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Is Fasting Necessary for the Assessment of Clinical Biochemical Parameters?

Berna KUS[1] · **Emre DIRICAN**[2] · **Serdar DOGAN**[3] · **Abdullah ARPACI**[3]
[1] *Department of Moleculer Biochemistry and Genetics, Hatay Mustafa Kemal University, Faculty of Medicine, Turkey*
[2] *Department of Biostatistics, Hatay Mustafa Kemal University, Faculty of Medicine, Turkey*
[3] *Department of Biochemistry, Hatay Mustafa Kemal University, Faculty of Medicine, Turkey*

Introduction: Blood tests are usually requested for patients visiting polyclinics in the morning following fasting. However, as hospitals provide services for 24 hours, blood is collected from patients at every hour of the day, and fasting status cannot be completely ensured. Therefore, our study aimed to compare the fasting/non-fasting biochemical parameters of individuals.
Materials and Methods: Our retrospective study examined 18 different most frequently requested blood tests of patients who visited certain polyclinics at our hospital only once as fasting and non-fasting values based on their hours of being requested.
Results: In our study which analyzed a total of 70,352 individuals, statistically significant differences were observed in the fasting/non-fasting values. However, when the effect size values were measured for the tests, they were not clinically significant (0.006-0.104).
Conclusion: There was no noticeable difference between the fasting and non-fasting parameters. It was concluded that, as long as there is no doubt on routine results, individuals could give blood samples throughout the day with or without fasting.
Keywords: Fasting, non-fasting, biochemistry parameters, effect size

Introduction

The in vitro reflection of nutrition in the clinic is the changes in the biochemical parameters in blood samples as a result of metabolic changes in the body. As a result of digesting food, serum glucose, amino acid, and triacylglycerol levels increase.1As a response to ingestion of food, the pancreas reduces glucagon secretion by increasing insulin secretion and tries to keep the blood glucose level in the reference range. This change in the insulin/glucagon levels triggers the anabolic stage in tissues (especially the liver, muscle, and adipose tissues). The liver forms glycogen and lipids from the substrates that are supplied. While the glycogen that forms is stored in the liver, lipids are transferred to the

Corresponding Author: Abdullah ARPACI, MD; Department of Biochemistry, Hatay Mustafa Kemal University, Faculty of Medicine, Turkey
ORCID: 0000-0002-6077-8258
E-mail: arpaci57@gmail.com
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blood via very-low-density lipoproteins (VLDL). Adipose tissue removes free fatty acids from lipoproteins, synthesizes triacylglycerols, and stores them in the form of insoluble droplets. When the metabolism is in the state of non-fasting, the brain and the heart use glucose as their energy source. Only after long-term fasting, they can use only ketone bodies (1, 2).

While transitioning from non-fasting to fasting, the body secretes the glucagon hormone from the α cells of the pancreas. The main purpose of glucagon is to try to keep the blood glucose level around normoglycemic levels in fasting (3). By regulating the metabolism, the insulin/ glucagon hormones regulate lipolysis/ lipogenesis and glycogenolysis or glycogen synthesis in a balanced way. Therefore, all bodily enzymes/hormones function in a way to keep the metabolism in balance despite fasting or non-fasting (4).

While the metabolism is conducting all these processes, a difference in blood biochemistry occurs between the states of fasting and non-fasting. However, this difference is on a negligible level for many tests (5-7). Importance is paid to the states of fasting and non-fasting in Turkey in terms of biochemical tests. For this reason, in our study, by taking the biochemical parameters of samples arriving at our laboratory, it was aimed to determine whether or not there were differences between the fasting and non-fasting blood samples in the statistical and clinical sense, and if any, how this was reflected on the clinic.

Materials and Methods

Ethical Statement & Subjects

The data that were retrospectively used in this study were obtained from the automation system (ENLIL) of the Hatay Mustafa Kemal University (HMKU) Health Application and Research Hospital. Ethics committee approval was obtained (19.YL.024). The groups of the study were formed by using the hospital database information of patients who visited the polyclinic between 01.01.2019-31.10.2019.

