Hourly Changes in Serum Cholesterol Concentration

Effects of the Anticipation of Stress

By John E. Peterson, M.D., Robert A. Keith, Ph.D., and Alan A. Wilcox, Ph.D.

Observations on the variability of serum cholesterol concentration at times of stress have been made by several investigators. In most cases the concentration of cholesterol has been found to increase during or shortly after a stressful experience, but there is evidence that the nature of the stressor and factors involved in the selection of experimental subjects may influence such responses.

During a previous study it was found that remarkable changes in serum cholesterol concentration could occur within a few hours when selected individuals were exposed to certain conditions of stress. In the case of cold, it was noted that serum cholesterol began to increase before the subjects entered the cold room and that it fell rather abruptly thereafter (fig. 1). This led us to question whether exposure to cold or the anticipation of this experience initiated the changes that were observed. The following report describes an effort to clarify this point.

Methods and Procedure

Selection of Subjects

For this experiment eight subjects were selected from a larger group of 82 freshman students at Loma Linda University. The larger group had volunteered for certain studies, which included the measurement of serum cholesterol on several occasions during the school year. At the time of our selection the 82 students had been under observation for a period of 4 months and serum cholesterol had been measured on five separate occasions. Two of the measurements had been made during semester examinations and the others were at times when the subjects were thought to be relatively free from stress.

The eight persons chosen for this particular study were those of the larger group who had shown the greatest variation in serum cholesterol concentration during the course of these five determinations. Selection was based on the difference between maximum and minimum concentrations regardless of whether these values were found during periods of examination or control. Table 1 gives the maximum and minimum cholesterol values on which the selection was made and also indicates how the subjects were paired for this experiment.

Plan of Study

During a spring vacation all eight subjects remained in a student laboratory on 3 consecutive days. Venous blood was drawn at hourly intervals from 9 a.m. until 6 p.m. No constant diurnal change was found in the course of a previous study, and it was therefore agreed to omit night sampling and to permit the subjects to return to their homes over night. The diet was unrestricted except that the subjects were given a standard luncheon together on each of the 3 days.

Day 1. Subjects reported to the laboratory at 8 a.m. and polyethylene catheters were placed in a brachial vein to facilitate collection of hourly blood samples. Venous samples of a physiologic salt solution were allowed to run slowly between withdrawals of blood to prevent obstruction of the catheter by clotting. During these initial proceedings the subjects were given instruction concerning the general plan of the experiment. Chairs and couches were provided and the subjects were told they might lounge about the laboratory throughout days 1 and 3, which were to serve as control periods. It was stated that they would be involved only in the collection of hourly blood samples on each of these 2 days. At the same time it was indicated that each subject would be exposed to cold—0 C. for 30 minutes—on the second day of the experiment. It should be noted that most of the subjects were at least somewhat acquainted with an earlier experiment in which other subjects had been somewhat similarly exposed to cold.

Day 2. All eight subjects again reported at 8 a.m. to the laboratory. Intravenous catheters were replaced and blood samples were obtained. The subjects were reminded that later during the day each would be taken to a cold room for exposure.
The morning was planned to heighten anticipation as much as possible without indicating when the experimental treatment might begin.

Starting at 2 p.m. subjects were taken in pairs to a cold room. This was a small insulated cubicle designed for thermoregulatory experiments and in which the environmental temperature could be closely controlled. As one subject was placed inside the room, the other was seated nearby to await his turn. After each person had been exposed for 30 minutes both subjects were taken to an adjacent room. The one exposed to cold was quickly warmed with blankets and both were then told that the design of the experiment involved sham exposure for one individual and actual exposure to cold for the other. There was no opportunity for communication between pairs exposed to the experimental situation and those awaiting it. After each pair had been similarly treated all eight subjects were returned to the laboratory and hourly blood sampling was continued until 6 p.m.

**Day 3.** Subjects returned to the laboratory at 8 a.m., venous catheters were inserted, and hourly blood samples were obtained with subjects lounging about the laboratory as on the first day of the experiment.

