CONCISE REPORT

Sulphate and osteoarthritis: decrease of serum sulphate levels by an additional 3-h fast and a 3-h glucose tolerance test after an overnight fast

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Background: Low sulphate levels in blood may contribute to osteoarthritis by decreasing cartilage chondroitin sulphation.
Objective: To measure serum levels of sulphate during 3 h of fasting or glucose ingestion after overnight fasts to determine how much sulphate lowering may occur during this period.
Methods: Sera from 14 patients with osteoarthritis who fasted overnight were obtained every 15–30 min during 3 h of continued fasting and during 3 h after ingestion of 75 g of glucose. Sulphate was assayed by high-performance liquid chromatography with a Metrohm-Peak 761 Compact IC and simultaneously assayed for glucose by high-performance liquid chromatography with a Metrohm-Peak 817 Bioscan.
Results: Continuation of overnight fasting for 3 h resulted in a near-linear 3-h decrease in levels for all 14 patients ranging from 3% to 20% with a mean drop of 9.3%, whereas the 3-h decrease after glucose ingestion ranged from 10% to 33% with a mean drop of 18.9%.
Conclusion: A 3-h continuation of fasting caused a marked reduction in serum sulphate levels, whereas ingestion of 75 g of glucose in the absence of protein resulted in doubling the reduction. This suggests that fasting and ingestion of protein-free calories may produce periods of chondroitin under-sulphation that could affect osteoarthritis.

Many of the functions of cartilage depend on the high charge of the sulphate substituents of chondroitin sulphate; hence, undersulphation may produce a modification that in turn may relate to the onset and severity of osteoarthritis.

Using cultures of human[1] and other animal[2] cell types, we and others[3] have found that sulphation of chondroitin in cultured cells is usually lowered when sulphate concentrations in culture medium are <0.2 mmol/l. Normal fasting sulphate concentrations in adult humans are lower than those in any animals that have been tested,[4] generally averaging between 0.25 and 0.4 mmol/l,[4–6] with a few people reported to have concentrations as low as 0.1 mmol/l.[5] This has suggested a potential for chondroitin undersulphation. Furthermore, there have been suggestions that glucosamine sulphate and chondroitin sulphate used for treatment of osteoarthritis may provide benefit by sulphate supplementation.[7 8]

We previously measured the serum sulphate levels in stored fasting serum samples collected over a 33-year period from participants of the Boston Veterans Administration Normative Aging Study who did and did not develop osteoarthritis during that period.[9] Although we found no differences in sulphate levels between the two groups, all the samples had been taken after overnight fasts; hence participants had not ingested any protein for ⩾10 h. In general, the major supply of sulphate in humans is provided by metabolism of the sulphur-containing amino acids cysteine and methionine in liver, whereas cartilage is incapable of forming sulphate.[10] As these are essential amino acids, a diet deficient in protein for even a short period may result in decreased sulphate levels. Sulphate levels in rats have been shown to drop by 30–50% after an overnight fast,[4] and rats fed a diet deficient in protein for 4 days have been shown to have 50% decrease in sulphate levels.[11] Although serum levels of sulphate in normal humans have been shown to increase dramatically from 3 to 8 h after ingestion of a single large amount of protein,[5] no studies of sulphate levels in humans during fasting or in the absence of dietary protein have been reported. Diurnal variations in serum or plasma sulphate in animals[4] and humans[6] have been described, but these studies do not seem to have been carried out under fasting conditions.

We have measured serum sulphate levels during a 3-h period of continued fasting after an overnight fast by 14 study participants, and during a 3-h period after glucose, but no protein ingestion by the same patients after another overnight fast. We found marked near-linear decreases in sulphate levels in all the patients during the 3-h fast, and twice as much decrease during the 3 h after glucose ingestion by the same patients.

METHODS

Patients

Fourteen adults with osteoarthritis of the hand, hip or knee were recruited (with approval from the Human Institutional Review Board of the Tufts-New England Medical Center) from the Tufts-New England Medical Center Rheumatology Clinic, Boston, Massachusetts, USA. People with other major diagnosed conditions or a fasting glucose concentration >110 mg/dl (6 mmol/l) were excluded.

Patient preparation and fasting

Patients fasted from 22:00 h the preceding night, without drugs, vitamins or smoking. At time 0 in the morning they continued their fast for 3 h, and after an overnight fast on another day took 75 g of glucose at time 0 with no additional ingestion for 3 h.