Materials

Our study covered a total of 70,352 individuals including 42,696 (60%) women and 27,656 (40%) men. Test results were obtained from outpatients who received treatment from the polyclinics of otorhinolaryngology, orthopedics, ophthalmology, dermatology, neurology, physiotherapy & rehabilitation, general surgery, endocrinology, infectious disease, hematology, internal medicine, and gastroenterology. Inpatient data and the data of the oncology service and polyclinic, pediatrics, emergency services, nephrology polyclinic, and intensive care unit were not included in the analysis.

Methods

The study included 18 different biochemical tests used most frequently by clinics. As we considered that the probability of blood samples taken at early hours of the morning to be fasting blood samples would be higher than the same probability for those taken towards the afternoon and in the afternoon, the patients' blood tests were categorized as fasting for 07:00-10:00 and non-fasting for 10.01-17:00. The blood samples that were transferred to the laboratory under appropriate conditions were kept for 20 min, centrifuged for 10 minutes at 4°C and 4000 rpm, separated into their serums, and analyzed with an ADVIA 1800 Clinical Chemistry System autoanalyzer.

Statistical Analysis

In studies in general, the significance of the difference between groups is statistically analyzed and expressed in terms of units as $p<0.05$. In the literature, it was stated that

the "p" value is affected by the size of the sample that is analyzed, and in studies with large samples, the p-value could turn out to be smaller than 0.05 even if there are very small differences between the means-medians belonging to the groups that are not clinically significant (8).

A measurement parameter showing whether or not the difference between the results of the groups in a study is clinically significant is the effect size. The Effect Size is defined as the minimum change at which we want to be able to accurately determine the test result or the minimum degree of difference that will be clinically significant. As a general proposition of Cohen, it is stated that d values of smaller than 0.2 indicate a weak effect size, a value of 0.5 indicates a medium effect size, and a value of higher than 0.8 indicates a strong effect size (9, 10). In our study, SPSS 21 was used to analyze. The outlier values in all parameters found in the dataset were examined by a Box Plot and excluded from the study. After summarizing the data with descriptive statistics, Student's t-test and Mann Whitney U test were used for the analyses. The level of significance for all tests was accepted as $p<0.05$.

Results

Our study covered a total of 70,352 individuals including 42,696 (60%) women and 27,656 (40%) men. Among these individuals, 46,731 (66%) were assessed as fasting, and 23,621 (34%) were assessed as non-fasting. As seen in Table 1, while there was no significant difference in Amylase (AMY), Iron, Lipase (LIP), Phosphor, Total cholesterol, High-density lipoprotein, Triglycerides, and Alkaline Phosphatase (ALP), some differences were observed in Albumin (ALB), Alanin Aminotransferase (ALT), Aspartate Aminotransferase (AST), Creatinine (CRE), Calcium (Ca), Gamma-Glutamyl Transferase (GGT), Glucose (GLU), Blood Urea Nitrogen (BUN), Total Protein (TP), and Creatinine kinase (CK) at $p<0.05$. As shown in Table 2, between the fasting and non-fasting values of the women, there was no significant difference in AMY, AST, Phosphor, Total cholesterol, CRE, iron, ALP, TP, Triglycerides, High-density lipoprotein and CK, while there were significant differences in ALB, ALT, Ca, GGT, GLU, BUN, and LIP at $p<0.05$.

Table 3 has demonstrated that, between the fasting and non-fasting values of the men, while there was no significant difference in amylase, Total cholesterol, CRE, Iron, GGT, BUN, LIP, Triglycerides, ALP, and High-densitylipoprotein, there were significant differences in ALB, ALT, AST, Phosphor, Ca, GLU, TP, and CK at $p<0.05$. However, according to the effect size values that were calculated, even the parameters that were found to be statistically significant were not significant (effect size min-max=0.006-0.104) (Table 2-3).