*CHANGES IN SERUM CHOLESTEROL*

**Figure 1**

*Initial cold room experiment. Arrow A indicates announcement of plan for exposure to cold. Arrow E indicates time of exposure to cold.*

**Table 1**

*Subjects Paired on the Basis of Maximum and Minimum Serum Cholesterol Concentrations during Previous Observation*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Exposed to cold</th>
<th>Not exposed to cold</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum serum cholesterol</td>
<td>Minimum serum cholesterol</td>
</tr>
<tr>
<td>W.K.</td>
<td>313</td>
<td>212</td>
</tr>
<tr>
<td>R.S.</td>
<td>282</td>
<td>194</td>
</tr>
<tr>
<td>J.P.</td>
<td>334</td>
<td>257</td>
</tr>
<tr>
<td>H.E.</td>
<td>247</td>
<td>176</td>
</tr>
</tbody>
</table>
Measurement of Cholesterol

Serum cholesterol was measured by the method of Pearson, Stern, and McGavack and all chemical determinations were made by one of us (AAW). Each blood sample was divided and all determinations were in duplicate. Each aliquot was given a code number and the samples were randomized. Technical error of measurement for the paired samples was 2.13.

As a further test of reproducibility, pooled serum was similarly divided and pairs from the same pool were run daily along with other samples. The mean value for these determinations was 229.8 mg./100 ml. and the technical error for these aliquots amounted to 3.40.

Hematocrit levels were measured on each blood sample as a means of discovering any significant changes in blood dilution that might account for variation in the concentration of serum cholesterol. Though some variation in the volume of packed red cells was observed, there appeared to be no consistent relationship between hematocrit level and varying concentrations of serum cholesterol during the course of this experiment.

Results

Hourly values for serum cholesterol concentrations for all eight subjects are shown in table 2, and patterns for the matched pairs are seen in figure 2. These data give further evidence of the fact that serum cholesterol concentration can vary widely during the course of a few hours in certain selected individuals.

A characteristic diurnal pattern is not evident but changes in the concentration of serum cholesterol are rather alike for most of the subjects on any one day of the experiment. This similarity of individual patterns on a particular day is noteworthy, and it is seen during periods of control as well as in conjunction with the experimental treatment.

Variations in the concentration of serum cholesterol recorded on control days of this study equal or exceed those occurring on the day in which subjects were exposed to cold.

This response was unexpected and we can only speculate as to the reasons for it. The changes in cholesterol concentration occurring on control days are not clearly correlated with other known events. It is also apparent that hourly cholesterol patterns of those persons entering the cold room are quite the same as those treated with sham exposure. A double classification analysis of variance, with use of a co-variance adjustment for the initial inequality of the two groups on day 1, showed no significant difference for days, experimental treatment, or their interaction.

Finally it should be noted that the changes in serum cholesterol occurring on day 2 appear to relate more nearly to the announcement that experimental treatment was to begin than they do to exposure itself. This point becomes more evident as the interval lengthens between the announcement and the actual exposure to cold. In most cases it will be noted that a maximum concentration of serum cholesterol was encountered within 2 or 3 hours after the announcement, regardless of the time at which the actual exposure occurred.

Discussion

It is generally recognized that the concentration of serum cholesterol can vary widely from one week or month to the next, but it is less well known that similar changes can also occur within a few hours in certain individuals. This fact must be taken into account when occasional measurements are made and the effects of experimental treatment are assessed. While the diurnal variability in some individuals is minimal, there are others in whom wide variations can be observed even at times when the subjects' environments would appear to be well controlled.

The data here reported, as well as those from an earlier experiment, are consistent with a hypothesis that changes in the concentration of serum cholesterol may relate quite

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*Technical error of measurement = \sqrt{2d^2/2k}
where d is the difference between duplicates and k equals the number of pairs.

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Figure 2

Response of paired subjects during anticipation and exposure to cold. Arrow A indicates announcement of plan for exposure to cold. Arrow E indicates time of exposure to cold.

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closely to the anticipation of a particular event as well as to the event itself. The occurrence of such a relationship does not establish that of cause and effect but it does point up how difficult it is to define a stressor event as to its composition, its timing, and its significance for the subject.

Perhaps we should also comment on two aspects of this experiment that were quite unexpected. Though an effort was made to find comparable subjects and to provide a similar exposure to cold, the changes in serum cholesterol concentration were clearly less striking in this experiment than in the earlier one. Quite apart from the chemical measurements, it was noted by each observer that the second group of subjects seemed much less disturbed by their exposure to cold. They displayed less apprehension and complained much less of cold discomfort. In contrast, the variations in cholesterol concentration occurring on control days of this experiment were greater than we had expected from our earlier experience.

