay procedure, 100 μl Assay Diluent RD1-19 (included with the kit) were added to each well of a microplate. Subsequently, 100 μl of the standard, control, or diluted sample was added to the respective wells. The plate was covered with an adhesive strip and incubated at room temperature for 2 h to allow antigen-antibody binding. Following incubation, each well was aspirated and washed four times with 400 μl Wash Buffer to remove unbound substances. Next, 200 μl of Human Leptin Conjugate (included with the kit) was added to each well, and the plate was incubated for 1 h at room temperature. Following another series of washes to eliminate excess conjugate, 200 μl Substrate Solution (included with the kit) were added to each well and incubated for 30 min at room temperature, protected from light to prevent the degradation of the substrate. The reaction was terminated by the addition of 50 μl Stop Solution (included with the kit) to each well, causing a color change from blue to yellow. The optical density of each well was measured within 30 min using SpectraMax Plus 384 microplate reader (Molecular Devices, LLC) set to 450 nm.

Data analysis and presentation. All data were analyzed using GraphPad Prism 8 (Dotmatics). The results for continuous variables, such as adiponectin and leptin levels, are presented as the mean ± standard deviation (SD) and as the median and interquartile range (IQR). The analyses were descriptive due to the exploratory pilot design and small sample size.

Results

As regards the demographic characteristics, the 10 study participants had a mean age of 28.4±10.1 years and a mean BMI of 23.1±5.4 kg/m^2 (Table I). Serum adiponectin levels,

Table I. Demographic data of the study participants.

Subject	Age	Body mass index
1	20	17.5
2	20	18
3	21	18.8
4	22	19.6
5	23	20.5
6	24	22.2
7	26	24
8	39	26.5
9	44	31
10	45	32.9
Mean ± SD	28.4±10.1	23.1±5.4

measured using the Acrp30 Human Adiponectin Quantikine ELISA kit, exhibited a mean concentration of 31.2±11.1 ng/ml (0.0312±0.0111 μg/ml), a median of 28.8 ng/ml (0.0288 μg/ml); IQR, 24.3-44.5 ng/ml (0.0243-0.0445 μg/ml), and a range of 16.1-46.4 ng/ml (0.0161-0.0464 μg/ml) (Fig. 1).

Serum leptin levels, measured using the Human Leptin Quantikine ELISA kit, exhibited a mean concentration of 681.6±261.7 pg/ml (0.6816±0.2617 ng/ml), a median of 674.4 pg/ml (0.6744 ng/ml); IQR, 450.7-893.4 pg/ml (0.4507-0.8934 ng/ml), and a range of 353.7-1045.8 pg/ml (0.3537-1.0458 ng/ml) (Fig. 2).

The leptin-to-adiponectin ratio was also calculated for each participant. The mean ratio was 22.57±8.65, with a median of 21.20 and a range of 15.31-43.93 (Table II). Individual BMI values alongside the corresponding adiponectin and leptin measurements are provided in Table SI to allow for the descriptive assessment of potential trends across participants.

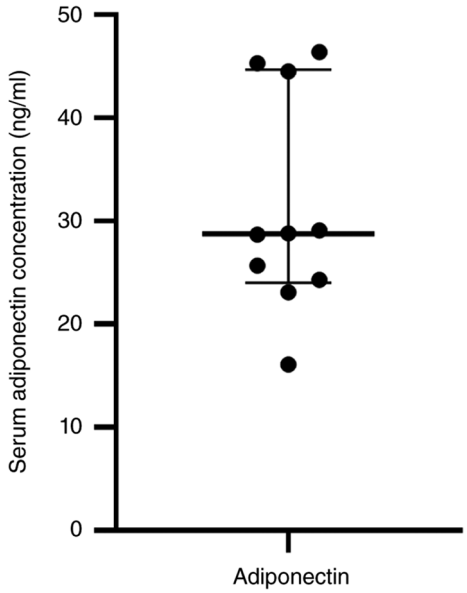

Figure 1. Human serum adiponectin concentration. Serum adiponectin was measured using the Acrp30 Human Adiponectin Quantikine ELISA kit. Individual data points are presented, with the median and interquartile range indicated. Data are representative of n=10 participants.

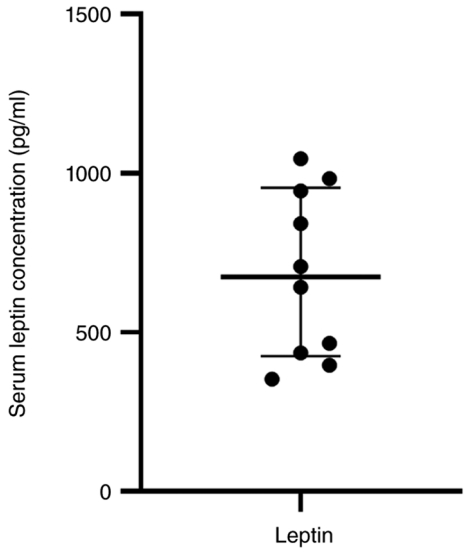

Figure 2. Human serum leptin concentration. Serum leptin was measured using the Human Leptin Quantikine ELISA kit. Individual data points are presented, with the median and interquartile range indicated. Data are representative of n=10 participants.