Additionally, while there was no significant difference among the women in AST, IRON, TP, ALP, High-density lipoprotein, and CK (Table 2), there was no significant difference among the men in IRON, GGT, BUN, ALP, and High-density lipoprotein (Table 3). As it may be seen here, although some parameters such as CK and AST were not statistically significantly different among the women between the fasting and non-fasting values ($p>0.05$), the differences in the men were significant ($p<0.05$) (Tables 2-3). Again, while parameters such as GGT and BUN were not significantly different among men, they were significantly different among the women. According to the effect size values that we measured to determine whether or not the parameters that turned out to be statistically

Table 1. Values of Parameters in Fasting/Non-Fasting States

	Fasting				Non-Fasting				p	Effect Size
	N	Mean±SS	Median	Min - Max	N	Mean±SS	Median	Min - Max		
Age	45929	49,49±16,96	50	18-98	23074	46,66±18,08	46	18-106	0,001	-
ALB	16356	4,31±0,35	4,35	3-5,66	7159	4,34±0,38	4,38	3-5,5	0,001	0,082
AMY	3670	70,05±27,5	66	7-185	2126	69,6±27,7	65	13-183	0,248	0,031
AST	26525	21,7±6,9	21	0-49	13221	22,04±7	21	1-49	0,001	0,037
ALT	38063	21,3±9,73	19	0-59	18936	21,62±10,08	19	0-59	0,007	0,023
Pi	6781	3,5±0,68	3,6	1,1-6,3	3233	3,61±0,66	3,6	1,1-6,2	0,164	0,03
Ca	20110	9,41±0,48	9,43	7,37-11,54	8379	9,46±0,49	9,48	7,3-11,54	0,001	0,103
CHOL	4880	188,2±43,06	184	35-379	2579	187,3±43,05	185	41-362	0,366	0,021
CRE	40471	0,743±0,22	0,7	0-1,64	19835	0,75±0,22	0,72	0,07-1,6	0,001	0,043
IRON	8807	63,69±37,54	58	1-225	3762	65,06±38,86	60	1-226	0,067	0,035
GGT	8114	21,82±13,42	18	1-72	5597	21,2±13,24	17	1-72	0,008	0,046
GLU	31376	93,89±16,09	90	36-150	15550	93,09±15,72	90	36-150	0,001	0,05
BUN	23778	14,21±5,79	13	1-36,9	14087	14,09±5,65	13	0,6-36,9	0,044	0,02
LIP	3387	37,41±12,37	35	12-84	1915	36,79±12,13	34	2-84	0,078	0,05
TP	9264	7,24±0,056	7,26	4,82-9,53	4187	7,27±0,057	7,29	4,89-9,66	0,002	0,053
TRIG	10082	148,56±78,46	129	0-469	4263	150,08±82,3	129	26-468	0,307	0,018
ALP	10070	74,05±25,83	70	8-179	4723	73,88±25,88	70	19-179	0,717	0,006
HDL	4089	47,08±12,69	45,5	7-103,9	2330	47,29±13,26	46	14-103,9	0,527	0,016
CK	7327	87,9±45,9	77	1,44-269,7	3115	92,78±47,8	81,35	9-269,7	0,001	0,104

Units: GLU, BUN, CRE, HDL, LDL, T-Cholesterol, TRIG, Ca, Pi: mg/dL; ALP, AMY, LIP, CK, ALT, AST: U/L; TP, ALB: g/dL; IRON: ug/dL