In seeking causes for these differing responses it was obvious that the two experiments had several supposedly minor differences in design despite the fact that they were intended to be comparable. One difference of possible importance is that the first group were taken together to a large walk-in refrigerator where they were kept until some began to complain of the cold. Prior to entering the cold room no time was set for the duration of exposure and they were told simply that they were to remain in the re-

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**Table 2**

*Hourly Values for Serum Cholesterol Concentration (Average of Duplicate Measurements) for Eight Subjects*

<table>
<thead>
<tr>
<th>Time</th>
<th>W.K.</th>
<th>R.S.</th>
<th>J.P.</th>
<th>H.E.</th>
<th>D.F.</th>
<th>P.B.</th>
<th>P.W.</th>
<th>J.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 a.m.</td>
<td>240</td>
<td>215</td>
<td>274</td>
<td>196</td>
<td>236</td>
<td>247</td>
<td>207</td>
<td>184</td>
</tr>
<tr>
<td>10 a.m.</td>
<td>242</td>
<td>205</td>
<td>274</td>
<td>178</td>
<td>252</td>
<td>252</td>
<td>194</td>
<td>167</td>
</tr>
<tr>
<td>11 a.m.</td>
<td>272</td>
<td>227</td>
<td>336</td>
<td>155</td>
<td>205</td>
<td>233</td>
<td>171</td>
<td>152</td>
</tr>
<tr>
<td>12 noon</td>
<td>276</td>
<td>228</td>
<td>295</td>
<td>194</td>
<td>192</td>
<td>245</td>
<td>201</td>
<td>163</td>
</tr>
<tr>
<td>1 p.m.</td>
<td>236</td>
<td>205</td>
<td>263</td>
<td>159</td>
<td>192</td>
<td>216</td>
<td>178</td>
<td>170</td>
</tr>
<tr>
<td>2 p.m.</td>
<td>236</td>
<td>197</td>
<td>263</td>
<td>178</td>
<td>231</td>
<td>239</td>
<td>183</td>
<td>159</td>
</tr>
<tr>
<td>3 p.m.</td>
<td>213</td>
<td>181</td>
<td>251</td>
<td>160</td>
<td>210</td>
<td>230</td>
<td>165</td>
<td>155</td>
</tr>
<tr>
<td>4 p.m.</td>
<td>213</td>
<td>192</td>
<td>258</td>
<td>168</td>
<td>214</td>
<td>238</td>
<td>165</td>
<td>142</td>
</tr>
<tr>
<td>5 p.m.</td>
<td>232</td>
<td>180</td>
<td>165</td>
<td>180</td>
<td>232</td>
<td>247</td>
<td>160</td>
<td>165</td>
</tr>
<tr>
<td>6 p.m.</td>
<td>237</td>
<td>187</td>
<td>...</td>
<td>181</td>
<td>208</td>
<td>248</td>
<td>...</td>
<td>163</td>
</tr>
</tbody>
</table>

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fridge until they were thoroughly chilled. They expected to become uncomfortable and when one began to complain others joined in. Subjects for the second experiment were exposed individually and in a more elegant insulated room. Temperature and time of exposure were identical with those employed in the first experiment, but these were announced and the subjects were already somewhat acquainted with the results of the earlier exposure. While it was known that the first group had been uncomfortable, it was also known that none had suffered unduly from the experience. Regardless of the reason, it is clear that signs of apprehension and discomfort were noticeably less evident among the second group.

A second difference concerns the control days. Subjects in the first experiment were hospitalized and indwelling venous catheters were left in place throughout the experiment. Occasionally a catheter had to be replaced because of technical difficulties, but usually the morning blood sample was obtained without disturbing the subject. Lack of a constant diurnal pattern in earlier experiments made it seem unnecessary to obtain night samples during the current study. On this account the intravenous catheters were removed in the evening, the subjects were allowed to return to their homes, and new catheters were re-inserted each morning. Although the procedure was not painful, there was some anticipation of it and also some apparent relaxation once the day’s activities were under way. In this connection the concentration of serum cholesterol was usually near its highest point when the first morning sample was obtained. This also is in contrast with our previous experience.

The foregoing comments are to emphasize the difficulty in comparing data from experiments that are not identical. They indicate again that stress is defined by the subject, that anticipation may alter his response, and that factors involved in selecting subjects must be considered when one attempts to estimate the significance of a particular measurement of the serum cholesterol concentration.

Summary

Further evidence has been obtained to indicate that the concentration of serum cholesterol can vary widely during the course of a few hours in certain individuals.

The data are consistent with a hypothesis that changes in the concentration of serum cholesterol may relate to the anticipation of an event as well as to the event itself.

Similarity in the hourly variation in serum cholesterol concentration occurring among eight carefully selected subjects, during control as well as during test periods, is noteworthy.

References

Hourly Changes in Serum Cholesterol Concentration: Effects of the Anticipation of Stress

JOHN E. PETERSON, ROBERT A. KEITH and ALAN A. WILCOX

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