Sample collection

Blood samples (3 ml) were obtained at time 0 and then at 15–30-min intervals during 3 h, collected into tubes containing serum separator and allowed to clot at room temperature for 30 min up to 2 h. Serum was obtained by centrifugation at 1500 rpm at 4°C for 15 min, and kept at −70 to −80°C until analysed.

Abbreviation: BMI, body mass index

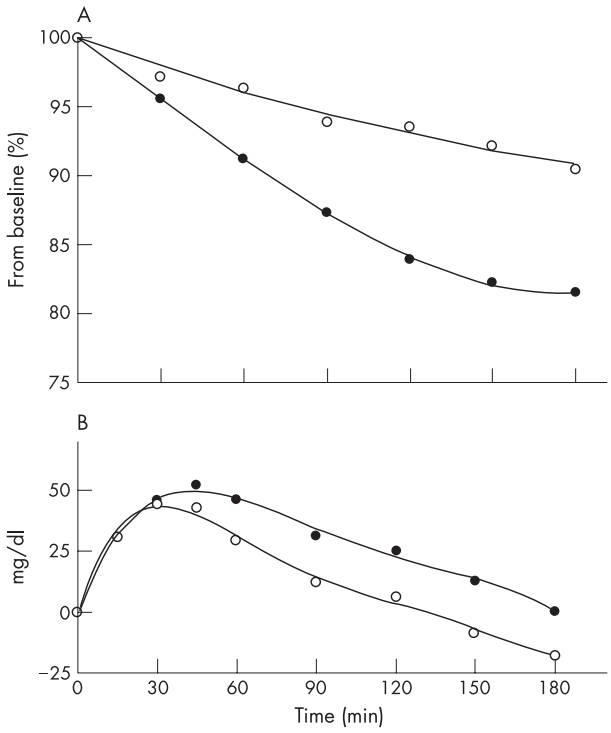

Figure 1 Mean serum levels at timed intervals. (A) Mean serum sulphate levels relative to baseline of all 14 patients during the 3-h fasting period (o) and during the 3 h after ingestion of 75 g of glucose (•). (B) Mean glucose levels of patients relative to baseline during the 3 h after ingestion of 75 g of glucose: (●) all 14 patients, (o) excluding the three patients with diabetes or glucose intolerance.

Sulphate and glucose analyses

Analyses of 0.05 ml of serum diluted 40-fold were carried out for sulphate by ion exchange high-performance liquid chromatography with a Metrohm-Peak 761 Compact IC (Metrohm-Peak, Houston, Texas, USA), and simultaneously for glucose by high-performance liquid chromatography for pulsed amperometric measurement with a Metrohm-Peak 817 Bioscan, as previously described.[9]

RESULTS

Table 1 shows the data on age, sex, weight and body mass index (BMI; weight (kg)/(height $(m)^2$)) along with the wide range of baseline serum sulphate levels after overnight fasting and after 3 h of continued fasting. By 3 h, levels for all participants had decreased continuously to varying degrees (table 1), with no apparent relationship to sex, age, chondroitin, weight or body mass. Mean percentage decrease of all 14 patients at each time interval (fig 1A) indicated near linearity for the entire 3 h. Levels for all participants after ingestion of 75 g of glucose decreased continuously to varying degrees (table 1), with mean decreases for all 14 patients at each time interval that were roughly twice as great as that with continued fasting (fig 1A). The decreases only remained near linear for 90 min. The mean baseline (0.41 mmol/l) for this portion of the study was somewhat higher than that of the mean baseline (0.37) for the totally fasted portion, possibly related to a different time of year (several months) that separated the performance times of the two portions.

The glucose AUC increment after glucose ingestion varied greatly among patients, with some having little or no rise (table 1); however, a difference between fasting and glucose ingestion was highly significant ($p<0.001$). Participants 5, 7 and 17 had patterns diagnostic of diabetes or glucose intolerance, although these abnormalities had not previously been known to the participants. The mean glucose levels for all participants (fig 1B) showed a peak at 30–60 min with a gradual return to baseline by 3 h, whereas omission of participants 5, 7 and 17 showed a lower, earlier peak that returned to baseline by 2 h, and then dropped below baseline. Mean decreases in serum sulphate levels of these three participants did not differ from the other 11 participants in linearity or amount.