Table 2. Fasting / Non FastingValues of Female Individuals

Variables	Fasting				Non-Fasting				p	Effect Size
	N	Mean±SS	Median	Min - Max	N	Mean±SS	Median	Min - Max		
Age	28697	48,05±16,45	48	18-98	13305	45,49±17,66	45,49	18-106	0	-
ALB	10003	4,29±0,32	4,33	3-5,66	4079	4,31±0,35	4,31	3,02-5,37	0,03	0,059
AMY	2090	67,19±25,48	64	7-182	1155	66,78±26,16	66,78	13-183	0,659	0,015
AST	16611	20,93±6,53	20	0-49	7474	20,92±6,48	20,92	1-49	0,86	0,001
ALT	24685	19,82±8,68	18	0-59	11166	19,47±8,78	19,47	0-59	0	0,04
Pi	4074	3,68±0,66	3,6	1,1-6,3	1830	3,68±0,64	3,68	1,1-6,2	0,908	0
Ca	12952	9,41±0,48	9,42	7,38-11,54	4898	9,43±0,49	9,43	7,38-11,54	0,01	0,042
CHOL	2701	193,61±43,19	190	35-376	1296	192,42±43,07	192,42	41-362	0,413	0,027
CRE	25463	0,66±0,18	0,63	0-1,64	11369	0,66±0,18	0,66	0,07-1,63	0,138	0
IRON	6208	59,2±35,29	54	2-225	2599	59,99±36,38	59,99	2-226	0,341	0,022
GGT	4943	18,83±12,19	15	1-72	3232	17,56±11,15	17,56	1-72	0	0,108
GLU	20905	92,43±15,27	89	38-150	9267	91,97±15,21	91,97	46-150	0,016	0,03
BUN	13327	13,20±5,68	12	1-36,9	7548	12,97±5,48	12,97	1-36,9	0,005	0,041
LIP	1878	37,30±12,23	35	12-84	1047	36,31±11,35	36,31	2-82	0,031	0,083
TP	5248	7,23±0,54	7,25	4,9-9,53	2212	7,23±0,55	7,23	5,2-9,66	0,917	0
TRIG	6205	139,98±73,42	122	15-469	2379	138,96±76,95	138,96	33-468	0,577	0,013
ALP	6306	72,11±25,75	68	15-179	2661	71,06±25,73	71,06	19-177	0,076	0,04
HDL	2150	51,91±12,71	50	7-103,9	1135	52,48±13,31	52,48	14-103,9	0,226	0,043
CK	5402	81,71±41,45	72,85	1,44-269	2013	82,97±40,55	82,97	9-269,28	0,239	3

Units: GLU, BUN, CRE, HDL, LDL, T-Cholesterol, TRIG, Ca, Pi: mg/dL; ALP, AMY, LIP, CK, ALT, AST: U/L; TP, ALB: g/dL; IRON: ug/dL

Table 3. Fasting/Non Fasting Values of Male Individuals

Variables	Fasting				Non-Fasting				p	Effect Size
	N	Mean±SS	Median	Min - Max	N	Mean±SS	Median	Min - Max		
Age	17232	51,9±17,52	54	18-94	9769	48,25±18,54	48,25	18 - 101	0	-
ALB	6353	4,34±0,39	4,39	3-5,49	3080	4,38±0,42	4,38	3 - 5,50	0	0,099
AMY	1580	74,86±29,52	69	16-185	971	73,02±29,07	73,02	14 - 183	0,123	0,062
AST	9914	23,21±7,49	22	0-49	5747	23,51±7,37	23,51	3-49	0,016	0,04
ALT	13378	24,27±10,85	22	2-59	7770	24,71±11	24,71	0 - 59	0,004	0,04
Pi	2707	3,46±0,7	3,4	1,3-6,3	1403	3,53±0,68	3,53	1,6 - 6,2	0,004	0,101
Ca	7158	9,42±0,49	9,44	7,37-11,41	3481	9,49±0,51	9,49	7,45 - 11,4	0	0,141
CHOL	2179	181,66±41,9	179	69-379	1283	182,18±42,4	182,18	71 - 332	0,722	0,012
CRE	15008	0,87±0,2	0,85	0,2-1,64	8466	0,88±0,19	0,88	0,15 - 1,64	0,378	0,051
IRON	2599	74,42±40,45	70	1-225	1163	76,39±41,72	76,39	1- 226	0,17	0,047
GGT	3171	26,49±13,9	23	26-72	2365	26,18±14,21	26,18	2-72	0,42	0,022
GLU	10471	96,82±17,25	93	36-150	6283	94,73±16,29	94,73	36 - 150	0	0,124
BUN	10451	15,49±5,68	14,2	1-36,9	6539	15,37±5,57	15,37	0,6 - 36,9	0,166	0,021
LIP	1509	37,55±12,55	35	13-84	868	37,37±12,98	37,37	12-84	0,749	0,014
TP	4016	7,25±0,58	7,27	4,82-9,42	1975	7,32±0,58	7,32	4,89 - 9,63	0	0,12
TRIG	3877	162,3±84,11	141	0-468	1884	164,1±86,71	164,12	26 - 467	0,445	0,021
ALP	3764	77,29±25,64	73	8-179	2062	77,53±25,64	77,53	19 - 179	0,735	0,009
HDL	1939	41,72±10,28	40,5	8-88,7	1195	42,37±11,17	42,37	16,6 - 98	0,108	0,06
CK	1925	105,29±52,82	94,83	6,82-269,75	1102	110,70±54,42	110,7	15,07-269,7	0,007	0,1