Discussion

In the present study, the mean serum adiponectin level was 31.2±11.1 ng/ml (0.0312±0.0111 μg/ml), with a median of 28.8 ng/ml (0.0288 μg/ml); IQR, 24.3-44.5 ng/ml (0.0243-0.0445 μg/ml) and a range of 16.1-46.4 ng/ml (0.0161-0.0464 μg/ml), in a pilot cohort of apparently healthy Saudi men. These values were lower than those presented in other studies, including external clinical reference ranges and previous Saudi data (6-8). However, such differences should be interpreted cautiously, as cross-study comparisons may be influenced by differences in assay platform, sample handling, participant selection and cohort characteristics. Given that the cohort had a mean BMI of 23.1±5.4 kg/m^2, with individual BMI values ranging from 17.5 to 32.9 kg/m^2, the relatively low adiponectin levels may reflect variations in adiposity, as well as other factors, including lifestyle, environmental exposures and broader participant characteristics that may affect adipokine regulation in this cohort. Moreover, the variability in adiponectin levels across participants highlights the need for larger studies with more detailed metabolic and clinical characterization.

For leptin, the mean serum level was 681.6±261.7 pg/ml (0.6816±0.2617 ng/ml), with a median of 674.4 pg/ml (0.6744 ng/ml); IQR, 450.7-893.4 pg/ml (0.4507-0.8934 ng/ml) and a range of 353.7-1045.8 pg/ml (0.3537-1.0458 ng/ml). These values fall within the broad reference interval cited from external clinical sources but appear lower than those in other studies (6,9). These values also appear lower than those reported in a previous study, as summarized in Tables SII and SIII (7). As with adiponectin, such differences should be interpreted cautiously, since they may reflect variations in participant characteristics, adiposity,

Table II. Leptin-to-adiponectin ratio in individual participants.

Subject	Leptin (pg/ml)	Adiponectin (ng/ml)	Leptin-to-adiponectin ratio
1	707.3	16.1	43.93
2	641.5	29.1	22.04
3	1045.8	45.3	23.09
4	353.7	23.1	15.31
5	396.7	25.7	15.44
6	944.4	44.5	21.22
7	842.3	28.7	29.35
8	982.7	46.4	21.18
9	435.8	24.3	17.93
10	465.5	28.8	16.16
Mean ± SD			22.57±8.65
Median (IQR)			21.20 (16.76-22.83)
Range			15.31-43.93

nutritional status, physical activity, stress and methodological factors rather than a distinct biological pattern. In addition, the present study calculated the leptin-to-adiponectin ratio as a supplementary descriptive measure, since this index has been proposed as a more integrated marker of adipose tissue dysfunction and insulin resistance than either adipokine alone, reflecting the balance between leptin-associated pro-inflammatory signaling and adiponectin-associated insulin-sensitizing effects (10). In the present pilot cohort, the ratio is reported descriptively and may be useful to include in future larger Saudi studies with broader metabolic characterization.

Several factors may have contributed to the differences between the findings of the present study and those of previous reports (6,7,9). These include assay-related variation, pre-analytical conditions, participant age, BMI, overall health status, diet, physical activity and other lifestyle-related variables. Genetic variation may also be a contributing factor; however, the present study was not designed to assess genetic influences directly and cannot determine their relative role (2,11,12). Furthermore, the relatively small sample size of the present study (n=10), together with the variability in participant age and BMI, limits the generalizability of the findings and may have contributed to the variation observed when compared with larger and more diverse populations in other studies.

These preliminary findings highlight the importance of generating standardized population-specific reference data for adiponectin and leptin in Saudi cohorts. Larger studies with broader clinical and metabolic characterization are required to determine how these biomarkers can be interpreted more reliably in local research and clinical contexts.

The present study has several limitations that should be considered when interpreting the findings. The small sample size (n=10) limits the generalizability of the results. In addition, the cohort included only male participants and was recruited from a single center, which further limits broader population representativeness. Although blood samples were collected within a defined morning window (8:00 to 11:00 a.m.), residual circadian variation in adipokine levels, particularly leptin, cannot be excluded. Adiposity was assessed using BMI only, and no direct measurements of body fat percentage or body composition were obtained. Moreover, inflammatory biomarkers such as C-reactive protein, interleukin-6 and tumor necrosis factor-α were not measured, limiting interpretation of the findings in relation to inflammatory status.

In conclusion, the present pilot study provides preliminary assay-specific data on serum adiponectin and leptin in apparently healthy Saudi men and highlights the importance of standardized sampling and analytical protocols in future work. The findings also support the feasibility of larger studies designed to generate population-specific reference data. The present study provides valuable information for future larger studies that will include both men and women and incorporate stratification by BMI.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MA and MLA conceptualized and planned the study, contributed to the experiments and analyses, and drafted and

critically revised the article. JA and BMA contributed to the design of the experiments, sample preparation, experiments, data collection, analyses, and verification of the analytical methods. MA and MLA confirm the authenticity of all the raw data. All authors discussed the results, contributed to the drafting of the manuscript, contributed to the critical revision of the manuscript, and have read and approved the final draft of the manuscript.

Ethical approval and consent to participate

The institutional review board (IRB) at KSAU-HS approved the present study (RC18/004/R). Written informed consent was obtained from all participants, including consent for the use of anonymized data in publications.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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