DISCUSSION

In a previous report,[9] we inferred that sulphate levels may not be related to osteoarthritis, as we found no differences in the overnight fasting levels of serum sulphate between men who developed or did not develop osteoarthritis. However, this previous study was limited to the single morning fasting level, in contrast with the current new study that shows linear decreases in all participants, varying from 3% to 20% after an additional 3 h of fasting (fig 1A). The linearity suggests that the decrease would be still greater if the fast were continued for longer. It also indicates that metabolism of mobilised endogenous protein was insufficient to maintain a level of cysteine and methionine that would provide a constant sulphate level in the absence of dietary protein.

Table 1 Participant characteristics and serum values at baseline and at 3 h

					Sulphate (mmol/l)						
					No glucose			With glucose			Glucose
ID no	Sex	Age (years)	Weight (kg)	BMI	0	3 h	% decrease	0	3 h	% decrease	3-h AUC (mg min/ml)
3	F	70	96	32	0.34	0.33	3	0.43	0.37	14	53
4	F	74	74	27	0.41	0.36	12	0.39	0.34	13	75
5	F	56	97	40	0.41	0.36	12	0.40	0.31	22	66
6	F	41	88	34	0.36	0.35	3	0.42	0.35	17	−19
7	F	61	42	22	0.31	0.28	10	0.30	0.24	20	237
13	F	63	60	23	0.45	0.40	11	0.49	0.41	16	8
14	F	49	74	31	0.34	0.30	12	0.46	0.37	20	30
17	M	69	95	31	0.40	0.38	5	0.36	0.30	17	131
18	F	59	65	27	0.44	0.35	20	0.58	0.46	21	39
19	M	71	73	25	0.37	0.33	11	0.60	0.49	18	27
20	M	46	132	40	0.35	0.32	9	0.32	0.27	16	6
21	F	52	97	36	0.37	0.34	8	0.39	0.35	10	13
22	F	67	72	26	0.26	0.24	8	0.29	0.21	28	23
23	F	70	118	38	0.34	0.32	6	0.33	0.22	33	16
				Mean	0.37	0.33	9.3	0.41	0.34	18.9	

AUC, area under the curve; BMI, body mass index.

Ingestion of 75 g of glucose (300 cal) changed the degree of sulphate reduction in all participants, doubling the mean decrease to 18.9% from the mean of 9.3% that had occurred during conditions of fasting (table 1, fig 1A). A probable explanation is that ingestion of carbohydrate provides sufficient energy to allow the maintenance of glucose levels and baseline metabolism, without a need for using endogenous protein that would have been mobilised under fasting conditions. The lower use of endogenous protein results in a deficiency of cysteine and methionine for formation of sulphate. The sulphate levels did not continue to fall linearly after glucose levels had returned to baseline, consistent with the possibility that this resulted in renewed mobilisation of endogenous protein with consequent provision of cysteine and methionine. Thus, dietary patterns, particularly omitting protein even for parts of a day, can diminish sulphate levels markedly in some people, resulting in discrete periods of undersulphation of chondroitin sulphate.

The variations in the baseline levels and decreases in percentage suggest that only a small portion of the population (such as participants 22 and 23) may have decreases to levels <0.2 mmol/l under fasting conditions. We conjecture that low levels occur intermittently, so that only some areas of undersulphation are formed, and remain for an extended period because of the slow turnover of cartilage chondroitin sulphate. These areas may be somewhat defective in function because of the lowering of negative charge and may be more susceptible to damage, owing to increased susceptibility to enzymatic degradation of the undersulphated chondroitin. It is particularly noteworthy that many drugs, including aspirin and acetaminophen, are conjugated with sulphate for excretion, and have been shown to lower sulphate levels in humans[6 12 13] and other animals.[11 14] This adds to the possibility that sulphate levels in some people lacking protein consumption under specific circumstances or being treated with drugs can affect osteoarthritis. We do not know whether sulphate changes with fasting would be similar with controls, as this has not been measured. If a difference were to be found, it would add to the importance of the result in patients with osteoarthritis.

Any relevance of sulphate levels to osteoarthritis requires future demonstration that chondroitin in osteoarthritic portions of human cartilage is undersulphated relative to portions in which cartilage is normal.

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