Units: GLU, BUN, CRE, HDL, LDL, T-Cholesterol, TRIG, Ca, Pi: mg/dL; ALP, AMY, LIP, CK, ALT, AST: U/L; TP, ALB: g/dL; IRON: ug/dL

significantly different at $p<0.05$ between the fasting and non-fasting groups were clinically significant, no clinically significant difference was observed in any of the parameters. While the smallest effect size value was 0.016 for High-density lipoprotein, the largest one was 0.104 for CK, and there was no clinical significance according to Cohen's d (Table 1).

Discussion

In the literature, while it had been accepted until the 2010s that the appropriate metabolic state is the fasting state since the length of stay is desired to be shortened and since the laboratory is asked for serving all applicant patients 24 hours a day, blood is taken from patients at any time of the day, and it is not possible to be sure of the fasting state of these patients (11,12). Due to the crowdedness of blood collection units in the early morning, patients who arrive at the hospital to provide blood samples at 8 am may have to wait until noon. The fact that this waiting process is difficult for diabetic, pregnant, elderly, or pediatric patients, in particular, or the fact that these people cannot wait on an empty stomach, may cause distress for phlebotomists and patients.

In fact, outside the hours of the day, one is required to fast, the body metabolism is in a state of non-fasting most of the day. It might not be the right thing to still assess individuals with fasting metabolism tests (6,13). While research on the difference between fasting and non-fasting values in humans is mostly on lipids, a limited number of studies covered other biochemistry parameters including albumin, bilirubin, and uric acid (13-15).

Langsted et al. assessed lipids, lipoproteins, apolipoproteins, and albumin in diabetic and non-diabetic individuals at different periods following their latest meal. As a result, they reported that the plasma Triglycerides amounts in a non-fasting state increased by only 0.2 mmol/L in comparison to fasting in diabetic and non-diabetic individuals, non-High-density lipoprotein and Apolipoprotein-B stayed constant before and after meals, LDL-CHOL and albumin amounts decreased in diabetic and non-diabetic individuals, and this was probably caused by hemodilution as a result of fluid intake. They also emphasized that the use of non-fasting blood in lipid profile measurements is more useful than fasting blood (16). Mora et al. assessed lipid profiles and reported changes in Triglycerides by 0.2 mmol/L, Total cholesterol by 0.1 mmol/L, and LDL-CHOL by 0.2 mmol/L, while there was no significant change in High-density lipoprotein (17). Similarly, in our study, no significant difference was seen between the fasting and non-fasting values for Total cholesterol, Triglycerides, and High-density lipoprotein ($p=0.366$, $p=0.307$, $p=0.527$, respectively) (Table-1). In the review by Nordestgaard et al. which was supported by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), according to the results of studies assessing fasting and non-fasting lipid profiles, it was proposed that non-fasting should be the standard, several countries (the United Kingdom, Canada, Scandinavian countries, etc.) have changed their clinical guidelines, and fasting is not required in lipid profile measurements in either diabetic or non-diabetic individuals, those with or without cardiovascular diseases and children or adults (5,18). They argued that fasting samples could be repeated for only patients with a doubt in routine results, and fasting samples could be collected based on their own guidelines in

patients with non-fasting triglyceride levels of >5 mmol/L (440 mg/dL) or chronic hyper triglyceridemia (5).

Plumelle et al. assessed the fasting and non-fasting results of 77 tests (37 biochemistry, 16 hematology, 3 coagulation, 21 endocrine parameters). They stated that, among the 37 biochemistry parameters, 29 were not affected by fasting or non-fasting (ALT, ALB, ALP, AMY, Apo A1, AST, B_2M, BIC, BUN, Ca, Total cholesterol, CK, GGT, HbA1c, High-density lipoprotein, Iron, K, LDH), the remaining were affected by food ingestion at $p<0.05$ (UA, TBIL, BNP, CREA, GLU), but as they did not affect the total change limit (TCL), they did not have clinical significance ($TCL=\sqrt{(2.77CVa)2+(0.5CVb)2}$).14 In our study, similar to the findings of the aforementioned studies, there were no differences in the ALT, ALP, AST, ALB, BUN, Ca, GGT, IRON, CK, TP, and Total cholesterol parameters.

The Bispebjerg study conducted by Sennels et al. prospectively examined the effects of fasting and non-fasting states on the circulating concentrations of 14 frequently used clinical biochemical parameters. Consequently, among the parameters common with ours in the 14 examined parameters, while there was no difference between fasting and non-fasting values in creatinine, there were differences in creatine kinase, ALT, and AST (19). Additionally, Plumelle et al. stated that there was no significant difference between fasting and non-fasting in ALT and AST values, and therefore, regardless of the hour of the day, measurements on the metabolism did not differ based on non-fasting or fasting states (14).

In our study, the p-value between the fasting and non-fasting values was 0.001 for creatinine, 0.007 for ALT, and 0.001 for AST (Table 1).

While studies on the effects of fasting and non-fasting on only the lipid profile among the 18 parameters that were examined in our study could be encountered in the literature, there is a limited number of studies regarding the other parameters we included. For this reason, we were not able to compare our results on some parameters to values reported in the literature.

Conclusion

Considering the mean values of the ALB, ALT, AST, Ca, CRE, GGT, Glu, TP, CK, and BUN parameters, it was found that they were close to each other between the statuses of fasting and non-fasting, but there were differences. However, when effect size analysis that is used in studies with large samples was applied, no clinical significance was found. In addition to this, no significant difference was found between the fasting and non-fasting values in the parameters of High-density lipoprotein, Triglycerides, Phosphor, Total cholesterol, AMY, Lipase, and ALP. Moreover, while there were differences in some parameters when this situation was assessed based on sex, again, clinically insignificant results were obtained.

In conclusion, biochemical values obtained by collecting blood at any time of the day may be used to make diagnoses of diseases without considering fasting/non-fasting status.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Contact Details

Berna KUS
Department of Moleculer Biochemistry and Genetics, Mustafa Kemal University, Faculty of Medicine, Turkey
E-mail: bernakus460@gmail.com
ORCID: 0000-0001-8279-0357

Emre DIRICAN
Department of Biostatistics, Hatay Mustafa Kemal University, Faculty of Medicine, Turkey
E-mail: emredir44@gmail.com
ORCID: 0000-0003-3550-1326

Serdar DOGAN
Department of Biochemistry, Hatay Mustafa Kemal University, Faculty of Medicine, Turkey
E-mail: drserdardogan@gmail.com
ORCID: 0000-0001-6854-2197

Abdullah ARPACI
Department of Biochemistry, Hatay Mustafa Kemal University, Faculty of Medicine, Turkey
E-mail: arpaci57@gmail.com
ORCID: 0000-0002-6077-8258

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